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Physicochemical and Microbiological Quality of Synbiotic Yogurt with The Addition of Hanjeli Flour (*Coix lacryma-Job*i L.) as a Natural Stabilizer

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Abstract. This research aims to determine the physicochemical quality of synbiotic yogurt with the addition of hanjeli flour (Coix lacryma-jobi L.) as a natural stabilizer and substrate for probiotic growth, besides being able to improve the quality of synbiotic yogurt by increasing the viscosity value, improving the texture and stability, and reducing the syneresis value. Research variables include viscosity value, syneresis, water holding capacity, pH value, total acid, and total lactic acid bacteria. The method used in this research was a Completely Randomized Design (CRD) consisting of 4 treatments, including P0 (hanjeli flour concentration 0% + skim milk 2%); P1 (hanjeli flour concentration 1% + skim milk 2%); P2 (2% hanjeli flour concentration + 2% skim milk); and P3 (3% hanjeli flour concentration + 2% skim milk). The data obtained were analyzed using Analysis of Variance (ANOVA); if there were real differences, then it was continued with the Duncan's Multiple Range Test (DMRT). The results of the analysis of variance showed that the addition of hanjeli flour with different concentrations had a significant effect ($P<0.05$) on the viscosity, syneresis, water holding capacity, pH value, total acid, and total lactic acid bacteria values. The conclusion from this research is that P3 is the best treatment with a viscosity value of 4250 cP, syneresis 17.09%, WHC 90.62%, pH 4.02, total acid 1.26%, and total lactic acid bacteria 7,42 log CFU/g.

1 Introduction

Yogurt is a fermented dairy product that features a unique sour taste due to the activity of lactic acid bacteria. These bacteria metabolize lactose, transforming it into lactic acid, which imparts yogurt's characteristic flavor. Yogurt is a type of processed fermented milk produced by adding bacterial cultures such as *Lactobacillus bulgaricus*, *Streptococcus thermophilus*,

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Lactobacillus acidophilus, and *Bifidobacterium*. This results in a product with a thick texture that has the potential to be developed as a functional food source due to its positive impact on human health. The inclusion of *Lactobacillus acidophilus* and *Bifidobacterium* enhances the functional value of yogurt, as these probiotic bacterial starters help maintain the balance of intestinal microflora [1].

Quality damage frequently occurring during the yogurt production process includes a reduction in binding capacity and an increase in syneresis, attributed to a decrease in the isoelectric point. These issues lead to a decline in the quality of the produced yogurt. Therefore, it is necessary to implement measures to enhance and maintain yogurt quality, specifically by adding stabilizers. Stabilizer is a food additive that functions to stabilize a liquid that cannot be mixed naturally. The purpose of adding stabilizers to synbiotic yogurt is to improve its quality and maintain its desired characteristics. The addition of stabilizers to yogurt serves to mitigate physical damage and provides a nutrient source for lactic acid bacteria, thereby promoting health benefits for humans [2]. The results of the study [1] showed that yogurt with the addition of stabilizer was able to produce yogurt with better quality compared to the treatment without the addition of stabilizer in terms of viscosity, syneresis, total acid, water content and water binding capacity. One of the food ingredients that can be added in making yogurt is the addition of natural stabilizers using hanjeli flour (*Coix lacryma-jobi* L.) to improve the quality of yogurt.

Hanjeli (*Coix lacryma-jobi* L.) is a type of cereal that possesses higher nutritional content compared to other cereals. However, its potential as a processed food product remains largely underutilized [3]. Hanjeli has a high carbohydrate content, so it is suitable for being processed into flour. [4] explains hanjeli flour has a high carbohydrate content of 72% as well as a high protein content of 13–14%, which comes from local raw materials of hanjeli grains, has a low fat content, and contains a high antioxidant activity of 91.35%. The prebiotic components in hanjeli flour can be utilized by probiotic bacteria as a carbon source in the colon, making it a highly potential alternative food ingredient. It can be employed as a stabilizer to enhance product quality and produce functional food that supports food security, thereby generating a synbiotic yogurt product that serves as a substrate for probiotic growth. Synbiotic yogurt, which is produced by combining probiotics and prebiotics, has an increased functional value due to its positive health impacts. Therefore, it is necessary to conduct research on the addition of hanjeli flour as a stabilizer to address issues in the production of optimal synbiotic yogurt, considering its physicochemical and microbiological properties.

