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Formulation of High Protein Enteral Synbiotics based on Tempeh Flour and Channa Striata Extract for Critical Patients

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ABSTRACT

Diarrhea is common in patients admitted to the Intensive Care Unit (ICU). Studies report that the incidence of diarrhea in the ICU ranges from 2-95%. Antibiotic therapy, antifungals, prokinetics, and enteral feeding may predispose to diarrhea in critically ill patients. The enteral route is the more commonly used feeding method in the ICU and is often thought to be the cause of diarrhea so enteral feeding is discontinued. This can exacerbate calorie and protein deficits. Probiotics are known to reduce the incidence of diarrhea in critical patients on ventilators. Enteral food with tempeh flour and cork fish flour containing synbiotics and high protein can be used as an alternative enteral food for critical patients. The purpose of this study was to determine the effect of tempe flour and channa striata extract formulations on hedonic of quality, energy density, macronutrient content, synbiotics, and viscosity. This study was conducted by hedonic quality testing of 3 enteral formula balances, proximate test, lactic acid bacteria and viscosity of the best formula. The results of the research on the hedonic quality of the three formulas concluded that there were significant differences with $p < \alpha$ (0.05) in taste, scent, consistency and overall, and there was no difference in color with p (0.125) > 0.05. F3 is the best formula that is favored by panelists. The proximate test results per serving (200 ml), namely energy 241.56 kcal, protein 9.64 grams, fat 7.16 grams, carbohydrates 34.64 grams, lactic acid bacteria (LAB) 1.42.106, inulin 5 grams, energy density 1.2 kcal/ml, viscosity 43.70 cP.

***Keywords*:** Enteral Formula, Synbiotics, Critical Patients, Tempeh Flour, Channa striata extract.

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1. INTRODUCTION

Diare sering terjadi pada pasien yang dirawat di ruang *Intensive Care Unit* (ICU). Penelitian melaporkan bahwa kejadian diare di ruang ICU berkisar antara 2-95% [1]. Hal ini disebabkan oleh adanya infeksi (misalnya *Clostridium difficile* atau norovirus), farmasi (misalnya antibiotik, anti jamur, pencahar dan enema) atau penyebab non-infeksi lainnya (misalnya insufisiensi pankreas, lamanya penggunaan ventilator, dan pemberian makanan melalui selang enteral terus menerus). Diare pada pasien kritis berdampak pada kondisi pasien, meningkatkan beban kerja perawat dan biaya ICU, serta memperburuk morbiditas pasien melalui cedera kulit, gangguan penyerapan enteral dan ketidakseimbangan cairan dan elektrolit berikutnya [2].

Diarrhea is common in patients admitted to the Intensive Care Unit (ICU). Studies report that the incidence of diarrhea in the ICU ranges from 2-95% [1]. This is due to the presence of infection (e.g. Clostridium difficile or norovirus), pharmaceutical (e.g. antibiotics, anti-fungals, laxatives and enemas) or other non-infectious causes (e.g. pancreatic insufficiency, prolonged ventilator use, and continuous enteral tube feeding). Diarrhea in critical patients impacts the patient's condition, increases nurse workload and ICU costs, and worsens patient morbidity through skin injury, impaired enteral absorption and subsequent fluid and electrolyte imbalance [2].

Rute enteral merupakan metode pemberian makanan yang lebih sering digunakan di ICU dan sering dianggap sebagai penyebab terjadinya diare sehingga pemberian enteral dihentikan. Hal ini dapat memperparah defisit kalori dan protein [3] (McClave et al, 2016). Pada pasien kritis dengan diare membutuhkan cairan dan elektrolit sebagai pengganti kehilangan saat diare, seng untuk membantu memperbaiki mukosa usus yang rusak dan mengurangi durasi diare, vitamin A untuk imunitas dan pemulihan mukosa usus, vitamin C untuk meningkatkan daya tahan tubuh, vitamin B kompleks untuk metabolism energi, magnesium untuk keseimbangan cairan, dan serat larut air untuk memperbaiki konsistensi tinja dan memperlambat transit makanan di usus. Selain itu, probiotik diketahui dapat mengurangi kejadian diare pada pasien kritis dengan ventilator dengan meningkatkan asam lemak rantai pendek dan menekan respon inflamasi sistemik dengan menstabilkan mikrobiota usus [4].

