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BIOCHEMICAL ACTIVITY OF *SACCHAROMYCES CEREVISIAE* YEAST IN THE FORMATION OF MANNOPROTEIN ON PHYSICAL PROPERTIES OF SAUSAGE PRODUCTS

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Abstract

Saccharomyces cerevisiae is a yeast that is rich in protein, carbohydrates and fat, can be used for human consumption. The alternative media can be used as S. cerevisiae growth media, with the condition that the contents in it, meet the requirements of yeast growing media, so it can reduce the use of commercial media. One alternative media is a formula consisting of bean sprouts and several other ingredients. S. cerevisiae growth curves on formula media based on optical density, pH and biomass of yeast, have a 0 to 5^{th} hour lag phase, log phase 5^{th} – 30^{th} hour, stationary phase 30^{th} – 60^{th} hours and death phase 60^{th}- 70^{th} hours. In the death phase at 60^{th}- 70^{th} hours, there was an overhaul of the cell wall. Mannoprotein precipitate produced from yeast cell wall extraction can be used as bio-emulsifier. Emulsification activity of mannoprotein from the yeast at the 1^{st} and 24^{th} hour is 50%. The appropriation of S. cerevisiae yeast mannoprotein has an effect on water holding capcity, while cooking loss and tenderness of sausage had no effect.

Key words**:** *Saccharomyces cerevisiae, growth curve, media formula, mannoprotein, sausage*

INTRODUCTION

Saccharomyces cerevisiae is included in yeast which contains lots of protein, carbohydrates, and fat, so it can be consumed by humans. *S. cerevisiae* is very easy to be cultivated in various media as long as there are sources of carbon, nitrogen, hydrogen, oxygen, sulfur, calcium, vitamins, minerals and water (Erna *et al.,* 2004) [7]. The medium commonly used to grow *S. cerevisiae* is Yeast Malt Extract Agar (YMEA), but it is also possible to grow this yeast on alternative mediums of easily available materials and at a more economical price and environmentally friendly. One ingredient that can be used is bean sprouts which have high nutritional content such as protein, fat, fiber, phosphorus, vitamin B, energy, calcium, iron, ash, water, and carotene. The nutritional content of the bean sprouts can be used as a yeast growth media with the addition of several other supporting materials.

*S. cerevisia*e consists of capsules, cell walls, cytoplasmic membranes, nucleus, vacuoles, fat globules, and mitochondria. In the yeast cell wall can be used for bioethanol and as an emulsifier. Yeast cell walls consist of glucan, mannan, protein, chitin, and lipids. The glucans in the cell walls will form microfibrils while the mannan, which is generally associated with proteins, will form mannoproteins. Mannoproteins are formed from polypeptide chain molecules with short links, while long links with mannose. The number of cell walls is influenced by the growth curve of yeast, where young yeast has thin cell walls, while old yeast has thick cell walls (Balia, et al., 2017) [2].

Mannoprotein can be extracted from the yeast cell wall has bioemulsifier properties used in food and beverage processing due to the nature of the active surface of mannoprotein, so that it can stabilize the emulsion process in a product, and produce bioemulsifier products which is safe (food grade), non-toxic and environmentally friendly. Food products that use emulsifiers are found in some products such as sausages, meatballs, ice cream, and mayonnaise.

Emulsifier is a substance that can be used to maintain the stability of a product (Pawlik *et*

al., 2014) [14]. Sausage is one product that uses an emulsion process. Problems that often occur in making sausages are emulsion rupture, low water holding capacity due to bad emulsification process, and texture that are not compact, too hard or too soft, so need another alternative to overcome the problem. Sausage quality can be improved by increasing the binding capacity of water and fat emulsions using appropriate binders and fillers (Dewi *et al.*, 2013) [5].

MATERIALS AND METHODS

Research Materials

Research materials used in this study are instant dry yeast, bean sprouts, sugar, antibiotics, vegemite, oil, beef, ice cubes, tapioca flour, skim milk, margarine, salt, sugar, garlic, pepper, nutmeg, aquadest, alcohol, spiritus, Yeast Malt Extract Agar (YMEA), Malt Extract Broth (MEB), tripotassium citrate ($C_6H_5K_3O_7$), and ethanol.

Research Methods

The study was conducted experimentally in a laboratory using Completely Randomized Design (CRD) with 3 treatments, namely sausages without mannoprotein, giving mannoprotein as much as 0.8% and 1.6% with each treatment repeated 6 times. All data obtained were analyzed using analysis of variance (ANOVA) and differences between treatments were analyzed by the Tukey test.