2 Materials and Methods

2.1 Material

The materials used in this study include synbiotic yogurt supplemented with hanjeli flour. The specific materials comprised of fresh cow's milk, yogurt starter, hanjeli flour, skim milk, Na_2SO_4, distilled water, 70% alcohol, pH 4 and pH 7 buffer solutions, 1% phenolphthalein (PP), 0.1 N NaOH, MRSA (de Mann Ragosa Sharpe Agar), NaOH 0.1 N, H_2SO_4, 50% NaOH, zinc granules, HCl, PP indicator, distilled water, spiritus, acetone, 80% trichloroacetate, PBS-Tween-PMSF, cold ethanol solution, Tris-HCl pH 6.8, Reducing Sample Buffer, and Coomassie Brilliant Blue (CBBR 250).

The equipment used in this research was a viscometer brand "Elcometer 2300," a centrifuge brand "Heratus Biofuge Stratus Centrifuge," a centrifuge tube, a scale brand "Precisa, "a “Pyrex” brand measuring cup, a test tube, a test tube rack, an Erlenmeyer, a vortex, a magnetic stirrer, a stirrer, a "Gilson" brand micropipette, an incubator, an autoclave, a marker, a blue tip, a "Pyrex" brand beaker, a dropper pipette, a Bunsen burner, a refrigerator, and a colony counter.

2.2 Method

This research employs a Non-Factorial Completely Randomized Design (CRD) with the incorporation of hanjeli flour as a natural stabilizer in the production of synbiotic yogurt. The study comprises four treatments: P0 (0% hanjeli flour concentration + 2% skim milk), P1 (1% hanjeli flour concentration + 2% skim milk), P2 (2% hanjeli flour concentration + 2% skim milk), and P3 (3% hanjeli flour concentration + 2% skim milk).

2.3 Research Variables

The variables observed in this research include viscosity test, syneresis, water holding capacity, pH value, total acid and total Lactic Acid Bacteria.

2.3.1 *Viscosity*

Viscosity measurements were carried out using a viscometer following the procedure of [5]. The tools and materials were prepared for viscosity testing by setting up a viscometer with spindle No. 4 and adjusting the rotor to 30 rpm for 60 seconds. A 250 ml sample of synbiotic yogurt was prepared, then the spindle was inserted into the sample. The motor was turned on, causing the spindle to rotate. Once the dial needle displayed a stable reading, the motor was turned off, and the reading shown by the dial needle was recorded. Each sample was measured four times, and the average was calculated. The average value, multiplied by the multiplying factor corresponding to the speed and spindle number used, represents the viscosity of the product being tested.

2.3.2 *Syneresis*

The measurement of syneresis was carried out using the drainage method following the procedure of [5]. The measurement of syneresis begins by weighing a 15 g sample of synbiotic yogurt in a special centrifuge tube. The tube is then placed in a centrifuge, which is set to 1535 rpm and operated for 20 minutes. After this period, the supernatant of the yogurt is separated and weighed, and the results are recorded. The final step involves calculating the yogurt syneresis using:

$$Syneresis\ (\%) = \frac{initial\ weight\ (g) - final\ weight\ \ (g)}{weight\ at\ the\ beginning\ (g)}\ x\ 100\% \quad (1)$$

2.3.3 *Water Holding Capacity*

Measurement of yogurt WHC is using the centrifugation method following the procedure of [6]. Measurement of yogurt WHC begins with weighing a 10 g sample of synbiotic yogurt in a centrifuge tube, then centrifuged at 4000 rpm for 10 minutes. The yogurt whey (supernatant) was separated and the residual sediment weighed. The percentage of WHC of synbiotic yogurt was calculated using the equation:

$$WHC\ (\%) = \frac{sediment\ weight\ (g)}{initial\ weight\ \ (g)}\ x\ 100\% \quad (2)$$

2.3.4 *pH*

The measurement of pH was conducted using a pH meter, following the procedure outlined by [5]. The pH meter was calibrated with pH 7 and pH 4 buffer solutions. The electrode was rinsed with distilled water and dried with a tissue. A 10 ml sample of synbiotic yogurt was placed in a beaker, then the electrode was immersed in the sample, allowing the pH value to be read on the pH meter screen.

