International Journal of Trend in Scientific Research and Development (IJTSRD)
International Open Access Journal | www.ijtsrd.com
ISSN No: 2456 - 6470 | Volume - 2 | Issue – 6 | Sep – Oct 2018

The Production of Instant Powder Fermented Soy Food Supplement Enriched with Rice Bran

Sri Kumalaningsih
Professor, Universitas Brawijaya, Malang, Indonesia

ABSTRACT
Fermented soy bean or tempe has been documented as source of vegetable protein and consumed mainly by most Indonesian people living in Java Island. However due to having short storage life and also lack of vitamin and mineral become the main hindrance to develop the utilization of this product. The processing of fermented soy bean into probiotic powder supplement enriched with rice bran has been searched. Three stages of experiments were carried out. The first stage was to find out the effect of fermentation time on the characteristic and total microbial load of fermented soy bean. A single factor with six levels (4, 6, 8, 10, 12 and 14 hours) with four replications were used to carry out this study. The second stage of experiment was to find out the effect of temperature and emulsifier concentration on the characteristic and storage life of the fermented powder food supplement. A randomize block design was used to carried out this study. The temperature at three levels (50, 55 and 60ºC) as the first factor and emulsifier (tween 80) concentration at two levels (0.3 and 0.4 w/w) as the second factor, all the treatments were repeated three times. Result from the first stage shown that the fermentation time of 12 hours has the highest total microbial load of 0.8x108 cells/ml while the protein content was 2.894% w/w, fat content 1.2209 % w/w, pH 3.6 and the ash content of 0.57% w/w. Result from the second stage shown that the temperature of 500C and tween 80 at a concentration level of 0.3% w/w, has total microbe of 0.24x108 cells/ml and 282.67 mg/100 g isoflavone. The powder enriched with rice bran has a significant role to increase the body weight of rats. After 15 days of feeding the rats body weight increased about 30% w/w.

Keyword: *Fermentation, Foam mat drying, Instant Powder, Rice Bran, Soy Bean.*

INTRODUCTION
soy bean is one of the most important horticultural crop, easy to cultivate and could grow at fertile land[1]. Furthermore, fermented of this soy bean into product which so call *tempe* has been familiar and consumed by most of Indonesian people due to it is tasty and also low price (…). However, this product having low storage life of only 48 hours and become rotten, and loss Vitamin B and mineral [2]. Under such circumstance approach to process this soy bean into probiotics powder enriched with rice bran is expected to develop further use of the natural herbal protein having high storage stability and could be transferred to another island.

Soy bean is well documented not only as protein and oil source but also content isoflavone[3] which is very important as phytoestrogen hormone and improve the palatability of children [4],could replace the shortage of cow milk because of its high protein content. Furthermore it is documented that 500 ml soy milk could fulfill 30% of the need of protein which should be consumed by children and also important being eaten by menopause woman due to its content high isoflavonr [5]. According to Patisaul and Jefferson [6] isoflavonewas found abundantly in the soybean tissue as glycoside attached with glucose. hydrolyzationthis linkage through fermentation caused the free isoflavone which is very important hormone mainly for menopause woman [7].

The inoculation of *Lactobacillus sp*. on to the soybean milk to release the glycoside through fermentation process is expected could produce three biotic namely dietary fibre (prebiotic), *lactobacillus* (probiotic) and isoflavone[8].However this product still lack of mineral (Ca, Fe) and also vitamin such as thiamine and riboflavin. The addition of rice bran on to the soy milk may improve the shortage of mineral as well as

the vitamin of soy milk and could be used as food supplement. Rice bran according [9] not only contained mineral and vitamin but also contained essential amino acid which promote lactic acid bacteria to grow [9]. However, the application of this product in the liquid form may face several problems due to not easy to perform and handle, but also difficult to transport. The processing of this product into instant probiotic flour is essential.

Processing of probiotic flour due to high protein and amino acid content should be dried using low temperature. Foam mat drying method is one of the most important dehydration involve a low temperature and not affected the biotic compound Jayaraman and Das Gupta [10]. The liquid was added with albumin and shake until a lot of foam was produced. Then the slurry was spreaded out on the try and dehydrated at low temperature [11].

