DETERMINATION OF ALCOHOL CONTENTS OF FERMENTATED BLACK *TAPE KETAN* BASED ON DIFFERENT FERMENTATION TIME USING SPECIFIC GRAVITY, REFRACTIVE INDEX AND GC-MS METHODS

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ABSTRACT

Tape ketan is the product of fermentation process using black glutinous riceas basic ingredients, which then fermented for two to three days and finally produced tape ketan, organic acid and alcohol. The purpose of this research was to determine alcohol contents of fermentated black tape ketan based on different fermentation time with three diferent methods. Methods used are specific gravity, refractive index and GC-MS. Alcohol concentration obtained by using specific gravity method at 3, 10, 17, 24, and 31 days of fermentation, respectively, are 3.17% v / v; 3.02% v / v; 3.63% v / v; 3 , 12% v / v; and 4.47% v / v, using the method of refractive index is 3.90% v / v; 3.69% v / v; 4.31% v / v; 3.80% v / v and 5.04% v / v, and using GC-MS method was 4.30 % v/v; 4.23 % v/v; 5.01 % v/v; 4.75 % v/v; and 5.34 % v/v.The variation of fermentation time obviously did not influence the produce of alcohol contents statistically.

Keywords: Tape ketan, specific gravity, refractive index, GC-MS methods, fermentation

INTRODUCTION

Fermentated food is agricultural product (**Akoh et al., 2008**) which has been processed action of microorganisms (bacteria, yeast, and mycelia fungi)into desired food product (**Nout, 2003**). This product will have more attractive characteristic compared with is original source. The advantages of processed fermentated food is that it can increase economical value. This food play an important role in the diet of people in many parts of the world, even not only provide important sources of nutrients but have also great potential in maintaining health and preventing diseases (**Kabak and Dobson, 2011**).

The types of fermentated food are various and has been famous since years in Indonesia (**Beuchat, 2008**). The processing of fermentated food mostly produced at home industries scale, because it is cheap and simple. One of this fermentated product is *tape ketan*.*Tape ketan* is a traditional food originated from West Java (**Sastramiharja, 1986**). *Tape ketan* is often made at the time of Eidfor a meal the guests who come to stay in touch in Sundanese people. It is the product of fermentation process using cooked black sticky riceas basic ingredients, which fermentated for two to three days and finally produced tape ketan, acid and alcohol (**Cronk et al., 1977**). Previous research of tape ketan by other country authors has been published since long time ago. **Cronk et al. (1979)** describe well of this product. Tape ketan is fermented by using culture *A. rouxii, S. cerevisiae*, and *P. Anomale* (**Cronk et al., 1977, Siebenhandl et al., 2001**). The other Inodnesian tape, the lactic acid bacteria (LAB) are among the integral microflora of yeast of Balinnese tape (**Sujaya et al., 2002**). Tape ketan ready for consumption after fermentation at 30°C for 36 to 48 h, however tape ketan will produce the highest concentration of ethanol until 8% (v/v) by the mold plus *E. burtonii* at 144 h, even Cronk et al.also detected isobutanol, amyl alcohol, and isamyl alcohol in tape ketan produced after 36 hours at 30°C. The level of alcohol total will reach up 600 mg/L (6 % v/v) after 8 days (**Cronk et al., 1979**).

The higly concentration of alcohol of side product is obtained as disadvantages of *tape ketan* process. Alcohol content is produced increasing, if the fermentation time is lengthened, but this alcohol of side product is not allowed to be consumed over 1 % by moslems in Indonesia. The purpose of this research was to determine alcohol contents of fermentated black *tape ketan* based on different fermentation time and three diffrent methods.

MATERIAL AND METHODS

Materials

Materials used in this research were black ketan rice *of Oryza sativa* forma *glutinosa* (determined by Department of Biology of FMIPA UNPAD). Yeast used for the process of fermentation were market yeast which consisted of *Amylomyces rouxii, Candida pelliculosa, Bacillus*, and *Sacharomyces* (determined by PT. Biofarma, Bandung), alcohol p.a (Merck), alcohol analysis grade, sulfuric acid p.a. (Bratachem), potassium dichromate (Bratachem), NaOH p.a. (Merck), and iodium solid p.a. (Merck).