Research procedures

Making Formula Media

The process of making media formulas using the method of Balia *et al.* (2018) [3] is as heats 3 liters of distilled water that has been added by 1 kilogram of bean sprouts as fas as 1 liter of water volume. Afterward, sugar, vegemite, and antibiotics were added. The mixture of ingredients sterilized at 121°C form 15 minutes. The media formula is ready to use.

Implantation Yeast on Formula Media

S. cerevisiae that has planted on MEB is taken as much as 1% (50 µl) then transferred to 5 ml of the media formula, incubated at 25°C with incubation time 0^{th} to 70^{th} hours, carried out testing of optical density. pH measurement and cell biomass in every 5 hours the incubation time.

The Growth Curve

The growth curve testing consists of optical density, pH, and biomass. The testing of optical density was perfomed by using a 600 nm spectrophotometer. The measurement of pH was carried out on *S. cerevisiae* isolates using a pH meter and biomass testing was carried out by drying at 40^{o}C for 12-16 hours (Garcia-Ochoa and Casas, 1999 modifications) [9].

Mannoprotein Extraction

Mannoprotein extracted from *S. cerevisiae* cells by the modified Torabizadeh, et al., (1996) [19] in Dikit *et al*., (2010) [6]. Extraction of mannoprotein was started by separating of precipitate using a centrifuge, the outcome of precipitate was added 0.1 M potassium citrate then sterilized by autoclaving at 121°C for 15 minutes to 2 hours. Furthermore, the isolate was centrifuged at 6,000 rpm for 15 minutes at 4°C, the supernatant was added with 90% chilled ethanol and stored at 4°C for 12-16 hours until the precipitation process completed. The results of precipitation obtained were centrifuged at 6,000 rpm for 15 minutes at 4°C followed by washed twice with chilled ethanol

Making Sausages

The procedure for making sausages uses the modified Suryaningsih Lilis (2006) [18] method. Prepare 2 kg of meat, each treatment 100 g, then ground in a food processor. Add ice cubes 5 g, tapioca flour 10 g, skim milk 3 g, margarine 3 g, salt 2 g, sugar 0.5 g, garlic 1.5 g, pepper 1 g, and nutmeg 0.5 g, milled until the dough becomes homogeneous. Add diluted mannoprotein according to treatment, milled again. Put the mixture into the sleeve and tie the end using a string. Then sausage boiled for 45 minutes at a temperature of 60^{o}C, when cooked drain.

Measurement of Emulsification Activity (EA)

Measurement of emulsification activity uses a modified method according to Cooper and Goldenbery (1987) [4], which is taking mannoprotein has diluted with 1 ml of

distilled water and oil into the Eppendorf tube, then conducted agitation using vortex for 3 minutes and stored for 24 hours.

Measurement of the Physical Quality of Sausages

Water-holding Capacity

Measurement of water-holding capacity by the method of Soeparno (2011) [17]. Sausage samples weighed as much as 0.3 g. Place the sample on Whatman filter paper No. 42 then the sample is given a weight of 35 kg between two glass plates for 5 minutes. After completion, mark the area covered by the sample and the area of the surrounding wet area on paper. Calculate the sample wet area, free water content, sample water content, and water holding capacity.

Cooking Loss

Cooking loss is an indicator of nutritional value related to meat water content, namely the amount of water bound in and between muscles (Soeparno, 2011) [17]. Sausage dough that has been put in a sheath weigh first for the initial weight, then boil the sausage at 60^{o}C for 45 minutes then drain it, and weigh it again as after cooking weight.

Tenderness

This test uses a penetrometer with the method according to Muchtadi and Sugiyono (1992) [13]. Sausages are chopped 3 cm long, 3 cm wide, and 2 cm thick. The sample is placed at the tip of the penetrometer needle. The needle is adjusted until it touches the sausage surface. Press the needle lock for 10 seconds, then released and press the scale slowly until it touches the penetrometer needle. The stabbing is done 10 times in 10 different places.

RESULTS AND DISCUSSIONS

Growth Curve

The growth curve can be obtained by either relating the incubation time to optical density or to biomass (Acourne *et al.,* 2007) [1]. The growth curve which based on optical density, pH, and biomass showed that the lag phase of *S. cerevisae* is relatively short (Fig. 1 (a,b,c). This phase was occurred at 0 to 5 hours of culture. At 5 hours of culture, it has an OD of 0.3394, a pH of 6.30, and a biomass of 0.0026 gr/ml. The lag phase is relatively short because the yeast grows on media containing a composition that is almost the same as the initial media, so self-adjustment to the new environment takes place quickly (Wahono *et al.*, 2011) [20].