The enteral route is the more commonly used feeding method in the ICU and is often thought to be the cause of diarrhea so enteral feeding is stopped. This can exacerbate calorie and protein deficits [3]. Critical patients with diarrhea require fluids and electrolytes to replace losses during diarrhea, zinc to help repair damaged intestinal mucosa and reduce the duration of diarrhea, vitamin A for immunity and restoration of intestinal mucosa, vitamin C to increase endurance, vitamin B complex for energy metabolism, magnesium for fluid balance, and water soluble fiber to improve stool consistency and slow food transit in the intestine. In addition, probiotics are known to reduce the incidence of diarrhea in critical patients on ventilators by increasing short-chain fatty acids and suppressing systemic inflammatory responses by stabilizing the gut microbiota [4].

Probiotics are thought to rebuild the disrupted gut microbiome and may provide health benefits through two main mechanisms. First, probiotics will inhibit the growth of pathogens or replace pathogenic bacteria with non-pathogenic bacteria (probiotics) and create a more favorable microbial environment in the stomach and intestines. Thus, colonization of the oropharynx by pathogenic bacteria can be prevented, thereby reducing the risk of pneumonia caused by micro-aspiration. In addition, translocation of gut bacteria into the blood and distant organs can be avoided by replacing pathogenic gut bacteria.

Secondly, the rebuilt microbiome may provide health benefits by influencing immune responses outside the gut.

Based on a study conducted on 128 patients in the Intensive Care Unit of Dr. Hasan Sadikin Hospital in 2023, probiotic administration can reduce the incidence of diarrhea by 29% [5]. A meta-analysis of 14 trials also reported that a total of 1233 critically ill patients experienced a reduction in infection after probiotic administration. In both outpatients and inpatients, not focusing only on ICU patients, probiotics reduced the risk of C. difficile infection and antibiotic-associated diarrhea. Probiotics and synbiotics are also safe to use in elective abdominal surgery and have few side effects. Both probiotics and synbiotics reduce the risk of postoperative infections, but the effect is greater for synbiotics than probiotics [4].

Tempeh is a fermented soybean product that is rich in nutrients and contains a number of probiotics and prebiotics (synbiotics). Tempeh is high in protein, water-soluble fiber, B- complex vitamins, and zinc minerals which can reduce the incidence of diarrhea in critical patients. Fermentation in tempeh also helps reduce anti-nutritional substances such as phytic acid, which can interfere with mineral absorption. The main raw material, soybean, is also relatively easy to find in Indonesia, making it an economical and sustainable option for synbiotic product development [6].

Protein requirements of critical patients of at least 1.3 g protein/kg/day should be targeted after the initial phase [7]. During the chronic ICU phase, and after the patient is discharged from the ICU, higher protein targets should be given. The catabolic response leads to muscle mass loss of approximately 1 kg per day in the first 10 days of ICU care while nitrogen loss increases fourfold in the first 24 hours [8].

Channa striata is an excellent source of protein, which is essential for the growth and repair of body tissues. Cork fish also contains essential amino acids such as lysine, methionine, and valine that are needed by the body. The high albumin content, as well as omega-3 and omega-6 fatty acids, can function as anti-inflammatories that are good for the body. Cork fish also contains various antioxidant substances that can help protect the body from free radical damage, as well as various vitamins and minerals.

Tempeh and Channa striata have nutritional content needed by critical patients. Both ingredients have a short shelf life and spoil quickly during storage [9]. To maintain the quality and shelf life of enteral products, flour processing is required. Processing of enteral formulas in the form of flour is most widely done because flour is an alternative form of semi-finished products that are more resistant to storage, easy to mix and easier to process, and have high solubility [10].

Currently, the most common enteral formula used in hospitals for critical patients is cow's milk-based enteral, with very few enteral formulas that contain probiotics and prebiotics as well as high protein. Even if they exist, the price of the formula is quite expensive, making it difficult to provide to middle to lower class patients. Therefore, the author wants to make enteral food that contains synbiotics and high protein content at a price that is affordable and easy to make by the community.

2. MATERIALS AND METHODS

2.1. Design

This research is an experimental research (True Experimental) with a research design of group randomized design using 3 levels of treatment, to determine the effect of enteral formulations on the content of nutritional values, lactic acid bacteria, and physical quality of enteral formulas.