2.3.5 *Total Acid*

The measurement of total acid was conducted using the titration method, following the procedure outlined by [5]. About 10 ml sample of synbiotic yogurt was measured and placed into a 250 ml Erlenmeyer flask. Before titration, 2 drops of 1% phenolphthalein (PP) were added to the sample. The sample was then titrated with 0.1 N NaOH until a persistent pink color was observed. The acid content is calculated using the following equation:

$$\text{Total Acid (\%)} = \frac{V_1 \; x \; N \; x \; B}{V_2 \; x \; 1000} x \; 100\% \qquad (3)$$

2.3.6 *Total Lactic Acid Bacteria*

Measurement of total lactic acid bacteria using the total plate count method following the procedure [7]. Sterilization of tools, diluents, and MRSA was carried out using an autoclave at a temperature of 121°C, with a pressure of 2 atm for 15-20 minutes. Test tubes labeled 1, 2, 3, 4, 5, and 6 containing 9 ml of sterile diluted solution, as well as petri dishes labeled with the final dilution value, were prepared in four replicates. The sample was shaken, and taken 1 ml placed into test tube #1 for the 10^{-1} dilution series. Subsequently, 1 ml of sample from tube 1 was transferred to tube #2 for the 10^{-2} dilution series, and this process was continued until the 10^{-6} dilution series. The sample from the final dilution was inoculated into a petri dish using the pour plate method. Approximately 15 ml of sterile MRSA media was poured into the petri dish, which was gently shaken making a figure 8. After the media solidified, the petri dish was inverted and placed in an incubator at 37°C for 24 hours. The counting number of colonies used Qubec Colony Counter.

2.4 Procedure for Making Synbiotic Yogurt

The procedure for making synbiotic yogurt followed the modified research procedure of [8] Fresh cow's milk was prepared, and hanjeli flour was added according to the treatment (P0 = 0%, P1 = 1%, P2 = 2%, and P3 = 3%) along with 2% skim milk. The mixture was then pasteurized at 72°C for approximately 15 seconds using the High-Temperature Short-Time (HTST) method, with the temperature monitored using a thermometer. The milk was cooled to 43°C, and then 3% of the milk volume of biocule starter was added. The mixture was incubated for 24 hours at room temperature, approximately 37°C. After incubation, the synbiotic yogurt with the addition of hanjeli flour was ready for testing.

2.5 Data Analysis

The data obtained in this study were tabulated using Microsoft Excel and subsequently analyzed using analysis of variance (ANOVA) based on a non-factorial Completely Randomized Design (CRD). If the treatment showed a significant effect ($P<0.05$), *Duncan's*

Multiple Range Test (DMRT) was performed. The study consisted of 4 treatments with 4 replicates each, resulting in a total of 16 experimental units.

3 Results and Discussion

3.1 Physicochemical and Microbiological Quality of Synbiotic Yogurt

The physicochemical and microbiological qualities observed in the research on the production of synbiotic yogurt with the addition of hanjeli flour include viscosity, syneresis, water holding capacity, pH value, total acid, and total lactic acid bacteria (LAB). The results of the physicochemical and microbiological testing of synbiotic yogurt are presented in **Table 1.**

Table 1. Mean values of physicochemical and microbilogical quality tests of synbiotic yogurt

Parameter	Treatments			
	P0	**P1**	**P2**	**P3**
Viscosity (cP)	1285 ± 25.17^{d}	1975 ± 50.00^{c}	2500 ± 81.65^{b}	4250 ± 57.73^{a}
Syneresis (%)	34.82 ± 0.61^{a}	24.00 ± 0.49^{b}	22.88 ± 0.33^{c}	17.09 ± 0.50^{d}
Water Holding Capacity (%)	52.44 ± 1.87^{d}	81.34 ± 0.56^{c}	83.70 ± 0.89^{b}	90.62 ± 0.57^{a}
pH	4.22 ± 0.02^{a}	4.17 ± 0.02^{b}	4.08 ± 0.03^{c}	4.02 ± 0.00^{d}
Total Acid	1.14 ± 0.02^{d}	1.19 ± 0.01^{c}	1.22 ± 0.00^{b}	1.26 ± 0.01^{a}
Total Lactic Acid Bacteria (LAB) (log CFU/g)	7.02 ± 0.00^{d}	7.28 ± 0.01^{c}	7.36 ± 0.01^{b}	7.42 ± 0.01^{a}

