Substitution of Rice Flour Components with Sago Flour in Yeast Career Media on the Growth of *Sacharomyces cerevisiae FNCC3049*

Abstract

Sago flour is a type of carbohydrate in the form of starch that can be converted into sugar using the amylase enzyme produced by amylolytic microorganisms. Saccharomyces cerevisiae is a unicellular fungus with amylolytic capabilities, and has been widely used in the food and beverage industry with a fermentation process from materials containing carbohydrates such as cassava tape, glutinous rice tape, bread, beer, wine and even as a bioethanol making agent. S. cerevisiae has been traded in the form of discs with the name of tape yeast. The disc-shaped carrier media is made from materials containing rice flour with the addition of spices. On this occasion, the effect of rice flour substitution in yeast carrier media on the growth of S. cerevisiae will be studied. The ability of fungi to grow on media containing sago flour, the ability to develop media dough, the number of yeast cells per gram of sago flour-based tape yeast and the texture of the substituted carrier media are parameters that will be determined. The results of the study showed that S. cerevisiae fungi can grow on yeast media containing sago flour. Growth in yeast medium made from rice flour showed better growth compared to yeast medium made from sago flour, this is because the carbohydrate composition of sago flour is more complex than rice flour so it takes more time and cell mass to use it as a carbon source. However, in medium B with a substitution of 50 grams of sago flour with 150 grams of rice flour, it still showed dense yeast colony growth similar to the growth of yeast colonies in medium A, as well as with the use of media there was a reduction in media weight which indicated the process of growth and multiplication of yeast colonies.

Introduction.

The carrier media is a medium for microorganisms in this case the yeast *Saccharomyces cereviciae* which is used during the shipping or sales process of yeast isolates. Generally in the form of a starter medium made in the form of flour or solids(1). The sales product for this yeast isolate is in the form of a disc circle known as yeast tape (2). This yeast isolate is not pathogenic but rather an agent in the manufacture of fermented foods and beverages, so that carrier media products containing this yeast isolate are freely sold on the market. The character of yeast isolate is amylolytic which means it can use starch as a substrate for its carbon source which will be converted into glucose, this is because yeast cells can produce the amylase enzyme which functions to convert starch into glucose(3). Glucose through the glycolysis process will be converted into 2 pyruvic acids and then through the ethanol fermentation pathway ethanol is produced(4). In addition, carbon dioxide is also produced in the fermentation process(5). The character it has, so that during the process of changing from starch to alcohol will produce a taste and flavor that can be enjoyed by humans(6), for example in making cassava tape(7), sticky rice tape(8), making bread, beer, wine and developing bread dough(9).

Making a carrier medium using materials consisting of rice flour and cassava flour if the carrier medium is made in an area that is not a rice producer will be a problem and finally never make it. Therefore, in this land of the birds of paradise Papua, there are natural resources containing starch including sago trees(10). The results of sago stem extraction produce around 300 kg of sago flour from a gross weight of grated sago stems of 500 kg(11). The abundant sago stem extraction product is very unfortunate only for ordinary food to replace rice when there is no rice. Therefore, to increase the selling price of sago flour, it must be modified in the form of other products including for *S.cerevisiae* yeast carrier media. The success of this research can provide an agent for converting food ingredients into food and drinks. So that it can improve the food and beverage industry using fermented yeast isolates as a form changer and flavor and aroma enhancer for food and beverage industry products. In addition, sago flour is known to have a low glycemic index so it is safe for diabetics when consuming fermented products with sago flour yeast(12). The substitution of glutinous rice flour with sago flour has been tried in making dodol (a type of traditional food) and at 50% substitution the best quality dodol was produced.(13). It is hoped that by substituting rice flour with sago flour in the manufacture of yeast carrier media, a quality yeast carrier media product will be obtained.

