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Providing variations in yeast types for proximate, antinutrient and organoleptic analysis of cowpea tempeh (*Vigna unguiculata* L. Walp.) brown eye varieties

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Abstract. The study utilised four types of tempeh starter cultures: RAPRIMA brand tempeh starter cultures (R0), tempeh starter cultures derived from soybean tempeh flour (R1), tempeh starter cultures grown on rice media (R2), and tempeh starter cultures grown on cassava media (R3). Proximate analysis was conducted to determine the water, protein, fat, ash, carbohydrate, and fibre content. Antinutrient analysis measured phytic acid levels, while the organoleptic properties, such as colour, texture, aroma, and taste, were evaluated by 25 panellists using a hedonic scale questionnaire. The study employed a Completely Randomised Design, repeated three times. Data analysis involved ANOVA at a 5% significance level. Results indicated that different tempeh starter cultures types significantly affected the proximate analysis of cowpea tempeh. Moisture content (%) was 33.17 for RAPRIMA tempeh starter cultures, 38.61 for soybean flour tempeh starter cultures, 45.22 for rice media tempeh starter cultures, and 51.5 for cassava media tempeh starter cultures. Fat content (%) was 33.36 for RAPRIMA tempeh starter cultures, 25.31 for soybean flour tempeh starter cultures, 23.08 for rice media tempeh starter cultures, and 15.27 for cassava media tempeh starter cultures. Protein content (%) was 28.56 for RAPRIMA tempeh starter cultures, 30.68 for soybean flour tempeh starter cultures, 26.27 for rice media tempeh starter cultures, and 26.91 for cassava media tempeh starter cultures. Regarding antinutrients, phytic acid levels (%) were 0.3957 on average, with RAPRIMA tempeh starter cultures at 0.5368, soy tempeh flour tempeh starter cultures at 0.5083, rice media tempeh starter cultures at 0.5602, and cassava media tempeh starter cultures at 0.5378. Sensory testing showed the tempeh was generally acceptable to panellists, with tempeh starter cultures type significantly affecting taste and aroma. It was concluded that tempeh starter cultures variation did not significantly affect proximate or antinutrient properties but had a significant impact on organoleptic qualities.

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1 Introduction

Tempeh is a traditional food made through a fermentation process using microorganism agents based on legumes The tempeh that is commonly known to Indonesians tempeh is made from soybeans, resulting in dense and white in color [2]. The Indonesian millennial generation takes pride in tempeh, and it remains a popular choice among the people.

In general, Indonesians recognise that tempeh is made from legumes, primarily soybeans. One of them is soybean tempeh. Indonesia's soybean production is currently decreasing. Indonesia's consumption of soybeans is currently increasing. This inverse relationship between production and consumption has led to an increase in the price of tempeh products, posing a threat to food security in the country [16]

According to Ganang and Syamsul (2022) soybean productivity continues to decline. National soybean consumption averaged 2,953,022 tons in the 2015-2020 period and soybean production was only able to obtain production results in the range of an average of 674,843 tons in the 2015-2020 period. Tempeh, as a source of vegetable protein with a distinctive taste, is popular among Indonesian people [1].

Apart from using soybeans, making tempeh can also use other legumes, one of which is cowpea. Cowpeas are easy to cultivate, contain quite high protein and are relatively affordable as an alternative to tempeh for food security. Cowpeas are a type of bean which has the scientific name (*Vigna unguiculata* L. Walp.). Cowpeas have developed in Indonesia, but many people do not know that the potential of cowpea seeds is quite high as a basic ingredient for tempeh [9,17] Cowpeas are one of the basic ingredients for making tempeh as a substitute for soybeans [5].

Apart from requiring cowpeas to make tempeh, tempeh starter culture plays a crucial role in the fermentation process. Tempeh starter culture is very important in influencing the quality of tempeh. The fungus that is often used in the tempeh fermentation process is *Rhizopus sp.* including *Rhizopus oligosporus* and *Rhizopus oryzae* [4]. Cassava and rice can be used as growing media for *Rhizopus oligosporus* and *Rhizopus oryzae* respectively.

The cowpea tempeh fermentation process can improve the physicochemical, nutritional and antioxidant properties [10]. It is necessary to carry out research on proximate analysis, namely a comprehensive analysis of nutritional content consisting of carbohydrate content, protein content, fat content, water content and ash content of cowpea tempeh and anti-nutrient content, namely phytic acid. Related research that provides quantitative information on proximate content and antinutrient content is still very lacking. Tempeh is a favourite food among Indonesians, making it necessary to test the quality of cowpea tempeh organoleptically, including its aroma, appearance, taste, colour, and texture.

