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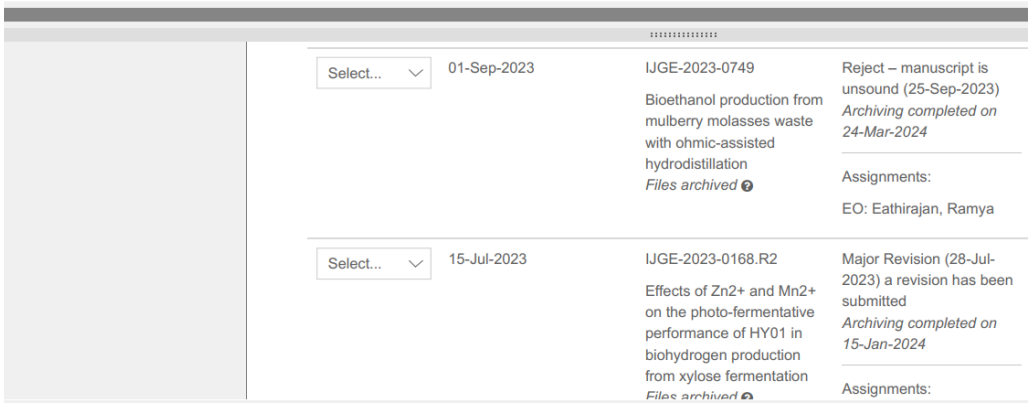

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Submitted review-Bioethanol Production from Mulberry Molasses Waste with Ohmic-Assisted Hydrodistillation

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03-Aug-2023

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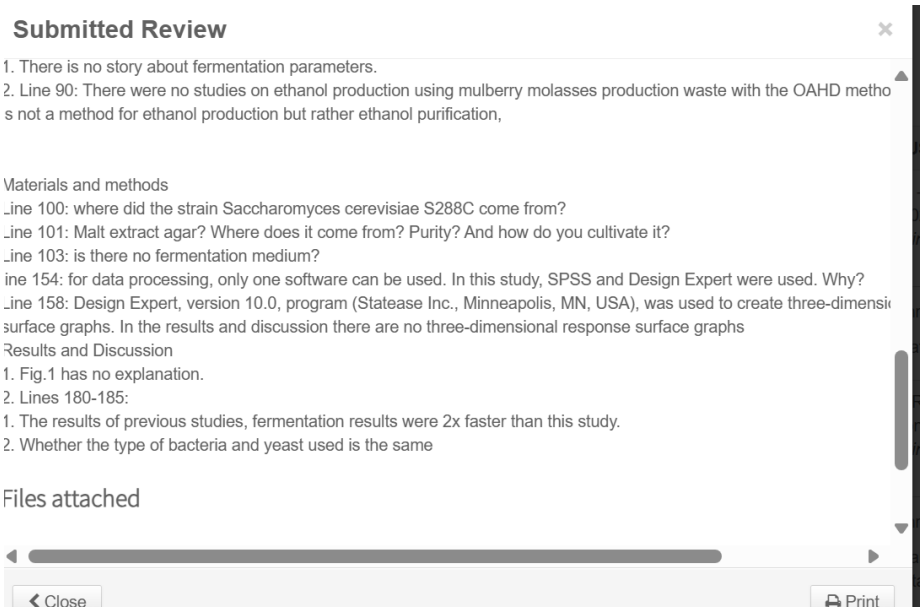

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1. There is no story about fermentation parameters.
2. Line 90: There were no studies on ethanol production using mulberry molasses production waste with the OAHD metho
s not a method for ethanol production but rather ethanol purification,

Materials and methods
Line 100: where did the strain Saccharomyces cerevisiae S288C come from?
Line 101: Malt extract agar? Where does it come from? Purity? And how do you cultivate it?
Line 103: is there no fermentation medium?
ine 154: for data processing, only one software can be used. In this study, SPSS and Design Expert were used. Why?
Line 158: Design Expert, version 10.0, program (Statease Inc., Minneapolis, MN, USA), was used to create three-dimensi
surface graphs. In the results and discussion there are no three-dimensional response surface graphs
Results and Discussion
1. Fig.1 has no explanation.
2. Lines 180-185:
1. The results of previous studies, fermentation results were 2x faster than this study.
2. Whether the type of bacteria and yeast used is the same

