FERMENTED *Moringa oleifera* SEEDS ENHANCED WITH *Euchema cottonii* AS AN ALTERNATIVE TEMPEH: ORGANOLEPTIC ANALYSIS, PROTEIN, AND FIBER CONTENT

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ABSTRACT

A study of new tempeh produced from *Moringa oleifera* seeds fermented with commercial yeast and the addition of *Euchema cottonii* was performed. The study was designed to utilize *M. oleifera* seeds as an alternative material for tempeh, to determine its protein and fiber content, as well as organoleptic analysis. Tempeh was produced with a variation ratio of commercial yeast and fermentation time. The ratio of *M. oleifera* seeds: *E. cottonii* was varied to explore the most suitable composition for consumers. Protein and fiber content analysis was determined following reference methods. Results indicated that 24 hours' fermentation using 1-gram commercial yeast produced the highest protein (20.57%). *M. oleifera* seeds: *E. cottonii* ratio of 70:30 was the most preferred tempeh with a hedonic value of 3.76. Forty-eight hours' fermentation with 3-grams commercial yeast yielded maximum fiber content. It can be concluded that this alternative tempeh is a good alternative for soybean tempeh as it contains high proteins and fibers.

Keywords: Tempeh, *Moringa oleifera*, *Euchema cottonii*, Fermentation, Protein and Fiber Content, Organoleptic Analysis.

RASĀYAN J. Chem., Vol. 14, No.1, 2021

INTRODUCTION

Food processing industry faces rising demands to develop diverse and healthy foods as new food products. Food can be processed in several ways, including fermentation, which involves the utilization of microorganisms.¹⁻³ Fermentation offers new food that varies with not only nutritional and functional values but also enriched taste as a must-have attribute. One of the well-known fermented food in Indonesia is Tempeh⁴. Tempeh is traditionally produced using soybean fermented with *Rhizopus*, but it can also be produced using other substrates, such as corn, rice, barley, and white beans.⁵⁻⁶

Moringa oleifera is an indigenous plant found in African and Asian countries, like India and Indonesia⁷. Roots, stems, leaves, flowers, and seeds of *M. oleifera* are consumed by humans. High amounts of protein, minerals, and antioxidant compounds contained in its leaves have attracted researchers to study and produce medicines. The seeds also contain high-quality fatty acids, protein, and carbohydrates.⁸⁻⁹ Nutritional content of *M. oleifera* seeds has been widely reported, but to date, little is known about fermented seeds of *M. oleifera* and their acceptance by the consumer as functional foods. The advantage of using moringa seed instead of soybean is cost-effective, since in Indonesia, mostly soybeans are imported from overseas.¹⁰

Euchema cottonii (*E. cottonii*) is carrageenan-producing seaweed with valuable nutritional content. It is high in ash and dietary fiber, but low in lipid. It is also high in vitamin C, alpha-tocopherol, and several minerals.¹¹ The utilization of seaweed enhances the fortification of food products, influencing the physical properties, chemical components, and organoleptic qualities of food.¹² Product development needs to be directed to create a new product that has more benefits and can be enjoyed by people from various circles.

In this article, new tempeh from *Moringa oleifera* seeds fortified with seaweed as additional material has been produced by fermentation to find out the nutritional values, particularly protein and fiber contents, and sensory acceptance.

EXPERIMENTAL

Materials

Commercial yeast (Raprima), distilled water, NaOH (Merck), ethanol (Merck), HCl (Merck), n-hexane (Merck), Whatman filter paper 41 (GE healthcare) were purchased. Seeds were collected from *M. oleifera* tree surrounding Tadulako University. *E. cottonii* was from coastal area of Morowali district.

Preparation of Moringa seeds Tempeh

M. oleifera seeds were prepared with or without the addition of *E. cottonii*. Seeds were soaked in clean and sterile water for 12 hours, peeled, boiled for 45 minutes, drained, and air-dried at room temperature. *E. cottonii* was washed with clean water and dried under the sun, washed again with running water and soaked for 1 week in lime water. It was washed again using clean water and cut into small pieces to a size of ± 0.5 cm. As much as 100 g of dried seeds were added with 1 g, 2 g, and 3 g of commercial yeast and stirred evenly. Mixtures of seeds and yeast at different ratios were fermented for varied periods of 24 h, 36h, 48 h, and 72 h.

Protein Content Analysis

Total protein content was measured using the *Kjeldahl* method as follows: 0.5 grams Tempeh sample was boiled in a Kjeldahl flask containing 1.2 grams of mixed catalyst (Kjeldahl powder) and 10 mL concentrated H_2SO_4 , until the solution turned greenish and clear. The solution was diluted 10 times. 5 mL of the diluted sample was added to 30% NaOH in a distilling flask and mixed with 4 drops of Phenolphthalein. The neutralization reaction was run for 5 – 10 minutes, and ammonia gas that was formed was collected in a receiving flask containing 2% boric acid. Nitrogen content was estimated by titration with 0.01 N HCl.

$$\text{Protein Content (\%)} = \frac{(V_1 - V_2 \times N \times 14.007 \times 6.25)}{W} \times 100\% \quad (1)$$

(V_1 and V_2 are titration volume of sample and blank; 14.007 gram is molecular weight (MW) of Nitrogen; N is normality of HCl; and W is the weight of the sample; 6.25 is protein factor for food.)