However, only the best formula based on hedonic quality was tested for nutritional value in the laboratory.

The treatment was conducted with 3 formulations: formula 1 (50% tempe flour, 50% channa striata extract), formula 2 (60% tempe flour, 40% channa striata extract), and formula 3 (70% tempe flour, 30% channa striata extract). The variables in this study are divided into independent (enteral formulation with substitution of tempe flour and channa striata extract) and dependent variables (organoleptic properties, viscosity, nutritional content of macronutrients, probiotics).

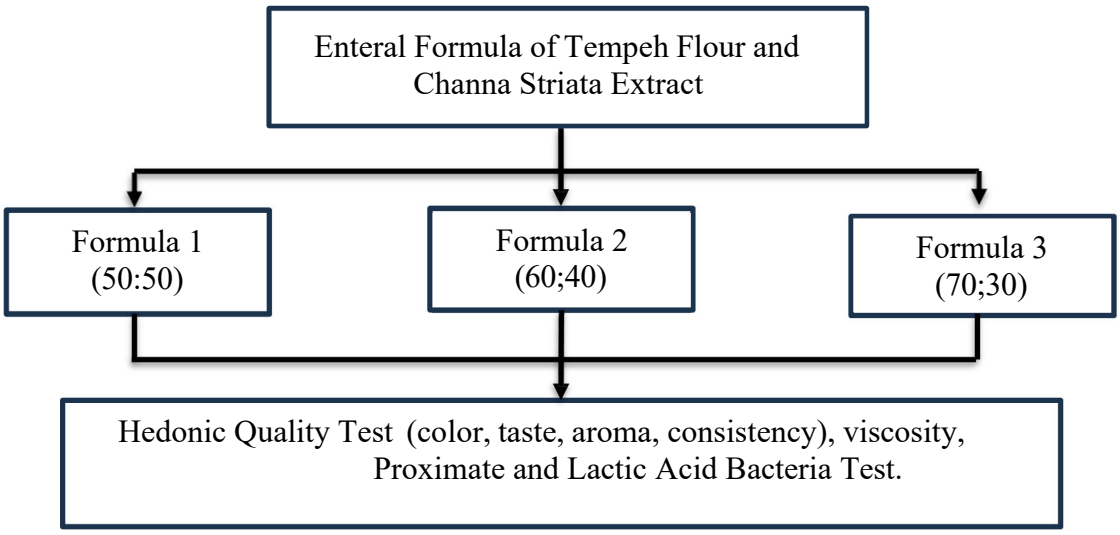

Figure 1. Schematic of Nutritional Content, Lactic Acid Bacteria, and Viscosity Tests.

2.2. Research Procedure

The formula was made by mixing tempeh flour, channa striata extract, mokaf flour, inulin and maltodextrin, powdered sugar. Then stirred until mixed using a mixer. Then add canola oil slowly while still stirring, after all the ingredients are united, add 40°c boiled water as much as 145 ml until the amount becomes 200 ml, then stir until all dissolve, then filter using a 100 mesh filter, and the enteral formula is ready for consumption.

