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Optimization production of dry yeast using mixture of pineapple solid waste and liquid waste of fermented soybean industry

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Abstract

A combined substrat consisting of solid and liquid waste produced during the course of pineapple and fermented soybean processing was used for the production of instant dry yeast. Two stages of experiment were conducted. The first experiment aimed to find out the best proportion beween solid pinneaple and liquid waste released from fermented soybean industry (1:0 ; 1:1.5 ; 1:1 ; 1:1.5; 0:1) as substrate for the growth of *Saccharomyces cerevisiae* and the second stage of experiment was to find out the optimum concentration of dextrin as filler and lecithin for the production of instant dry yeast. A central composit design with two factor was used to carry out the second stage experiment. Dextrin concentration (50%; 60%; 70%) as the first factor and lecithin concentration (0.5%:1.0% 1.5%) as the second factor. The response analysed are the water content, solubity, cell viliability and ethanol content. Results obtained from the first stage shows that the best substrate for the growth of *S. cerevisiae* was the ratio of pineapple waste and liquid soybean waste (1 : 0.5), incubation time 12 hours, temperature incubation 30^{o}C, and agitation rate 150 rpm. The production of *S. cerevisiae* is 4.35 x 10^{8} cell/ml. The shortest adaptation time is 4 hours. Results obtained from the second stage experiment are computed with Design Expert 7.1.3. The optimum condition obtained were dextrin concentration of 63.59 % (w/v) and lecithin concentration of 1.57 % (w/v), producing product having water content 6.25%, solubility 77.56%, cell viliability is 62.6%, and ethanol concentration is 4.21 %.

Introduction

Instant dry yeast is one of the most important product for the production of bread and alcoholic beverages. Presently the demand of dry yeast in Indonesia increased substantially. According to Statistic Indonesia (2012) import of it this commodity from overseas reached up to 1.76 - 3.2 tons, which is considered too costly. Under such circumstances production of instant dry yeast in country is urgently required. Processing of dry yeast could be carried out by the inoculation of *Saccharyomyces cerevisiae* on to substrate containing glucose and nitrogenous compound, the yeast cells are from the broth by centrifugation. The cells are then washed by dilution with water and recentrifuged until they are light in color (Bernardeau and Vernoux, 2013)

In East Java, pineapple is found abundantly and consumed as fresh beverages drink and also as canned fruit. Part of them in Indonesia pine apple can use for selling street and snack foods (Fellows and Hilmi, 2011). Processing of this comodity may release about 30 % of solid waste which presently not being utlitized and used as animal feed of low price. Pineapple solid waste still contains sugar and mineral which favoured for the growth of microorganism but lack of nitrogenous compound (Ketnawa *et al*., 2012). The addition of liquid waste effluent produced during the course of fermented soy bean processing is expected could provide high potential substrate for making dry yeast. The utilization of pineapple waste and liquid waste released during soybean processing as substrate through fermentation for the production of dry yeast is claimed may reduce the operational cost (Pradana *et al*., 2011; Ketnawa *et al.*, 2012).

Materials and Methods

Inoculum preparation

Strain of *Sacharomyces cerevisiae* was obtained from Food Microbiology Laboratory of Brawijaya University, East Java, Indonesia. Dry Yeast with trade name of SAF was purchased from local chemists. Pineapple core extract from pineapple chips industry, Malang regency, East Java, Indonesia. Liquid waste was purchased from small scale tradisional fermented soybean industry Malang, East Java province. The stock culture was inoculated on to sterilized Nutrient Broth, incubated at 30^{0}C for 24 hours. The inoculum (10 % v/v) was transfered on to solution containing

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YEPD broth (Atlas, 2013)

Media preparation for biomass production

Pineapple core was cleaned, washed then blanched at 80^{0}C for 5 minutes and pressed with manual hidrolic pressure, extracted and filtered with filter material then centrifuged in 3,500 rpm for 10 minute. The supernatant was decanted off and used as substrate. The liquid waste from the fermented soybean industry was filtered and centrifuged in 3,500 rpm for 10 minutes, the supernatant was decanted off.

