

Determination of Alcohol of Broth of Fermented Black Sticky Rice at Refractive Index and GC-MS Methods

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ABSTRACT

The determining of alcohol contents of fermentations process in making black tape ketan on different fermentations time by means specific gravity, refractive index and GC-MS methods had been carried out. The results of determination of alcohol contents with specific gravity methods at 3, 10, 17, 24, and 31 day fermentations time were 3.17 % v/v; 3.02 % v/v; 3.63 % v/v; 3.12 % v/v; and 4.47 % v/v, respectively, with recovery 82 %. Determination of alcohol contents with refractive index methods at 3, 10, 17, 24, and 31 day fermentations time were 3.90 % v/v; 3.69 % v/v; 4.31 % v/v; 3.80 % v/v; and 5.04 % v/v, respectively, with recovery 88 %. Determination of alcohol contents with GC-MS methods at 3, 10, 17, 24, and 31 day fermentations time were 3.99 % v/v; 4.20 % v/v; 4.42 % v/v; 5.23 % v/v; and 5.30 % v/v, respectively, with recovery 95 %. The length of fermentations time obviously did not influence the produce of alcohol contents.

INTRODUCTION

Type of food fermentation of amount of a lot of and have been recognized by since some times ago in Indonesia. Making of this food fermented have been done many at household environment, this matter is caused by the process of simple enough him and do not cost money the bigness. One of the the product fermented is *tape ketan*. *Tape ketan* is traditional food which comes from West Java (Sastramiharja, 1986). *Tape ketan* is food product of fermentation process constructively yeast using elementary materials soft rice, where final product obtained food felt beloved, a few acid, and alcoholic (Mallouchos *et al.*, 2003). Result beside from making of this *tape ketan* is alcohol. Fermentation time take place during 2-3 days, in the making of *tape ketan*. Alcohol content is expected increasing, if the fermentation time is lengthened.

The main objective of this study was to determine alcohol content of fermentation result of making of black *tape ketan* with different fermentation time.

MATERIALS AND METHODS

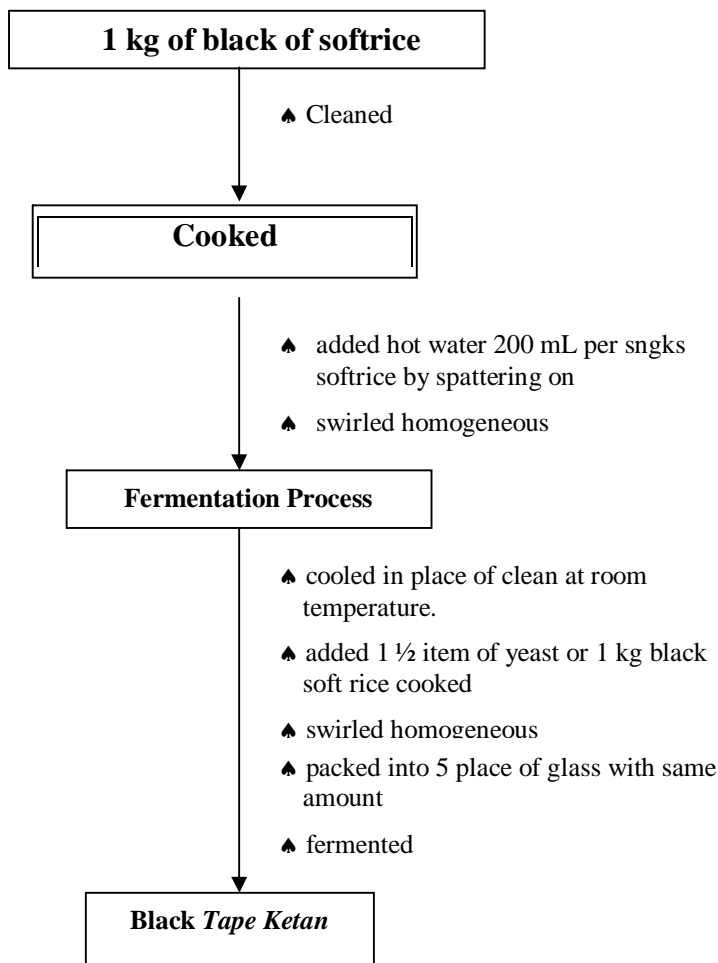
Materials

In this research, materials were used black soft rice of *Oryza sativa* forma *glutinosa*. Yeast used for the process of fermentation was market yeast. Result of determination of yeast at PT. Biofarma that it was consisting of *Amylomyces rouxii*, *Candida pelliculosa*, *Bacillus*, and *Sacharomyces*. Yeast used counted one item halves to one black soft rice kilograms. In this research, three of black *tape ketan* sample (A, B, and C) which made with same treatment to be divided to become five shares differing from is same amount pursuant to differences of

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fermentation time, so that as a whole there are fifteen sample. The fermentation time used was 3, 10, 17, 24 and 31 days.