The process of making black *tape ketan*

Three kilograms of black sticky rice (*Oryza sativa* forma *glutinosa*) were used as the basic ingredient to produce three samples (A, B, C, and D) with same treatment to purpose repeating sample of black *tape ketan* with the same treatment, but it was produced at different time and at 30 (A, B, and C) and (D) 25°C (room temperature). *Amylomyces rouxii, Candida pelliculosa, Bacillus*, and *Sacharomyces* were used as the yeasts. Each sample was divided into five different parts and was treated with various fermentation time (3, 10, 17, 24, 31 days).

Separation of alcohol with distillation methods

For specific gravity and refractive index methods :Black *tape ketan* obtained was extracted using cloth filter, then pipetted an aliquot of 25 mL into distillation flask. Water was added (25 ml), further distilled to obtain 25 mL of distillate. The temperature of distillate was arranged until it was equal to the temperature of the sample during the pipetting.For GC-MS : Distillate was extracted each with diethyl ether for five times. Every extraction was done using 25 mL of diethyl ether. Distillates were filtered off and then added by diethyl ether 25 mL, and were homogenized by shaking.

Qualitative analysis of alcohol in the distillate

Qualitative analysis of alcohol were conducted by : a) color reaction using potassium dichromate 3,5 % in sulphuric acid, b) iodoform reaction, c) mass spectrometry at 70 eV, 40-550 amu.

Quantitative analysis of alcohol in the distillate

Specific gravity methods

This method was conducted at 20 $^{\circ}$C. Alcohol in the distillate was determined using tables expressing relation between specific gravity with alcohol rate (Association Official Agricultural of & Analytical Chemists International, 2000, New York, USA). To be able to determine alcohol content in sample, alcohol content in the distillate has to be converted before. The distillation methods was showed as mention above (Horwitz, 2000).

Refractive index methods

The refractive index method was based on condensation refractive index. The instrument used was refractometer (Abbe) with measurement area between 1,3000 up to 1,7000. Alcohol content in the distillate was obtained by measuring the refractive index of distillate which then was interpolated to make calibration curve.Determination of the refractive index which is not carried out at 20 ° C should be corrected first by using the equation:

n^{20}_{D} = Refractive Index Sample (n) + 0,0004 (T- 20°C)   (Horwitz, 2000)

n^{20}_{D} -refractive index correction

T   - observation temperature

GC-MS

Measurement was performed using a HP 5890 series II Gas Chromatography 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 $50^{o}C/4$ minutes to $150^{o}C/5$ min ($10^{o}C/$ 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.

Recovery

Recovery of the three methods were calculated on the basis of comparation between alcohol standard and alcohol in distillate (25 mL alcohol standard was added into 25 mL distilled water, then the mixture was distilled). All treatment from distillation to separation of alcohol was conducted in similar conditions.

pH

The pH range of black *tape ketan* samples were 3.83 to 4.56, thereby optimal pH which needed by yeast for growth which lied between 4.0-4.5 had been fulfilled (Siebenhandl et al., 2001).

RESULTS AND DISCUSSION

All distillates gave positive results when reacted with $K_2Cr_2O_7$-H_2SO_4 which produced greenish blue colour. There showed the existence of alcohol compound. (Thompson, 1990). Identification of alcohol using iodoform reaction showed positive result which proven by the arousing of characteristic iodoform odour and the formation of colored precipitation. When the precipitate was placed under microsope we could obtain hexagonal crystal or flowered form crystal. Pursuant to result identify with reaction of $K_2Cr_2O_7$ - H_2SO_4 and reaction forming of iodoform, hence it could be concluded that there were alcohol compounds in black *tape ketan* samples. Fragmentation of Mass Spectrometry showed that peak number2 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^+).

The result of determination of alcohol with specific gravity method can be seen at Tables 1.

