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Characteristics of Tempeh Edamame Fermented *Rhizopus Oligosporus*: Effect of Fermentation Time and Inoculum Concentration

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Abstract— *Edamame is one type of soybean with large legumes and has a high nutritional. Utilization of edamame afkir into tempeh is expected to increase nutritional value through the fermentation process by the inoculum (Rhizopus Oligosporus). The study aimed to determine the interaction between concentration with fermentation time on the chemical characteristics of tempeh edamame. The study used the RAK method. The first factor, the ratio of inokulum concentration (0.5%, 1%, 1.5%) and the second factor in the length of fermentation time (36, 48, 60 hours). The data was analyzed using the GLM variety analysis with Tukey's advanced test. The results of the analysis showed tempeh edamame with an inoculum concentration of 0.5% and a 48 hours fermentation time produced tempe edamame treatment that had a water content of 36.29% bk, ash of 3.85% bk, protein of 48.28% bk, fat of 21.67 bk, fiber of 27.25% bk and isoflavones of 8.72µg/g.*

Keywords— *Edamame, Rhizopus Oligosporus, Tempeh.*

I. INTRODUCTION

Edamame *(Glycine max (L) merr* or known as green vegetable soybeans is a species of soybean that can be used as a vegetable and the seeds are cooked either with pods or not. Edamame has the same nutritional content as soybeans (Shurtleff and Aoyagi, 2009). Edamame contains nutrients that are good for the body and a large amount of production but has not been balanced with its large utilization. PT. Mitra Tani 27 Jember is an Indonesian company that is an exporter of agricultural commodities, namely edamame *(Glycine max L. Merr)* or green vegetable soybeans (Shurtleff and Aoyagi, 2009). Of the amount export, as much as 12.8 tons per year is rejected edamame (Wibisono and Warsito, 2021). Edamame that does not pass export standards is categorized as edamame afkir. This edamame has a high content of protein, isoflavones and amino acids (Zeipina *et al*., 2017), which acts as an antioxidant, lowers the risk of cardiovascular disease and is beneficial for Dietary Approaches to Stop Hypertension (DASH) for people with hypertension (Vasdev and Stuckless, 2010).

Tempeh is a fermented soy food inoculated using *Rhizopus oligosporus* which is rich in amino acids and peptides (Astawan *et al*., 2016). The high popularity of tempeh increases the demand for tempeh from various countries around the world. This makes Indonesia the largest exporter of tempeh in Asia. High tempeh production is directly proportional to the needs of soybeans, where 60% of the total national soybean consumption is processed into tempeh (Soim, 2013). Until now, there are no other nuts that can rival soybeans as raw materials for tempeh. This is because soybeans are rich in isoflavones and their derivates (Atun, 2009).

II. METHOD

A. Time and place

The study was conducted for 7 months, starting in January 2021-July 2021, from the preparation of the material to the analysis of the product. The research was carried out at the Quality Processing and Supervision Laboratory, the Laboratory of Chemical and Biochemical Agricultural Products Department of Agricultural Product Technology, Faculty of Agricultural Products, Brawijaya University, Malang City, East Java.

B. Materials and Research Tools

The raw material edamame samples came from PT. Mitra Tani 27 Jember, and commercial inokulum obtained from LIPI which has been circulating in the market under the name raprima yeast.

The tools used to know the nutrition in edamame and tempeh : Kjeldahl gourd, oven 105℃, distillation set, cabinet dryer, M-310 digital scales, test tube, measuring glass, micro pipette, burette, drip pipette, erlenmeyer, beaker glass, cup glass, microtube, measuring pipette, bulp, glass stirrer, spatula, aluminum foil, paper wrap, electric stove, porcelain cup, furnace, desikator, funnel, filter paper, cotton, yarn, pumpkin *evaporator, rotary evaporator,* a set of fat extraction tools (soxhlets), a set of reflax tools and a spetrophotometer.

C. Experimental Design

The experimental design used in this study was a completely randomized design with 2 factors and 3 replications. The comparative treatment of inoculum concentration and fermentation time from this study were: Factor I inoculum concentrations 0.5%, 1% and 1,5%. Factor II fermentation time 36 hours, 48 hours and 60 hours. The parameters observed in this study were chemical composition, water content, protein content, total fat content, ash content, fiber content. The data obtained is analyzed using diversity fingerprint analysis (ANOVA). If one or both have a real or very real different effect then tukey further test with a confidence interval of 95%. The best treatment is determined by zeleny method.

