

# 2019 Characterization of yeast hydrolysate enzymatic -yhe-

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## 9 Characterization of yeast hydrolysate enzymatic (yhe) from yeast fermented in the variation of rice flour

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**Abstract.** Research has been conducted to determine the chemical and physical characteristics of Yeast Hydrolysis Enzymatic (YHE) based on its fermentation media. The fermentation medium used is white, red, and black rice. The procedures carried out include the sample preparation, the fermentation, making pineapple crude and the enzymatic hydrolysis stage. In this study measured  $\text{Cr}^{3+}$  content using voltammetry method, and measurement of protein content using Kjeldahl method. There are three kind of YHE: YHE from yeast which is grown in white rice (YHE-Er), red rice (YHE-Rr) and black rice (YHE-Br). YHE from yeast grown in various growth media has different  $\text{Cr}^{3+}$  content. The lowest yield of  $\text{Cr}^{3+}$  was YHE-Wr which was  $32 \times 10^{-5}\%$  while YHE-Rr was  $37 \times 10^{-5}\%$  and YHE-Br was  $40 \times 10^{-5}\%$ . The protein content of YHE-Wr, YHE-Rr and YHE-Br were respectively 38.45; 38.48; and 39.23%.

### 1. Introduction

Yeast Hydrolysate Enzymatic (YHE) is a yeast extract that is processed through an enzymatic hydrolysis process. Yeast is a unicellular fungus, it is known to have several types and one of them is *Saccharomyces cerevisiae*. *Saccharomyces cerevisiae* is one of the yeasts that has been widely known and widely used, especially in the fermentation process. *Saccharomyces cerevisiae* is a microorganism contained in baker's yeast and there are no other microbes in it [1]. Based on market research, it is known that the world yeast extract needs approximately \$ 1.000 million in 2009 and this need continues to increase until now. Over the next five years, projects that Yeast Extract will register a 4.3% CAGR in terms of revenue, reach US\$ 1920 million by 2023, from US\$ 1490 million in 2017 [2]. On the other hand, the price of this product is relatively expensive. Yeast extract can be used for various needs, among others: as a medium for microbial growth, as a basic ingredient of pesticides and fertilizers or bio fertilizer which is very useful in agriculture, and as a flavour-enhancing ingredient that can be used by the food industry and for home needs, preparing family food menu every day [3], it can be used as a supplement to meet the nutrition of people who experience poor nutrition, Food & Beverages (soups, processed foods, dairy products, bakery, sauces & savory flavours), animal feed, and pharmaceuticals). The yeast extract is known to contain peptone and acid various amino acids have been used for centuries as a major nutritional component in culture media. This compound is a source of carbon, minerals, vitamins and growth factors. The results showed that YHE from fermented yeast using tapioca waste and sucrose then hydrolyzed enzymatically as follows: total nitrogen (NT) 25.97%,  $\alpha$ -amino nitrogen (N- $\alpha$ ) 20.07%, price (N- $\alpha$ ) / (NT) 0.77%, fat content 1.18%, pH (2% in

solution) is 5, total carbohydrate is 58.99%, moisture content is 7.19%, vitamin: thiamine, riboflavin, pyridoxine, and niacin) is 10.307% proven to be used as microbiological media material [4].

One of the efforts to meet the needs of the global market for yeast extract is one of the alternatives is the production itself using materials available in the surrounding environment, including rice flour. Rice flour can be used as a fermentation medium. One of the good fermentation media for fermentation with *Saccharomyces cerevisiae* is flour whose main content is starch [5]. In addition to meeting needs, it can also increase the economic value of rice. These foodstuffs are available abundantly in Indonesia, Thailand, Korea, Japan, and other countries. This ingredient is known to be rich in carbohydrates, namely starch. There are several kinds of rice, including white rice, brown rice, and black rice. Several studies reveal that these three types of rice have different chemical content, including protein, starch, minerals, and antioxidants [6-7]. Black rice shows higher amounts of minerals, higher than anthocyanins, faster hydrolysis levels, higher amino than white rice acids. Black rice also has the highest mineral content when compared to brown rice or white rice, such as iron, zinc, manganese, and phosphorus [6]. Mashed brown rice contains 7.30% protein, 4.20% iron, and 0.34% vitamin B1 [8]. Amylum is a complex compound that cannot be directly used by yeast as a source of energy and growth. This compound must be degraded into a simple compound, glucose. This degradation process can be done in many ways, one of which is enzymatical. Enzymatic hydrolysis is known to be most effective than others. The enzymes used in the process are  $\alpha$ -amylase and glucoamylase. Amylase starch will be degraded into simpler constituent parts such as dextrin, isomaltose, maltose, and glucose, and then degraded by glucoamylase to produce glucose. Glucose is used for yeast growth.