Description: P0 (0% hanjeli flour concentration + 2% skim milk); P1 (1% hanjeli flour concentration + 2% skim milk); P2 (2% hanjeli flour concentration + 2% skim milk) and P3 (3% hanjeli flour concentration + 2% skim milk) and Notation ^{a,b,c,d} on the same line indicates significant effect (P<0.05).

3.1.1 Viscosity

Viscosity is the amount of resistance of a liquid to flow and stirring [1]. The results of the analysis of variance indicated that the addition of hanjeli flour at different concentrations had a significant effect (P<0.05) on the viscosity of synbiotic yogurt, as can be seen in **Table 1**. This difference was influenced by the inclusion of hanjeli flour in the production of synbiotic yogurt. Hanjeli flour can bind water due to its carbohydrate content in the form of amylose, which is easily soluble in water and therefore leaches out of the granules during the heating process [32].

The results of research by [9] explained that the more the concentration of stabilizer, the water capacity will also increase. Amylose content has a good water binding ability in the process of making yogurt. Starch granules absorb water and swell, thus increasing viscosity [10].

The average viscosity value of the resulting synbiotic yogurt ranged from 1285 cP to 4250 cP. The highest average viscosity value was obtained in the P3 treatment (3% hanjeli flour concentration + 2% skim milk), at 4250 cP, while the lowest average viscosity value was observed in P0 (0% hanjeli flour concentration + 2% skim milk), at 1285 cP.

An increase in the addition of hanjeli flour in the preparation of synbiotic yogurt corresponds with an increase in viscosity. This is likely because hanjeli flour acts as a natural stabilizer that can increase the total solids content, leading to protein coagulation due to organic acids produced by bacterial starters. The prebiotic component in hanjeli flour serves as a carbon source for probiotic bacteria in the colon, supporting probiotic growth. Additionally, the addition of skim milk provides soluble solids that can enhance the texture of synbiotic yogurt. Lactic acid bacteria break down some of the lactose from milk into lactic acid, which enhances the yogurt's texture. Lactic acid reacts with calcium from casein, causing it to precipitate due to the coalescence of different casein molecules. This is a result of the acidic pH, which causes calcium to dissociate from casein, allowing ionic charges in the milk to bind together and cause clumping [11]. The results of research by [12] explains that the addition of solids in yogurt making increases the viscosity, texture and thicker yogurt.

3.1.2 Syneresis

Syneresis is a complex phenomenon involving the contraction of protein gels due to the increase in protein interaction but decreasing hydration, resulting in curd formation and whey separation. The analysis of variance indicated that varying concentrations of hanjeli flour significantly affected ($P<0.05$) the syneresis value of synbiotic yogurt, as presented in **Table 1**. This effect is attributed to the differing percentages of hanjeli flour incorporated into the yogurt formulation. Hanjeli flour, when added to synbiotic yogurt, enhances texture, thereby reducing syneresis and increasing viscosity. The reduction in syneresis is associated with an increase in viscosity, which leads to decreasing whey separation. Research by [13] explains that syneresis is the event of water coming out of the gel, where the higher syneresis value indicates the instability of the gel bond. Sineresis is one of the quality assessments of yogurt, the higher the syneresis value, the lower the quality [14].

The average value of syneresis of synbiotic yogurt produced ranged from 17.09% to 34.82%. The highest syneresis value was obtained in P0, which was 34.82%, while the lowest syneresis value was obtained in P3, which was 17.09%. [1] explains that the more the addition of stabilizer, the sineresis value decreases. The control treatment (P0) has the highest average value of sineresis which is 29.63%, while the lowest average value of sineresis is obtained in P3 which is 22.01% with the addition of 3% stabilizer. The decreased sineresis value resulted in a better texture of the yogurt produced, this is because if the sineresis is too high it can the texture of the yogurt. Research by [15] reports that the higher the total solid content, the lower the syneresis value of yogurt produced due to shrinkage of the protein structure which resulted in a lack of protein binding ability resulting in the separation of whey.

