



Application of Fermentation Technique to Antioxidant Activity of Soybeans (*Glycine max* (L.) Merr) Incorporated in Gel

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ABSTRACT

Soybeans (*Glycine max* (L.) Merr) was known to contain flavonoids that have antioxidant activity which can inhibit the formation of free radicals (Reactive Oxygen Species) causes' premature aging. This study was aimed to determine the effect of fermented soybeans antioxidant activity and determine physically stable gel formulation. The fermentation process used *Lactobacillus plantarum*. Determination of antioxidant activity was measured by DPPH method. The results showed fermented soybeans has higher antioxidant activity of IC₅₀ value 87.17 mg/mL compared to unfermented soybeans with IC₅₀ value 59.26 pg/mL ($P < 0.05$). The fermented soybeans were incorporated to the gel formulation with HPMC concentration variations. Based on the evaluation for 28 days is known that gel preparation is physically stable, homogeneity result showed the similarities of color, spreadability test result over a range of 6 cm - 4.1 cm, the measurement of pH value of $P < 0.05$ and viscosity measurements $P < 0.05$.

Keywords: Soybeans (*Glycine max* (L.) Merr); Antioxidant; Fermentation; DPPH

INTRODUCTION

Premature aging is a structural change and physiological function of the skin layer progressively characterized by a change in skin appearance [1]. There are two main factors that affect the aging process, namely intrinsic factors, such as genetic and hormonal conditions in the body; And extrinsic factors resulting from exposure to sunlight/ultraviolet, cigarettes, pollution and other sources containing free radical elements. Free radicals are an atom that has one or more unpaired electrons that cause the compound is very reactive, so that if free radicals in the body is not immediately addressed it will cause various negative effects [2]. One way to inhibit the effects of premature aging is with the use of antioxidants. Antioxidants can contribute to reducing and eliminating oxidative damage caused by reactive compounds and can protect cells from damage to cell death [3]. Based on sources, antioxidants are grouped into two, namely natural and synthetic. The use of synthetic antioxidants such as Butylated Hydroxyanisole (BHA) and Butylated Hydroxytoluene (BHT) has been widely prohibited in some countries because in many cases it has negative effects such as an increased risk of cancer.

The condition has prompted scientists to develop antioxidants derived from nature, one of which is soybeans. Soybeans contain phenolic compounds especially the isoflavone compounds that contribute to free radical capture and absorption [4]. In addition, IC₅₀ extract of soybean extract is known to have an IC₅₀ of 90.43 ppm [5]. Antioxidants from a plant can be increased antioxidant activity by using fermentation techniques. This is because fermentation can alter and produce more phenolic compounds from a plant that has a correlation to the increase in antioxidant activity [6]. Increased antioxidant activity may be due to the β -glucosidase enzyme produced by starter cultures used during the fermentation process [7]. The antioxidant activity obtained from the fermentation product may be phosphorylated to a pharmaceutical preparation such as a gel preparation in order to facilitate the process of its use. The gel preparation was chosen because ceramide SLN incorporated in gel preparation had a better effect when

compared to the cream preparations. This is because the gel contains a high enough moisture content to have effect hydration on the skin that can interfere with brick and mortar conformation in the stratum corneum so as to produce better penetration rate of the atif. Based on this background, a study was conducted to investigate the effect of fermentation on soybean (*Glycine max* (L.) antioxidant activity Merr) and obtain the best formulation of soybean (*Glycine max* (L.) Merr).

MATERIALS AND METHODS

Starter Culture Preparation

Sterilization of 10 ml MRSB media using autoclave at 121°C for 15 min, then add 1 ose *Lactobacillus plantarum* then incubation in incubator at 37°C for 18 hours [3].

Sterilization of Soy Milk

Fresh soy milk is put into a glass bottle covered with aluminum foil and plastic, then sterilized by using pasteurization method at 60°C for 30 min. This pasteurisasi process was conducted for two days [8].

Making Non Fermented Soybean Milk and Fermentation

Non fermented soy milk is made by inserting 500 ml of sterile soy milk into a glass bottle. As for fermented soy milk is made by entering 500 ml of sterile soy milk into glass bottles then add 1% *Lactobacillus plantarum* starter culture (v/v). Further incubation of non-fermented soy milk and fermentation into the incubator at 37°C for 24 hours [3].

Freeze Drying

Non fermented soy milk and fermented soy milk are fed into freeze dryer glass then frozen at -20°C until freezing, then dry in freeze dryer at -52°C for 38-48 hours or until soybean powder [9].

Antioxidant Activity Test

Testing is done by using DPPH method. The interpretation of the results of the antioxidant activity testing of this method is IC50 (Inhibition Concentration). IC50 is the concentration of test sample which will cause the reduction of DPPH radical activity by 50%.