The process of making *Black Tape Ketan*



Separation of alcohol with distilation methods

For specific gravity and refractive index methods : *Black Tape ketan* was extracted by using cloth filter, then pipetted a number of 25 of mL last in distillation flask, and added water of 25 ml, then it were distilled until get of distillate of 23 mL. Temperature of distillate arranged so that is equal to temperature of sampel at the time of pipette.

For GC-MS : Distillate was extracted with each diethyl ether five times. Every extraction has been done by 25 ml diethyl ether. Distillate were placed funnel appliance apart, then added by diethyl ether 25 ml, funnel apart to be closed, to be shacked. To prevent strong pressure in funnel filter, to be released the by gas of

opened spillway a few on course heel. Result of him formed by 2 coat, where ethanol located in diethyl ether coat or flake. Diethyl ether fraction was accommodated in gourd of erlenmeyer close.

Qualitative Analysis of Alcohol of Result Destilation

Qualitative analysis of alcohol which to consist of a) reaction of colour by using reagen of Potassium of Dichromate 3,5 % in Sulphate acid solution 50 % mass reaction, b) forming of iodoform, c) mass spectrometry at 70 eV, 40-550 amu.

Quantitative of Alcohol Content of Distillate

1. Specific gravity methods

In this research was conducted at temperature 20 °C. Content alcohol in distillate determined by using tables expressing relation between specific gravity with alcohol rate to adaptation of Association Official Agricultural of & Analytical Chemists (AOAC) International. To be able to determine alcdhol content in sample, hence alcohol content in distillate has to be converted beforehand.

2. Refractive index methods

Quantitative of alcohol content by using method of refractive index was based on condensation refractive index. In this research, Instrumentation was used refractometer (Abbe) having measurement area between 1,3000 up to 1,7000. Alcohol content in distillate was obtained by measuring refractive index of distillate which later, then interpolated to curve calibrate.

3. GC-MS Methods

Measurements was performed using a HP 5890 series II gas chromatograph coupled to a HP 5970 series Mass Spectrometer at 70 eV, 40-550 amu, fused silica capillary column (HP-5 (Crosslinked 5% phMe Silicone), 25m x 0.2 mm), helium as carrier gas and temperature programming from 50°C/4 minutes to 150°C/5 min (10°C/ min). Identification of the substances was carried out by mass

spectral data with those from the MS data system Willey-229 lib., Nist-62 lib., and Nist-12 library.

4. Recovery

Recovery of three methods was calculated on the basis of comparison between alcohol p.a and alcohol p.a in distillate (25 ml alcohol p.a was added 25 ml aquadest, then was distilled. All treatment from distillation until to separation of alcohol was same.

RESULT AND DISCUSSION

Black *tape ketan* sample have to range pH 3.83 until 4.56, thereby optimal pH which needed yeast for growth of that is between 4.0-4.5 (Waluyo, 1984) had been fulfilled.

Qualitative Analysis of Alcohol

All distillate give reactions which are positive with reaction of $K_2Cr_2O_7 - H_2SO_4$ productively condensation which is blue greenness. There showed the existence of alcohol compound. (Thompson, 1990). Identifying of alcohol compound with reaction of ioddform showed result of reaction which was positive. Result of reaction forming of iodoform yield typical aroma and formed by rust colored sediment him. If the sediment perceived by using microscope, hence obtained to crystallize flower or wake up hexagon. Pursuant to result identify with reaction of $K_2Cr_2O_7 - H_2SO_4$ and reaction forming of iodoform, hence can be concluded that there are alcohol compound in black soft rice *tape* sample. Fragmentation of mass spectrometry showed that peak of numberd of 2 (can be seen in the Figure 3) was ethanol with ion molecule m/z 46 ($C_2H_5OH^+$), 31 (CH_2OH^+), 29 ($CH_3CH_2^+$), 28 (CH_3CH^+), and 18 (H_2O^+).