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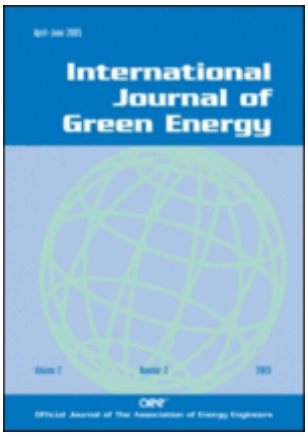

Bioethanol production from mulberry molasses waste with ohmic-assisted hydrodistillation

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Keywords:	bioethanol, Energy consumption, Fermentation, Mulberry molasses waste, Ohmic assisted hydrodistillation

Bioethanol production from mulberry molasses waste with ohmic-assisted hydrodistillation

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Abstract

In this study, the fermentation process of bioethanol production conditions was optimized by response surface methodology. Mulberry molasses production waste was used as the only carbon source for yeast fermentation to produce bioethanol. Hydrodistillation (HD) and ohmic heating assisted hydrodistillation (OAHD) methods were employed to concentrate the bioethanol. Fermentation time (48-168 hours), waste matter rate (5-45%) and pH (5-7) were selected as independent variables. Alcohol concentration was treated as the response. Optimum fermentation conditions were obtained as 96.894 hours fermentation time, 45% waste ratio and pH 7. At these optimum conditions, alcohol concentration was determined as 3.77±0.33%. While the distillate obtained in the HD method contained 22.50±1.89% alcohol, it showed 27.72±0.24% in the ODHD method. The energy consumption values of HD method were 53.24±1.74 Wh/mL ethanol and for the OAHD method was 2.92±0.51 Wh/mL ethanol.

Keywords: Bioethanol, Energy consumption, Fermentation, Mulberry molasses waste, Ohmic assisted hydrodistillation.

1. Introduction

Molasses (Pekmez) is a syrup-like food product that is produced from sugar-rich fruits [1]. Mulberry molasses is a traditional food in Turkey [2]. Although molasses is produced commercially, its traditional production is also quite common in Turkey. In the production of molasses, after the fruit sugar is passed into the syrup, the pulp should be pressed. The remaining part after pressing is waste. This mulberry molasses production waste still contains some amount sugar. Agricultural, food and industrial wastes have economic and ecological importance because of their high organic matter, perishability, high-water and high salt content [3, 4].

Bioethanol is the most promising biofuel these days [5]. Its net CO_2 emission is zero and because of this ethanol has been accepted as a cleaner biofuel [6]. According to reports, when compared to gasoline, corn ethanol can reduce greenhouse gas emissions by 39 to 52% [7]. Although, some countries have been used sugarcane and corn for bioethanol production in industrial scale, it is not suitable to cultivate sugarcane because of climatic limitation. Thus, utilization of food waste is promising resolution for bioethanol production [8]. Ethanol production has been carried out mostly from food wastes which contain carbohydrates such as corn, cassava, sugar beets, and other plants that have high sugar content [5, 6]. Over 90% of the ethanol production is carried out by fermentation in the ethanol industry [6]. Distillation is used to separation from broth and concentration of ethanol. About 40% of total energy consumed in ethanol production is used in the distillation stage [9]. Hydrodistillation (HD) as a traditional method, is consume high energy and time for ethanol concentration. Due to its higher heating rate and lower energy usage when compared to conventional heating techniques, ohmic heating has the potential to be employed for hydrodistillation [10]. Ohmic heating is the process by which food produces heat when an electric current is passed through it due to its electrical current. As a result, thermal energy is produced when an electric current flows through a

substance [11]. Ohmic assisted hydrodistillation (OAHD), which combines ohmic heating with hydrodistillation, produces a greater yield than traditional hydrodistillation methods while taking up less energy and shorter extraction time [12]. Previous researches have reported that in comparison to the conventional HD system, the OAHD approach uses less energy and extracts materials faster [9, 10, 12-14].