Table 1 Determination of Alcohol Levels with Specific gravity method

Black *tape ketan* sample*	Fermentation Duration[**]	Specific gravity in distillate	Specific gravity in sample	% v/v alcohol distillate	% v/v alcohol sample[***]	Recovery
A	0	$0.9950 \pm 2.0 \ 10^{-4}$	$1.1743 \pm 1.5 \ 10^{-4}$	3.4 ± 0.10	4.01 ± 0.02	
	1	$0.9947 \pm 1.5 \ 10^{-4}$	$1.1749 \pm 1.1 \ 10^{-4}$	3.61 ± 0.09	4.26 ± 0.02	
	2	$0.9949 \pm 2.2 \ 10^{-4}$	$1.1762 \pm 2.4 \ 10^{-4}$	3.47 ± 0.12	4.10 ± 0.03	
	3	$0.9955 \pm 3.1 \ 10^{-4}$	$1.1798 \pm 1.4 \ 10^{-4}$	3.05 ± 0.19	3.61 ± 0.03	
	4	$0.9938 \pm 5.0 \ 10^{-4}$	$1.1682 \pm 3.2 \ 10^{-4}$	4.26 ± 0.21	5.01 ± 0.08	
B	0	$0.9971 \pm 1.5 \ 10^{-4}$	$1.1834 \pm 1.5 \ 10^{-4}$	1.95 ± 0.11	2.31 ± 0.01	81 %
	1	$0.9975 \pm 3.2 \ 10^{-4}$	$1.1866 \pm 3.5 \ 10^{-4}$	1.68 ± 0.18	2.00 ± 0.02	
	2	$0.9956 \pm 2.4 \ 10^{-4}$	$1.1742 \pm 2.1 \ 10^{-4}$	2.98 ± 0.13	3.51 ± 0.02	
	3	$0.9966 \pm 4.1 \ 10^{-4}$	$1.1833 \pm 1.8 \ 10^{-4}$	2.29 ± 0.20	2.72 ± 0.03	
	4	$0.9940 \pm 1.6 \ 10^{-4}$	$1.1640 \pm 1.2 \ 10^{-4}$	4.11 ± 0.12	4.81 ± 0.01	
C	0	$0.9960 \pm 3.1 \ 10^{-4}$	$1.1794 \pm 1.7 \ 10^{-4}$	2.70 ± 0.17	3.20 ± 0.02	
	1	$0.9965 \pm 3.4 \ 10^{-4}$	$1.1795 \pm 1.4 \ 10^{-4}$	2.36 ± 0.15	2.79 ± 0.03	
	2	$0.9959 \pm 2.3 \ 10^{-4}$	$1.1761 \pm 3.8 \ 10^{-4}$	2.77 ± 0.13	3.27 ± 0.01	
	3	$0.9962 \pm 5.5 \ 10^{-4}$	$1.1784 \pm 2.5 \ 10^{-4}$	2.57 ± 0.24	3.04 ± 0.09	
	4	$0.9955 \pm 2.2 \ 10^{-4}$	$1.1746 \pm 4.1 \ 10^{-4}$	3.05 ± 0.18	3.60 ± 0.01	

* A, B, and C was three sample of black tape ketan which produced with the same treatment,