Gel Formulation

The soybean milk powder that has the highest antioxidant activity was then incubated on gel preparation using HPMC gel base concentration variations of 1.5%, 1.75% and 2% (Table 1).

Table 1: Selected gel formulations

Material	Formula % (w/v)		
	FT1	FT2	FT3
Soybean Powder	0.87	0.87	0.87
HPMC	1.5	1.75	2
Propylenglycol	15	15	15
Glycerin	5	5	5
Microcare®	0.3	0.3	0.3
Aquadeion add	100	100	100

The HPMC is dispersed into hot glycerin at 50°C and stirred until HPMC expands by using a magnetic stirrer and then mixes microcare®, propylene glycol and soybean powder into a homogeneous mixture. The mixture is incorporated into previously developed HPMC and then adds aquadeion to 100 ml and stir until homogeneous using a magnetic stirrer at 200-300 rpm until gel preparation is formed. After the gel preparation is formed then it is stored in a sealed container and evaluate [10,11].

Evaluation of Gel Preparation

Examination of stability of the preparation was carried out at room temperature on days 1, 3, 5, 7, 14, 21 and 28 for organoleptic, homogeneity, spreading, pH and viscosity tests to determine the stability of the preparation during the storage process.

Organoleptic Test

The organoleptic test is performed to view the physical appearance by observing the shape, color and aroma of the prepared preparation [12].

Homogeneity Test

Homogeneity testing is done by placing gel between two glass objects then seen in direct rays. Homogeneity is shown in the absence of coarse grains [12].

Spreading Power Test

The gel preparation is weighed as much as 0.5 g then placed on a spherical glass. On top of the gel and then placed other transparent materials and weights so that the weight of glass round and ballast of 150 g, let stand for 1 min then then recorded the diameter of its spread [12].

pH measurement

Evaluation of pH value is done by using pH meter tool. Prior to use, the electrode is calibrated first by dipping the electrode in a standard buffer of pH 4 and pH 7. The pH measurement of the preparation is carried out by dipping the pH meter electrode into the gel preparation. The pH value reading is viewed on the screen when the pH value has shown a stable value.

Measurement of Viscosity

The viscosity measurement is done by placing the sample in the Brookfield viscometer until the spindle is submerged. Set the spindle and the speed to be used then the tool runs. Observe and note the numbers indicated by the constant pointing needle.

Hedonic Test

Hedonic test was performed on 20 panelists by applying the test formula on hand and left for ± 5 min. At each panelis observed comfort, safety and irritation reactions such as itching, redness, and swelling.

Data Analysis

The method used in the processing of evaluation of preparation from research of Fermentation Technique Application to Antioxidant Activity of Soy Bean (*Glycine max* (L.) Merr) which is incorporated Gel preparation that is using Statistics software spss 16.

RESULTS AND DISCUSSION

Fermentation Process

One indicator of the occurrence of fermentation is the decrease of soy milk pH. How to know the decrease of pH then at the time before addition of starter culture in soybean milk done pH checking first and at the end of fermentation process do check back again, it is necessary to know whether there is difference of pH before and after fermentation. The result of the measurement showed that during the fermentation process there was a decrease of pH value of soy milk which previously had pH 6 to about pH 5 (non fermented soy milk) and pH 4 (fermented soy milk). Decrease in pH can be caused at the time of fermentation process occurs, lactic acid bacteria used is *Lactobacillus plantarum* will hydrolyze the sugar contained in soy milk. All types of sugar contained in soy milk, used *Lactobacillus plantarum* as a source of energy to meet the growth process of bacterial cells and organic acids, so that the organic acid will cause a decrease in pH value during the fermentation process [8].

According to some researchers, the decrease in pH during the fermentation process may be related to the metabolic activity and growth ability of the microorganism itself [4]. In addition, the decrease in pH in the fermentation process has several positive effects, such as preventing the growth of other pathogenic microorganisms that may interfere with the development of microorganisms used as starter cultures [4]. The process of soy milk fermentation is done for 24 hours. After 24 hours, soy milk is transferred into the freezer at -20°C . Storage of soy milk in freezers serves to stop the fermentation process because *Lactobacillus plantarum* cannot survive at low temperatures or not optimal temperature growth [4].

Measurement of Antioxidant Activity

Before the measurement of antioxidant activity, first non-fermented soy milk and fermented soy milk dried by using freeze dryer. It aims to test the antioxidant activity by using the DPPH method which is unstable when the sample used contains water. Drying method with freeze dryer is also chosen because it has a lower degradation risk of test compounds, because the temperature used in this tool is so low that it is suitable for thermolabile compounds such as antioxidant compounds. The antioxidant activity test parameter of this method is the IC₅₀ value. IC₅₀ value of antioxidant activity of non-fermented soybean milk powder and fermentation (Table 2).