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D. Research Procedure

The research procedure was the effect of inoculum concentration and fermentation time to the tempeh edamame into two, namely the preparation of the ingredients and the manufacture of tempeh.

a. Material preparation

The main ingredient in making tempeh edamame is edamame soybean and inoculum. While the tools needed are scales, plastic containers, pans and stoves.

b. The Process of Making Fruit Juice

The procedure for making tempeh edamame, sorting, stripping the skin, washing, soaking with clean water for 12 hours, boiling with a temperature of 100°C for 5 minutes, stripping the epidermis, washing thoroughly, slicing then dredging. Addition of commercial inoculum to raw materials according to the treatment predetermined. Stirring until evenly mixed and filling into perforated plastic, glue the plastic tip so that it is tightly closed. Store at 28-30°C on a shelf so that there is air circulation during fermentation for 36 hours, 48 hours and 60 hours.

E. Analysis Method

Data analysis of this study used ANOVA General Linear Model and Tukey's advanced test used MINITAB version 3.1.2 software to find out the significant relationship between factors and responses.

III. Results and Discussion

A. Characteristics of Edamame

The raw material used in the manufacture of tempeh edamame is fresh edamame. The chemical composition analyzed in raw materials is water content, ash content, protein levels, fat content, and fiber content. The results of analysis of the chemical composition of edamame can be seen in Table 1.

TABLE 1. Comparison of Chemical Composition Edamame Results of USDA Analysis and Standards

Parameters	Unit	Analysis Results	Edamame*
Water	g/100g	67.54±1.09	67,50
Protein	g/100g	12.63±0.30	12,95
Total fat	g/100g	6.79±0.13	6,80
Ash	g/100g	1.74±0.01	1,70
Carbohydrates	g/100g	-	11,05
Total dietary fiber	g/100g	6.71±0.73	4,20

The results of the analysis of fresh edamame water content in this study are in accordance with the edamame water content from the USDA (2019) which is 67.50 g / 100g. In addition, the results of the analysis on this study are in accordance with the fresh edamame water content in the Yu *et al study.* (2021) which ranges from 63.10-73.22%. This high water content makes it easier for edamame to be processed compared to soybeans whose water content is lower.

The level of fresh edamame protein in this study is in accordance with the edamame protein content from the USDA (2019) which is 12.95 g / 100g where the level of edamame protein is higher than the content of soy protein from the USDA (2019) which is 9.86 g / 100g. However, the results of the analysis of fresh edamame protein levels in this study are different from guo *et al research.* (2020) which states that the protein content of edamame ranges from 36-45%. This difference is thought to be due to the conditions and location of planting between the two studies, where edamame in this study was planted in tropical regions with high environmental temperatures while edamame in Guo *et al research.* (2019) cultivated in subtropical regions with environmental temperatures lower than tropical country environmental temperatures.

The total content of fresh edamame fat in this study is in accordance with the total edamame fat from the USDA (2019) which is 6.80 g / 100g, which is lower than the content of soy fat from the USDA (2019) which is 25.35 g / 100g. However, the results of the fat level analysis in this study are different from the Xu *et al study.* (2016) reports that the fat content of the two varieties of edamame (Asmara and Mooncake) ranges between 17.2% and 18.9%. Nevertheless, the fat content in the edamame of this study is better, because according to Zhang et *al.* (2017), lower fat content is generally preferred for food products from legumes, especially soy and edamame.

The ash levels in this study were lower than the levels of soy ash in the (Xu *et al.*, 2016) is 3.58-5.49% and (Yu *et al*., 2021) is 4.29-5.83%. Lower ash levels in edamame are thought to be due to a 1-1.5% phytic acid content (dry weight) binding to important minerals, including calcium, iron, zinc, phosphorus, and copper. So that the measurable ash level becomes lower.

The level of fresh edamame fiber in this study is higher than the content of edamame fiber according to the USDA (2019) which is 4.20 g / 100g. The results of this study are in accordance with the research of (Yu *et al*., 2021) which is 7.14-9.81%. In addition, the results of this study are also supported by research Ciabotti *et al.* (2016) which states that soybean seeds contain fiber by 6.7-10.7% as well as jiang et al *research.* (2018) which shows that the fiber content of soybean seeds is 5.53-8.04%.