Biomass production experiment

This experiment was aimed to determine the best proportion of pineapple waste and liquid waste from fermented soybean processing to produce maximum biomass. These two supernatants (pineapple and soybean industry waste) after being processed and sterilized were mixed together with the ratio of pineapple and soybean waste supernatant of: 1:0 ; 1:1.5 ; 1:1 ; 1:1.5 ; and 0:1. The mixture of these two supernatants were arranged so that the total volume was 200 ml and then was transfered to 500 ml Erlenmeyer to get a large room avoiding the floaded of the fermented liquid. The pH of these mixture was adjusted by the addition of acetic acid so that the pH became 4.5, and sterilized for 15 minutes. The mixture was then cooled and inoculated with 10% of Saccharyomyces cerevisiae, and after which it was incubated at 30^{0}C for 24 hours. These treatments were arranged in a complete randomized block design with one factor.

The proportion between pineapple waste and liquid soy bean waste (1:0 1:1.5.1:1 0:1) and repeated three times. Analyses the total yeast found was carried out by plate count method and the best media favoured for the growth of yeast was used for the second experiment. The best media having high total yeast count obtained was centrifuged at 3500 rpm for 15 minutes. The sedimen cells were washed three times with buffer pepton 1% and mixed with lecitin and dextrin. A central composit design with two factors was used. Dextrin concentration (50%; 60%; 70%) as the first factor and lecithin concentration (0.5%: 1% and 1.5%) as the second factor .

Dry yeast production experiment

This experiment was aimed to determine the optimal dextrin and lecithine concentration to produce Dry Yeast. A Respons Surface Method from Software Design Expert 7.1.1. was used to run this study. The first factor was dextrin concentration (50% (b/v); 60% (b/v); 70% (b/v) and the second factor was lecithin concentration with 3 level : (0.5% (b/v); 1.0% (b/v); 1.5 % (b/v).

Microencapsulation process

About 100 ml solution containing dextrin and sterilized aquadest was added with lecithin in concentration according to treatment. Shaked until homogen and pasteurized. The biomass produced from first stage of experiment is mixed with 100 ml pasteurized then poured in the petridish, and dried at 40^{o}C for 24 hours, grinded for making instant dry yeast.

Results

First stage of experiment

The chemicals composition of pineapple waste and liquid fermented soybean waste is indicated in Table 1. From Table 1 it could be seen that pineapple and liquid fermented soybean waste contain micronutrien and the pH is below 5 which is favoured for the growth of *Saccharomyces cerevisiae*. In the pineapple and liquid fermented soybean waste, it was found sugar as carbon source about 55% and 2.32% respectively. Liquid fermented soybean waste also contain enough nitrogen (1.077%), while pineapple waste also contain nitrogen but only 0.149%.

Table 1. Chemicals composition of pine apple waste extract and liquid fermented soybean waste

Parameter	Pineapple waste extract	Liquid fermented soybean (%)
Total Sugar (%)	9.75	3.32
Reducing Sugar (%)	8.2	2.32
Nitrogen (%)	1.66	1.077
Pantothenic Acid (mg/100 ml)	0.33	0.211
Thiamine (mg/100 ml)	0.056	0.103
Magnesium (Mg) (mg/100 ml)	44.979	11.611
Zink (mg/100 ml)	1.983	0.19
Manganese (mg/100 ml)	0.275	5.641
Calcium (mg/100 ml)	91.787	64.21
Kalium (mg/100 ml)	1426.197	748.883
Ferro (mg/100 ml)	4.135	1.678
Cupprum (mg/100ml)	0.287	0.110
Sulfur(mg/100 ml)	172.55	3.731
Phosphate (mg/100 ml)	306.096	57.393
pH	4.5	4.9

From the yeast growth profile above it could be seen that the highest productivity and shortest adaptation phase was obtained in media C wereas the smallest productivity was found in media A. In media C the adaptation phase was 4 hours and reached the stationer phase after 8 hours of incubation and stated to decrease slowly after 20 hours of incubation. In Media A the adaptation phase was 8 hours and reached the stationer phase after 18 hours after of

incubation and decrease after 22 hours of incubation. So that media C is the best proportionfor the growth of *Saccharomyces cerevisiae*.