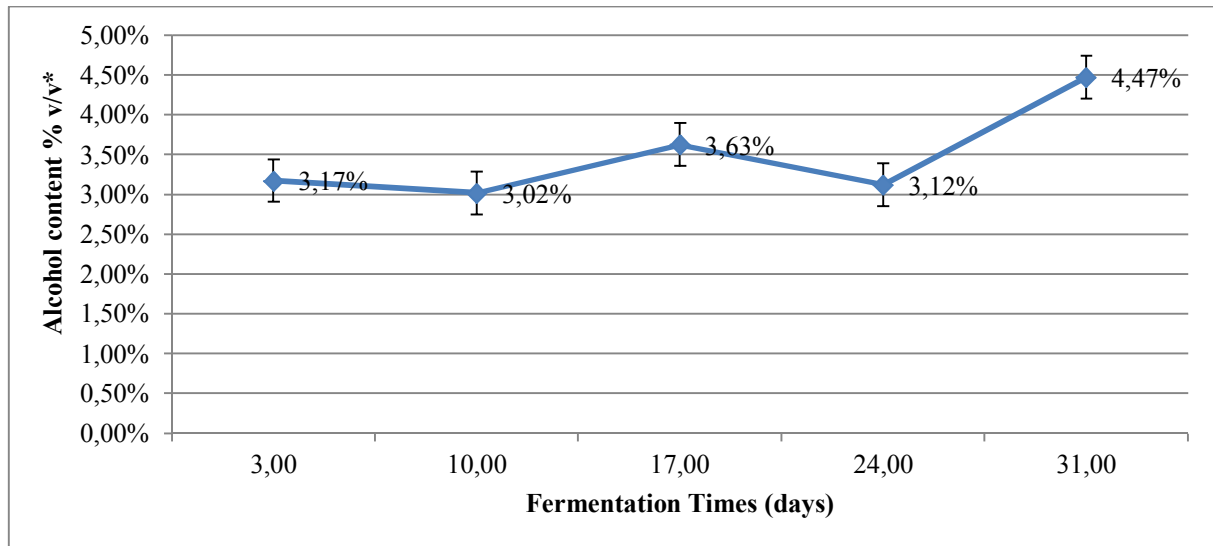But it was produced at different moment

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

*** % alcohol in sample was determined according to equation as mention below:

$$\% \text{ v/v alcohol in sample} = \frac{(\% \text{ v/v alcohol in distillate}) \text{ x (specific gravity in sample)}}{(\text{Specific gravity in distillate})}$$

In the Table 1, black *tape ketan* A contained alcohol in the range of 3.61 % *v/v* to 5.01 % *v/v*, with the highest alcohol content equal to 5.01 % *v/v* when fermentated for 31 days. In the sample B, the concentration of alcohol ranged from 2.00 % *v/v* to 4.81 % *v/v*, with the highest alcohol content equal to 4.81 % *v/v* when fermentated for 31 days, while sample C were 2.79 % *v/v* until 3.60 % *v/v* with the highest alcohol content equal to 3.60 % % *v/v* when fermentated for 31 days

This research proved that the highest alcohol content in the three black *tape ketan* samples obtained in 31 days fermentation time. Curve showing relation between alcohol content in black tape ketan to fermentation time can be shown at Fig. 1.

* Alcohol contents (%) are mean averages of samples A, B, and C in same fermentation times

Figure 1 Curves of Alcohol Content to Fermentation Time with Specific Gravity

In the Fig. 1, determination of alcohol content with specific gravity method showed the existence of enough difference mean from content alcohol differed to fermentation time, but result of processing of statistical showed that (α= 0.01) there were not significant difference between alcohol content with fermentation time. This matter indicated that obtained alcohol content of fermentation process of making of black *tapeketan* 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 **(Belitz and Grosch, 1999)**.

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 (7th day) 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 (1987) 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)**.

Determination of alcohol content using refractive index methods pursuant to refractive index of sample, then it was interpolated into calibration curve. The result of analysis was shown at Table 2.

Table 2 Determination of Alcohol Levels with Refractive Index Methods*

Sample	Fermentation Times*	Temperature	Refractive Index Correction n^{20}_D	% v/v Alcohol in distilate	% v/v Alcohol in sample	Recovery
	0	1.1743	1.33560	3.75 ±0.040 %	4.43 %	
	1	1.1749	1.33546	3.53 ±0.026 %	4.17 %	
A	2	1.1762	1.33542	3.48 ±0.016 %	4.11 %	
	3	1.1798	1.33520	3.12 ±0.066 %	3.70 %	
	4	1.1682	1.33603	4.45 ±0.018 %	5.23 %	
	0	1.1834	1.33503	2.84 ±0.018 %	3.37 %	
	1	1.1866	1.33486	2.57 ±0.016 %	3.06 %	
B	2	1.1742	1.33572	3.95 ±0.046 %	4.66 %	89 %
	3	1.1833	1.33516	3.05 ±0.045 %	3.62 %	
	4	1.1640	1.33620	4.72 ±0.016 %	5.53 %	
	0	1.1794	1.33531	3.29 ±0.055 %	3.90 %	
	1	1.1795	1.33528	3.24 ±0.026 %	3.83 %	
C	2	1.1761	1.33546	3.52 ±0.040 %	4.16 %	
	3	1.1784	1.33540	3.44 ±0.096 %	4.07 %	
	4	1.1746	1.33556	3.69 ±0.048 %	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 + 1.3327; Coefisisen corelation : r = 0,99

*** Percentage of alcohon in sample was determined according to equation as mention below:

$$\% \text{ v/v alcohol in sample} = \frac{(\% \text{ v/v alcohol in distillate}) \text{ x (refractive index in sample)}}{(\text{refractive index in distillate})}$$