B. Characteristics of Tempe Edamame

TABLE 2. Chemical Content of Tempeh Edamame

Parameters		Water	Ash	Protein	Fat	Fiber
Inoculum	hours					
0,5%	36	36.61	5.32	52.99	44.41	59.94
1%	36	33.30	6.67	40.23	53.01	68.08
1,5%	36	31.49	7.44	38.81	58.73	73.64
0,5%	48	40.71	3.85	57.65	18.63	27.25
1%	48	37.81	4.53	60.16	25.80	32.73
1,5%	48	36.27	4.95	57.55	36.25	40.42
0,5%	60	36.18	5.41	52.07	36.95	41.81
1%	60	35.18	5.80	48.65	42.17	49.11
1,5%	60	34.59	6.56	44.83	47.44	56.11

Based on data on the chemical content obtained, among them the highest water content of 40.71% in dry weight is found in the treatment of long fermentation 48 hours and inokulum concentration of 0.5%, while the lowest water content is 31.49%/bk is found in the old fermentation treatment of 36 hours and inokulum concentration of 1.5%. The water content in tempeh edamame is still in accordance with the tempeh water content standards set by the USDA (2019) which is 59.65 g/100g anin accordance with SNI Tempe 3411-2009, namely a maximum water content (b / b) of 65%. In edamame whose seed water content is higher than soybeans, which is 67.54±1.09 g/100g (USDA standard of 67.50 g/100g), during the process of soaking water content decreases. Another factor is the fiber content in tempeh, the higher the fiber content in tempeh edamame, then its ability to bind water is higher (Riyanto, 2014).

The ash content of tempeh edamame ranges from 3.85-7.44%/bk. The highest ash level of 7.44% was found in the prolonged treatment of 36 hours immersion and inokulum concentration of 1.5%, while the lowest ash level of 3.85%/bk was found in the 48-hour fermentation long treatment and 0.5% inokulum concentration. Low ash levels are caused by processing such as washing and immersion. This is because the minerals will dissolve and be wasted with water during the process. According to Nzewi and Egbuonu (2011) ash levels can decrease significantly due to boiling, due to the dissolution of minerals into the immersion medium that is accelerated by heating.

The protein content of tempeh edamame ranges from 60.16-38.81%/bk. The highest protein content was 60.16% found in the 48-hour fermentation long treatment and 1% inokulum concentration. The content of soluble proteins in tempeh increases sharply due to the activity of protease enzymes produced during fermentation. Therefore, the quality of tempeh protein is higher than the quality of edamame protein that is not fermented. According to Damanik *et al.* (2018), the increase in protein content is thought to be caused by the enzyme urease from *Rhizopus oligosporus* which is used to break down urea into ammonia and CO^2 acids.

The highest fat content of 58.73%/bk was found in the long treatment of 36 hours of immersion and an inokulum concentration of 1.5%. While the lowest fat content of 18.63%/bk is found in the old treatment of 48 hour fermentation and the inokulum concentration of 0.5%. In the treatment of inoculum conentration 0,5% and fermentation time 48 hours, the process of degradation of fat into fatty acids takes place optimally. So that the measurable fat content is lower than other treatments. This shows that a fermentation length of 48 hours is the optimal time to degrade fat, in addition the inokulum concentration of 0.5% is the right concentration of inokuum to obtain maximum results. This is in accordance with the statement of (Handoyo and Morita, 2006) where at the time of fermentation 48 hours there is maximum growth and requires fatty acids in large quantities so that the fat content decreases drastically.

The fiber content of tempeh edamame ranges from 27,25-73,64%/bk. The highest fiber content is 73,64% found in the old fermentation treatment of 36 hours and the inokulum concentration of 1.5%. While the lowest fiber content is 27,25% found in the old fermentation treatment of 48 hours and the concentration of inokulum 0.5%. According to Riyanto (2014), the higher the fiber content, the tempeh's ability to bind water the better, so the higher the water content.

IV. Conclusions and Recommendations

A. Conclusion

Tempeh edamame can be developed as a product development because it has good nutritional value. The best tempeh edamame treatment is found in the old 48-hour fermentation treatment and the inokulum concentration of 0.5%. The results of the chemical analysis of edamame tempeh obtained are water content 36.29% bk, ash content 3.85% bk, protein content 48.28% bk, fat content 21.67 bk, fiber content 27.25% bk and isoflavones content 8.72μg /g.

B. Recommendations

Based on the research results, suggestions for future research to additions about the benefits of amino acids and the content of bioactive peptides of tempeh edamame in experimental animals as a preliminary step in preclinical testing.

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