The use of foam mat drying method is considered beneficial not only could protect the bioactive compound but also produce product having tasty flavor. Dehydration method with foam mat drying has been practiced by Sulaksono, Kumalaningsih [12], for the processing instant yoghurt. According to Molnes [13] the effectiveness of this method is affected by the interaction between filler and emulsifier. Dextrin and maltodextrin had been reported as a gentle and nontoxic filler [14]. Furthermore Kumalaningsih, Padaga [15] stated that maltodextrin was more promising than that of dextrin due to it is soft and easy to be enzymatically altered by the organism to increase the moisture content for supporting the cell growth. The use of emulsifier to accelerate the evaporation of the free water is important. Tween 80 as emulsifier has been utilized by Kumar, Subas [16] which is excellent to enlarge the surface area. Narsih, Kumalaingsih [17] stated that this product could push the drying rate. The dry product is therefore should blend, screened in the 60 mesh to get a very tiny powder. To convince the quality of the dry product it is an urgently required to evaluate storage stability of the product stored at room temperature. Finally the implementation of the product, a feeding trial should be carried out on the effect of the probiotic protein on the weight of rats. The objective of this study was to find out the best treatment for the production of “instant probiotic powder food supplement” enrich with rice bran.

Materials and Methods:

Materials Preparation

Soy milk

Soybean purchased from the Food Crop Institute Malang East Java province was weight 1000 g washed, cleaned and soaked for 16 hours in water. The peel was removed and the bean was added with boiling water in a ratio of 1:6. The peel was discarded, and the soybean was added with hot water, blended. The soybean slurry was screened and separated between soybean milk and the soybean waste, mixed with 5% (w/v) rice bran, stirred, then was added with 5% (w/v) skim milk and 5% (w/v) sucrose, pasteurized at 100^{o}C for 30 minutes, then cooled.

The rice bran purchased from the rice mill at Malang region east java province was screened with a screener at 60 mesh and packaged in Aluminum Foil.

Maltodextrin and tween 80 were purchased from local chemical shop and used for the second experiment.

Fermented Soy Milk and Rice Bran Preparation

The soybean milk prepared above was mixed with 5% (w/v) of rice bran and aseptically added with 5% (w/v) skim milk and 5% (w/v) sucrose placed on reaction tube (10 ml) inoculated with culture of *Lactobacillus sp.* incubated at 30^{o}C for 24 hours then delivered to 990 ml pasteurized soy milk and rice bran and incubated for 18 hours, pasteurized for 3 minutes and cooled.

Culture preparation

Pure culture of *Lactobacillus sp* was purchased from PAU (Intern-University Centre) Gajah Mada University Central Java Province Indonesia. This microbes were cultured on MRS broth incubated at 30^{0}C for 48 hour and harvested by centrifugation. The sediment cells were washed three times with 0,1% buffer peptone and used immediately in the experiment. One ml of the fresh culture *Lactobacillus sp.* was added on to 9 ml of MRS broth, incubated at 30^{o}C for 24 hours and then delivered to 990 ml of the substrate.

Methods

Experiment1. The effect of time of fermentation on the characteristic of fresh fermented soy milk

A randomized block design was used to carry out this study. Time of fermentation at six level (4, 6, 8, 10, 12 and 14 hours) with four replications was carried out for this study. The fresh culture was diluted with

10 ml of aquadest shaked and one ml of the culture was inoculated on 9 ml of the prepared substrate, incubated at 30^0C for 24 hours then delivered to 990 ml of prepared mixed substrate, incubated at 30^0C for 18 hours.

Experiment2. The effect of temperature and emulsifier concentration on the characteristic of instant powder Fermented Supplement
The fermented supplement consisting of soy milk, rice bran, sucrose and skim milk was then treated with 8% (Maltodextrin) and Tween 80, according to the treatment pasteurized at 80^0C for 15 minutes. The slurry was then spreaded out on a tray and dehydrated at 50^0C for 18 hours at cabinet dryer.

Chemical composition analyses was determined by AOAC series method [18], moisture content [18], protein [18], Fat Content [18], Isoflavone was determine by HPLC method [19].

Experiment3. Feeding Trial of instant powder fermented supplement
To convince the quality of food supplement a feeding trial was carried out. The albino rats purchased from the Department of research East Java Province were used.

The albino rats were selected and divided into two groups.
Group1. The rats fed with standard Feed
Group2. The rats fed with standard feed and the granulated flour.

The standard feed is a mixture purchased at local animal feed shop at Malang Region contained of corn grit, rice bran and fish meal. The feeding trial was carried out for 15 days and each group was fed with 20 g feed twice a day. Prior to feeding the pH of the mixture should be adjusted to neutral.

Statistical Analysis
Data were analyzed by performing analysis of variance (ANOVA) using the statistical software package NCSS (version 5.1; Kaysville, UT). Significant difference were detected by performing a Tukey multiple range test, considering a significance level (α) of 0.05.