Material and Methods

1. Tools and materials.

The equipment used in the study was a set of cooking utensils (basins, bowls, graters, blenders, cloths, trays, stirrers, and ovens). While the materials used were (50 grams of sago flour, 200 grams of rice flour, 80 ml of water, 2 cloves of garlic, 1 segment of galangal, 1 teaspoon of granulated sugar, and ½ lime) as well as Potato Dextrose Agar (PDA) medium (Oxoid CM0139) and *S. cerevisiae FNCC 3049* came from *Food and Nutrition Culture Collection* (FNCC) Gadjah Mada University.

2. Rejuvenation and making of *S.cereviciae* starter

Rejuvenation of the isolate was carried out by making a suspension of pure isolate culture by pouring sterile distilled water into pure isolate culture flour. Furthermore, inoculating into PDA solid medium, after growing inoculated into starter medium in the form of Potato Dextrose Broth (PDB) Oxoid CM0962 medium. After incubation for two days, the number of cells in ml of medium is counted.

3. Procedure for making sago flour-based tape yeast.

S.cereviciae starter with a cell density of 10^6 is mixed with rice flour in a bowl and set aside temporarily; spices in the form of galangal, garlic, sugar and sago flour plus water are blended until smooth, poured into a basin containing a mixture of rice flour with yeast cell suspension, stirred and lime juice is added, the basin containing the media mixture is covered with a thin cloth and incubated at room temperature for three days, after three days there is visible growth in the form of colonies on the surface of the medium, the water in the medium is removed by squeezing it using a cloth cover, the squeezed results are weighed and formed into flat balls then dried for two days, after drying it is weighed.

4. Experimental procedure

This study uses an experimental method by substitution 200gram rice flour a parts of yeast carrier medium recipe with the use of sago flour, namely by adding 0 grams of sago flour (control) A, 50 grams B, 100 grams C, 150 grams D and 200 grams E with the composition of other ingredients the same as in (tape yeast making procedure). The parameters measured were the number of yeast cells in each gram of sago tape yeast, the amount of media usage by yeast and the dry weight of the yeast media.

Results and Discuss

Results

In this study, the addition of sago flour with varying weights was attempted in the composition of the growth media for tape yeast (*S.cerevisiae*). The difference in composition lies in the substitution of rice flour in the composition of yeast media in general. The composition of tape yeast media A, 200 grams of rice flour without the addition of sago flour, media B 150 grams of rice flour and the addition of 50 grams of sago flour, media C 100 grams of rice flour and the addition of 100 grams of sago flour, media D 50 grams of rice flour and the addition of 150 grams of sago flour, and media E contains 0 grams of rice flour and 200 grams of sago flour. In various variations of growth media, it appears that S. cerevisiae grows well in media without sago flour. In media with partial substitution of rice flour also showed growth and the growth was relatively good in the substitution of rice flour with 50 grams of sago flour, as seen in **Figure 2.**

Figure 2. Growth of *S.cerevisiae* yeast in variations in the composition of sago flour content medium.

The growth of S.cereviciae can be proven by the use of the medium as shown in Figure 3. The growth medium without sago flour appears to be used immediately, there is an increase in cell mass at T0 to T1 and the weight of the medium appears to decrease along with the growth of fungal cells in the form of increasingly dense colonies up to T6 as in Figure 2. Likewise in the variation of medium B with the addition of 50 grams of sago flour and 150 grams of rice flour, while the use of the medium by yeast with variations of medium C, D and E shows the growth and use of the medium by yeast cells.