This research aims to examine the proximate analysis of cowpea tempeh (*Vigna unguiculata* L. Walp.) brown eye variety with various types of yeast before and after fermentation, Antinutrient analysis of cowpea tempeh (*Vigna unguiculata* L. Walp.) brown eye variety with various types of yeast before and after fermentation, Differences in sensory tests (color, aroma, taste, texture) of cowpea tempeh (*Vigna unguiculata* L. Walp.) brown eye variety with variations in Tempeh starter culture.

2 Materials and Methods

2.1 Place and time of research

Exploratory research on the brown eye variety of cowpea in Rembang, Central Java. Analyzing proximates, antinutrients and organoleptics before and after fermentation, namely at the Microbiology Laboratory, Faculty of Science and Mathematics, Diponegoro University. The research time is September - November 2023.

2.2 Materials

Making cowpea tempeh involves soaking, boiling, dehulling, filtering, and fermenting. The process uses containers, stoves, pans, steaming equipment, rubber or plastic gloves, a large sieve, and an Erlenmeyer flask. Ingredients for brown eye cowpea variety, RAPRIMA brand tempeh starter cultures (R0), tempeh starter cultures derived from soybean tempeh flour (R1), tempeh starter cultures grown on rice media (R2), and tempeh starter cultures grown on cassava media (R3).

2.3 Metode

In this research, cowpea tempeh was made through a fermentation process using tempeh yeast, through several stages of treatment, namely sorting and cleaning, the beans were cleaned and rinsed using water. Boil the brown eye variety of cowpeas for 30 minutes. Draining and cooling to reduce the water content in the seeds, dry the seeds and reduce the temperature of the seeds. Soaking is done in water for 24 hours. Dehulling the epidermis on cowpeas is done manually. Peel the epidermis to facilitate fungal growth and then dry it [11]. Inoculation is carried out with RAPRIMA brand tempeh starter culture, tempeh starter culture from tempeh flour, tempeh starter culture using rice growing media and tempeh starter culture using cassava growing media by sprinkling yeast on the surface of the tempeh. In this stage there are variations of tempeh yeast that will be sprinkled. Packaging uses plastic that has been perforated for tempeh fermentation. Fermentation is carried out For approximately 24 hours at room temperature ranging 25-37°C or 32°C it should be consisten.

Proximate testing consists of tests for water content, protein content, fat content, ash content, fiber content and carbohydrate content. Antinutrient testing for phytic acid levels using the titration method and sensory testing analyzed the quality of tempeh based on panelists' preferences. The quality tested is the texture, color and aroma of the tempeh produced. 25 panelists gave assessments using a hedonic scale questionnaire, giving scores ranging from 1-4 with the following assessment criteria: 1 = Very dislike, 2 = Dislike, 3 = Like, and 4 = Very like (Soekarto, 1985).

This research used a Completely Randomized Design (CRD) experimental method with 4 treatments and 3 replications, so there were 24 treatment units. Treatment includes cowpeas and tempeh cowpeas added with RAPRIMA brand tempeh starter cultures (R0), tempeh starter cultures derived from soybean tempeh flour (R1), tempeh starter cultures grown on rice media (R2), and tempeh starter cultures grown on cassava media (R3).

2.3.1 Water content

Porcelain sample cups weighing 2 grams were dried in an oven at 105 °C for 3 hours. The sample was crushed and weighed in a desiccator repeatedly until it reached a constant weight.

2.3.2 Protein content

Analysis of protein levels using the Micro-Kjeldhal method. A 30 mL Kjehdal flask was added to the sample as much as 0.1-0.25 grams, followed by 1.9 grams of K_2SO_4, 40 mg HgO, and 3.8 ml of H_2SO_4, then the sample was boiled for 1-1.5 hours until the liquid became clear. The sample is then cooled, a little water added slowly, and cooled again. The liquid in the Kjehdal flask was transferred to a still state and 8-10 ml of 60% NaOH-5% Na2S203 solution was added. In the distillation apparatus, a 250 ml Erlenmeyer flask containing 5 ml of H3BO3 solution and 2-4 drops of methylene red-methylene blue indicator is placed under the condenser, and the end of the condenser tube is immersed in the H3BO3 solution. Distillation is continued until approximately 15 distillates are collected. including. The resulting distillate was then titrated with a standardized 0.02 N HCl solution until the color of the distillate changed to gray.