Yeast which is successfully grown can be further processed, which is extracted through an enzymatic hydrolysis process. Enzymatic hydrolysis is the most effective process compared to others [3]. Yeast is known to be rich in protein. Protein is an essential molecule for the structure and function of all living things. This compound is composed of various amino acids which are linked to peptide bonds and form various complex structures [9]. This protein can be degraded into a simpler component, namely peptone and various amino acids enzymatically, using bromelain. Amino acids are needed for cell growth.

Yeast besides being known to contain iron, zinc, manganese, and phosphorus also contains chromium. The content of chromium in yeast every 1 oz (28.35 grams) contains 3.3 microgram [10]. This mineral is needed by the body, even though it has to be fulfilled a little. Chromium has several types, including chromium (III) or  $\text{Cr}^{3+}$ . Valence mineral (III) is the most stable and is known to be the safest to consume. Chromium serves to help lower glucose in body cells. Glucose can enter the body's cells with the help of the hormone insulin. Regulation of blood glucose levels by chromium is not widely understood. It is recently known that chromium interacts with the low-molecular-weight chromium (LMWCr) [11]. The interaction of chromium with LMWCr will activate the role of insulin. If the amount of hormone insulin in a person's body is insufficient or if the body's cells do not respond to insulin, there will be a build up of glucose in the blood (hyperglycemia) and the person suffering from DM. Therefore, the content of chromium in food is very important to know.

## 2. Experimental Section

### 2.1 The Process of Making Bromelin

Pineapple fruit is washed, cut and mashed using a blender. The crushed pineapple is filtered with a cloth and then centrifuged at 1500 rpm for 10 minutes. The resulting filtrate was added with 35% ammonium sulfate and stirred slowly using a magnetic stirrer in cold conditions for 45 minutes and allowed to stand for 24 hours in the refrigerator. The mixture was centrifuged at a speed of 6000 rpm for 15 minutes; the resulting residue was a crude bromelain extract. The residue is ready to be used for yeast hydrolysis [12].

## 2.2. The Process of making YHE

The initial stage in making YHE is to prepare flour as fermentation medium, namely red, white, and black rice flour which passes the 100 mesh sieve. Each of flour was dissolved in distilled water in a ratio of 1: 5, then gelatinized at 100°C and cooled. The formed gel was hydrolyzed using  $\alpha$ -amylase and glucoamylase at 50°C for 24 hours. Hydrolysate is added with yeast bakery with a ratio of 1: 5, then fermented at 37°C for 36 hours. After fermentation, plasmolysis was done by adding 35% NaCl, incubated at 60°C for 48 hours, then centrifuged at 1500 rpm for 10 minutes. The residue obtained is fermented Yeast from rice flour, namely yeast-white rice, yeast-brown rice, and yeast black rice added bromelain crude and incubated for 48 hours at 37 ° C [12]. Hydrolysates obtained were YHE-white rice, YHE-brown rice, and YHE-black rice which were ready to be tested for water content, Cr<sup>3+</sup> content, and protein.

## 2.3. Determination of trivalent chromium levels (Cr<sup>3+</sup>).

The first step is to make a standard Cr<sup>3+</sup> solution. The standard solution used is CrCl<sub>3</sub>.6H<sub>2</sub>O with variations in concentrations of 10, 20, 40, and 100 ppm. The next stage, preparing samples of YHE-white, YHE-red, and YHE-black rice flour. Each sample was cooled in a furnace at 500 °C for 4 hours cooled and each dissolved using 1 mL concentrated HCl and 1 mL concentrated HNO<sub>3</sub>. The mixture was allowed to stand for 24 hours, then each one was dissolved with 25 mL of aqua-demineral then taken 10 mL of sample and added 5 mL of citrate buffer pH 3, and also added 10 mL of KCl 0.1N. Each standard solution and samples were tested for Cr<sup>3+</sup> using voltammeter. The obtained voltammogram was analyzed using the originPro7.0 application so that the current values of the standard solution and the sample were known [13].