Table 2: IC₅₀ value of non-fermented soy milk and fermented soy milk

Sample	IC ₅₀ Value
Non Fermented 1	87.77 µg/ml
Non Fermented 2	86.82 µg/ml
Non Fermented 3	86.92 µg/ml
Fermented 1	59.44 µg/ml
Fermented 2	58.83 µg/ml
Fermented 3	59.50 µg/ml

From IC₅₀ value of non-fermented soybean milk powder and fermentation obtained statistically significant different result with value of $P < 0.05$ so that result can be interpreted that existence of process of fermentation in soybean milk can significantly influence antioxidant activity of soybean milk. This is because during the fermentation process responsible for the increase of polyphenol compounds are specific enzymes produced by microorganisms during the fermentation process ie enzyme β -glucosidase [4]. The β -glucosidase enzyme is a secondary metabolite of lactic acid bacteria, especially the bacteria used for this fermentation, *Lactobacillus plantarum* which has the function to hydrolyze the isoflavones glycosides ie genistin, daidzin and glycine which are conjugated with one molecule of sugar to isoflavone aglikon such as genestein, daidzein and glisetein [4].

Gel Formulation and Evaluation

In the three formulations are added antioxidant active substances from fermented soy milk of 0.87% w/v. The percentage was obtained from dosing calculations for topical preparations between IC₅₀ soy milk and IC₅₀ Vitamin C.

Organoleptic Test

The antioxidant gel preparation of fermented soybean powder showed clear and transparent color, but in the three selected formulas having different concentrations of gelling agent HPMC of 1.5%, 1.75% and 2% did not affect the aroma and color Of the gel preparation formed.

Homogeneity Test

Evaluation of homogeneity is one important factor because it aims to determine whether all the ingredients in the gel formulation have been mixed evenly [13] and may affect the drug distribution of a preparation. The gel preparation is said to be homogeneous if it has color equations in all parts and the absence of other visible and palpable particles or raw materials. From the results of homogeneity evaluation showed the existence of color equations and the absence of coarse particles so that it can be said that all gel preparations homogeneous.

Spreading Power Test

Spreading power evaluation conducted aim to know how gel ability can spread at location of usage. Spreading capacity for a good semi-solid preparation is used topically with a diameter of about 3-5 cm [13]. Data on gel thickness test results decreases in the range of 6-4.1 cm. In FT1, FT2 and FT3 have different concentrations of gelling agent HPMC used so that it can affect the viscosity value of a dosage. An increase in the viscosity value of a dosage may result in a decrease in the resulting generating power.

pH Measurement

Data of pH result of gel preparation which then processed by using statistic method of non-parametric Kruskal-Wallis test and Test Statistic The result of processing at FT 1, FT2 and FT showed significantly different result with FT1 P value of 0.018 < 0.05 , FT 2 P value of 0.045 < 0.05 and FT 3 P values of 0.030 < 0.05 . From all data it is found that during the 28 day evaluation period on the three selected formulas show significantly different or can be interpreted that the pH of the stock is unstable.

Measurement of Viscosity

The purpose of evaluation of viscosity measurement is to know the consistency of dosage which can influence the process of topical dosage application. From observation data, the higher the concentration of gelling agent the greater the viscosity value and vice versa. Overall, each formula shows the length of the dosage storage proportional to the increase in viscosity. The increase is due to the fact that HPMC does not have the strength to retain water so that evaporation processes occur which cause a decrease in water content and increase the viscosity of the stock [14]. The data of viscosity measurement of statistical data processing of FT1, FT2 and FT3 showed significantly different with P value of $0.000 < 0.05$, the result showed that the viscosity of the three formulas selected during the 28 day period was unstable.

Hedonic Test

Organoleptic test:

From the results of data processing the three selected formulas have a value of $0.661 > 0.05$ or not significantly different which can be interpreted that the three formulas are all favored by the panelists.

Irritation test:

Test results on 20 panelists found no indication of irritation or skin to be scaly, so it can be concluded that gel preparations have been safe to use.

CONCLUSION

Fermented soy milk has IC₅₀ 87.17 µg/ml greater than IC₅₀ non fermented soy milk of 59.26 µg/ml. The result of the evaluation revealed that the gel preparation was physically stable, homogeneity showed the existence of color equation, spreading test in range 6-4.1 cm, pH value of $P < 0.05$ and the viscosity of P value < 0.05 .

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