Second stage of experiment

Based on result obtained from the first experiment Media C is used for the production of dry yeast using encapsulation method consisting of lechitin as emulsifier and dextrin as filler. The media C containing 6.44% reducing sugar and nitrogen 0.52%. The inoculation of *Saccharomyces cerevisiae* on this media and incubated 8 hours has shown promising results. The optimum solution obtain from software design expert 7.1.3 is depicted in Table 2.

Tabel 2. Optimum solution of design expert 7.1.3

X_1 (%)	X_2 (%)	Water content (%)	Solubility (%)	Cell viability (%)	Ethanol content (%)	Desirability	Status
70	2.47	6.121	78.249	62.342	4.194	0.965	Selected

Water content

The respon curve could be seen in the Figure 1. The optimum water content of the encapsulated dry yeast was 6.248% with dextrin content 60% and Lecithin content 1.50%. The addition of lecithin concentration significantly affect the water content of instant dry yeast. This is due to the molecular structure of lecithin is very effective to combine the liquid phase and the oil phase. This condition cause the solution which contain dextrin aquadest and the yeast cell has the stabilized condition which is promoted the rate of water during evaporation. The water content of dry yeast could be detected through the following equation:

$$\text{Water content } (Y_1) = 3.8 + 0.07X_1 - 0.14X_2 + 0.0001X_1X_2 - 0.001X_1^2 - 0.04\,X_2^2\,,\ R = 92.70\%$$

Where Y_1 is the water content, X_1 is the dextrin concentration and X_2 is the lecithin concentration. Based on the above equation, it is found the maximum water content is depend on the lecithin and dextrin concentration

Solubility

The respon curve of the solubility could be seen in the Figure 2. The optimum solubility was 77,476% with dextrin content 60% and Lecithin content 1.50%. The concentration of lecithin significantly affect the solubility of instant dry yeast power. The solubility of dry yeast could be detected through the following equation:

$$\text{Solubility } (Y_2) = 48.006 + 0.857X_1 - 2.984X_2 + 0.024X_1X_2 - 0.006X_1^2 - 0.463X_2^2\,,\ R^2 = 94.44\%$$

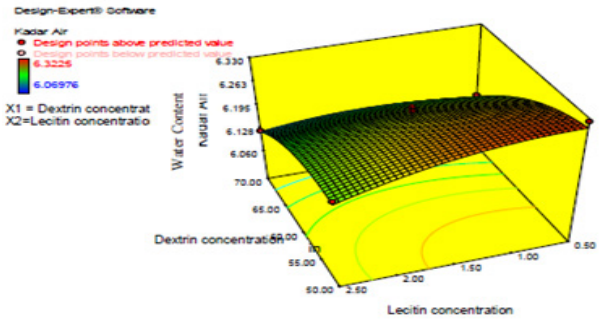

Figure 1. The respon curve of water content

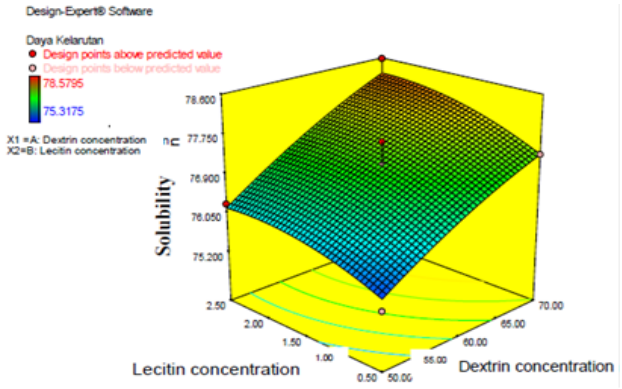

Figure 2. The respon curve of solubility

Where Y_2 is the solubility, X_1 is the dextrin concentration and X_2 is the lecithin concentration. Based on the above equation, it is found the maximum solubility of dry yeast is depend on the lecithin and dextrin concentration