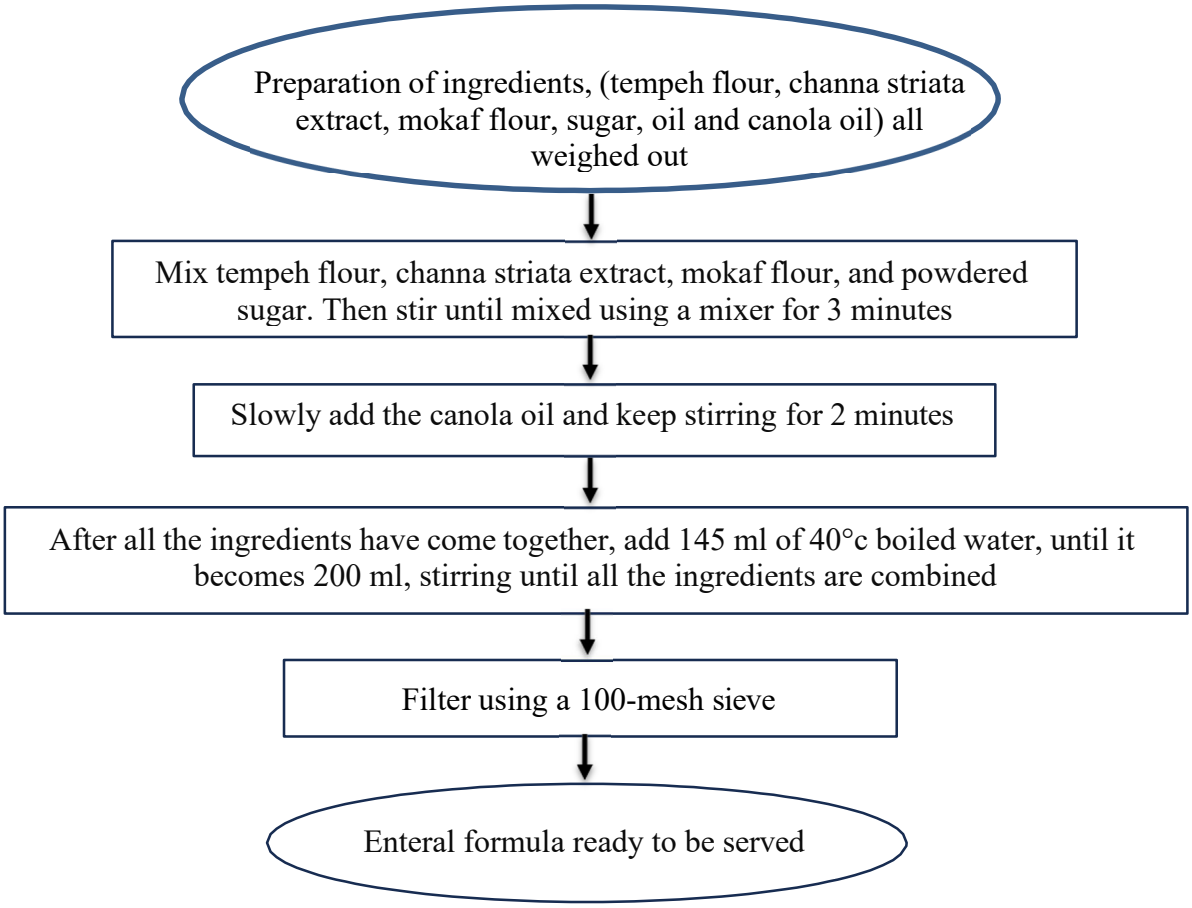

Figure 2. Flowchart of Enteral Formula Preparation.

3. RESULT AND DISCUSSION

3.1. Hedonic Quality Test Results

Enteral formula made from tempeh flour and channa striata extract is a formula development that contains probiotics and prebiotics for critical ill patients. Hedonic quality test was conducted to determine the quality of the enteral formula, it were conducted on 30 panelists using the hedonic test method with 5 scales (1 = strongly dislike, 2 = dislike, 3 = neutral, 4 = like, and 5 = strongly like). Based on the normality test, $p < \alpha$ (0.05) was obtained for data on the aspects of taste, scent, color, and texture which were not normally distributed. Thus, the statistical test used for the five aspects is the Kruskal Wallis test.

3.1.1. Taste

Panelists' assessment of the hedonic quality of color aspect showed that formulas 3 and 1 were preferred by panelists compared to formula 2. A total of 16.7% of panelists really liked formula 3 while 63.3% liked the enteral formula, and 20% of panelists were neutral.

Table 1. Percentage of Hedonic Quality Test for Taste.

Formula	Taste Rating Scale											
	Strongly Dislike		Dislike		Neutral		Like		Strongly Like		Total	
	n	%	n	%	n	%	n	%	n	%	n	%
F1	0	0	1	3.3	11	36.7	14	46.7	4	13.3	30	100
F2	0	0	2	6.7	16	53.3	12	40.0	0	0	30	100
F3	0	0	0	0	6	20.0	19	63.3	5	16.7	30	100

The enteral formula with 70:30 formulation of tempeh flour and channa striata extract produced a formula with a fish flavor that was not too strong compared to the other formulations. Enteral formula F1 with tempeh flour and channa striata extract formulation of 50:50 had the lowest level of panelist preference. Although the processing has used the smoking method with the addition of curcuma and ginger, as well as drying, the fish flavor is still strong. Previous research reported that the heating process including steaming and drying can increase the intensity of umami perception of channa striata [11].

3.1.2. Scent

Table 2. Percentage of Hedonic Quality Test for Scent.