Results and Discussion:
Experiment 1: Fresh Fermented mixture of Food Supplement (Soy milk and rice bran)

Prior to fermentation, the chemical composition of the soy bean flour enrich with rice bran and the composition was shown in Table 1.The protein content was 4.53% w/w, fat 1.46% w/w, isoflavone 0.05% w/w and moisture content 10%. After fermentation, that was significance decreased of the protein and fat, but the isoflavone content increased. This condition due to the fact that the protein and fat content was used by the organism for supporting the growth and started to hydrolyze the linkage of glucose and isoflavone.This caused the isoflavone increased substantially. This result consistent with the finding obtain by Nakajima, Nozaki [4].

The result of the characteristic of fresh fermented food supplement after fermentation was shown in Table 2.

Protein and Fat Content
As shown in Table 1 prior to fermentation of 12 hours, the product has protein and fat content 2.801% w/w and 1.222% w/w respectively. After the inoculation of microorganism the protein and fat content decrease due to the organism used up the protein and protein and fat as source as energy. It is well documented that soy milk has low protein and fat content in compared with cow milk. In this study, the soy milk was diluted with water in a ratio of 1:6, hence obviously the initial chemical composition was very low. [20, 21]. The microbial enzymes degrade the high molecule weight to support their grow[22].

The enrichment of 5% rice bran and also the addition of 0.5% sucrose on to the soy milk, supported the LAB growth. Zubaidah, Nurcholis [9] stated that inoculation of LAB on to rice bran producing high valuable probiotic drink, as shown in Table 1. The indicator of evaluation for making probiotic drink base on the total bacterial count. This result has pH of 3.0 and thought that this low pH was favored by the organism and protect the spoiled organism to grow and only acidophilic organism could grow[8].The fermentation time of 12 hours was chosen for the next further study. The addition of rice bran stabilized the substrat and improve its valuable compound such as glucose and amino acid. Glucose is formed due to the hydrolyzation of glycoside. Rice bran not only contained high dietary fibre but also mineral and amino acid. Fermentation stabilized rice bran and even improve its valuable compound [23]. The carbohydrate content as well as amino acid increase during fermentation.

Experiment2. The effect of temperature and emulsifier on the characteristic of supplement and the LAB growth

The characteristic of food supplement powder was shown in Table 3 and the LAB (Lacto bacillus) growth curve was depicted in Figure 1.

There was a significant interaction between treatments on the characteristic of food supplement powder. As the main indicator for evaluation was the pH. Temperature of 50^0C and 0.3% Tween 80 concentration showed that the pH was 3.37 the protein 35.56%, fat content was 1.86%, and the total microbe $0.8x10^8$ cells/ml and isoflavone content was 282.67 mg/100 g. Apparently tween 80 as emulsifier could enlarge the area of the slurry to push the drying rate and keep the survival of the LAB. This is consistent with the finding obtain by Narsih, Kumalaingsih [17] that tween 80 at 0.3% was the best emulsifier for drying liquid product. On the hand, the formation of isoflavone is a promising end product. The addition of rice bran could increase the isoflavone content by 282.67 mg/100g.As reported by Dewell, Weidner [24]isoflavone has been used as phytohormone and mostly used hormone therapy for menopause women [5]. Furthermore this probiotic powder could be consumed by children to increase the body weight.

Experiment 3: Effect of Granulated Food Supplement on the Weight of rats

The addition of 20% (w/w) of granulation powder on to the standard feed affected significantly the weight of rat. After 15 days of feeding trial the rats with mixture of granulated food supplement and standard feed increased the body weight of rats from 100 g to 130 g. The result is given in Figure 2.

From the results it could be seen that the feeding trial could increase the body weight of rats by 30%. This is due to the fact that the digestibility of the product as well as the quality of the granulated flour which contain isoflavone and also amino acid may support the body weight of rats [25, 26, 27].

The use of modified foam mat drying could produce excellent granulated product. The enrichment of 5% (w/v) rice bran increased the total bacterial count and also increased the body weight of rats. The food supplement is recommended for children and menopause women as herbal food.

Conclusion:

After 12 hours of fermentation the addition of rice bran prior to dehydration is very important because the rice bran may stabilize the fermented lignin and increase not only the water content but also mineral and amino acid. The addition of rice bran can stabilized the consistency of the product so evaporation in low temperature can occur until the product become dry. Grinding and screening will produce an instant flour of high quality and useful to be consumed by woman and also children. Socialization this process for the development of instant food mainly at rural region is very important.