Determination of Alcohol Content

1. Specific gravity Methods

Result of determination can be seen at Tables 1.

Table 1. Determination of Alcohol Content with Specific gravity Methods

Black Tape Ketan	Fermentation Time*	Specific gravity of Distillate	% v/v Alcohol in Sample	Recovery
A	0	0.9950	4.01 %	81 %
	1	0.9947	4.26 %	
	2	0.9949	4.10 %	
	3	0.9955	3.61 %	
	4	0.9938	5.01 %	
B	0	0.9971	2.31 %	83 %
	1	0.9975	2.00 %	
	2	0.9956	3.51 %	
	3	0.9966	2.72 %	
	4	0.9940	4.81 %	
C	0	0.9960	3.20 %	82 %
	1	0.9965	2.79 %	
	2	0.9959	3.27 %	
	3	0.9962	3.04 %	
	4	0.9955	3.60 %	

*Fermentation Time : 0= 3 days ; 1= 10 days ; 2= 17 days ; 3= 24 days ; 4= 31 days.

In the Tables 1, black *tape ketan* had alcohol content with range of 3.61 % v/v until 5.01 % v/v, with highest alcohol content equal to 5.01 % v/v when ferment fourth. In the black *tape ketan* of B, obstetrical of alcohol range from 2.00 % v / v until 4.81 % v / , with highest alcohol content, when ferment fourth, that is equal to 4.81 % v / v, while black *tape ketan* of C had alcohol content ranging from 2.79 % v / v until 3.60 % v / v, obstetrical of highest alcohol, when ferment fourth.

It was shown in this research, as a whole indicate that highest alcohol content for the black *tape ketan* of A, B, and C obtained by when ferment fourth or 31 days Curve showing relation between alcohol content in black *tape ketan* to ferment time can be seen at Figure 1.

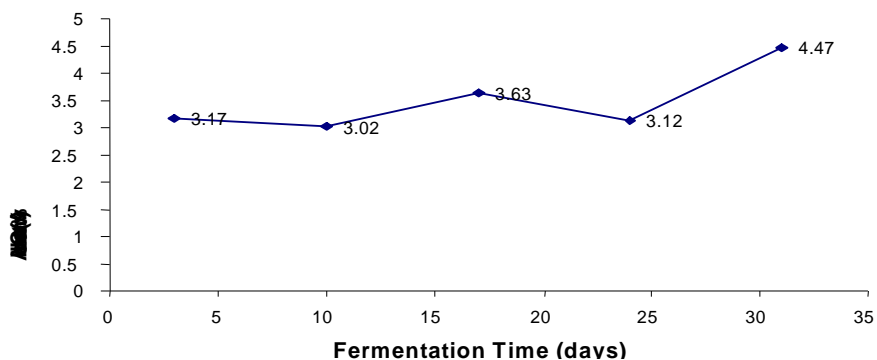


Figure 1. Curve of Alcohol Content to Fermentation Time with Specific gravity

In the Figure 1, Determination of alcohol content with specific gravity method showed the existence of enough difference mean from content alcohol differed to fermentation time him, but result of processing of statistical showed that ($\alpha= 0.01$) there were not significantly between alcohol content with fermentation time. This matter indicated that obtained alcohol content of fermentation process of making of black *tape ketan* had rate which was constant relative, when fermentation of 3 days. So that though, the fermentation time was lengthened, yielded alcohol content did not increase significantly. Belitz and Grosch (1999) explain that the primary fermentation starts within a day and reaches its maximum after 3-4 days, and after the end of main fermentation, which last 5-7 days.

On the basis of previous researches (Sumarmi, 2002), Fermentation time of 0 until 48 hour (day second) do not identify existence of alcohol content. However, when among 72 hours (third day) until 168 hours (day of 7) obstetrical of alcohol range from 1.52 % v/v until 4.22 % v/v. Pursuant to the mentioned, the result of this research correlated with research result conducted by Sumarmi (2002).

Hartoto and Sastramiharja (198) explain that alcohol content periodically reach constant rate, this matter is caused decreasing of glucose content and tolerance of microorganism the lowness to yielded alcohol, so that activity of microorganism become to be pursued. The inhibition of activity of microorganism at fermentation process, possibility is caused by alcohol accumulation at shares intra cell able to yield poisonous cell and cause change of cell membrane structure causing to degradation of cell membrane function (Torija *et al.*, 2003).

