International Journal of Nutrition Sciences

Journal Home Page: ijns.sums.ac.ir

SHORT COMMUNICATION

Comparison of IC_{50} Antioxidant Analysis of Local Soybean Tempeh and Imported Soybean Tempeh in Indonesia

Fery Lusviana Widiany[1*], Metty Metty[1], Rahayu Widaryanti[2], Shafira Nur Azizah[1]

1. Department of Nutrition, Universitas Respati Yogyakarta, Yogyakarta, Indonesia
2. Department of Midwifery, Universitas Respati Yogyakarta, Yogyakarta, Indonesia

ARTICLE INFO

Keywords:
Antioxidant activity
Antioxidant IC_{50}
Imported soybean tempeh
Local soybean tempeh

**Corresponding author:*
Fery Lusviana Widiany, PhD;
Department of Nutrition, Universitas Respati Yogyakarta, Yogyakarta, Indonesia.
Email: fery_lusviana@respati.ac.id
Received: August 10, 2022
Revised: November 11, 2022
Accepted: November 15, 2022

ABSTRACT

Background: Antioxidants contain in tempeh are free radical scavengers that can inhibit the process of lipid oxidation and prevent several diseases. Types of tempeh sold in the Indonesian market include local soybean tempeh and imported soybean tempeh. This study aims to compare and analyze the differences in the antioxidant activity of local soybean tempeh and imported soybean tempeh.

Methods: The research was conducted from June to November 2022 in Yogyakarta, Indonesia. This study used objects in the form of local soybean tempeh and imported soybean tempeh. Tempeh was floured first, then its antioxidant activity was analyzed in the laboratory. Antioxidant activity was defined as the amount of IC_{50} antioxidant in tempeh flour studied in units of mg/mL, with a ratio scale. IC_{50} antioxidant analysis used the diphenylpicrylhydrazyl (DPPH) method. The data obtained were compared and analyzed statistically using the Independent t-test.

Results: It was shown that the IC_{50} antioxidant level in both types of tempeh was significantly different (p=0.0001). The difference in the mean of the two groups showed a higher IC_{50} antioxidant score in local soybean tempeh of 9.96 mg/mL.

Conclusion: Based on the IC_{50} antioxidant value, imported soybean tempeh had significantly a higher antioxidant activity than the local soybean tempeh.

Please cite this article as: Widiany FL, Metty M, Widaryanti R, Azizah SN. Comparison of IC50 Antioxidant Analysis of Local Soybean Tempeh and Imported Soybean Tempeh in Indonesia. Int J Nutr Sci. 2022;7(4):241-244. doi: 10.30476/ijns.2022.97526.1213.

Introduction

Tempeh is a traditional food that has long been famous in Indonesia. This food can be made by fermenting soybeans for a certain time using the fungus *Rhizopus sp.* (1). Mushrooms that grow on red beans can hydrolyze the complex compounds present in the beans as the basic ingredients of tempeh such as carbohydrates, fats, and proteins into simple compounds in the form of glucose, fatty acids, and also amino acids which are compounds that are easily digested by the human body and have an impact on the fulfilment of nutrition (2, 3). Besides, due to the fermentation process, antioxidant components will be formed which function as free radical scavengers (4).

Tempeh has many advantages compared to other food ingredients, including high nutritional content, antioxidants in the form of isoflavones namely

genistein, daidzein, and 8 hydroxy daidzein, super oxide dismutase and vitamin E (3, 5, 6). Tempeh is reported to have a low glycemic index and has the potential to be used as nutritional support for patients who require a high-protein diet such as hemodialysis patients, so it is hoped that it can increase the patient's nutritional intake (7-10). Tempeh also has good taste and the price is cheap, so it has a great opportunity to be used in the context of fulfilling family nutrition.

The antioxidants contain in tempeh are free radical scavengers that can inhibit the lipid oxidation process and prevent many diseases. The role of antioxidants is very important in neutralizing and destroying free radicals that can cause cell damage and also biomolecular damages, such as deoxyribonucleic acid (DNA), proteins, and lipoproteins in the body which can eventually trigger degenerative diseases. The degenerative diseases appear due to the presence of antioxidants in the body that are unable to neutralize the increased concentration of free radicals (11). Types of tempeh sold in the Indonesian market include local soybean tempeh and imported soybean tempeh. This study aimed to compare and analyze the differences in the antioxidant activity of local soybean tempeh and imported soybean tempeh.

Materials and Methods

The research was conducted from June to November 2022 in Yogyakarta, Indonesia. This study used objects in the form of local soybean tempeh and imported soybean. The local soybean tempeh used in the study was the Grobogan soybean variety. Imported soybean tempeh was purchased from a producer at a traditional market in Yogyakarta. Tempeh was floured first, then its antioxidant activity was analyzed in the laboratory.

Antioxidant activity in this study was defined as the amount of IC_{50} (Inhibition Concentration 50) antioxidant in tempeh flour in units of mg/mL, with a ratio scale. The antioxidant activity in this study was based on the IC_{50} antioxidant, while testing was done with a Duplo system. IC_{50} antioxidant analysis used the diphenylpicrylhydrazyl (DPPH) method. IC_{50} value wass the concentration of antioxidants that could reduce free radicals by 50% when compared to controls through a linear line equation. IC_{50} was the concentration of the sample solution required to inhibit 50% of free radicals. The smaller the IC_{50} value, the stronger the antioxidant in counteracting free radicals or it can be said to have stronger antioxidant activity. The data obtained were analyzed statistically using the independent T Test. This research obtained permission from the Health Research Ethics Committee, Faculty of Health Science, Universitas Respati Yogyakarta, Indonesia, with Ethical Clearance number of 116.3/FIKES/PL/VII/2022.