## 2.4. Determination of protein content using the Kjeldahl method

The method of determining the N-total method of Kjeldahl was adopted from AOAC [14]. YHE-black, white and red rice samples were weighed about 5 grams each and put into the Kjeldahl flask. A sample of 1.4 grams of catalyst and boiling stone was added. 5 ml of concentrated H<sub>2</sub>SO<sub>4</sub> is added (in a fume hood). The sample is reconstructed until the color becomes green then left to cool. The sample was added 60 ml of aquadest, shaken and put into Erlenmeyer 300 ml. In the distillation stage, the sample solution was diluted by adding 40% NaOH, the flask was placed on the distillation apparatus. Distillation is stopped if all N has been caught by sulfuric acid in the Erlenmeyer flask. In the Titration process, the excess H<sub>2</sub>SO<sub>4</sub> is used to capture N titrated with Sodium Hydroxide. Titration is stopped if the solution changes from purple to turquoise. Blank solutions are also made, namely replacing the sample with distilled water. The final step is to calculate the nitrogen-based content on the result of the titration. The results of this calculation will get a percent value of TKN (Total Kjeldahl Nitrogen). Here is the calculation formula:

$$\%TKN = \frac{(\text{Tr. Sample} - \text{Tr. Blank}) \times 14.007 \times N}{\text{Mg. Sample}} \times 100\%$$

Note:

- Tr. Sample = number of titrant for sample (ml)
- Tr. Blank = number of titrant for blanks without sample (ml)
- N = titrant normality
- mg. Sample = sample weight (mg)

## 3. Discussion

The initial stage carried out in this study is menumbuhkan yeast dalam media tepung beras. Yeast yang digunakan adalah *bakery yeast*, yang diketahui mengandung *Saccharomyces cerevisiae*.

Fermentation is a way to convert a substrate into a certain desired product by using microbial assistance [15]. Before stepping into the fermentation process, first, do the gelatinization process. Gelatinization is a process when starch granules are heated with enough water to develop starch granules and produce a gel form [16]. This process occurs the breakdown of intermolecular bonds from starch in the presence of heat and water given [17]. The heat and water used in the gelatinization process cause high granule freezing and amylose is able to diffuse out of the granule [18]. Each gel-shaped rice is easily accessible and degraded by  $\alpha$ -amylase and glucoamylase so that glucose is produced which is used by yeast for cell growth. Degradation is characterized by decreasing the level of viscosity. The sample which was originally in the form of a gel turned into liquid after the process of degradation or enzymatic hydrolysis. This is due to the breaking of the glycosidic bond from starch. Then, the hydrolyzate obtained is used for yeast fermentation. Yeast hasil fermentasi selanjutnya didegradasi secara enzimatik menggunakan bromelin nanas sehingga diperoleh YHE dan dikarakterisasi. Characterization includes: moisture content, protein content and  $\text{Cr}^{3+}$  content.

Determination of water content was carried out by the gravimetric method using a Moisture Analyzer. Moisture Analyzer utilizes infrared or halogen lamps as heat sources. The results of flour-13, flour-Rr, and flour-Br water content are found in table 1. In this research also determined the water content of rice flour and yeast as raw material of YHE.