There are studies about ethanol production from food wastes such as home food waste [3, 7], expired cookies [8], date wastes [4] and pineapple fruit peel [5] in literature. In the literature, there are some studies in which the OAHD was used to concentrate beer and pure ethanol [9, 14, 15]. There were no studies on ethanol production using mulberry molasses production waste with the OAHD method. Thus, this work aims to optimize ethanol fermentation using mulberry molasses production waste and thereafter to concentrate ethanol by the OAHD method.

2. Material and methods

2.1. Raw material and microorganisms

Mulberry molasses production waste was collected from local people from Gümüşhane, Turkey. The part remaining, after traditional molasses production, was taken and kept at -18°C until use. The composition of the mulberry molasses production waste was determined as 22.33±0.38% dry matter, 4.83±0.16% reducing sugar, 5.11±0.20% total sugar, 6.78±0.46% fiber according to the AOAC methods.

The strain *Saccharomyces cerevisiae* S288C was used for bioethanol fermentation in this study. Malt extract agar was used for the cultivation of *S. cerevisiae* S288C and the strain was grown at 26°C for 24 hours to activate the stock culture.

2.2. Optimization and ethanol fermentation

To optimize the amount of ethanol for optimization, the three-level Central Composite Design with three parameters was used to identify the ideal values of fermentation period (48-168 hours, A), waste ratio (5-45% minutes, B), and pH (5-7) (Table 1). Design Expert software,

version 10.0 (Statease Inc., Minneapolis, MN, USA), was used to perform analysis of variance (ANOVA) tests for lack of fit, determine regression coefficients, and generate three-dimensional graphs.

Ethanol fermentation was carried out in 100 mL conical flasks with different waste ratios and pH at anaerobic conditions. Fifty mL of waste solution was inoculated with seed culture (100 µL) of *S. cerevisiae* S288C (18 hours old culture) and incubated at 26±2°C for different fermentation times. The sample was centrifuged at 10,000□xg at 4°C. The supernatant was used for chromic acid test and gas chromatography analysis.

2.3. Chromic acid test

Chromic acid test was performed to calculate the alcohol content as %. For preparing chromic acid solution potassium dichromate ($K_2Cr_2O_7$) (5 g) was dissolved into 5 mL distilled water, and 100 mL concentrated sulphuric acid was then slowly added into the mixture. After the acid addition, the temperature of mixture was increased and it was cooled to 40°C and stored in a glass bottle. First, 0.1 mL of the fermentation samples at different concentrations were placed in the test tubes and the volume in each tube was completed to 5 mL with distilled water. Then, 5 mL of chromic acid solution was put into the test tubes were incubated at 60°C for 20 min in a water bath [16]. At the end of the incubation, the absorbance for each sample was measured at 584 nm. Different concentrations of pure ethyl alcohol solutions (1, 3, 5, 7, 10, 20 and 30%) were prepared to obtained a standard calibration graph (calibration curve having $R^2 = 0.9930$). Using the calibration curve, the alcohol content under different conditions were determined.

2.4. Alcohol concentration with different distillation methods

Classic Hydrodistillation: Classic hydrodistillation was carried out with all glass condenser using laboratory heater and circulating water bath. Two hundred mL supernatant (with

3.88±0.33 % alcohol), containing 0.5% NaCl, was heated in an apparatus flask. The comparison of the OAHD system was done using a 0.5% NaCl solution.

Ohmic Assisted Hydrodistillation: The OAHD system design is described in previous work [11]. Data loggers were used to record temperature, voltage, and current data at 5-second intervals. 200 mL supernatant containing 0.5% NaCl was used for hydrodistillation. The NaCl solution acquired adequate electric conductivity. The distillation process was maintained until the temperature reached 97ºC. The alcohol amount in the distillate was calculated by the chromic acid test.