Cell viability

The respon curve of the cell viability could be seen in the Figure 3.The optimum cell viability was 62.50% with dextrin content 60% and Lecithin content 1.50%. The relationship of cell viability and the dextrin and lecithin concentration is showed in the following equation.

$$\text{Cell viability } (Y_3) = 28.434 + 1.021X_1 - 1.841X_2 + 0.008X_1X_2 - 0.007X_1^2 - 0.391X_1^2\,,\ R^2 = 92.40\%$$

Where Y_3 is the cell viability, X_1 is the dextrin concentration and X_2 is the lecithin concentration. Based on the above equation, it is found that the maximum cell viability of dry yeast is depend on the lecithin and dextrin concentration.

Ethanol content

The respon curve of the ethanol content could be seen in the Figure 4. The optimum ethanol content was 4.203% with dextrin content 60% and Lecithin content 1.50%. The addition of dextrin concentration and lecithin affect on the cell viability and also the ethanol content after the resolution of the instant dry

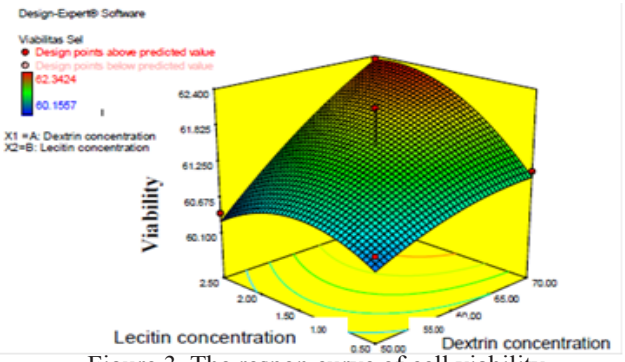

Figure 3. The respon curve of cell viability

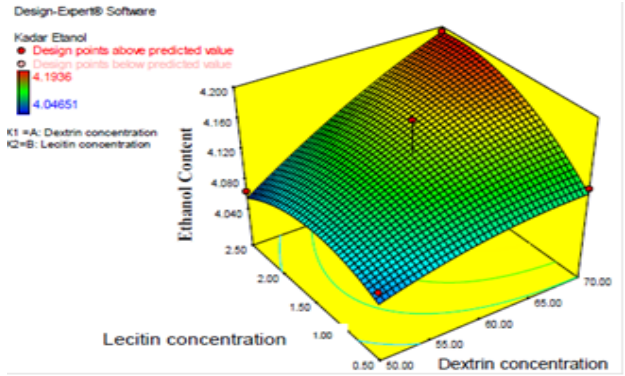

Figure 4. The respon curve of ethanol content

yeast on to water containing sugar. The encapsulation of the cell with lecithin and dextrin may protects the cells from the disruption during the drying process. The viability of the instant dry yeast is promising indication the utilization of dry yeast for making bread as well as beverage drink. The relationship of ethanol content and the dextrin and lecithin concentration is showed in the following equation:

$$\text{Ethanol content } (Y_4) = 2.183 + 0.058X_1 - 0.201X_2 + 6.3310\text{-}6\ X_1X_2 - 0.000465X_1^2 - 0.064X_2^2 ,$$

$$R^2 = 87.70\%.$$

Where Y_4 is the ethanol content, X_1 is the dextrin concentration and X_2 is the lecithin concentration. Based on the above equation, it is found the maximum ethanol content of dry yeast is depend on the lecithin and dextrin concentration.