3.1.3 Water Holding Capacity

Water holding capacity is a crucial indicator for determining the quality of synbiotic yogurt. The results of the analysis of variance demonstrated that varying concentrations of hanjeli flour significantly influenced ($P<0.05$) the water holding capacity of synbiotic yogurt, as can be seen in **Table 1**. This significant difference was attributed to the addition of hanjeli flour at different concentrations and the inclusion of 2% skim milk. Hanjeli flour contains starch which consists of two components, namely amylose and amylopectin which are able to bind water in synbiotic yogurt. Consequently, this absorption reduces the water content and increases the viscosity of the resulting synbiotic yogurt, thereby enhancing its water holding capacity. [16] explains that the more the concentration of stabilizer, the higher the ability to bind water. Skim milk has a high content of protein, minerals and lactose so that it can be utilized by bacteria in the fermentation process and increase the water absorption process so that the texture of yogurt will be thicker [17]. According to [18] the increased water binding capacity prevent and reduce free water molecules that come out through the pores between casein molecules so that the value of water holding capacity will increase.

The average water holding capacity of the synbiotic yogurt produced ranged from 52.44% to 90.62%. The highest average water holding capacity was observed in sample P3, at 90.62%, while the lowest was in sample P0, at 52.44%. Water holding capacity is a key parameter for determining the quality of synbiotic yogurt. A higher water holding capacity indicates better yogurt quality, as it can retain a greater amount of free water within the yogurt [19]. The average value of water holding capacity in this study increases as the concentration of hanjeli flour increases, this is because hanjeli flour increases the amount of total solids in synbiotic yogurt. The higher the total solids, the more the ability to hold water in yogurt so that the texture of the yogurt increase and the value of syneresis decrease [20].

3.1.4 pH

The pH value is a measure used to express the acidity of the resulting synbiotic yogurt. The analysis of variance indicated that varying concentrations of hanjeli flour significantly affected ($P<0.05$) the pH value of synbiotic yogurt, as presented in **Table 1**. This effect is attributed to the addition of hanjeli flour during the yogurt production process. The decrease in pH is due to the growth and development of lactic acid bacteria, which is enhanced by the increased availability of substrates, particularly glucose. As lactic acid bacteria metabolize these substrates, they produce more lactic acid, thereby lowering the pH. The primary substrate from milk is lactose, while hanjeli flour provides amylose and amylopectin, which are broken down into glucose by enzymes from lactic acid bacteria and subsequently converted into lactic acid [8].

The mean pH value in this study ranged from 4.02 to 4.22. The highest average pH value was observed in the P0 treatment, at 4.22, while the lowest average pH value was found in the P3 treatment, at 4.02. Increasing the addition of hanjeli flour up to 3% resulted in a reduction in the pH value of synbiotic yogurt. [22] indicates that the addition of corn starch as a stabilizer also decreased the pH. Specifically, the addition of corn starch at a concentration of 0.5% resulted in a pH value of 4.42, which is lower than the pH value of 4.50 observed without the addition of corn starch. [21] explains that changes in pH value are influenced by the activity and growth of lactic acid bacteria which are capable of producing pectinolytic and cellulolytic enzymes that degrade starch cell walls. This enables lactic acid bacteria to utilize carbohydrate sources as an energy source, leading to the hydrolysis of starch and lactose. The pH value in this study is still in accordance with the Indonesian National Standardization (SNI) 2981: 2009 pH of yogurt is 4-4.5, then yogurt is suitable for consumption [23].

3.1.5 Total Acid

Total acid is an indicator used to determine the acidity level in food. The analysis of variance results showed that varying concentrations of hanjeli flour significantly affected ($P<0.05$) the total acid content of synbiotic yogurt, as can be seen in **Table 1**. This significant difference was attributed to the varying percentages of hanjeli flour used. The use of hanjeli flour at different concentrations demonstrates a consistent effectiveness in increasing the total acid content of synbiotic yogurt. This is evidenced by the increasing total acid values corresponding to the higher percentages of hanjeli flour used in the production of synbiotic yogurt. [1] explains that the higher increase in total acid value is caused by an increase in the activity of lactic acid bacteria in breaking down lactose into lactic acid. The addition of stabilizer in yogurt increases the glucose used by LAB as a substrate and convert it into lactic acid so that it will affect the total acid. [24], the addition of stabilizer stimulate the metabolic activity of LAB so that it increase the acidity of yogurt.