The results were 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 black *tap eketan* 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 data of Table 2 indicated that highest alcohol content of black *tapeketan* 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 Fig. 2.
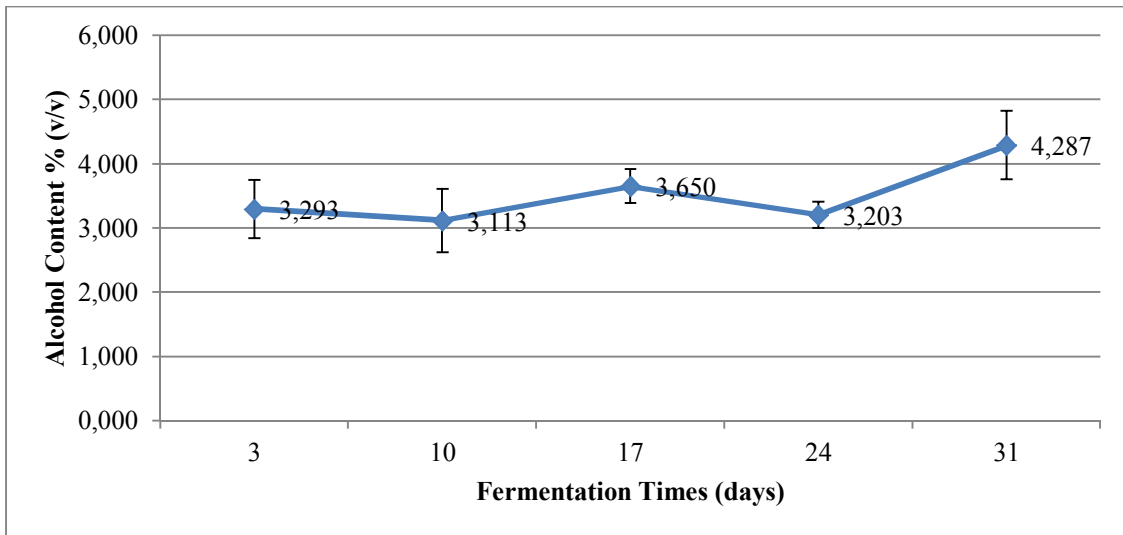
Figure 2 Curve of Alcohol Content to Fermentation Time with Refractive Index Methods

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

Prior to determine of alcohol by using GC-MS methods, standard curve was obtained. The peak area were exposed to different concentrations of alcohol (% v/v) (10ppm or 0.001 %, 25ppm or 0.0025 %, 50ppm or 0.0050 %, 75 ppm or 0.0075 %, 100 ppm or 0.010 %, 125 ppmor 0.013 %, 150 ppmor 0.015 %, and 200 ppm or 0.020 %. Alcohol content in sample was calculated according to linear regression equation from the data (Peak Area = 3832.4(ppm) + 753433, r2 = 99.38 %) by Microsoft office XL 2007 as shown on Fig. 3.
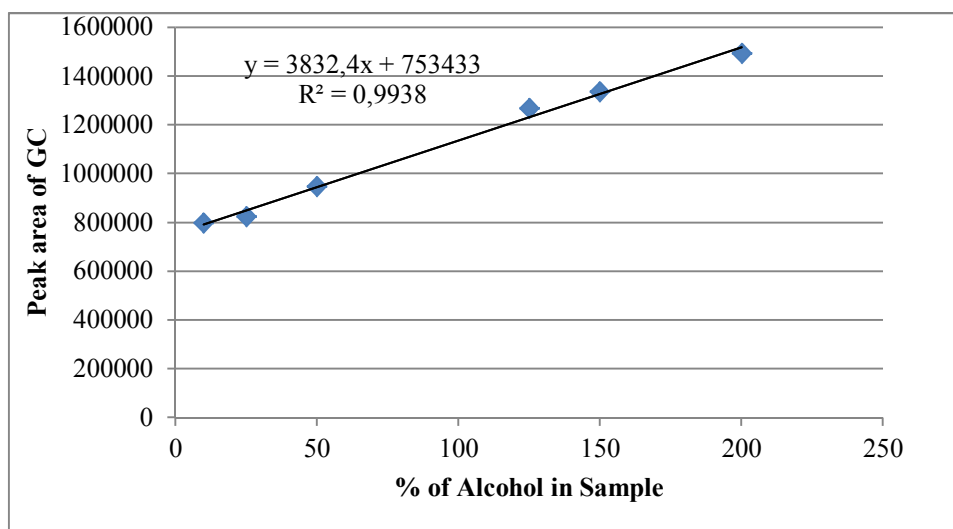