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Table1. Characterization of soy bean flour enrich with rice bran prior to fermentation

Component	Percentage
Protein	4.534
Fat	1.463
Isoflavon	0.052
Moisture contain	10

Table2. Effect of Fermentation Time on the Characteristic of Food Supplement

Treatments (hours)	Protein Content (%)	Fat (%)	Log Cells/ml	Mh	Ash (%)
4	3.167 c	1.3435 c	0.35×10^3	5.5 c	0.519 a
6	3.004 b	1.282 b	0.7×10^4	5.0 b	0.531 ab
8	2.944 ab	1.259 b	0.7×10^5	4.0 b	0.544 b
10	2.904 ab	1.241 ab	0.8×10^6	3.2 ab	0.55 b
12	2.894 a	1.220 a	0.8×10^8	3.0 a	0.57 b
14	2.801 a	1.219 a	0.7×10^9	3.0 a	0.58 b

Table3. Effect of Emulsifier concentration (tween 80) and temperature on the characteristic of granulated food supplement

Temperature (^{0}C)	Tween 80 (%)	Total Micro oganism (log 10^8 CFU/ml)	Protein (%)	pH	Mois-ture Content (%)	Fat (%)	Isoflavone (mg/100 g)
50	0.3	0.24 c	35.56 d	3.37 a	11.79 f	1.81 f	282.67 c
	0.4	0.20 bc	27.70 cd	4.10 ab	9.58 e	1.73 e	275.33 c
55	0.3	0.11 ab	21.63 bc	4.60 bc	7.32 d	0.99 d	267.00 b
	0.4	0.10 ab	19.93 ab	4.57 bc	6.55 c	0.82 c	255.00 b
60	0.3	0.05 a	18.53 ab	4.82 cd	5.52 b	0.79 b	244.33 a
	0.4	0.04 a	15.79 a	4.83 cd	5.10 a	0.73 a	237.33 a

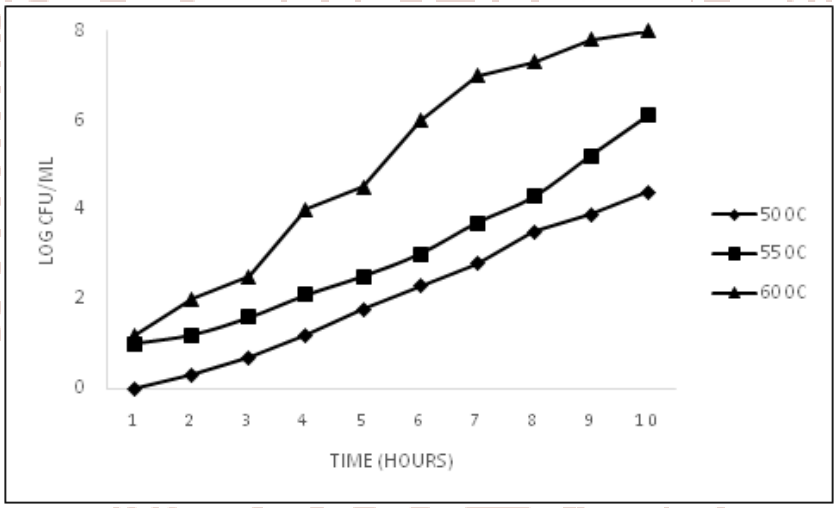

Figure1. The LAB curve of instant flour

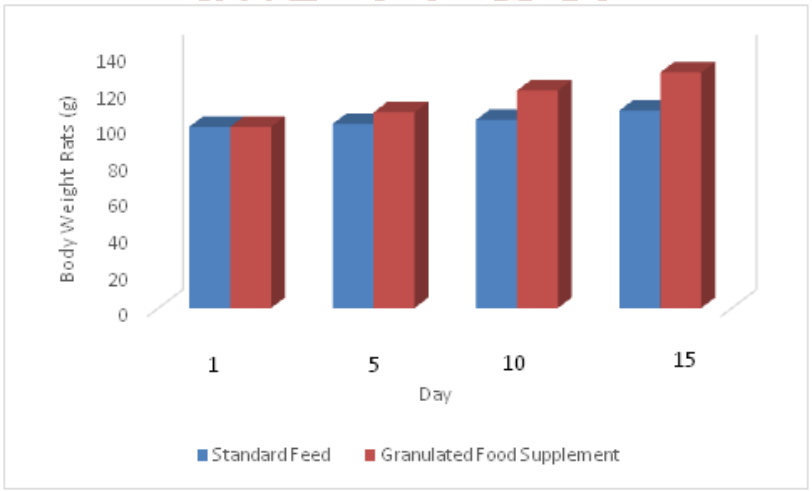

Figure2. The increasing weight of rats during Feeding Trial