2.3.3 Fat content

To know the fat content of the product, a bottle of fat is used which is dried in the oven and left in a desiccator and weighed with a standard weight. A sample weighing 5 grams is packed in filter paper, then put into a soxlet and filled with hexane solution, the bottom of the soxlet is connected to the top of the soxlet with an oil bottle, and covered with a cooler. The sample was then refluxed for 5 hours, after 5 hours the used solvent was collected and the fat bottle containing the extracted fat was dried in an oven at 105°C until the weight remained constant. Cooled in a desiccator before weighing, then the flask containing fat is weighed.

2.3.4 Ash content

2 gram sample was added to a dry porcelain cup and fired in an electric furnace at temperatures up to 550°C until complete ashing. The burned sample is then cooled in a desiccator and weighed until a constant weight is reached.

2.3.5 Carbohydrate content

Analysis of carbohydrate content is carried out using the average method, namely 100% of the nutritional value of the sample minus water, ash, protein and fat content.

2.3.6 Fiber content

The raw fiber samples were ground until homogeneous and weighed 2 grams, then put into a 300 mL Erlenmeyer flask and 100 mL of boiling 0.3 N H2SO4 was added. The Erlenmeyer flask containing the sample was placed in a cooler and heated for 30 minutes while shaking occasionally. The sample is then filtered with filter paper and the remaining residue is washed with boiling water until the washing water is no longer acidic. The residue was then returned to the Erlenmeyer flask while the residue remaining on the filter paper was washed with 200 mL of boiling NaOH solution until all the residue entered the Erlenmeyer flask. The sample was heated for 30 minutes using an inverted condenser while shaking occasionally. The sample was then filtered through filter paper of known weight while washed with 10% K2SO4. The residue remaining on the filter paper is washed with boiling water and then with 95% alcohol. The filter paper is then dried in an oven at 110°C for 1-2 hours, then cooled in a desiccator for approximately 15 minutes, then the sample is weighed until the weight is constant. The remaining mass of crude fiber is obtained from the difference between the mass of the sample and filter paper and the mass of dry paper.

2.3.7 Phytic acid content

Weighed 2 grams of cowpeas and tempeh, then put them in a 250 mL Erlenmeyer flask, soaked in 2% concentrated HCL for 3 hours, filtered and 50 mL of the filtrate was taken.

After getting the phytate, put it in a 250 mL beaker for titration, add 107 mL of distilled water. The results of the titration were added to a standard FeCl2 solution containing 0.00195/mL. Next, calculate the phytate percentage

Measurement data were analyzed using ANOVA at a 5% level of significance and if there were differences between treatments, it was continued with Duncan's Multiple Range Test (DMRT) using SPSS software.

3 Results and discussion

The local people of Rembang, Central Java only know that the cowpea seed plant is used as an ingredient for cooking vegetables. In order to verify food, cowpea seeds can be used as an alternative ingredient for making tempeh. This research examines tempeh made from cowpeas as an alternative ingredient to replace soybeans by providing various types of yeast. The following is a picture of the brown eye variety of cowpea plants *(Vigna unguiculata* L. Walp.) in the rice fields of Rembang, Central Java:

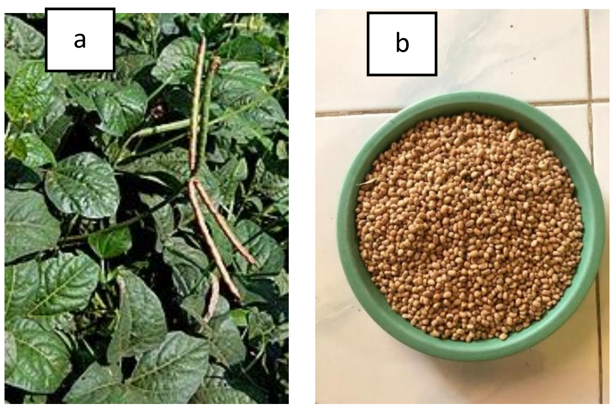

Fig. 1. (a) Cowpea plants in the Rembang area (b) Cowpea which has been peeled from the skin

The cowpea plant in the Fig. 1. can survive in any habitat conditions such as dry land habitats, so it is easy to plant anywhere. Cowpea plants are planted by preparing a hole in each soil used for cultivation. Caring for cowpea plants by watering them with water. Harvest cowpeas at 76 days after planting. The relatively short harvest time is an alternative for producing cowpea seeds as a substitute for soybeans for making tempeh.

