Conference Paper

The Processing Model in Making Tempeh Benguk [*Mucuna pruriens* (L.) DC] Containing High L-Dopa

Sri Winarni and Yudhy Dharmawan

Public Health, Diponegoro University, Prof Soedharto SH, Tembalang, 50239, Semarang, Central Java, Indonesia

Abstract

"*Koro benguk*" [*Mucuna pruriens* (L.) DC] seed contains L-dopa which is able to repair spermatozoa quality. The aim of this experiment is to analyze L-Dopa using HPLC in engineering manufacture of tempeh benguk. There are four engineering model's variants of making tempeh benguk. The engineering process begins with selection of *koro benguk* seed, tools required, immersion, boiling, humidity, temperature and pH. The result shows that the process in making tempeh benguk which contains the highest concentration of L-Dopa (11 346.85 mg · kg^{-1}) is white-colored and intact *koro benguk* seed; one time immersion with replacing the water for 4 to 5 times/day (two days and two nights); one time boiling (< 1 h until *koro benguk* seed can be opened); mixed with 10 mL lime juice/1.5 kg, with concentration of 0.83% until pH 5, added with mashed teak leaves, and ended with the process of making tempeh benguk at room temperature (30°CC), relative humidity room (65%). Tempeh *benguk* which is produced is cleaner, whiter, nonperishable, and more tender.

Keywords: HPLC; L-dopa; modified process of making; seeds benguk [*Mucuna pruriens* (L.) DC]; tempeh.

Corresponding Author:
Sri Winarni
wiwin.undip@gmail.com

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1. Introduction

One of the herbs that can improve fertility is *koro benguk* [*Mucuna pruriens* (L.) DC] seed, which 96% ethanol fraction of *koro benguk* seed contain 14.7% L-dopa and 56.47% isolates that could affect the functioning of reproductive system [1].This is consistent with previous research that claimed that the seeds have been proven through a research in animals that can improve spermatozoid's quality. In the animal research, data showed that koro benguk can increase sexual activity among normal white male rats [2], increase the motility and morphology of exposed spermatozoid to toxic substance of 2-ME [1].

According to Sukhla, *koro benguk* can increase spem quality [3], repairing semen profile, seminal plasma biochemical parameters [4], and increase male fertility [5]. Separately in Indonesia's region, especially in Wonogiri, Central Java, *koro benguk* is processed as tempeh benguk's ingredient, and in some UMKM—*Usaha Mikro, Kecil dan Menengah* (Small and Medium Enterprises) is processed as tempeh *benguk* chips. The

L-Dopa content in tempeh benguk has been studied within the first year of this study, which is as much as 9 781.55 mg · kg^{-1}. To increase L-Dopa content in tempeh *benguk*, modification is needed to either materials, eqipment, and processing. Therefore, it is necessary to test the content of L-Dopa active ingredient in modified tempeh *benguk*. The modification refers to the research of Misra and Wagner [6], which claimed that by increasing ascorbic acid will improve tempeh benguk's quality. Ascorbic acid serves as protector and has a maximum antioxidant activity (EC 50 = 2.5 μg) against DPPH radicals and toxic ion of MPP+ (l-methyl-4-phenylpyridium) [6].

2. Material and Method

This study is a laboratory study by measuring the L-Dopa content in modified tempeh *benguk* using HPLC techniques. The modification of tempeh benguk processing include the selection of koro benguk seeds, boiling and immersing, tempeh dough processing, temperature, and room relative humidity. At the stage of making tempeh dough process, there are several model group, one group was given by the mixture of lime as much as 10 mL for every 1.5 kg koro benguk seed with concentration of 0.83% until pH 5 and the other group was not given the lime mixture. At the stage of wrapping tempeh, the tempeh dough, which has been wrapped, is stored in two different conditions, one group is stored in a room with relative humidity of 70% to 80% and in the temperature of 30°C and the other group is stored in a room with relative humidity of 60% and in the temperature of 30°CC. Four groups of different tempeh was tested the L-Dopa content with HPLC. Before the L-Dopa content is being tested, those four groups of tempeh *benguk* is dried, mashed, and fractionized by using kinetic maceration with 96% ethanol applying MWELL-1299 techniques [6]. In general, kinetic maceration using 80% ethanol within 1 h and the formed residue will be repeated the process three times. This is aimed to maximize all of the substance to dissolve into the fractionation solution [7]. The kinetic maceration in this process is using 96% ethanol mixture with distilled water. Set of extraction solution is mixed and then concentrated using rotary evaporator [7]. This research has been granted permission worthy of ethics (ethical clearance) at the Faculty of Public Health, Diponegoro University, No. 113/EC/FKM/2015.

3. Results and Discussions

The result showed that the highest L-Dopa content (9 781.55 mg · kg^{-1}) is through the process of *koro benguk* seed which is boiled one time (<1 h until the seed can be opened) and immersed one time with water substituted 4 to 5 times/ day (two days two nights) added with mashed teak leaves.

Researcher have helped UMKM of tempeh benguk in Wuryorejo Sub-District and Tirtomoyo Sub-District to obtain a business license of PIRT—*Pangan Industri Rumah Tangga* (Small Scale Home Industry) in Health Department.