Figure 3 Curve standard of ethanol level was determined by using GC-MS

The result data of analysis alcohol in sample can be seen at Table 3. The peak area of alcohol in sample was insided into regresion equation as mention above thus obtain level of alcohol in sample. Recovery of GC analysis method (89 %) showed that this method has a fairly good accuracy, where the method has capable to be extracted well alcohol from sample.

Table 3 Determination of alcohol content with GC-MS Methods

Black *TapeKetan*	Fermentation Time**	Peak Area	% v/v Alcohol in Sample**	Recovery
A	0	3043213255	4.04	
	1	3263296009	4.33	
	2	3430255339	4.55	
	3	4022202058	5.34	
	4	4113270784	5.46	
B	0	3020446074	4.01	91 %
	1	3157049162	4.19	
	2	3301241312	4.38	
	3	3984256755	5.29	
	4	4128448905	5.48	
C	0	3020446074	4.01	
	1	3141871041	4.17	
	2	3407488158	4.52	
	3	3900777089	5.18	
	4	3840064606	5.10	

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

In GC-MS methods, alcohol was detected at retention time of range 2-3 minutes, while alcohol content determined at 3, 10, 17, 24, and 31 day fermentations time were 4.295 % v/v; 4.23 % v/v; 5.005 % v/v; 4.747 % v/v; and 5.344 % v/v, respectively. Recovery analysis of this method was found be higher than in other methods. In this study, GC-MS is most reliability tools for analysis alcohol. In the previous research, GC also is applied in analysis of alcohol in wine fermented by Okunowo and Osuntoki (2007). They determine that ethanol is predominant in fermented product (**Okunowo and Osuntoki, 2007**).

In fact, the other methods analysis of alcohol in fermented food have been developed very advanced. Preparation methods in alcohol analysis of fermented food also develops very advanced. The recent method of alcohol analysis (in fermented food) explained by

Pietsworski (2012). SPE-headspace could trap volatile compounds such as alcohol thus headspace iss more efficient in the extraction of the volatile compounds of fermented apple when taking into consideration the cost effectiveness **(de Arruda Moura Pietrowski et al., 2012).**
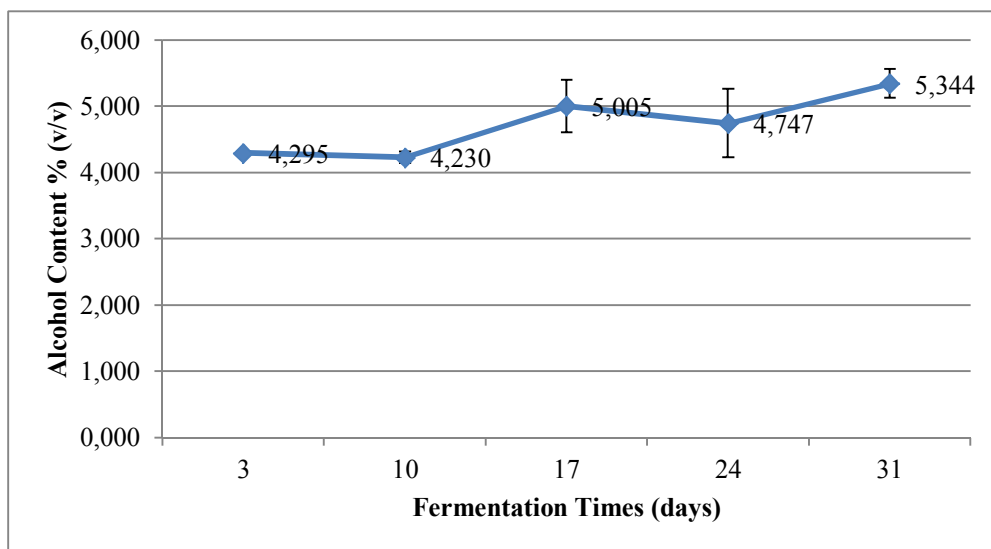