2.5. Energy consumption

The OAHD method's energy usage was calculated as kWh/mL for each run. Eq. (1) denotes Q_{ohmic}, whereas Eq. (2) denotes energy consumption;

$$Q_{Ohmic}(j) = \int V \cdot I \cdot dt \qquad \text{Eq. (1)}$$

$$Energy\ consumption\left(\frac{kWh}{mL\ ethanol}\right) = \frac{Q_{ohmic}}{V_{ethanol}} \cdot \frac{1\ hour}{3600\ s} \qquad \text{Eq. (2)}$$

The power meter (TT T-ECHNI-C, China) was used to calculate the amount of energy consumption by the CHD method and was calculated with the Eq. (3).

$$Energy\ consumption\left(\frac{kWh}{mL\ ethanol}\right) = \frac{Q_{heater}\ (kWh)}{V_{ethanol}\ (mL)} \qquad \text{Eq. (3)}$$

2.6. Gas chromatography-mass spectrometry

The essential oils were examined using a Gas chromatography (Agilent GC 7890A) coupled with a mass spectrometry detector (Agilent 5975C VL) and an HP-5MS capillary column (30 m length, 0.25 mm diameter, and 0.25 µm film thickness). Helium gas was used as a carrier gas at a constant flow rate of 1 mL/min. The injector and detector were maintained at a temperature of 250°C. The GC conditions were as follows; the initial temperature was 45°C and with an

increase of 3°C/min, the temperature was increased to 230°C. A sample volume of 2 µL was injected with a 1/25 split ration into the GC/MS.

2.7. Statistical analyses

Three duplicates were used for all analyses. The data were statistically analyzed via SPSS software (version 24, SPSS inc) for ANOVA (analysis of variance). Post hoc-Duncan tests with a 95% confidence level were applied to compare differences in dependent variable means. Design Expert, version 10.0, program (Statease Inc., Minneapolis, MN, USA), was utilized to creating three-dimensional response surface graphs.

3. Results and Discussion

3.1. Optimization

Response surface methodology was carried out for statistical analysis of the relationship between the responses and input variables at fermentation. Table 1 shows the experimental results and fermentation process parameters. Alcohol ratio values were changed in range 0.21 - 4.53 %. The model significances were evaluated using ANOVA (Table 2). The F-test was used to examine the significance of the model, parameters, and lack of fit, as shown in Table 2. The regression coefficient (R^2) of the model for alcohol content was 0.8263. R^2 value implied that fitness of the model between experimental and predicted values. Lack of fit value was not significant ($P>0.05$) indicating that the model was adequately fitted the experimental data. The differences in alcohol concentration compared to independent factors are illustrated in Fig. 2. Increasing waste ratio increased the alcohol content. Although increasing pH and fermentation time was decreased the alcohol content, these parameters were not significantly ($P>0.05$) important for fermentation (Table 2). Process conditions of fermentation were optimized for maximum alcohol content. Optimal process condition for fermentation was found as 96.894 hours for fermentation time, 45.000 % for waste ration and 7.000 for pH. The desirability value

was found as 0.929. alcohol content was found 3.77±0.33% for optimum fermentation conditions. Under optimal process parameters, measured and estimated dependent variables are displayed in Table 3. There was an 11.93% difference between the estimated and measured alcohol content.

Casabar, Unpaprom and Ramaraj [5], produced bioethanol from pineapple wastes at 24, 48 and 72 hours. They were reported that 48 hours of incubation having highest bioethanol production. Anwar Saeed, Ma, Yue, Wang and Tu [6] reported that 44 hours fermentation gives higher ethanol production and they were expressed to this is beneficial for an industrial-scale application. In a study in which fermentation was carried out from food waste, it was reported that high waste ratio provided high alcohol production [17].

3.2. Comparison of distilled product quantity, quality and chemical composition

The initial concentration of alcohol samples used in distillation systems was determined 3.77±0.33%. It was observed that the concentrated alcohols were clear. Distilled ethanol concentration of alcohol samples was found 22.50±1.89% and 27.72±0.24% for the HD and OAHD methods, respectively. Although the alcohol was distilled in a shorter time with the OAHD system, it is seen that the alcohol concentration is higher than the HD system. However, there was not any significant difference between the HD and OAHD's distilled ethanol concentrations ($P>0.05$). Similarly, studies have reported that there is no statistical difference in alcohol samples distilled using the HD and OAHD systems [9, 14, 15].

Fig. 3 shows the GC chromatograms of distilled ethanol samples obtained from the OAHD and HD methods. Main component ethyl alcohol was determined 95.57% and 96.66% in the diluted alcohol for HD and ODHD methods, respectively. In addition, a low amount of isoamyl alcohol, a product of fermentation, was detected (Table 5).