Formula	Scent Rating Scale											
	Strongly Dislike		Dislike		Neutral		Like		Strongly Like		Total	
	n	%	n	%	n	%	n	%	n	%	n	%
F1	0	0	3	10.0	16	53.4	10	33.3	1	3.3	30	100
F2	0	0	5	16.7	16	53.4	7	23.3	2	6.6	30	100
F3	0	0	0	0	7	23.3	17	56.7	6	20.0	30	100

Panelists' assessment of the organoleptic properties of the scent aspect showed that formula 3 was preferred by panelists compared to formulas 1 and 2 and formula 2 was preferred to formula 1. A total of 20.0% of panelists really liked, 56.7% liked formula 3, and 23.3% of panelists were neutral towards formula 3.

F3 enteral formula is the treatment with the lowest formulation of channa striata extract, so it has the best scent. The use of less channa striata extract resulted in a less dominant fish scent in this treatment. The scent of channa striata is influenced by the volatile compounds contained in it. These volatile compounds come from primary and secondary metabolites, as well as the degradation of proteins, lipids, and other compounds during the metabolic process or processing. Some of the main volatile compounds that are usually found in Channa striata include: aldehydes, alcohols, ketones, volatile fatty acids, esters, sulfur, amines, and hydrocarbons [12].

3.1.3. Color

Panelists' assessment of the organoleptic properties of the color aspect showed that formula 3 was preferred by panelists compared to formula 1 and formula 2, and formula 1 was preferred to formula 2. As many as 23.3% of panelists really liked it while 66.7% liked it, and 10% of panelists were neutral towards formula 3.

Tabel 3. Percentage of Hedonic Quality Test of Color.

Formula	Color Rating Scale											
	Strongly Dislike		Dislike		Neutral		Like		Strongly Like		Total	
	n	%	n	%	n	%	n	%	n	%	n	%
F1	0	0	0	0	5	16.6	20	66.7	5	16.7	30	100
F2	0	0	0	0	7	23.3	21	70.0	2	6.7	30	100
F3	0	0	0	0	3	10.0	20	66.7	7	23.3	30	100

3.1.4. Consistency

Panelists' assessment of the organoleptic properties of the consistency aspect showed that formula 3 was preferred by panelists compared to formulas 1 and 2 and formula 2 was preferred to formula 1. 80.0% of the panelists liked, 13.3% really liked, and 6.7% were neutral towards formula 3.

Tabel 4. Hedonic Quality Test Percentage of Consistency.

Formula	Consistency Rating Scale											
	Strongly Dislike		Dislike		Neutral		Like		Strongly Like		Total	
	n	%	n	%	n	%	n	%	n	%	n	%
F1	0	0	0	0	12	40.0	16	53.3	2	6.7	30	100
F2	0	0	0	0	7	23.3	20	66.7	3	10.0	30	100
F3	0	0	0	0	2	6.7	24	80.0	4	13.3	30	100

Based on the table above, the highest notation is shown by the third treatment enteral formula, 70% of panelists said they liked it and 30% said they really liked it. Therefore, it can be concluded that the third treatment is declared as the best treatment that is most accepted organoleptically.