Results

The test results of the antioxidant activity based on the IC_{50} value were presented in Table 1. The results of the independent t-test analysis showed that the IC_{50} antioxidant levels in the two types of tempeh had a significant difference (p<0.0001). The mean difference between the two groups showed that the IC_{50} antioxidant score of local soybean tempeh was 9.96 mg/mL that was higher than that of imported soybean tempeh, meaning that the antioxidant activity of imported soybean tempeh was higher than that of local soybean tempeh.

Discussion

Low molecular weight antioxidants, including vitamins C, and E, coenzyme Q, carotene, glutathione, and trace elements, are responsible for deactivating reactive radicals. Some of these, including glutathione, ubiquinone, albumin and metallothioneins, and uric acid, are produced in the body, but most are exogenous compounds derived from natural sources such as plants (flavonoids, phenolic acids, carotenoids, stilbenes, coumarins, lignans, organosulfur, vitamins) or minerals (selenium, zinc, manganese) taken with food. When endogenous antioxidants involved in free radical defence cannot protect the body against reactive oxygen species, exogenous antioxidants are needed (12).

The activity can be evaluated by determining the IC_{50} value, which corresponds to the concentration of the mushroom sample which can scavenge 50% of the free radicals present in the reaction mixture. A high IC_{50} value indicates low antioxidant activity (13). IC_{50} or half the maximum inhibitory concentration of a compound is the number of antioxidants needed to reduce DPPH concentration by 50% (14). The method commonly used to test the antioxidant activity of a substance is the free radical 1,1-diphenyl-2-

Table 1: Differences in the IC_{50} antioxidant of local soybean tempeh and imported soybean tempeh.

Variable	Unit	Local soybean tempeh	Imported soybean tempeh	*p* value	Mean differences
		Mean±SD	Mean±SD		
IC_{50} Antioxidant	mg/mL	25.67±0.14	15.72±0.09	0.0001*	9.96

*Analyzed by using *independent t-test.*

picrylhydrazil (DPPH). DPPH is a free radical that is stable and active by dislocating free electrons in a molecule so that the molecule is not as reactive as other free radicals. This dislocation process is indicated by the presence of a deep purple (violet) color, which can be characterized in the absorbance band in ethanol solvent at a wavelength of 520 nm (15).

The antioxidant capacity of this test depends on the chemical structure and antioxidants. The reduction of DPPH radicals is dependent on the number of hydroxyl groups present in antioxidants, so this method indicates the structural dependence or antioxidant ability of biological antioxidants. Measurement of antioxidant activity with the DPPH method uses the principle of spectrophotometry. The dark purple DPPH compound (in methanol) is detected at a visible wavelength of around 517 nm. A compound can be said to have antioxidant activity if the compound can donate its hydrogen atom to bind to DPPH to form reduced DPPH, marked by the loss of purple color (turning pale yellow) (15).

Antioxidants will donate protons or hydrogen to DPPH and then form new radicals which are stable or unreactive (1,1-diphenyl-2-pykrylhid Razin). Parameters for interpreting test results with the DPPH method include IC_{50} (inhibition concentration), which is the concentration of the sample solution required to inhibit 50% of DPPH free radicals (16). The results showed that imported soybean tempeh had higher antioxidant activity than local soybean tempeh. This could have happened because the kinds or types of antioxidants present in tempeh were determined by the length of the fermentation they experienced (5).

Biochemically, the process of making tempeh is a fermentation process of legumes using inoculum from the *Rhizopus sp.* group, which is a mixture of *Rhizopus oligosporus* and *Rhizopus orrizae* in powder form. The application of the fermentation process in the field varies greatly including the length of fermentation time used by tempeh producers in the process of making tempeh that varies greatly with a period of between 36-48 hours and even up to 60 hours. The difference in the length of fermentation time can affect the antioxidant content too. This is because fermentation is a metabolic process or an oxidation-reduction process from the work of microorganisms. Microorganisms, in this case, *Rhizopus sp.*, are tempeh fungi that can produce the enzymes amylase, lipase, and protease, in which the enzyme production is determined by the length of time (5).

Antioxidant activity is classified as very strong if the IC_{50} value is less than 50 ppm, strong if the IC_{50} value is 50-100 ppm, moderate if the IC_{50} is 101-150 ppm, and weak if the IC_{50} is 150-200 ppm (15). The results of this study indicated that the antioxidant activity of local and imported soybean tempeh was very weak. This could be caused by the heating applied to the samples in this study. Tempeh was given repeated heating treatments during the drying process, namely steaming and drying in an oven (drying cabinet) at 50°C for 10 hours. The drying process affects the antioxidant activity and can reduce the radical scavenging capacity of the samples (17, 18).

Conclusion

Based on IC_{50} antioxidant value, imported soybean tempeh showed a significantly higher antioxidant activity than the local soybean tempeh. It is necessary to analyze the IC_{50} antioxidant in tempeh in its raw form, which has not undergone a heating process beforehand.

Acknowledgment

The authors would acknowledge Universitas Respati Yogyakarta, which has supported this research through internal funding, with contract number of 01/Pen/Hibah.Int/PPPM/VI/2022, so that this research was completed properly.

Conflict of Interest

None declared.

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