3.3. Comparison of distillation kinetics of hydrodistillation methods

The distillation kinetics parameters of ethanol distillation with different the HD methods are presented in Table 4. Distillation for the HD and OAHD techniques began 97°C, since there was 0.5% salt in both samples. It was essential to heat the feed mixtures 2.22±0.10 min and 8.80±1.10 min for OAHD and HD respectively in order to achieve the boiling point (97 °C) and assemble the first droplet of ethanol. This time difference was found to be statistically significant ($P<0.05$). Distillation duration times were found 0.79±0.16 and 3.58±0.46 minutes for the OAHD and HD systems, respectively. For the same ethanol concentration, the OAHD method needed 2.85±0.31 and HD method 12.38±1.30 minutes, which show that OAHD method almost 4 times faster than the HD method ($P<0.05$). According to Gavahian and Farahnaky [18], ethanol distillation process time might be decreased by up to 34% by changing the HD to OAHD. Rate of ethanol distillation of the OAHD method (1.91±0.21 mL/min) were significantly ($P<0.05$) higher than HD method (10.70±1.93 mL/min). Ohmic heating system enables the heat foods rapid rates while the electric current passes through the foods, generating thermal energy faster than conventional methods. [12, 18, 19]. Therefore, kinetic parameters related to the time, give more advantageous results in the ohmic system. A similar results were reported on using the OAHD for ethanol concentration of fermented broth in comparison to the traditional the HD [9]. Researchers indicated that at the rate of temperature rise, OAHD was about 1.8 times greater than HD. They were also reported that beginning of distillation time, distillation duration and total distillation time of OAHD method were significantly shorter than HD method [9]. It is seen that the ohmic assisted hydrodistillation system gives advantageous results in different hydrodistillation studies. In various ohmic assisted hydrodistillation system studies, it is seen that the OAHD method is more advantageous than the classical method [11, 12, 14, 15, 20, 21].

3.4. Energy consumption and cost comparison of HD methods

Table 4 gives the energy consumption and CO_2 emissions data. The HD was shown to have significantly ($P<0.05$) greater energy usage and CO_2 emitted values than the OAHD.

Energy consumption results for distillation the same quantity of ethanol were lower for the OAHD as compared to conventional HD, because the OAHD method converts 100% of the input energy from electrical to thermal [11]. Consumers pay a lot of attention to green technology because of their low energy usage, clean environment, inexpensive cost, and lack of chemicals. The amount of energy to distillation of ethanol was reduced by 94.52% while using the OAHD technique instead of to the HD method. This finding shows that the OAHD approach, when compared to the HD method, significantly reduces the cost of extracting essential oils. The combustion of fossil fuels will result in the release of 800 g of CO_2 into the atmosphere for every 1 kWh of energy, as described by Seidi Damyeh and Niakousari [22]. OAHD and HD methods emitted 2.34±0.41 and 42.59±1.39 g CO_2/mL ethanol, respectively.

CO_2 emission and energy consumption values have changed in proportion to each other. The shorter distillation time and efficient heating mechanism reduced energy consumption in the OAHD [13]. There are similar findings about energy consumption and CO_2 emission values for the OAHD method for essential oil and alcohol distillation [9, 10]. Researchers indicated that OAHD method consumed lower energy and emitted lower CO_2 than the HD method. In light of these results, an alternative to the conventional HD method for ethanol distillation that is greener and more ecologically friendly is the ohmic assisted hydrodistillation technique.

Conclusion

In the study, bioethanol production was carried out in order to evaluate the mulberry molasses production waste. For this purpose, the fermentation conditions were optimized with three variables (waste rate, fermentation time and pH) and a response (alcohol rate). Optimum fermentation conditions were obtained as 96.894 hours of fermentation time, 45% waste ratio

and pH 7. At the optimum point, 3.77±0.33% alcohol was produced. The alcohol rates which were concentrated by HD and OAHD methods were determined as 22.50±1.89% and 27.72±0.24 %, respectively. Alcohol distillation with the ohmic assisted hydrodistillation provided less energy requirement, less CO_2 emission rate and therefore it can be expressed as a green technology. Bioethanol production has been successfully carried out by using mulberry pulp, which is the production waste of a traditional product. It is seen that cooperation with the industry can be made with regard to future studies and the utilization of these wastes in the industry. Multistage distillation application can be expressed as a technique that can be used in further research in order to reach higher alcohol rates of the obtained distillate.

