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Preparation of Tempeh Spore Powder by Freeze Drying

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***Abstract*—**Study production of tempeh inoculums powder by freeze-drying comparison with dry at 50°C and the sun bask for developing efficient tempeh inoculums for tempeh producing. *Rhizopus oligosporus* in PDA slant cultures was incubated at 30°C for 3-5 days until spores and mycelium. Preparation spores suspension with sterilized water and then count the number of started spores. Fill spores suspension in Rice flour and soy flour, mixed with water (in the ratio 10: 7), which is steamed and sterilized at 121°C 15min. Incubated at room temperature for 4 days, count number of spores. Then take the progressive infection and full spore dough to dry at 50°C, sun bask, and lyophilize. Grind to powder. Then pack in plastic bags, stored at 5°C. To investigate quality of inoculums which use different methods, tempeh was fermented every 4 weeks for 24 weeks of the experiment. The result found that rice flour is not suitable to use as raw material in the production of powdered spores. Fungi can growth rarely. Less number of spores and requires more time than soy flour. For drying method, lyophilization is the least possible time. Samples from this method are very hard and very dark and harder to grind than other methods. Drying at 50°C takes longer time than lyophilization but can also set time use for drying. Character of the dry samples is hard solid and brown color, but can be grinded easier. The sun drying takes the longest time, can't determine the exact time. When the spore powder was used to fermented tempeh immediately, product has similar characters as which use spores that was fresh prepared. The tempeh has normal quality. When spore powder stored at low temperature, tempeh from storage spore in weeks 4, 8 and 12 is still normal. Time spending in production was close to the production of fresh spores. After storage spores for 16 and 20 weeks, tempeh is still normal but growth and sporulation were take longer time than usual (about 6 hours). At 24 week storage, fungal growth is not good, made tempeh looks inferior to normal color, also smell and texture.

***Keywords*—**Freeze drying, preparation, spore powder, tempeh.

I. Introduction

TEMPEH is native fermented food of Indonesia [16], [22]. It usually made from soybeans and fermented with the fungus, *Rhizopus oligosporus* [4], [5], [8], [14]. The characteristics of tempeh are white mold mycelia combines soybean seed fix together. [11] The fungus is not harmful to human. The flavor of tempeh are different from others consumption soybeans [6], [7]. The fermentation also increases the nutritional value of fermented soybeans [1], [9], [10]. The ability to digest and absorb nutrients in soybeans has increase (and no flatulence) because fungi build many kinds of enzymes to digest proteins [3]. Nutrients Analysis found that the quantity of soluble nitrogen in tempeh is higher compared with soybeans [18], [20]. The amount of free fatty acid and vitamins such as vitamin B1 (riboflavin), niacin and vitamin B12 are also more than in soy beans. In addition, mold can also create antioxidant compound which prevent rancidity and destroy free radicals in the body. Tempeh can be used as a substitute for meat protein as well [13]. It is an Ideal source of protein for those who do not eat meat or those who low income. There may also be an approach to generate income because the production process isn't complicated. So this research studied the production of tempeh spore powder made by freeze - drying to develop highly effective inoculums for tempeh production.

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II. Procedure for Paper Submission

A. Culture preparation of R. oligosporus

1. Culture *R. oligosporus* in PDA slant. Incubated at 30° C 3-5 days until the fungal mycelium and spore are full filled. [2]
2. Add sterile water to PDA slant to prepare spore solution
3. Count number of spores in solution.

B. Spore Powder Preparation

1. Mix rice flour and soy flour with water (ratio 10: 7). Sterilized at 121°C 15 minutes.[21]
2. Add the solution spores to the sterilized flour.
3. Incubated at room temperature for 4 days or until media full filled with fungal mycelium and spore.[12],[17]
4. Count number of spores.
5. Bring the fermented flour to dry by [19]

5.1 hot air oven at 50°C
5.2 sunbathing.
5.3 lyophilization.

6. Grind dried flour and pack in plastic bags. Store at 5°C.

C. Verify the Quality of Inoculums

Verify the quality of inoculums using different drying methods by applying to ferment tempeh every 4 weeks for 24 weeks [15].