The process of making tempeh by soaking cowpeas for 8 hours with fermentation of cowpea tempeh carried out for 48 hours at a 25-37°C or 32°C it should be consistent. RAPRIMA brand tempeh starter cultures (R0), tempeh starter cultures derived from soybean tempeh flour (R1), tempeh starter cultures grown on rice media (R2), and tempeh starter cultures grown on cassava media (R3). The results of cowpea tempeh are shown in the Fig. 2.

Fig. 2. (a)RAPRIMA brand tempeh starter cultures, (b)tempeh starter cultures derived from soybean tempeh flour, tempeh starter cultures grown on rice media (c), and tempeh starter cultures grown on cassava media (d).

A different treatment in the process of making tempeh is the provision of variations in starter during the fermentation process. The purpose of the tempeh starter cultures is to hydrolyze complex compounds into simple compounds using enzymes.

3.1 Proximate analysis of cowpea tempeh (*Vigna unguiculata* L. Walp.) brown eye variety

Analysis of the results of proximate test levels plays an important role in determining food quality as a basis for determining nutritional value and overall consumer acceptance [15]. Proximate test analysis is carried out to determine the main components of a material. Proximate analysis for water content, fat content, protein content, carbohydrate content, ash content and fiber content.

The results of the proximate analysis for cowpeas and cowpea tempeh are presented in the Table 1. The water content of tempeh corresponds to a maximum of 65%, the fat content of tempeh corresponds to a minimum of 7%, the protein content of tempeh corresponds to a minimum of 15%, the carbohydrate content and ash content of tempeh corresponds to a maximum of 1.5%, according to the SNI quality criteria, but Tempe fiber content has a value that does not meet the quality criteria required by SNI with a maximum limit of 2.5%.

Table 1. Proximate Test Results of Cowpeas and Cowpea Tempeh

Parameter (%)	Cowpea Tempeh Variations in starter culture							
	R0	**SD R0**	**R1**	**SD R1**	**R2**	**SD R2**	**R3**	**SD R3**
Water content	33,17	± 0,48	38,61	± 5,35	45,22	± 1,51	51,5	± 5,95
Fat content	33,36	± 1,13	25,31	± 0,86	23,08	± 0,41	15,27	± 0,26
Protein content	28,56	± 0,02	30,68	± 0,15	26,27	± 0,05	26,91	± 0,17
Ash content	0,1	± 0,017	0,07	± 0,018	0,08	± 0,018	0,07	± 0,018
Carbohydrate content	4,81	-	5,33	-	5,35	-	6,25	-
Fiber content	17,54	± 2,62	22,22	± 0,15	19,94	± 0,13	22,22	± 1,16

Information
R0 = RAPRIMA brand tempeh starter cultures

R1 = Tempeh starter cultures derived from soybean tempeh flour
R2 = Tempeh starter cultures grown on rice media
R3 = Tempeh starter cultures grown on cassava media

Anova test on calculated f water content (0.64) < f table 5% (4.06) means there is no real effect of giving variations of yeast on the water content of cowpea tempeh. anova test on fat content f count (2.05) < f table 5% (4.06) then there is no real effect of giving variations of yeast on the fat content of cowpea tempeh. Anova test on protein content f count (0.08) < f table 5% (4.06) then there is no real effect of giving variations of yeast on the fat content of cowpea tempeh. Anova test on the ash content f count (0.33) < f table 5% (4.06) then there is no real effect of giving variations of yeast on the ash content of cowpea tempeh. Anova test on the calculated f fiber content (0.37) < f table 5% (4.06) then there is no real effect of giving variations of yeast on the fiber content of cowpea tempeh.

The water content in cowpeas and cowpea tempeh experiences water absorption that enters the cowpea seeds so that it will increase the weight of the water in them and the water content will increase. In cowpea tempeh, the increase in water content results from the breakdown of carbohydrates by microbes and as the fermentation time increases. The water content in cowpeas increases as water molecules are absorbed into the tempeh.

According to Septiani et al (2004) fat content has decreased in cowpea tempeh due to the activity of the lipase enzyme from the mold which hydrolyzes fat (triglycerides) into free fatty acids and glycerol. The boiling process for making tempeh damages the protein structure contained in cowpeas, boiling using high temperatures results in protein denaturation so that the protein levels in cowpeas decrease. The longer the boiling, the more protein will be damaged [6].