2. Refractive Index Methods

Determination of alcohol content by using refractive index methods pursuant to refractive index of sample, then it was interpolated into curve calibrate. The Result of analysis can be seen at Table 2.

Table 2. Determination of alcohol content with Refractive Index Methods

Black Tape Ketan	Fermentation Time*	Refractive index N ²⁰ D	% v/v Alcohol in Sample	Recovery
A	0	1.3356	4.43 %	85 %
	1	1.3354	4.17 %	
	2	1.3354	4.11 %	
	3	1.3352	3.70 %	
	4	1.3360	5.23 %	
B	0	1.3350	3.37 %	90 %
	1	1.3348	3.06 %	
	2	1,3357	4.66 %	
	3	1.3351	3.62 %	
	4	1.3362	5.53 %	
C	0	1.3353	3.90 %	89 %
	1	1.3353	3.83 %	
	2	1.3355	4.16 %	
	3	1.3354	4.07 %	
	4	1.3356	4.35 %	

*Fermentation Time : 0= 3 days ; 1= 10 days ; 2= 17 days ; 3= 24 days ; 4= 31 days.

Linear Equation: $Y = 0,00062 X + 1,33327$; Coefisisen corelation : $r = 0,98903843$

The results given in Table 2 indicated that black *tape ketan* of **A** had alcohol content of range from 3.70 % v/v until 5.23 % v/v with highest alcohol content equal to 5.23 % v/v obtained when ferment fourth. Alcohol contained by balck *tape ketan* of **B** were 3.06 % v/v until 5.53 % v/v with highest alcohol content obtained by when ferment fourth, that is equal to 5.53 % v / v, while *tape ketan* of **C** had alcohol content ranging from 3.83 % v / v until 4.35 % v / v. The file of Table 2 indicated that highest alcohol content of black *tape ketan* of **A**, **B**, and **C** were obtained by when ferment fourth or 31 day. Curve showing relation between alcohol content in black *tape ketan* to ferment time was showed at Figure 2.

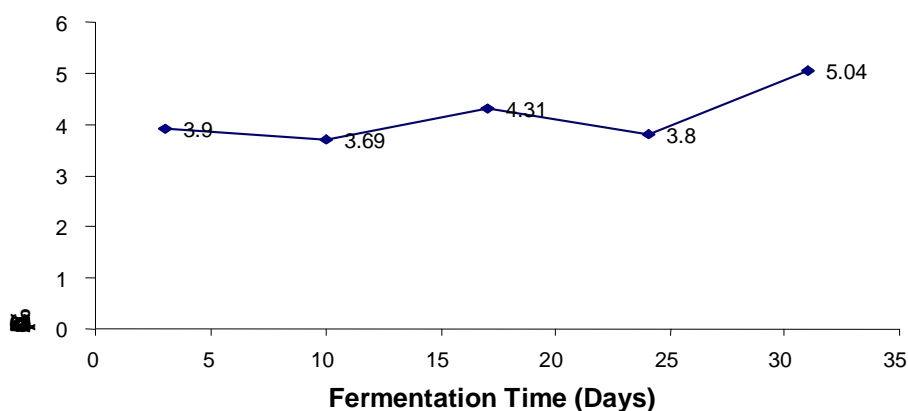


Figure 2. Curve of Alcohol Content to Fermentation Time with Refractive Index Methods

As does at specific gravity methods, descriptively, alcohol content with refractive index method showed the existence of difference of obstetrical result of enough alcohol mean differed time him of fermentation, but statistically indicate that difference of ferment time did not give difference significantly to yielded alcohol content.

3. GC-MS Methods

The Result of analysis can be seen at Table 3.

Table 3. Determination of alcohol content with GC-MS Methods

Black Tape Ketan	Fermentation Time**	Peak Area**	% v/v Alcohol in Sample**	Recovery
A	0	3043213255	4.01 %	85 %
	1	3263296009	4.30 %	
	2	3430255339	4.52 %	
	3	4022202058	5.30 %	
	4	4113270784	5.42 %	
B	0	3020446074	3.98 %	90 %
	1	3157049162	4.16 %	
	2	3301241312	4.35 %	
	3	3984256755	5.25 %	
	4	4128448905	5.44 %	
C	0	3020446074	3.98 %	89 %
	1	3141871041	4.14 %	
	2	3407488158	4.49 %	
	3	3900777089	5.14 %	
	4	3840064606	5.06 %	

* Fermentation Time : 0= 3 days ; 1= 10 days ; 2= 17 days ; 3= 24 days ; 4= 31 days.