III. RESULT

A. Culture Preparation of R. Oligosporus

On PDA slant culture in day 3-5, *R. oligosporus* produces white mycelium and black spore (Fig. 1). Dilute to about 10^6 spores per ml for using as inoculums.

Fig. 1 White mycelium and black spore of *R. oligosporus*

B. Spore Powder Preparation

Comparative study of growth and sporulation of *R. oligosporus* in rice flour and soy flour found that *R. oligosporus* growth and sporulation on soy flour faster and better than on rice soy flour. Since fungi do not grow well on rice flour, therefore spore production chose to use only soy flour.

Fig. 2 Growth of *R. Oligosporus* on rice flour and Soy flour

TABLE I
COMPARISON OF THE GROWTH OF *R. OLIGOSPORUS* ON RICE FLOUR AND SOY FLOUR

feather	Rice flour	Soy flour
Mycelium growth	Growth is very slow. Growing spotty. Mycelium not covered substrate. (Little progress).	Grow rapidly and can be covered substrate in around 18 hours
The duration of sporulation	Take longer than 72 hours, but most will not produce spores	Sporulation within 48 hours and spores full not over 96 hours.
color of the spores	Black	Black

Fig. 3 Spores powder from rice flour after grinding

Fig. 4 Spores powder from soy flour after grinding

TABLE II
CHARACTER OF SOY FLOUR AFTER DRYING

	DRYING METHOD		
Result	Dried in hot air oven at 50°C	Sunbathing	lyophilization
Characteristics of spores powdered	Solid lump	Solid lump	Solid lump, very hard
Color	Dark brown	Dark brown	Dark brown
Period of drying	Over 48 hours	Over 72 hours	Over -night but not more than 18 hours
Duration of spore germination	Not over 18 hours	Not over 18 hours	Not over 18 hours

Fig. 5 Character of the sample after lyophilization

C. Verify the Quality of Inoculums

When use fresh spore powder produces tempeh, tempeh product has normal quality in both color and flavor. Spore powder which stored in the refrigerator at 5°C was used to produce tempeh every 4 weeks for 24 weeks. The properties of tempeh were still good in all character. Spores can germinate and grow to mycelium covered soy bean in 18 hour. Even in the latter weeks, Germination is quite noticeable slow but it also has developed to be tempeh.

IV. Conclusion

From the results found that rice flour is not suitable to be used as raw material in the production of powdered spores because the fungus didn't grow well. They produced few spores and consumed long time, which may cause contamination. Unlike the use of soy flour, fungus can grow quickly. Mycelium and spores produced in less than 18 hours that can be easier to control contamination by other microorganisms. The fungi grow well in soy flour is probably because there are more nutrients, especially protein.

When incubate inoculated soy flour at room temperature for 4 days, the mycelia and spores were mature completely. Get it dry by using hot air oven at 50°C, sun bathing and lyophilization. Lyophilization took the least time and easy to control drying conditions. But the character of the drying sample is quite not good. It is very dark and hard, very difficult to grind than product from other methods. Drying at 50°C took longer time than Lyophilization but also can control drying condition and contamination. Character of the sample when dry is brown solid but not difficult to grind. Sun bathing is uncontrollable drying because of unpredictable temperature and weather each day, while contamination potential is relatively high. Character of the sample after drying is darker and looks solid. It takes the longest time to dry, can't determine the exact time. But if there is no device use for drying, sun bathing can be applied.

When use fresh spore powder to produce tempeh immediately, products have alternatives property to ones which use fresh spores from media. The tempeh has normal quality, both color and flavor. Using spores powder stored at low temperature (in the refrigerator) produce tempeh, in weeks 4, 8 and 12 found that tempeh is still normal. Time spent in production was close to the production from fresh spores. After 16 and 20 weeks storing, the tempeh is still normal but growth and sporulation time were longer than usual (about 6 hours). In Week 24, it was found that the growth of the mycelium was not good. This make tempeh looks inferior from normal tempeh. This tempeh had poorer in color, flavor and texture.

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