Crude Fiber Content Analysis

Crude fiber content was determined by a gravimetric method based on ISO 5498:1981.¹³

Ten grams of sample was dried at 80 °C for 1 h. Lipid content was extracted using hexane for 6 hours using a Soxhlet extractor. After distillation, extracted lipid was dried at 105 °C. Lipid-free sample was boiled for 30 minutes, with 25 mL 1.25% sulphuric acid. Twenty-five mL 3.25% NaOH was added, and the boiling process continued for 30 minutes. Next, the mixture was filtered using Buchner funnel containing Whatman filter paper no 41. Sediment was rinsed with 1.25% H_2SO_4 , hot aquadest, and 96% ethanol, respectively. Sample in filter paper was placed in a petri dish and dried at 105 °C for 1 hour.

$$\text{Fiber Content (\%)} = \frac{W_2 - W_1}{W} \times 100\% \quad (2)$$

(W is sample initial weight; W_1 is the weight of petri dish and filter paper; W_2 is the weight of petri dish and filter paper + dried residue.)

Organoleptic Analysis

An organoleptic test was performed to test the aroma, texture, and color of tempeh. The test was carried out using the hedonic test method by giving 4 tempeh samples (3 treatment samples and 1 control sample). As many as 25 people (students, age of 20-23, and lecturers, age of 30-50) were asked to provide an assessment of the tempeh quality characteristics presented by writing their level of preference on a numerical scale of 1 (dislike) to 5 (like very much).

RESULTS AND DISCUSSION

Protein Content

M. oleifera seeds were fermented for 24, 36, 48, and 72 hours with various amounts of commercial yeast (Raprima), which contains *Rhizopus oligosporus*. Kjeldahl method was performed as generally used for protein analysis in foods.¹⁴ The highest protein content (20.57%) was obtained at 24 hours of fermentation time with 1 g yeast (Fig.-1). The lowest protein content (7.60%) was obtained at 72 hours

of fermentation with 3 g yeast. According to the Indonesian National Standard (SNI 3144:2009) soybean tempeh quality requirements should have a minimum protein content of 16%¹⁵. Fermented tempeh with yeast weight as much as 0.5 g was also tested, but during the 24-hour fermentation, mycelia grew only slightly and unevenly due to inadequate substrate used by yeast to grow (data not shown). Besides, 72 hours of fermentation of tempeh yielded unpleasant odor and softer texture, caused by damage to the fungus found in tempeh and the action of lipoxygenase enzymes.¹⁶

Fig-1: Total Protein Content of Fermented *M. oleifera* seeds.

Seaweed, *E. cottonii*, was incorporated at varied ratios to increase the quality of tempeh. Seaweeds, like *E. cottonii* are used in the production of carrageenan that is applied in pharmaceuticals, cosmetics, and food industries.¹⁷⁻¹⁸ Carrageenan has a high viscosity and gelling properties, which are essential as texture modifiers.¹⁹ Furthermore, seaweeds also contain fibers and minerals that aid in reducing the utilization of salt. The incorporation of seaweed extract will help as a natural preservative for tempeh.²⁰

Fig-2: Total Protein Content of *M. oleifera* seeds:*E. cottonii*.

The results indicated that the more the seaweed, the lower was the protein content produced. Likewise, the longer the fermentation, the lower was the protein content produced. A decrease in protein content in tempeh is caused by several factors, specifically the addition of ingredients, heating (boiling/steaming), and fermentation time.²¹ In these findings, the addition of seaweed affects the protein content produced by *M. oleifera* seed (Fig. 2). The highest protein contents were obtained at the ratio of *M. oleifera* seeds to *E. cottonii* 100: 0 (26.51%), and at 24-hour fermentation time (25.705 %). During the fermentation process, generally, proteins are degraded into simple peptides. The longer fermentation, the more proteins degraded. Also, there is an increase in the number of amino acids, and many molds are actively breaking down complex compounds.²² After the fermentation process, the total content of amino acids decreases, but free amino acids will increase sharply; this is because mold from commercial yeast uses amino acids as a source of nitrogen for its growth.²³ A decrease in the content of tempeh seeds of *M. oleifera* and seaweed before reaching the optimum value can be due to the high carbohydrate content of seaweed, which consists of seaweed polysaccharide components, such as agar and carrageenan, which can form a gel affecting the fermentation process.