**Peak area of standard etanol p.a (100 %) = 75890604863

$$** \text{ Calculated of equation} = \frac{\text{peak area sample} \times 100 \%}{75890604863}$$

In GC-MS methods, alcohol was detected at retention time of range 2-3 minutes (Figure 3), while alcohol content determined at 3, 10, 17, 24, and 31 day fermentations time were 3.99 % v/v; 4.20 % v/v; 4.42 % v/v; 5.23 % v/v; and 5.30 % v/v, respectively. Recovery analysis of this method was found with higher recovery excess than other methods.

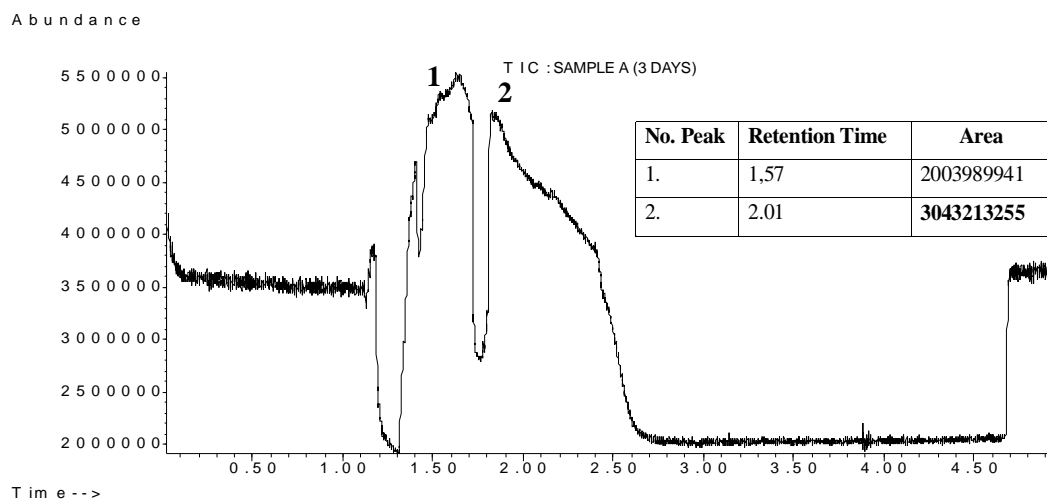


Figure 3. Total ion chromatogram of black tape ketan sample after distillation for sample of 3 days fermentation. No. peak 1 (1,57 minutes) was diethyl ether (solvent) and no. 2 (2,01 minutes) was ethanol.

Alcohol content obtained of fermentation result at making of black *tape ketan* had rate which is constant relative. Alcohol content of constant reached when ferment 3 day.

The result of determination by specific gravity methods yielded alcohol content of range 3.02 % v/v until 4.47 % v/v; whereas the concentration of alcohol identified by refractive index methods was 3.69 % v/v until 5.04 % v/v, and GC-MS methods determined of alcohol with range 3.99 % v/v until 5.30 % v/v.

Ardhana and Fleet (1989) showed that in the yeast of *tape* are *Amylomyces rouxii*, *Candida pelliculosa*, *Saccharomyces cerevisiae*, and bacteria from family of *Bacillus* and *Acetobacter*. Microorganism identified of yeast of *tape* in be used this research correlated with results of Ardhana and Fleet (1989) researched, but it did not find *Acetobacter*.

CONCLUSION

The result of determination of alcohol content with specific gravity methods was obtained that alcohol content in black *tape ketan* with ferment time 3, 10, 17, 24, and 31 days successively, that was : 3.17 % v/v; 3.02 % v/v; 3.63 % v/v; 3.12 % v/v; and 4.47 % v/v; with recovery analysis was 82 %, while with refractive index methods of alcohol in black *tape ketan* successively, that was : 3.90 % v/v; 3.69 % v/v; 4.31 % v/v; 3.80 % v/v; and 5.04 % v/v, with recovery 88 %, and with method of GC-MS each 3.99 % v/v; 4.20 % v/v; 4.42 % v/v; 5.23 % v/v; and 5.30 % v/v, with recovery 95 %.

Highest alcohol content to three types of determination methods obtained at fermentation for 31 days. Result of processing of statistical obtained conclusion that the duration ferment time in the reality did not influence yielded alcohol content.

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