Table 1 shows that by adding lime and slightly lower the pH leads to an increase of L-Dopa content in tempeh *benguk*. The highest L-Dopa content in tempeh *benguk*

Sample	Treatment	L-Dopa (mg · kg^{-1})
1	Without lime addition, stored in 30°C, humidity 70% to 80%	5 021.37
2	Without lime addition, stored in 30°C, humidity 60%	3 595.83
3	Added with lime, stored in 30°C, humidity 70% to 80%	6 094.06
4	Added with lime, stored in 30°C, humidity 60%	11 346.85

Table 1: L-Dopa content variation in four treatments.

is at the treatment of adding lime as much as 10 mL for every 1.5 kg koro benguk seed or with the concentration of 0.83% which given before the tempeh dough is wrapped by leaves. This is consistent with the study of Haq et al. [8], which stated that the effective concentration of lime juice to preserve ± 1.8 kg rice which stored in rice cooker is 0.93%, and the exact time to give the lime juice is before cooking rice [8]. The result of tempeh benguk in this study is cleaner, whiter, nonperishable, and more tender.

The model of adding ascorbic acid refers to the fractionation process with M-WELL 1299 method. In the method of M-WELL 1299, there is an addition of 2.5 g of ascorbic acid in distilled water and 96% ethanol (1 : 1) (1 250 mL distilled water : 1 250 mL ethanol 96%). The addition of ascorbic acid (the content of lime) causes an increase in L-Dopa content in Tempeh *benguk*. Based on the study of Misra and Wagner [6], showed that to gain maximum L-Dopa, the *benguk* seed need to be extracted by using the mixture of EtOH-H_2O (1 : 1) and applying ascorbic acid as a protector, which known as M-WELL 1299. *Benguk* which used the extract of M-WELL 1299 will show a maximum antioxidant activity (EC 50 = 2,5 μg) against radical DPPH. In addition, M-WELL 1299 showed a result of opposing the toxic MPP^+ion *(l-methyl-4-phenylpyridium*) [6]. When compared to the previous study, tempeh *benguk* processing was not using vitamin C, therefore the measured L-Dopa content in tempeh *benguk* in this study is 9 781.55 mg · kg^{-1}. Vitamin C can increase the absorption of Levodopa in the body. It has been proven by Nagayama et al. [9]. With the addition of ascorbic acid of 200 mg · d^{-1} within a week, the Levodopa absorption is increasing in the body of Parkinson's patient [9].

In this study, ascorbic acid which is used, is from natural materials, which is lime juice. There are a lot of chemical compounds which are useful in lime such as citric acid, amino acids (tryptophan and lysine), essential oils (limonene, linalin acetate, acetic geranil, fellandren, sitral, lemon campher, kadinen, aktialdehid and anildehid), vitamin A, B1 and vitamin C. Rough lime extract has benefit of antimicrobial and inhibit anaerobic bacteria and gram-positive bacteria in the range of minimum inhibitory concentration (MIC) 32 g · mL^{-1} to 128 g · mL^{-1}. Citric acid in lime has the benefit of inhibit the growth of *Saccharomyces cerevisiae* and *Zygosaccharomyces bailii* [10].

Vitamin C is a type of vitamin which can repair damaged cells and can help increasing the L-Dopa production from amino acid tyrosine. The amino acid tyrosine is converted into L-Dopa aided by the tyrosine hydroxylase enzyme. L-Dopa is a precursor to

monoamine or catecholamine neurotransmitters dopamine, norepinephrine (noradrenaline), and epinephrine (adrenaline). Dopamine is formed by the decarboxylation of L-Dopa. The chemical structure of L-Dopa or Levodopa is $C_9H_{11}NO_4$. The molecular weight is 197.2. Its chemical name is 3-*hydroxy*-L-*tyrosine* or (-) 3-(3,4-*dihydroxyphenyl)*-L-*alanine*. Its color is white or almost white, will be change if exposed to air and light, shaped like a crystalline powder, odorless, tasteless, slightly soluble in water, soluble in HCl and formic acid, is not soluble in ethanol, chloroform, benzene, and ethyl acetate [11]. The dose of vitamin C which should be drinked by human for this process is approximately 100 mg to 300 mg (1 to 2 times a day).

The processing model in making tempeh *benguk* containing high L-Dopa are:

- Selecting koro *benguk seed*

  The selected *koro benguk* seed are the white seed, still intact and not deflated.

- Boiling and immersing

  The *koro benguk* seed is immersed once for two days two nights. The water is replaced for 4 to 5 times per day. The *koro benguk* seed is boiled one time for <1 h until the seed could be opened. The average time in this stage until the seed could be opened is 30 min, due to the utilization of standardized utensils for the immersion and boiling (stainless steel pan).

- Tempeh dough processing

  Clean *koro benguk* seed will be measured the pH until it reached pH of 6. The dough will be mixed with yeast/mashed teak leaves. In this stage, the dough was given by the mixture of lime as much as 10 mL for every 1.5 kg *koro benguk* seed with concentration of 0.83% until pH 5. The dough which was mixed by lime will be attempted to reach the pH of 5.

- The tempeh making with conditioned room temperature and humidity

  The *koro benguk* seed is wrapped by teak leaves. In this stage, this product is stored in a room with relative humidity of 60% and in the temperature of 30°C.

4. Conclusions

The modification of tempeh *benguk* which has the highest content of L-Dopa is by the process of selecting white and intact *koro benguk* seed, immersed one time with water replacement as much as 4 times/day to 5 times/day (two days two nights), boiled one time (<1 h until the seed can be opened), mixed by 10 mL lime juice for every 1.5 kg or with the concentration of 0.83% until the pH reaches 5 and given before the tempeh dough is wrapped by leaves, added with yeast/mashed teak leaves, and ended with the tempeh making process in room temperature of 30°C, room relative humidity of 65% with the result of L-Dopa content as much as 11 346.85 mg $\cdot$ kg^{-1}), which is higher than the tempeh *benguk* without any modification (the initial L-Dopa in tempeh *benguk* is 9 781.55 mg $\cdot$ kg^{-1}). In addition, the modified tempeh *benguk* is cleaner, whiter, nonperishable, and more tender.

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