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Author Contributions

Merve Tuğçe Tunç: Conceptualization; funding acquisition; supervision; data curation; formal analysis; investigation; methodology; writing-original draft. **Berna Genç:** Methodology, writing-original draft. **Şeyda Merve Karataş:** Methodology, writing-original draft.

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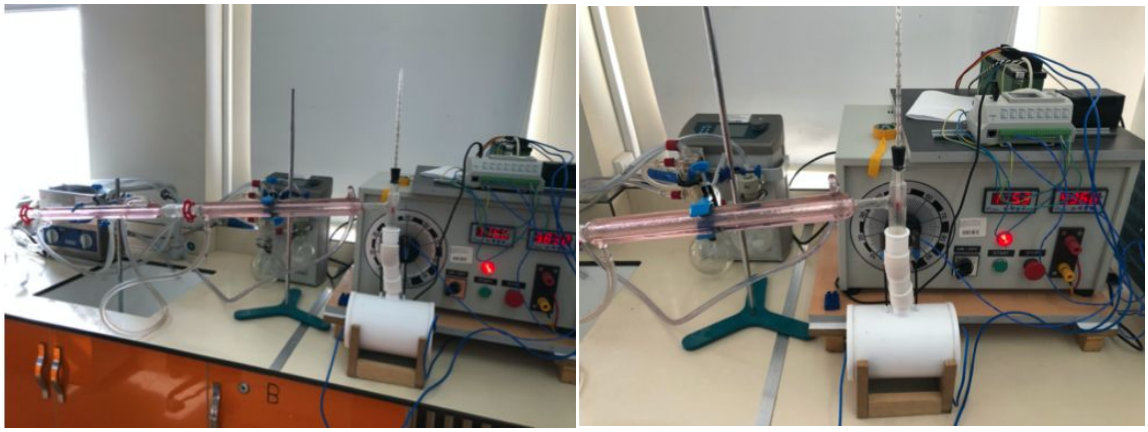