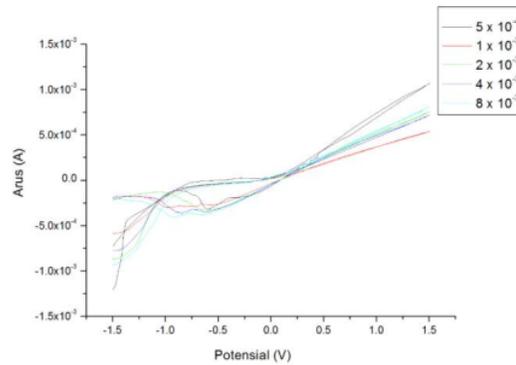
**Table 1.** Water content of rice flour, yeast, and YHE

Sample	Water content (%)
White rice flour (flour-Wr)	14.04
Red rice flour (flour-Rr)	11.67
Black rice flour (flour-Br)	12.86
Yeast-White rice (yeast-Wr)	77.00
Yeast-Red rice (yeast-Rr)	74.06
Yeast-Black rice (yeast-Br)	72.21
YHE-White rice (YHE-Wr)	52.95
YHE-Red rice (YHE-Rr)	58.29
YHE-Black rice (YHE-Br)	62.67

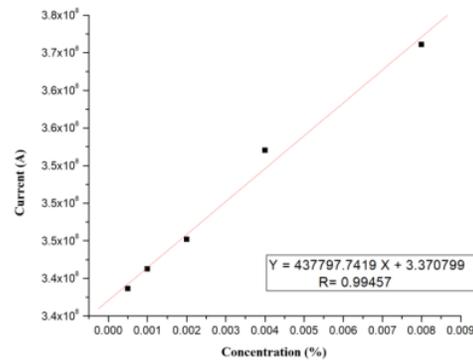
The data in table 1 shows that white rice flour has the highest water content when compared to brown and black rice, which is 14.04%. Water content of YHE is smaller when compared to yeast both YHE-Wr, YHE-Rr and YHE-Br. YHE-Br has higher water content than YHE-Wr and YHE-Rr. Water content of material affect the shelf life. High moisture content causes susceptibility to microbial activity. The water content in the ingredients is the growth medium of mold and fungus.

$\text{Cr}^{3+}$  levels on YHE were determined using the voltammetry method, cyclic voltammetry. The voltammetry provides analysis of various metals and provides both qualitative and quantitative information [19]. The first step taken to determine  $\text{Cr}^{3+}$  is to dispose of  $\text{Cr}^{3+}$  standard solution with variations in concentrations of 5, 10, 20, 40, 80, and 100 ppm. The next step of each standard solution is measured by its current strength with a voltmeter and will produce a voltammogram. The data obtained were analyzed using originPro 7.0 and the current value of the current will be known. Figure 1 shows the voltammogram of the  $\text{Cr}^{3+}$  standard solution in various concentrations.

According to the Nerst law, strong currents are directly proportional to concentration [19], the greater the concentration of the solution the greater the current strength. Based on the voltammogram results in figure 1, the current strength of each solution can be determined. Figure 2 shows the relationship between current strength and the concentration of  $\text{Cr}^{3+}$  standard solution.



**Figure 1.** Voltammogram of Cr<sup>3+</sup> standard solution in various concentrations



**Figure 2.** The relationship between current strength and the concentration of Cr<sup>3+</sup> standard solution

The mapping between concentration and current strength of each standard solution can be obtained by the equation  $Y = 437797.7 X + 3.370799$ . Furthermore, measurements of YHE-Wr, YHE-Rr, and YHE-Br sample current strength were measured. The results are entered into the equation  $Y = 437797.7 X + 3.370799$  and the Cr<sup>3+</sup> concentration of each sample will be known. Table 2 shows the results of measuring Cr<sup>3+</sup> on YHE from yeast grown in various types of rice flour.

**Table 2.** Results of Cr<sup>3+</sup> measurement in YHE

Sample	Cr <sup>3+</sup> content (%)	Difference of Cr <sup>3+</sup> between flour and yeast	Difference of Cr <sup>3+</sup> between yeast and YHE
White rice flour (flour-Wr)	20 x 10 <sup>-4</sup>		
Red rice flour (flour-Rr)	19 x 10 <sup>-4</sup>		
Black rice flour (flour-Br)	20 x 10 <sup>-4</sup>		
Yeast-White rice (yeast-Wr)	101 x 10 <sup>-4</sup>	81	
Yeast-Red rice (yeast-Rr)	103 x 10 <sup>-4</sup>	84	

1 Yeast-Black rice (yeast-Br)	112 x 10 <sup>-4</sup>	92	
YHE-White rice (YHE-Wr)	32 x 10 <sup>-4</sup>	12	69
YHE-Red rice (YHE-Rr)	37 x 10 <sup>-4</sup>	18	66
YHE-Black rice (YHE-Br)	40 x 10 <sup>-4</sup>	20	72