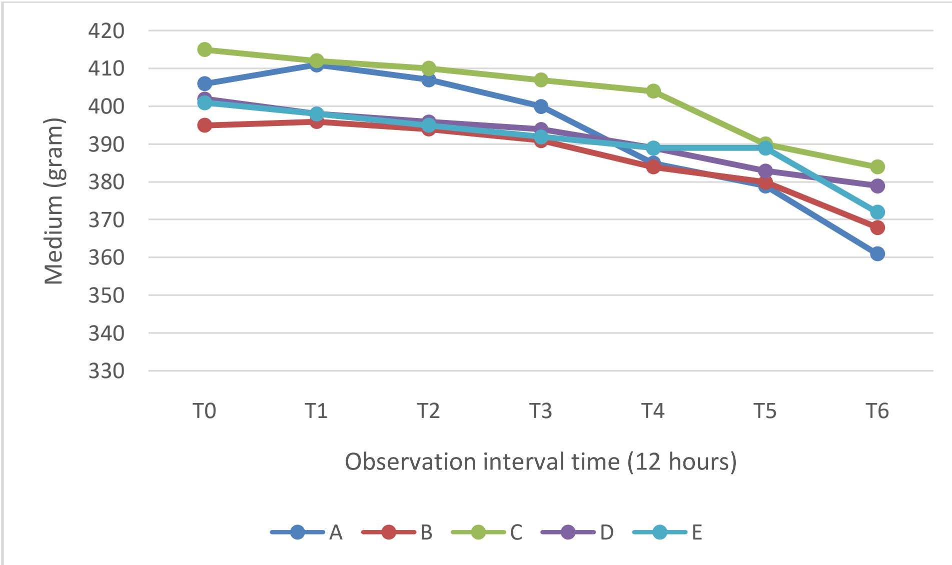

Figure 3. Growth and using of yeast medium variations by *S. cerevisiae*

The percentage of dry weight of the yeast tape carrier media after being dried for two days under the sun, as seen in Figure 4, shows that media A can dry with a water content of 11.5%, followed by media B, C, D and E. Medium with rice flour substitution with sago flour in drying for two days, the dry weight process is not like medium A, this is possible because in sago flour there are still fibers from the pith that still retain water, so the drying time needs to be increased.

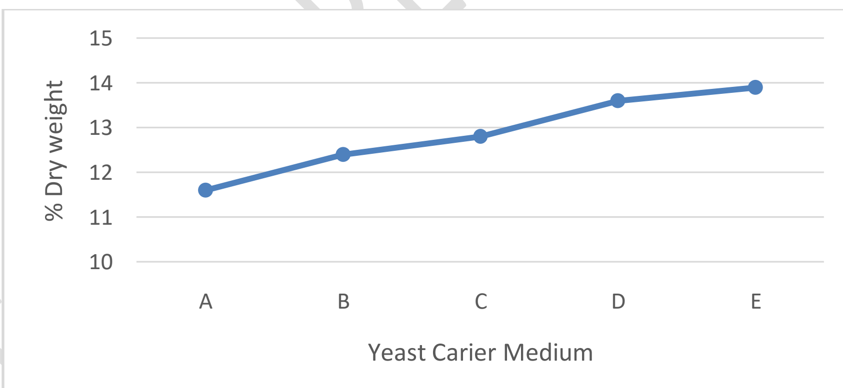

Figure 4. Percentage of dry weight of yeast medium variations after 2 days of drying.

The product of the dry tape yeast carrier media is shown in Figure 6. The tape yeast carrier media A is whiter than the other four variants, this is because the media composition does not contain sago flour. While Variants B, C, D and E are almost white, slightly white, slightly brown and brown, light brown sago flour affects the color of the tape yeast carrier media.

Figure 5. S. cerevisiae yeast carrier media with five variants

The dry yeast tape media is then used to determine the morphological shape of the yeast cells as shown in Figure 6, which is oval in shape and there are visible buds at the ends of the cells.

Figure 6. Morphology of yeast cells (M. 10 x 40)

Next, the density of the number of yeast cells/gram of medium was also calculated, it appears that medium A and B contain 10^8 CFU/gram of yeast media, medium C contains 10^7 CFU/gram and medium D and E contain 10^6 CFU/gram of yeast media, as shown in Figure 7.