Fig. 1. Ohmic assisted hydrodistillation system

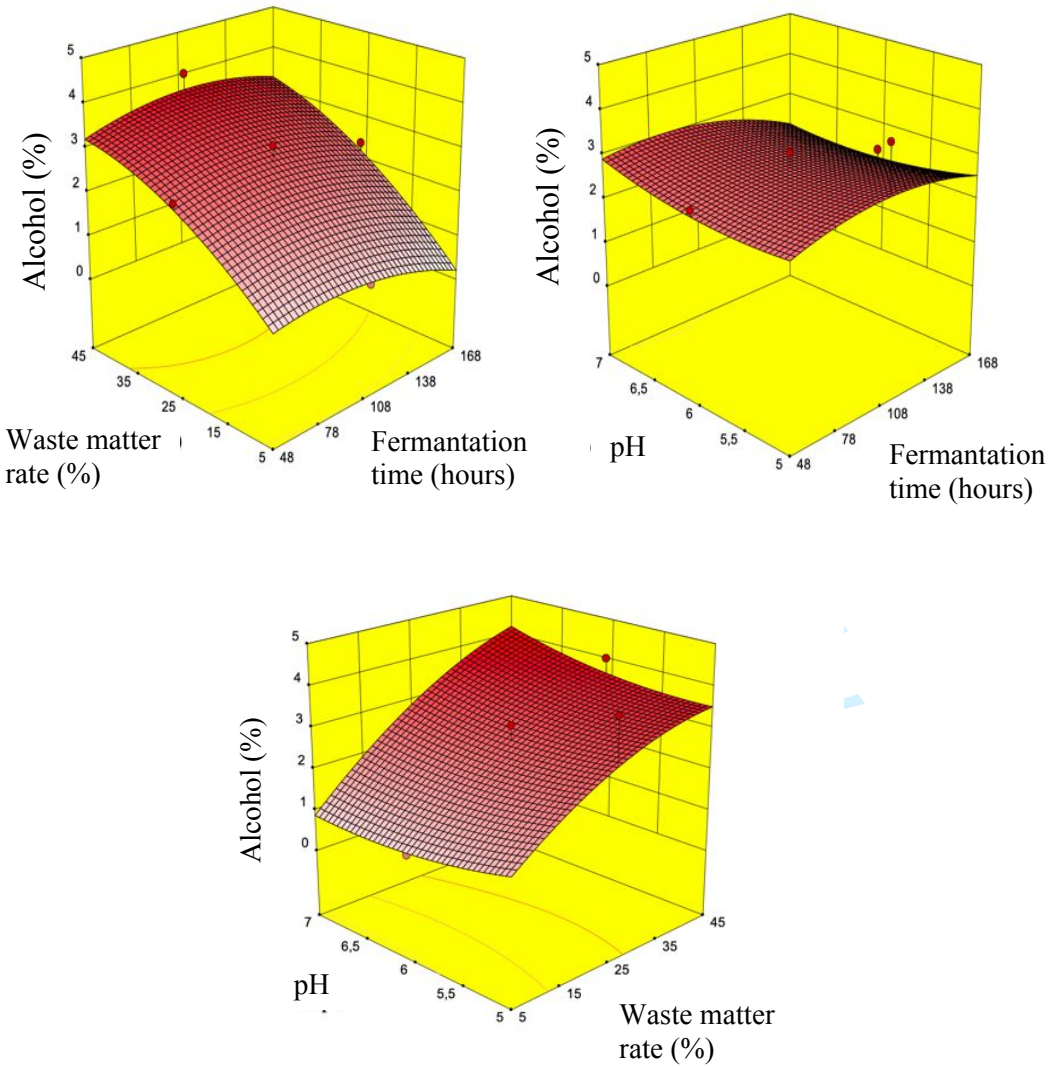

Fig. 2. The change in ethanol ratio of OAHD method versus independent variables.