Figure 4 Curve of alcohol levels to fermentation time with GC-MS methods

Alcohol content obtained of fermentation result at making of black *tape ketan* had rate which is constant relative. Alcohol content of constant reached when fermented 3 day. Cronk et al. described that alcohol level of *tape ketan* would be increase more than 2 % after 2 days (48 hours), and reach up to more than 4 % after 4 days (96 hours). In other their publication, Cronk et al explained that total alcohol of tape ketan reach up 6 % or 600 mg/L after 8 days **(Cronk et al., 1979)**.

Our results of determination by specific gravity methods yielded alcohol level of range 3.02 % v/v until 4.47 % v/v; whereas the concentration of alcohol identified by refractive index methods was3.69 % v/v until 5.04 % v/v, and GC-MS methods determined of alcohol with range 4.29 % v/v until 5.30 % v/v. Whereas, Cronk et al. claimed that alcohol level will increase to 8% after 6 days and we found that alcohol level was around 4-5 % after 10 days **(Cronk et al., 1977)**. However, the alcohol content depends on some factor including microorganism types and environment research

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* which be used this research correlated with previous research **(Ardhana and Fleet, 1989, Cronk et al., 1977, Siebenhandl et al.,**

2001), however it did not find *Acetobacter.* In Balinnese tape, yeast or *ragi starter* consist Pediococcus, Weissella and Enterococcus consistently during the tape fermentation; however the Lactobcillus sp. started to grow after 24 h fermentation then its population likely decrease afterwards (**Sujaya et al., 2010)**. It is similar microorganisms contained in Vietnamese tape or *banh men* (**Thanh et al., 2008**). In this study, bacteria from family LAB (lacto bacillus) also was detected.

We found clearly that alcohol level of *tape ketan* after more than 3 days was more than 1 %. Previous and our study might provide answers to some hitherto unsolved questions about *halal* status of tape ketan. Alcohol of tape ketan is categorized as khamr, its use could be regarded haram, regardless if it could intoxicate or not. The ruling concerning 'khamr' is that even the most very small amount of it is *haram*, regardless of whether it intoxicates or not **(Kramer et al., 2007)**. However, it is not fair if we decide that the *tape ketan* is haram. Previous study showed that alcohol level of *tape ketan* less than 1 % between 2-3 days, furthermore Muslim can consume tape ketan at this 2-3 days fermentation. In other hand, we determined alcohol content in tape ketan of sample D in different time with sample A, B, and C that found alcohol level in the sample D less than 1 % at 25°C of 1-3 days fermentation using *Amylomyces rouxii, Candida pelliculosa, Bacillus*, and *Sacharomyces* as shown Table 4 using GC-MS method, even this data is supported by Cronk et al. studies that levels of alcohol in the tape ketan does not reach over 1% at 25 °C with a fermentation time of 2-3 days **(Cronk et al., 1977)**.

Table 4 Alcohol Levels of Tape Ketan (Sample D) Using GC-MS methods in Making 25 °C

Fermentation Time	Peak Area	% v/v Alcohol in Sample
24 hours	81982302	0.1078
36 hours	156475883	0.2067
48 hours	398875460	0.5284
60 hours	657407302	0.8715
72 hours	2008875460	2.6653

CONCLUSION

The result of determination of alcohol content with specific gravity methods was obtained that alcohol content in black tape ketan with fermentation time 3 until 31 days successively, that was 3.17 % v/v until 4.47 % v/v; while with refractive index methods of alcohol in black tape ketan successively, that was : 3.90 % v/vuntil 5.04 % v/v, and with method of GC-MS each 4.295until 5.34 % v/v, respectively. Based on recovery data, the third methods has good accuracy to determine alcohol level in black tape ketan. Highest alcohol content to three types of determination methods obtained at fermentation for 31 days.

REFERENCES

AKOH, C. C., CHANG, S. W., LEE, G. C. & SHAW, J. F. 2008. Biocatalysis for the production of industrial products and functional foods from rice and other agricultural produce. in *J Agric Food Chem,vol.* 56**,** 2008, p. 10445-51.