Long boiling causes the mineral content in cowpea seeds to dissolve into the water. The longer boiling causes more minerals to dissolve into the water, causing the ash content of cowpea seeds to decrease. The decrease in carbohydrate levels was also caused by some of the oligosaccharide content in cowpeas being lost in the soaking and boiling process. The fiber content in the process of making cowpea tempeh fluctuates. Fiber levels fluctuate due to microbial activity during the tempeh fermentation process. Fiber is the part of food that cannot be hydrolyzed by strong acids and strong bases.

3.2 Antinutrient analysis of cowpea tempeh (*Vigna unguiculata* L. Walp.*)* brown eye variety

Table 2. Results of Phytic Acid Levels Tempeh Cowpeas

Parameter (%)	Cowpea Tempeh Variations in starter culture			
	R0	**R1**	**R2**	**R3**
Phytic Acid Levels	0,5368	0,5083	0,5602	0,5378

Information
R1 = RAPRIMA brand tempeh starter cultures
R2 = Tempeh starter cultures derived from soybean tempeh flour
R3 = Tempeh starter cultures grown on rice media
R3 = Tempeh starter cultures grown on cassava media

The Table 2 above the phytic acid levels in samples with variations of tempeh yeast, namely RAPRIMA brand yeast, soybean tempeh flour yeast, rice medium yeast and cassava medium yeast. Tempeh yeast, which is generally made from *Rhizopus* mold, produces the enzyme phytase which can hydrolyze phytic acid into inositol and orthophosphate. Cowpea seeds that have been soaked, boiled, and steamed, inoculated with tempeh yeast, wrapped in plastic with a hole in it, and incubated at room temperature (32 ºC) for 48 hours. Anova test on phytic acid f count (4) < f table 5% (4.06) then there is no real effect of giving variations of yeast on phytic acid in cowpea tempeh.

Phytic acid is very resistant to heating during processing of cowpea tempeh. This is evident from the phytic acid levels obtained during the research, with different variations of yeast being given, the results were different phytic acid levels. Cowpea results showed lower levels of phytic acid than cowpea tempeh in (Table 4.2). During tempeh fermentation, yeast has carried out metabolic activity and the phytase enzyme produced has begun to hydrolyze phytic acid.

In principle, the mechanism for peeling cowpeas separates the seed coat and the aleurone layer of the cowpea seeds, then the phytic acid contained in the aleurone layer of the cowpea seeds will be separated, thus the phytic acid content in the cowpea seeds after the peeling process decreases. The presence of phytic acid in cowpea tempeh affects enzyme activity.

3.3 Sensory analysis of cowpea tempeh (*Vigna unguiculata* L. Walp.) brown eye variety

Sensory testing on cowpea tempeh (*Vigna unguiculata* L. Walp.) which had been treated with a variety of yeasts was carried out by 25 panelists. The organoleptic properties tested include color, aroma, texture and taste parameters. The following is data on the results of sensory analysis for cowpea tempeh (*Vigna unguiculata* L. Walp.) with variations of starter culture:

Assessment criteria :

1 = Very dislike	3 = Like
2 = Dislike	4 = Like very much (Soekarto, 1985).