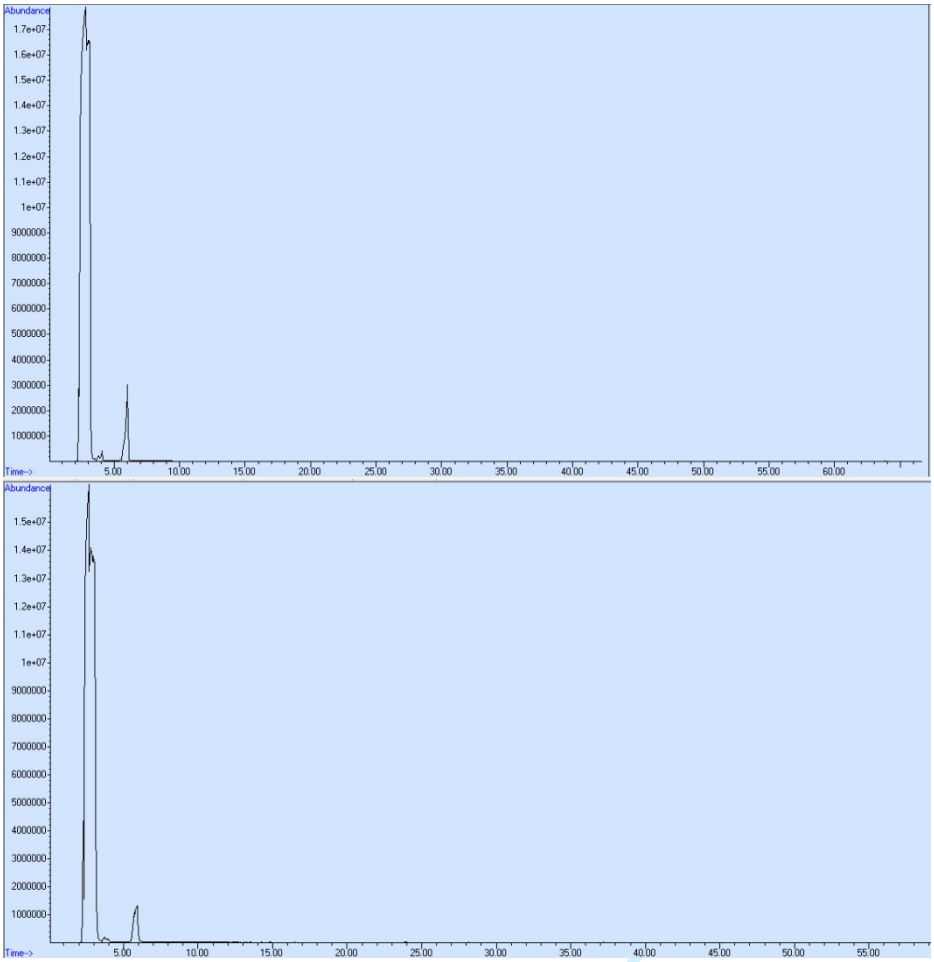

Fig. 3. GC chromatograms of distilled alcohol samples by HD (above) and OAHD (under)

Table 1. Central composite design for fermentation of ethanol production with grape molasses waste and process data.

Std	A: Fermentation time (hour)	B: Waste ratio (%)	C: pH	Ethanol (%)
1	48	5	5	1.27
2	168	5	5	0.21
3	48	45	5	2.18
4	168	45	5	3.41
5	48	5	7	0.85
6	168	5	7	0.66
7	48	45	7	4.53
8	168	45	7	3.43
9	48	25	6	2.60
10	168	25	6	2.38
11	108	5	6	0.85
12	108	45	6	4.01
13	108	25	5	4.05
14	108	25	7	2.15
15	108	25	6	2.65
16	108	25	6	2.44
17	108	25	6	2.12
18	108	25	6	3.07

Table 2. ANOVA table showing the variables as linear, quadratic and interaction terms on response variable

	Sum of squares	**F value**	**p value**
Model	21.96	4.23	**0.0273**
A-Fermentation time	*0.18*	*0.31*	*0.5922*
B-Waste ratio	*18.82*	*32.62*	***0.0004***
C-pH	*0.025*	*0.043*	*0.8403*
AB	*0.24*	*0.41*	*0.5387*
AC	*0.27*	*0.46*	*0.5160*
BC	*0.68*	*1.19*	*0.3079*
A^2	*0.41*	*0.71*	*0.4232*
B^2	*0.55*	*0.95*	*0.3586*
C^2	*0.13*	*0.23*	*0.6456*
Residual	4.62		
Lack of fit	*4.14*	*5.22*	***0.1021***
Pure error	*0.48*		
Total	26.57		
R^2	0.8263		

A- *Fermentation time*, B- *Waste ratio*, C-*pH.*

Table 3. Optimum condition values of CEO according to desirability function.

No	Fermentation time (hours)	Waste ratio (%)	pH	Alcohol	Desirability
1	96.894	45.000	7.000	4.224	0.929
2	96.911	45.000	7.000	4.224	0.929
3	96.207	45.000	7.000	4.223	0.929
Measured values	96.894	45.000	7.000	3.77±0.33	
Differences (%)				11.93	

Table 4. Comparison of different hydrodistillation methods.

	HD	OAHD
Distillation temperature (°C)	97	97
Come up time (beginning of distillation) (min)	8.80±1.10[a]	2.22±0.10[b]
Distillation duration (min)	3.58±0.46[a]	0.79±0.16[b]
Total process time (min)	12.38±1.30[a]	2.85±0.31[b]
Rate of ethanol distillation (mL/min)	1.91±0.21[a]	10.70±1.93[b]
Distilled ethanol concentration (%)	22.50±1.89[a]	27.72±0.24[a]
Distilled ethanol appearance	Clear	Clear
Consumed energy (Wh/mL ethanol)	53.24±1.74[a]	2.92±0.51[b]
Emitted CO_2 (g/mL ethanol)	42.59±1.39[a]	2.34±0.41[b]

In each row, means with different letters are significantly different ($P<0.05$).
HD, hydrodistillation; OAHD, ohmic assisted hydrodistillation.

Table 5. Chemical composition of alcohol concentrated with HD and OAHD methods.

Retention time	Compound	Peak areas (%)	
		HD	ODHD
2.848	Ethyl alcohol	95.565	96.655
6.027	Isoamyl alcohol	4.435	3.345

HD, hydrodistillation; OAHD, ohmic assisted hydrodistillation.