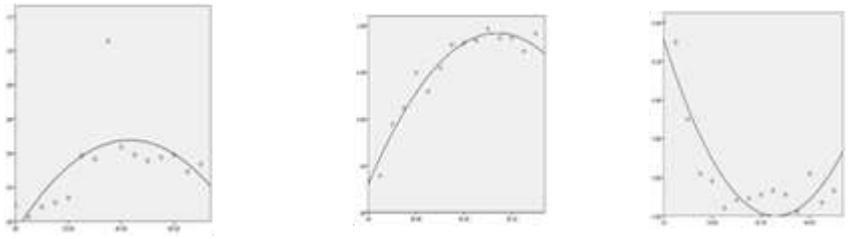
The growth curve also shows that at 5 to 35 hours of culture, the log phase has been characterized by significant growth, and the log phase lasts for 30 hours. At 35 hours of culture, it has the OD of 1.7950, pH of 5.49, and biomass of 0.1057 gr/ml. At the log phase, the microbes grew rapidly. The cell log phase produces many metabolic substances needed to meet their needs in the context of growth (Kosim and Putra, 2010) [1].

At the 35 to 60 hours of growth, *S. cerevisae* experienced a relatively fixed growth phase because it began to enter the stationary phase. At 60 hours of culture, it has the OD of 1.8694, pH of 5.62, and biomass of 0.0388 gr/ml. The size of the cells in the stationary phase will be smaller because the cells continue to divide even though the media nutrients have started to run out (Setyati *et al*., 2015) [16].

Figure 1 present the growth curve of *Saccharomyces cerevisiae* yeast on biomass (a), OD (b), and pH. (c).

The next phase of death occurs at 60 to 70 hours of culture, with a decrease in the rate of growth caused by a lack of growth nutrients such as vitamins and mineral elements, and a reduction in some essential nutrients in the media or due to the accumulation of autotoxins in the media, or a combination of both (Gaman and Sherrington, 1994) [8].

In the death phase, which is at 60 to 70 hours of culture, there is an autolysis process that degrades mannoprotein from the cell wall by glucanase and proteinase enzymes so that the mannoprotein is released from the cell wall.



(a) Biomass (b) OD (C) pH

Fig.1. The growth curve of *Saccharomyces cerevisiae* yeast on biomass (a), OD (b), and pH. (c).
Notes: The vertical direction shows the time of growth and the horizontal direction shows the results of growth.
Source: Own results in the laboratory.

The cell wall of newly formed yeasts is very thin and when it gets older it will get thicker (Madigan *et al.*, 2012) [12]. Mannoproteins that have been released from cell walls can be used as bioemulsifiers. The presence of a mannose hydrophilic polymer binding to a protein provides mannoproteins with an amphiphilic structure for its surface. This amphiphilic structure is effective as emulsifiers (Dikit *et al.*, 2010) [6].

In this study, the extraction process of *S. cerevisiae* yeast mannoprotein was using a 500 ml medium formula with an incubation period of 70 hours at 25^{o}C. Mannoprotein sediment which was obtained from the extraction process were 3.2 grams which were then diluted with a ratio of 1:20 ml. The results obtained from EA 1 hour is 50% while the 24-hour time is 50%. Mannoprotein which has been diluted and tested for its emulsification activity is then used as an emulsifier in sausages.

Sausages

Fresh meat can be processed into ready to serve products, such as sausages (Prayitno *et al.*, 2009) [15]. The main component of sausages consists of meat, fat, and water, while other additives such as salt, phosphate, preservatives (nitrites/ nitrates), coloring agent, ascorbic acid, protein isolates, and carbohydrates (Soeparno, 2011) [17]. The results of using *S. cerevisiae* yeast in the formation of mannoprotein on the sausages physical quality in various treatments are presented in Table 1.

Table 1. Average Physical Quality of Sausages in various Treatments

Treatments	**Average**		
	Water-holding Capacity (%)	Cooking Loss (%)	Tenderness (mm/g/10s)
P_0	27.39	1.45	77.02
P_1	25.91	1.79	67.83
P_2	23.26	1.57	77.17

Notes:
P_0: Sausages without mannoprotein addition (control)
P_1: Sausages with 0.8% of mannoprotein addition
P_2: Sausages with 1.6% of mannoprotein addition
Source: Own results in the laboratory.