Organoleptic Assay

An organoleptic assay was performed to assess the quality of moringa seeds tempeh.²⁴ Organoleptic tests on *M. oleifera* seeds tempeh with different seaweed ratios can be evaluated by the appearance,

aroma, texture, and color of the product. Panel response to this organoleptic test was obtained from 25 panelists (Table-1). In the food industry, testing of aroma is considered vital because it can quickly provide an assessment of the product regarding whether or not the product is accepted. Besides, it also serves as an indicator of damage to the product.²⁵ Hence, the aroma was one of the analysis parameters used to clarify the level of tempeh preference. The ratio of seeds and seaweed can change the level of tempeh preference, so the tempeh aroma test is required to determine the tempeh with the preferred aroma. Tempeh at the ratio of 70:30 was the most chosen by the panelists, with a hedonic value of 3.76 which was slightly liking the aroma of tempeh. The pungent aroma produced in seeds is pure from the distinctive aroma of *M. oleifera*.

Table-1: Organoleptic Test of Tempeh

Panelist	Seeds: Seaweed Ratio (g)								
	80:20			70:30			60:40		
	Score (1-5)								
	Aroma	Color	Texture	Aroma	Color	Texture	Aroma	Color	Texture
1	4	3	4	4	4	4	2	3	3
2	3	3	3	4	4	4	3	3	3
3	3	4	3	4	5	4	2	3	3
4	3	3	3	4	4	5	3	2	4
5	4	3	3	4	5	4	3	2	3
6	4	4	4	4	5	5	2	2	3
7	3	4	4	4	4	3	2	3	2
8	3	4	4	4	3	4	3	3	2
9	5	4	3	4	3	4	3	3	2
10	3	4	3	3	4	5	2	4	3
11	3	5	4	4	4	4	2	3	3
12	4	4	4	4	5	4	4	3	3
13	3	4	4	4	4	4	2	3	3
14	3	4	4	4	4	5	3	4	4
15	4	4	3	3	4	3	3	3	2
16	5	4	4	3	4	5	2	3	3
17	4	4	4	3	5	4	3	2	3
18	3	3	4	5	4	4	2	3	3
19	3	3	4	4	4	4	2	3	3
20	3	3	4	4	3	4	2	2	3
21	4	4	3	3	3	4	3	3	2
22	4	3	4	5	4	4	3	4	2
23	3	4	4	3	5	5	4	3	3
24	4	4	5	3	4	4	3	3	3
25	4	4	4	3	4	4	3	3	3

(1 dislike, 2 dislike slightly, 3 like slightly, 4 like moderately, 5 like very much)

Texture has complex properties and is related to the structure of the material, which consists of three elements, namely mechanical (hardness, elasticity), geometric (sandy, crummy), and mouthfeel (oily, watery). The dense texture of tempeh is influenced by the percentage of the addition of seaweed. The greater percentage of seaweed can form more mycelium so that mold hyphae grow intensively and evenly to form a braid that binds one seed to another so that it becomes compact, dense, and hard.²⁶ Carrageenan present in *E. cottonii* is a hydrocolloid compound with the ability to bind water. So higher the percentage of seaweed, the harder is the texture of tempeh.²⁷ Tempeh at the ratio of 70:30 yielded a hedonic value of 4.16 which is considered as moderately liked.

Table-2: Hedonic Test of Tempeh

Organoleptic	Seeds: Seaweed (g)		
	80:20	70:30	60:40
	Hedonic Value		
Aroma	3.56	3.76	2.64
Color	3.72	4.08	2.92
Texture	3.72	4.16	2.84

Determination of the quality of food, in general, is dependent on several factors, including aroma, texture, and color. However, before other factors are considered, first visual appearance and color greatly determine consumer acceptance and also provide a clue about chemical changes in food. Typically, tempeh has a compact white color and dense mushrooms.²⁸ This formation of the white color is due to the dense mycelium braid formed by mold species *Rhizopus sp.* in the tempeh. Good quality tempeh has clean white characteristics that are evenly distributed on its surface. The organoleptic analysis showed that the ratio of 70:30 was the most preferable, with an average value of 4 (Table-2).

Fiber Content

After determining that the ratio of 70:30 was the most preferable the tempeh, investigation was continued to calculate its fiber content. The longer the fermentation time, the higher was the fiber content produced (Fig.-3). Due to processing soybeans into tempeh, nitrogen levels and cellulose levels increase.²⁹ The increasing amount of commercial yeast also produced higher fiber content in seeds.³⁰ As seen in Fig.-3, the highest amount of fiber produced was by 3 grams of commercial yeast fermented for 48 hours.

Fig-3: Total Fiber Content of Fermented *M. oleifera* Seeds: *E. cottonii*

CONCLUSION

The current study reports the successful preparation of novel tempeh using *M. oleifera* seeds as primary material and *E. cottonii* as a food additive. The protein and fiber contents meet the national standard of Indonesia (SNI) and were moderately liked by consumers. The best condition to obtain the preferable tempeh was 24-hour fermentation with 1 g commercial yeast and addition of 3 g seaweed with seeds and seaweed ratio of 70:30. Further studies are required to find out its amino acid profiles and bioactive compounds.

ACKNOWLEDGMENT

Special gratitude to the Faculty of Mathematics and Natural Sciences, Tadulako University, for supporting facilities and to Nurdiana, Oktavita, and Meldiana for their technical assistance.

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[RJC-6014/2020]