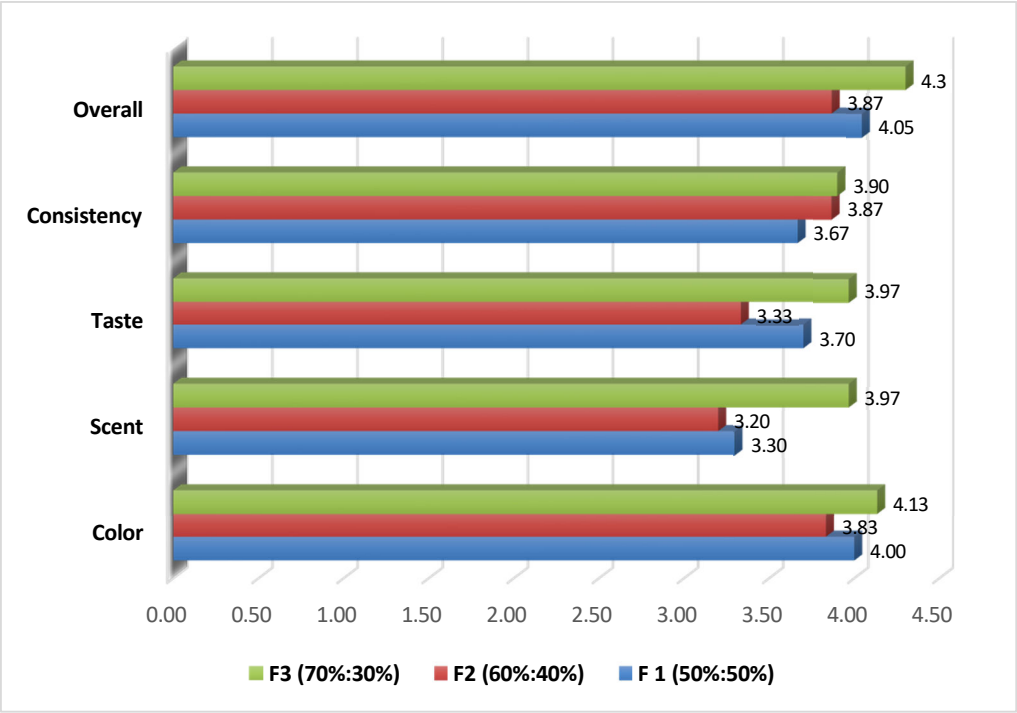

Figure 3. Hedonic Quality Test Results of Enteral Formula.

The average value of the level of liking of each formula can also be seen in Figure 3. Based on the average value of all aspects of taste, color, scent, consistency, and overall, it shows that formula 3 is more preferred than formulas 1 and 2.

3.2. Effect of Tempeh Flour and Channa Striata Extract Formulation on Hedonic Quality

The effect of tempeh flour and channa striata formulation on the hedonic qualities of enteral formula was analyzed using statistical tests. The results of the data normality test using the Shapiro Wilk test showed that in all organoleptic aspects (taste, aroma, color, consistency, and overall) the data were not normally distributed ($p < 0.05$). Therefore, the next analysis used the Kruskal Wallis test. The test results can be seen in table 5.

The data in Table 5 shows that there is an effect of tempeh flour and cork fish formulation on taste, aroma, consistency, and overall. However, there was no effect on the color of the formula. Further test results with the Mann Whitney test showed that the taste, aroma, and overall of enteral formula 2 and formula 3 were different, while formula 1 and formula 3 were different in the aspects of aroma and consistency ($p < \alpha$).

Table 5. Effect of Tempeh Flour and Channa Striata Extract Formulation on Hedonic Quality of Enteral Formula.

Formula	Taste		Scent		Color		Consistency		Overall	
	Median (Min-Max)	p value	Median (Min-Max)	p value	Median (Min-Max)	p value	Median (Min-Max)	p value	Median (Min-Max)	p value
F1	4 (2-5)	0,002 *	3(2-4)	0,000 *	5(3-5)	0,125	4(3-5)	0,019 *	4(3-5)	0,007 *
F2	4(2-4)		3(2-5)		4(3-5)		4(3-5)		4(3-5)	
F3	4(3-5)		3(3-5)		4(3-5)		4(3-5)		4(4-5)	

***) p<α (0,05)**

Based on the color aspect, panelists preferred formula 3, but the Kruskal Wallis test results showed no effect of formulation on the color aspect. This is because the three formulations have almost the same color. The two main ingredients, both tempeh flour and cork fish flour have almost the same color, so the color produced by the three formulas is not too different.

3.3. Nutritional and Lactic Acid Bacteria (LAB) Content

To determine the quality of this enteral formula product, Proximate and Lactic Acid Bacteria tests were conducted. The following are the results of the Proximate and Lactic Acid Bacteria test of the best treatment, namely F3 (70:30) as follows.

Table 6. Proximate and Lactic Acid Bacteria Test Results.

Parameter Analysis	Proximate and LAB Test* per 200 ml
Energy (kcal)	241,56
Protein (g)	9,64
Fat (g)	7,16
Carbohydrate (g)	34,64
Lactatic Acid Bacteria (cfu)	$1,42 .10^6$

*** Source:** Laboratory Proximate and Lactic Acid Bacteria Test Results, SIG Saraswanti, Bogor No. SIG.MARK.F.XI.2024.039884

Based on the table above, it can be seen that the nutrient content of the enteral formula per 200 ml is 241.56 kcal of energy, 9.64 grams of protein, 7.16 grams of fat, and 34.64 grams of carbohydrates, and 1.42,106 log cfu of lactic acid bacteria. Tempeh is a source of plant-based protein that is rich in essential amino acids. Fermentation of tempeh also increases protein absorption and reduces the content of antinutrients such as phytic acid.