Discussions

The growth profile of yeast in media C with proportion of pinneapple waste and fermented soybean waste in ratio of 1:1 has the highest and shorthest adaptation phase. This may be due to the media composition containing high nutritionous compound, which favoured the organisms to grow (Ketnawa *et al*., 2012). Furthermore, Pradana *et al*. (2011) stated that media for growth must contain 0.5% sugar and 0.5% nitrogen. In the media A containing the proportion of pineapple waste and liquid fermented waste in a ratio of 0:1 has the smallest productivity. This condition may be due to the sugar content of the media is low and this consistent with the finding obtained by Pradana *et al*. (2011). As stated by Waites *et al*. (2009), carbon source used for energy process during the course of the growth of organisms. Carbon support the organisms to produce ATP (Adenosine Triphosphate) and whereas nitrogen is needed for enzim synthesis in metabolisms.

The optimum water content of the encapsulated dry yeast was 6.248% with dextrin content 60% and Lecithin content 1.50%. This is due to the present of emulsifier and filler will stabilized the cell and accelerate the evaporation of water during drying (Sohail *et al*., 2013).

The concentration of lecithin significantly affect the solubility of dry yeast power, this is caused by clusters that exist in lecithin. It is caused by various factors, including the process of agglomeration or dissolution instant product depends on the material surface encapsulate (Sohail *et al*., 2013). Encapsulate material comes from a variety of materials from carbohydrates such as starch, dextrin, maltodextrin. The use of maltodextrin for encapsulation of Lactobacillus sp. has been reported by (Collin *et al*., 2010). Another filler such as gum arabic or whey and skim also has been searched (Rizqiati *et al.,* 2009). Dextrin has a hydroxyl group that serves to bind water. The higher the concentration the higher dextrin hydroxyl groups so that during the process of mixing with water it will rapidly bind dextrin water solubility so that power will be high (Waites *et al*., 2009)

According to Pradana *et al*. (2011) media suitable for microbial growth must contain macro and micronutrient and also environmental condition such as pH which appropriate for microbial growth. In the Media C, the substrate contain high reducing sugar of 55% and nitrogenous compound 2.32%. This condition is favoured for the growth of organisms (Morgan *et al*., 2009). Consequently, the cells having promising condition, so this healthy cells could withstand during drying. On the other hand, the encapsulation of the viable cells with dextrin and also lecithin may protect the cell. According to Collin *et al*. (2010) , dextrin is enkapsulat material that is widely used in the food industry because it has a few advantages that easily absorbs water when mixed with water without causing an increase in viscosity and be able to have high porosity so may protect microbial cells. It is caused by factors enkapsulat materials and materials emulsifier (lecithin).

However, about 37% of the death cells was observed. The death of cell occured may be due

to the exposure of the cell at high temperature since *Saccharomyces cerevisiae* is a mesofilic cell (Jarolim, 2013). This microbes are not stable at high temperature. If the cell is not good encapsulated, the protein will demage and cause the cell being desrupted. Cell with well encapsulated could with stand the high temperature applied during dehydration (Rizqiaty *et al.*, 2009).

The utilisation of instant dry cell for the production of bread or beverage drink could be detected through the ability of the yeast to produce ethanol. The dissolution of the instant dry yeast on the water containing sugar could produce ethanol of 4.203% with dextrin content 60% and Lecithin content 1.50%. This is due to the fact that the encapsulation could protect the viability and the properties of the cells. So, the cells still has the optimum fuction to produce ethanol

Conclusion

Solid waste and liquid waste of fermented soybean processing in a ratio of 1:0.5 is promissing substrate for the production of biomass The best yeast production is 4.35 x 10^8 cell/ml. The processing of this biomass in to be dry instant yeast shown promissing results. The optimmum condition for making encapsulated dry yeast is dextrin concentration 63.45% and lecithin emulsifier 1.57%. The characteristic of the product are water content 6.253%, solubility 77.563%, viability 62.609% and ethanol concentration 4.209%.

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