The average value of total acid of the synbiotic yogurt produced ranged from 1.14% to 1.26%. The highest average value of total acid was obtained in P3, namely 1.26%, while the lowest average value of total acid in P0 was 1.124%. The more the addition of hanjeli flour in making yogurt, the more the total acid value produced increases. The results of this study are in accordance with [25] that the addition of taro starch in making yogurt with a concentration of 2% has the highest total acid value of 1.08%, while the lowest total acid value is P0 without the addition of taro starch which has an average value of 0.94%. The standard value of total acid in yogurt ranges from 0.5% to 2.0% [23]. The total acid value is opposite to the pH value, the lower the total acid value, the higher the pH value [26]. [27] explains that the more glucose is metabolized, the more lactic acid production increases. A high amount of lactic acid can increase acidity and decrease the pH value.

3.1.6 Total Lactic Acid Bacteria

The total lactic acid bacteria (LAB) count is a critical factor in assessing the viability of synbiotic products as functional foods by determining the total amount of LAB produced. The analysis of variance results indicated that varying concentrations of hanjeli flour significantly affected ($P<0.05$) the total LAB count in synbiotic yogurt, as presented in **Table 1**. This significant difference was attributed to the different percentages of hanjeli flour used. The use of hanjeli flour at varying concentrations consistently enhanced the total LAB count in synbiotic yogurt. This is evidenced by the increasing LAB values corresponding to higher percentages of hanjeli flour used in the yogurt production. Hanjeli flour serves as a thickening agent due to its high carbohydrate content. Hanjeli flour has a carbohydrate content of 72% and has an amylose content of 8.36% [28]. [29] explains that the strategy that is often applied in making yogurt to increase the growth of probiotic bacteria is by adding prebiotic compounds. The hanjeli flour is used by LAB as a nutrient to grow and develop so that producing lactic acid indicates increasing number of LAB. The addition of skim milk in making synbiotic yogurt aims to utilize the high levels of lactose and protein in skim milk so as to make the growth of LAB grow optimally.

The mean value of total LAB of synbiotic yogurt produced ranged from 7.02 log CFU/g to 7.42 log CFU/g. The highest total LAB value obtained in P3 was 7.42 log CFU/g, while the lowest total LAB value obtained in P0 was 7.02 log CFU/g. The results of this study are in accordance with the research of [8] that the higher addition of stabilizer increase the number of LAB which found ranging from 7.20 log CFU/ml to 7.35 CFU/ml. The control treatment (P0) exhibited the lowest mean total LAB value at 7.20 log CFU/ml, whereas the highest mean total LAB value was observed in P3, with the addition of kimpul starch at a concentration of 3%, reaching 7.35 log CFU/ml. The total LAB in synbiotic yogurt with the addition of hanjeli flour complies with the Indonesian National Standard (SNI) 2981: 2009, which requires a minimum of 10^7 colonies per gram or 7 log CFU/ml of LAB in yogurt. Bacterial growth is divided into four phases: the lag phase, exponential phase, stationary phase, and death phase. The lag phase is the period during which bacteria adapt to their environment. Once adaptation occurs, the bacteria enter the exponential phase, characterized by rapid growth and cell division. Following this, the bacteria transition into the stationary phase, where the rate of bacterial growth equals the rate of bacterial death. The onset of the stationary phase is marked by a decline in the rate of bacterial growth. The last phase in bacterial growth is the death phase. In the death phase the bacterial growth rate decreases [30]. Factors that affect bacterial growth include pH, temperature, and nutrients available at the place of growth [31].

4 Conclusion

The research results showed that synbiotic yogurt with the addition of hanjeli flour at different concentrations was able to increase the viscosity, water holding capacity, total acid, and total LAB values, while the syneresis and pH values decreased further. The conclusion of this research is that P3 is the best treatment with a viscosity value of 4250 cP, syneresis 17.09%, WHC 90.62%, pH 4.02, total acid 1.26%, and total lactic acid bacteria 7.42 log CFU/g.

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