ARDHANA, M. M. - FLEET, G. H. 1989. The microbial ecology of tape ketan fermentation. In *Int J Food Microbiol* vol. 9**,** 1989, p. 157-65.

BELITZ, H. D. & GROSCH, W. 1999. *Food Chemistry,* Berlin, Springer-Verlag, p. 916.

BEUCHAT, L. R. 2008. Indigenous Fermented Foods. In *Biotechnology.* Wiley-VCH Verlag GmbH, chapther. 13, p. 528-529

CRONK, T. C., MATTICK, L. R., STEINKRAUS, K. H. & HACKLER, L. R. 1979. Production of higher alcohols during indonesian tape ketan fermentation. In *Appl Environ Microbiol,* vol. 37**,** 1979, p 892-6.

CRONK, T. C., STEINKRAUS, K. H., HACKLER, L. R. & MATTICK, L. R. 1977. Indonesian tape ketan fermentation. In *Appl Environ Microbiol,* vol. 33**,** 1977, p. 1067-73.

DE ARRUDA MOURA PIETROWSKI, G., DOS SANTOS, C. M., SAUER, E., WOSIACKI, G. & NOGUEIRA, A. 2012. Influence of Fermentation with Hanseniaspora sp. Yeast on the Volatile Profile of Fermented Apple. In *J Agric Food Chem,* vol. 60**,** 2012, p. 9815-21.

HARTONO & SASTRAMIHARJA, I. Pembuatan etanol dengan "immobilized cell" Saccharomyces cerevisiae dan bahan baku molase di dalam reaktor unggul terfluidakan. National Seminar "Teknologi Fermentasi", 1987 Bandung. PAU Bioteknologi, ITB, p. 1-18.

HORWITZ, W. 2000. Official Methods of Analysis of AOAC International. New York: AOAC International. vol. 1(17)

KABAK, B. & DOBSON, A. D. 2011. An introduction to the traditional fermented foods and beverages of Turkey. In *Crit Rev Food Sci Nutr,* vol. 51, 2011, p. 248-60.

KRAMER, A., BELOW, H., BIEBER, N., KAMPF, G., TOMA, C. D., HUEBNER, N. O. & ASSADIAN, O. 2007. Quantity of ethanol absorption after excessive hand disinfection using three commercially available hand rubs is minimal and below toxic levels for humans. In *BMC Infect Dis,* vol. 7, 2007, p. 117.

SASTRAMIHARJA, I. 1986. *Pengantar Rekayasa Mikroba Bagian II,* Bandung, ITB, p. 58-60

SIEBENHANDL, S., LESTARIO, L. N., TRIMMEL, D. & BERGHOFER, E. 2001. Studies on tape ketan--an Indonesian fermented rice food. In *Int J Food Sci Nutr, vol.* 52**,** 2001, p. 347-57.

SUJAYA, I. N., AMACHI, S., SAITO, K., YOKOTA, A., ASANO, K. & TOMITA, F. 2002. Specific enumeration of lactic acid bacteria in ragi tape by colony hybridization with specific oligonucleotide probes. In *World Journal of Microbiology and Biotechnology,* vol. 18**,** 2001, p. 263-270.

SUJAYA, I. N., NOCIANITRI, K. A. & ASANO, K. 2010. Diversity of bacterial flora of Indonesian ragi tape and their dynamics during the tape fermentation as determined by PCR-DGGE. In. *Int J Food Res,* vol. 17**,** 2010, p. 239-245.

SUMARMI, A. 2002. *Penetapan kadar etanol dalam tape ketan menggunakan metode kromatografi gas.* Thesis, ITB.

THANH, V. N., MAI LE, T. & TUAN, D. A. 2008. Microbial diversity of traditional Vietnamese alcohol fermentation starters (banh men) as determined by PCR-mediated DGGE. In *Int J Food Microbiol,* vol. 128**,** 2008, p. 268-73.

THOMPSON, S. 1990. *Small Scale Experiment for General Chemistry,* New Jersey Prentice Hall.

TORIJA, M. J., ROZES, N., POBLET, M., GUILLAMON, J. M. & MAS, A. 2003. Effects of fermentation temperature on the strain population of Saccharomyces cerevisiae. In *Int J Food Microbiol,* vol. 80**,** 2003, p. 47-53.