Table 2 shows that kandungan Cr<sup>3+</sup> in rice flour, yeast or YHE differ in all measurements. The highest Cr<sup>3+</sup> levels in YHE from yeast grown in flour-Br are 40x10<sup>-5</sup> %, while Cr<sup>3+</sup> in YHE from yeast grown in red rice flour has almost the same value and even close to the yield YHE –Br is 37 x 10<sup>-5</sup> %. YHE from yeast grown in a type of flour-Wr has the smallest level of 32 x 10<sup>-5</sup> %. The research results also showed that there were also differences between yeast and YHE, when compared with yeast, the Cr<sup>3+</sup> content was smaller both in YHE-Br, YHE-Re, and YHE-Br, respectively 69, 66, and 72%. This is probably due to Cr<sup>3+</sup> being released during the centrifuge process. The following table 3 shows YHE protein levels of sample.

**Table 3.** YHE protein levels from yeast grown in variations of rice flour

Sample	Protein content (%)	Difference of protein between flour and yeast	Difference of protein between flour and YHE	Difference of protein between yeast and YHE
White rice flour (flour-Wr)	7.45			
Red rice flour (flour-Rr)	9.06			
Black rice flour (flour-Br)	10.60			
Yeast-White rice (yeast-Wr)	40.00	32.55		
Yeast-Red rice (yeast-Rr)	38.66	29.60		
Yeast-Black rice (yeast-Br)	40.19	29.59		
YHE-White rice (YHE-Wr)	38.45		31.00	1.55
YHE-Red rice (YHE-Rr)	38.48		29.42	1.71
YHE-Black rice (YHE-Br)	39.23		28.63	0.96

Table 3 show that the highest protein content in YHE is YHE-black rice which is 39.23%. When compared with the fermentation media, namely flour-Wr, flour-Rr, and flour-Br, the YHE-Br has increased from 10.60% to 39.23%. YHE-Wr also experienced an increase compared to its fermentation media, from 9.06% to 38.45%. Whereas YHE-Br also experienced an increase compared to the fermentation media, from 7.65% to 38.48%. Increased protein levels in YHE-red, white and black rice occur due to the addition and growth of *Saccharomyces cerevisiae* during the fermentation process [20]. Cr<sup>3+</sup> is one of the minerals that is needed for cell metabolism, for growth. Growth is strongly related to protein metabolism. Chromium increases the rate of protein synthesis [21].

YHE black rice has a greater binding ability to Cr<sup>3+</sup> than YHE-Wr and YHE-Rr. Variations in the type of rice flour give a difference in the results of chromium content because it has a different composition. Crude fiber is known to have a good mineral binding ability, so it can be said indirectly that black rice has the ability to scrape Cr<sup>3+</sup> larger than others. Thus the impact on Cr<sup>3+</sup> black rice content is higher than the others. The highest crude fiber content is black rice [20], this supports the research findings which state that the ability to bind the highest minerals, Cr<sup>3+</sup> is YHE black rice (YHE-Br).

Based on Table 3, it is known that the protein content of each YHE is different. The highest protein level is YHE-Br. The difference in levels of this protein is likely to have something to do with the Cr<sup>3+</sup> content contained in rice flour. Cr<sup>3+</sup> is a mineral that is needed by yeast for growth. The highest

content of Cr<sup>3+</sup> black rice compared to the others. This will affect yeast growth in black rice. If yeast growth is high at the time of fermentation, it will affect the high levels of yeast protein produced. Similarly, when the yeast was processed into YHE-Br, the protein content was also the highest, which was 39.23%.

#### 4. Conclusion

Based on the research that has been carried out, the conclusions of this study are as follows: 1) the content of Cr<sup>3+</sup> from YHE-Wr by 32x10<sup>-5</sup> %, YHE-Rr by 37x10<sup>-5</sup> %, and YHE-Br by 40x10<sup>-5</sup> % ; and 2) YHE-Wr, YHE-Rr, and YHE-Br content respectively 38.45; 38.48; and 39.23%.

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