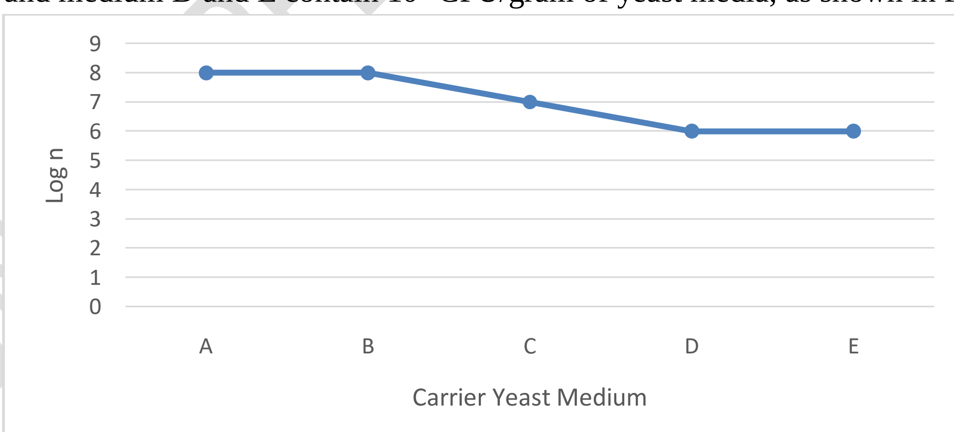

Figure 7. Number of yeast cells/gram of medium ($\log^n$)

Discussion

Yeast cells can be grown well on organic materials containing carbohydrates such as rice flour, cassava flour and sago flour. In addition to flour, they can also be grown on dregs or solid waste during processing to produce flour, such as corn stalks in ethanol production(14).

The use of sago flour to replace some or all of the rice flour used in making yeast carrier media has shown results with the growth of colonies in all variants of yeast media with various weight doses of sago flour added. In the medium usage graph (Figure 2), the adjustment phase to start yeast cell growth in all media variants shows that the medium is not used much and in the growth phase it shows quite significant use until the third day of the incubation period is complete (Dabija et al., 2017). The growth of yeast cells which is marked by a decrease in the weight. The weight of the medium can occur slightly increase because the composition of the medium contains sugar which is a source of carbon that is easily used by yeast cells for growth in order to increase the number of cells (Canché-Collí et al., 2021) due to glucose effect(15). The increase in the number of cells also means an increase in the number of individual yeast cells, and can then use sago flour or rice flour for the growth process (dos Santos et al., 2012). In figure 6, yeast cells that grow in carrier media show a pseudohypal (16) and an oval cell shape with some of the ends having buds as a tool for their reproduction by budding(17), using Scanning Electron Microscopy (SEM), yeast cells appear oval in three dimensions (Karimy et al., 2020). The number of yeast cells from the carrier media variants ranges from 10^8 to 10^6, and the highest is obtained from the yeast carrier medium with a composition without the addition of sago flour and also in the medium with the addition of 50 grams of sago flour. The growth of yeast cells in a medium containing sago flour does not seem to be very good. This is due to the carbohydrate content of sago flour is higher than that of sago flour(18), aside from that the presence of lignin wood content that is still present in sago flour, apart from being bad for the growth of yeast cells, it also causes the media to appear brownish(19).This requires optimal conditions to be found. With optimal conditions, yeast cell growth will be achieved as expected (20), likewise cell multiplication will be optimal(21). In general, the manufacture of food and industrial products made from sago still uses a mixture of other types of flour, for example in making bread, noodles, biscuits and cakes so that the taste, aroma, smell, color and texture can be accepted by consumers(22). From the research results, it can be recommended that rice flour be substituted with sago flour by using 50 grams of sago flour with 150 grams of rice flour in the recipe composition for making yeast carrier medium.

CONCLUSION

The yeast carrier media of Sacharomyces cerevisiae with rice flour substitution with sago flour in the nutrient composition of the carrier media can be used for the growth and storage of yeast isolates. Growth was clearly seen in media without sago flour substitution as well as in media with rice flour substitution with sago flour as much as 50 grams. The dry weight of each variant of yeast carrier media varied from 11% to 14%. The yeast cells used have characteristics similar to yeast cells in general, namely round oval with some tips that sprout.

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