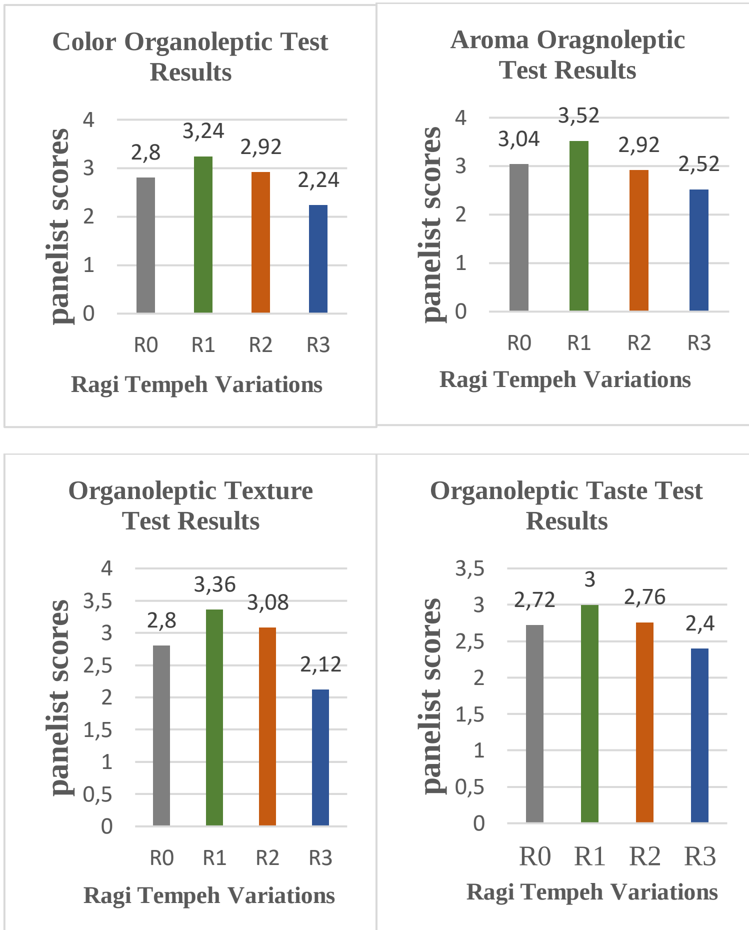

Fig. 3. Sensory analysis results for color, aroma, texture and taste

3.3.1 Color

Anova analysis of color f count (6.38) > f table 5% (3.008) means there is a real effect of giving variations of starter culture on the color of cowpea tempeh. The white cowpea tempeh shows the process The fermentation runs perfectly and produces good quality tempeh good. The white color is obtained from the presence of fungal mycelia that grow on the material raw tempeh during the fermentation process, thus creating a smooth tempeh texture compact (compact). Fermented cowpea tempeh is included in this category aerobic fermentation that requires oxygen in the process. Condition white tempeh indicates that the tempeh packaging conditions are satisfactory. Conditions/suitability for the growth of the fungus Rhizopus sp. Color plays an important role in product acceptability for both unprocessed and manufactured foods. Color can provide clues about chemical changes in food [13].

3.3.2 Aroma

Anova analysis of aroma f count (3.85) > f table 5% (3.008), means that there is a real effect of giving variations of starter culture on the aroma of cowpea tempeh. The difference in tempeh aroma occurs because each tempeh mold produces different enzymes. During 48 hours of fermentation, the mold hydrolyzes the complex compounds of cowpea seeds into simple compounds, resulting in an increase in the degree of unsaturation of the tempeh fatty acids, and producing a distinctive taste and aroma of tempeh. The aroma of cowpea tempeh produced during the fermentation process occurs due to the enzyme activity of the mold which produces the components 3-octanone and 1-octen-3-ol. This is confirmed by research by [8] who stated that the more carbohydrate content contained in tempeh, the less unpleasant the aroma will be.

3.3.3 Texture

Anova analysis of texture f count (6.53) > f table 5% (3.008) means that there is a real effect of giving variations of starter culture on the texture of cowpea tempeh. The very dense texture of tempeh is influenced by the percentage of added cowpea seeds. A larger percentage of cowpea seeds can form more mycelium so that the fungal hyphae grow intensively and evenly to form a network that binds the cowpea seeds to one another so that they become compact and dense. So it is proven that the percentage of added cowpea seeds in making tempeh greatly influences the texture of the cowpea tempeh.

3.3.4 Taste

Anova test of taste f count (28.17) > f table 5% (3.008) means there is a real influence of giving variations of starter culture on the taste of cowpea tempeh. Good quality tempeh has a distinctive aroma, generally tasty, this is because of the high protein and fat content in cowpeas which are then hydrolyzed into simpler compounds. Tempe has a bitter taste because it is influenced by compounds resulting from the degradation or oxidation of triglycerides caused by the hydrolysis process of amino acids that occur in the Maillard reaction, which can cause a bitter taste, such as: lysis, arginine, proline, phenylalanine and valine [7].

4 Conclusion

Cowpea tempeh produced from "brown eye" variety meets SNI quality criteria for water, protein, fat, carbohydrate, and ash content. However, the fiber content does not meet SNI requirements. Antinutrient Analysis**:** Phytic acid levels increased in cowpea tempeh with different yeast strains; however, these differences were not statistically significant ($p > 0.05$).Sensory Evaluation**:** Panelists found cowpea tempeh acceptable across all yeast treatments, with no significant differences ($p > 0.05$) in color, aroma, texture, or taste. Overall sensory acceptance was high.

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