In addition, tempeh also contains soluble fiber which is good for digestive health, as well as probiotics from the fermentation process. The content of vitamin B complex, iron, magnesium, and calcium in tempeh further provides more benefits for energy metabolism and fulfills the body's mineral needs.

Channa striata is famous for its high albumin content, which is important for accelerating wound healing and repairing body tissues. It is also rich in omega-3 and omega-6, which support immune system function and improve inflammation. The combination of albumin protein from cork fish and vegetable protein from tempeh can help tissue regeneration in critical patients, especially those with surgical wounds or pressure ulcers. Fat content such as omega-3 from cork fish and probiotics from tempeh can improve the body's immune response, helping to fight infection in critical patients, as well as gastrointestinal disorders such as diarrhea due to long-term administration of more than one type of antibiotic.

This enteral formula is specially designed with tempeh and cork fish supplemented with inulin as a prebiotic. Recent studies on a larger scale found that probiotics and prebiotics are a good complement to antibiotic therapy in critically ill patients [13]. Probiotics, with or without a combination of prebiotics, are suggested as a beneficial intervention in critically ill patients. The reason is that due to the widespread use of antibiotics, acute dietary changes, and disease- induced stress, the homeostasis of the patient's gut microbiome may be disrupted. Under these conditions, probiotics can maintain the patient's gut microbiota and prevent opportunistic infections that can exist in the absence of protective gut microorganisms [14].

3.4. Viscosity

Based on the viscosity test, it was found that the viscosity of the formula was 43.70 cp (appropriate if the viscosity is 3.5-10 cp). This result shows that the viscosity of the formula is above normal or too thick.

The resulting formula still has quite a lot of sediment. This is caused by various factors, including ingredient composition, production process, water solubility, pH, and system stability, and storage. Tempeh flour and channa striata extract have fat content that tends to separate, high fiber and protein, so they tend to form deposits. The production process that lacks homogenization, drying, and grinding can also lead to the formation of sediment. The content of water insoluble fiber and unstable pH is also the cause of the formation of deposits, and the longer storage will form deposits.

This enteral formula had an energy density of 1.12 - 1.13 Kcal/ml from all three treatments. These results have met the quality requirements of the standard enteral formula, which is the energy content per mL of 1 - 1.2 kcal [15]. All formulations had a protein content above 20%, F1 had a protein content of 23%, F2 contained 22% protein, and F3 contained 21%. This protein content is classified as high according to Queensland Health (≥20%). This is in accordance with dietary principles in critical patients who require high protein, especially conditions in sepsis, postoperative, and wound healing conditions. The albumin content found in fish also contributes to improving inflammatory conditions in critical patients.

This enteral formula contributed high fat in all three treatments (>30%). In critical patients with respiratory failure, there is often an increase in CO2 production, so giving high fat and low carbohydrates can reduce CO2 production. A fat:carbohydrate ratio of 20-50%:50-80% is recommended in critical patients [16].

Probiotics were obtained from tempeh flour. When compared to commercial probiotic enteral formulas, this formula has a much lower probiotic content. The probiotic content in the formula will be further reduced if heating is done, because lactic acid bacteria are not heat- resistant. The appropriate storage temperature for probiotics is around 2-8^{0}C [17], so storage is in the refrigerator. If forced to store at room temperature, it should be consumed in less than 3 hours. One way to increase the probiotic content in enteral formulas is by adding lactic acid bacteria. However, there are no studies that mention how much probiotics are recommended for critical patients.

4. CONCLUSIONS

There are significant differences in taste, aroma, consistency, and overall in enteral food with $p < \alpha$ (0.05). There is no significant difference in the aspect of color in enteral food with $p= 0.125$ ($p>0.05$). The proximate test results of the formula per serving (200 ml) were energy 241.56 kcal, protein 9.64 grams, fat 7.16 grams, and carbohydrates 34.64 grams. The content of lactic acid bacteria in the formula in 200 ml was 1.42,106 cfu and 5 grams of inulin. The resulting formula is too thick with a formula viscosity of 43.70 cP, so further formulation is needed.

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