Water-holding Capacity

Table 1 shows that the sausage at P_0 group had the highest water-holding capacity compared to other treatments (27.39% vs 25.91% of P_2 and 25.91% of P_1). Furthermore, to find out what extent the water-holding capacity of sausage is affected by the addition of mannoproteins, statistical analysis with variance, showing that mannoproteins had a significant effect ($P<0.05$) on the binding capacity of sausage water. To find out the differences between treatments, the Tukey Test was carried out.

The significant difference found in sausages with mannoprotein addition of 1.6% (P_2) compared with sausages without mannoprotein (P_0) might be caused by the addition of mannoprotein which played a role

as an emulsifier. The emulsifier itself has both hydrophilic and lipophilic groups or also called amphyphilic molecules (Dikit *et al.*, 2010) [6]. The hydrophilic group has water-holding properties which results in the more water contained therein being bound by the emulsifier (meat protein and mannoprotein) used.

Water-holding capacity is the ability of meat to hold water or water added during external treatment such as cutting, heating, grinding and processing. The less free water that comes out of meat might indicate that the meat sample has a high water-holding capacity, and vice versa (Soeparno, 2011) [17].

Table 2. Tukey Test of Water-holding Capacity of Sausages in Various Treatments

Treatment	Average	Significance
	……%.....	
P_2	23.26	A
P_1	25.91	AB
P_0	27.39	B

Notes: Different superscripts vertically in significance column shows a significant difference
Source: Own results in the laboratory.

The significant difference found in sausages with mannoprotein addition of 1.6% (P_2) compared with sausages without mannoprotein (P_0) might be caused by the addition of mannoprotein which played a role as an emulsifier. The emulsifier itself has both hydrophilic and lipophilic groups or also called amphyphilic molecules (Dikit *et al.*, 2010) [6]. The hydrophilic group has water-holding properties which results in the more water contained therein being bound by the emulsifier (meat protein and mannoprotein) used.

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Cooking Losses

Table 1 shows that the average sausage cooking loss in various mannoprotein treatments. Sausages without mannoprotein addition (P_0) had the lowest cooking loss percentage (1.45%). The addition of mannoprotein as much as 0.8% (P_1) seemed to increase the cooking losses (1.79%). Furthermore, the addition of mannoprotein by 1.6% (P_2) decreases the sausage cooking loss to the point of 1.57%. To find out what extent sausage cooking losses are affected by addition of mannoproteins, statistical analysis was performed by analysis of variance, showing that the mannoproteins did not have a significant effect ($P>0.05$). Sausage with mannoprotein does not have a significant effect on cooking losses, which is caused by the use of mannoprotein in liquid form, which it might cause more water evaporation by increasing water content in sausages.

Cooking loss is an indicator which shows the amount of component that is lost during cooking. During the cooking process, the loss of water is affected by the water holding capacity of the meat protein. The more water that can be retained by protein during cooking, the less water comes out, causing low cooking losses. Meat with a low amount of cooking loss has good quality because it loses less nutrients when cooked (Soeparno, 2011) [17].

Tenderness

Table 1 shows that the average sausage tenderness in various mannoprotein treatments. P_2 had the highest level of tenderness (77.17mm/g/10s), while the lowest level found in P_1 (67.83mm/g/10s). Furthermore, to find out what extent sausage tenderness is affected by mannoprotein addition, a statistical analysis was performed with an analysis of variance, showing that addition of mannoprotein had no significant effect ($P>0.05$) on sausage tenderness.

Sausage with mannoprotein did not have a significant effect on sausage tenderness due to the use of liquid form mannoprotein. Increased water content seemed to affect the water holding capacity of sausage, which seems to have a direct relation with meat tenderness. The use of mannoprotein as an emulsifier is expected to help the protein binding process with water which will affect

the water holding capacity which will then help the process of meat tenderization.
Meat tenderness is influenced by several factors, including antemortem factors, postmortem factors, meat storage temperature, and methods for increasing tenderness (Soeparno, 2011) [17]. The tenderness of processed meat products is influenced by the type of fillers used, water, fat, and protein content. Increased protein levels will increase the tenderness of processed meat products because it will increase the level of bound water (Lukman, 1995) [11].

CONCLUSIONS

In conclusion, the media formula can be used as a growing medium of *Saccharomyces cerevisiae*. Mannoproteins are produced from the *S. cerevisiae* cell wall in the death phase (hours 60 to 70), resulting in sediment as much as 3.2 grams and have a value of emulsification activity for one and 24 hours by 50%. The addition of *S. cerevisiae* yeast mannoprotein has a significant effect on the water holding capacity, while it has no effects on cooking loss and sausage tenderness.
Further testing is needed regarding the type of mannoprotein extracted from *Saccharomyces cerevisiae*.

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