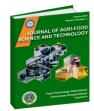
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# Quality Characteristics of Jack Bean (*Canavalia ensiformis L.*) Tempeh Milk with Addition of Ajwa Date (*Phoenix dactylifera L.*) and Various Types of Stabilizers

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# ABSTRACT

Jack bean tempeh was processed into jack bean tempeh milk. The addition of Ajwa date can improve the organoleptic and nutritional value of jack bean tempeh milk. Moreover, stabilizers must be added while making jack bean tempeh milk to enhance its quality. This study contributed to determining the effect of various stabilizers on jack bean tempeh milk quality. The treatments were (F1) the addition of iota carrageenan stabilizer (0.1%), (F2) the addition of gum Arabic stabilizer (0.1%), (F3) the addition of pectin stabilizer (0.1%), and (F4) the addition of carboxy methyl cellulose (CMC) stabilizer (0.1%). The results of this study showed that the stabilizer types had no significant effect on the organoleptic color, texture, and taste of the jack bean tempeh milk. The selected formulation was based on the hedonic quality test in the parameters of color and aroma, namely formula F4, which was jack bean tempeh milk with the addition of a CMC stabilizer. The results of the chemical characteristics of F4 jack bean tempeh milk was protein content of 0.65%, fat content of 2.48%, and antioxidant activity (IC50) of 7760 ppm. The physical characteristics of F4 jack bean tempeh milk was a pH value of 5.8, total dissolved solids of 20.7°Brix, a viscosity of 13.43 cP, L\* value of 60.61, a\* value of -0.12, and b\* value of 19.56.

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#### **1. INTRODUCTION**

The rise in veganism, lactose intolerance, and concern for animal welfare have led to a significant increase in the consumption of plant-based milk. Plant-based milk has been defined as an aqueous liquid obtained from the extraction of plants, which resembles animal milk. Water is used as an extractant, followed by separating the liquid part from the solid particles (Romulo, 2022). Plant-based milk comes in various forms, each with a unique flavor and

nutritional profile. Some of the most popular plant-based milks are soy, almond, oat, and other varieties. Soy milk is made from soybeans, while almond milk is made from almonds and oat milk from oats (Sharma, 2023). Different materials can be used to make plant-based milk, and these materials can be categorized based on the raw ingredients that are used, including legumes (soybean, chickpea, kidney bean, lupin, pea, cowpea, and peanut), cereals (corn, rice, spelled, sorghum, rye, wheat), nuts (almond, coconut, pistachio, walnut, and hazelnut), seeds (flax, sunflower, hemp, and sesame), and pseudocereals (teff, amaranth, and quinoa) (Wijaya & Romulo, 2021).

Jack beans can potentially be developed as a plant-based milk substitute because of their protein content, almost comparable to soybeans. According to Purwandari et al. (2023), jack beans are an inexpensive source of protein (22–35 g/100 g), carbohydrates (45–65 g/100 g), and dietary fiber (4–17 g/100 g). Jack bean proteins contain high amounts of essential amino acids such as leucine, isoleucine, and threonine and are considered a good source of lysine. Jack beans are rich in nutrients and contain toxic substances in the form of hydrogen cyanide (HCN). Ramli et al. (2021) determined that the HCN content of raw jack beans is 52.78 mg/kg or 52.78 ppm. The content of HCN can be completely or partially removed by processing. One is fermented into jack bean tempeh (Puspitojati et al., 2019). The added tempeh yeast inoculum, *Rhizopus oligosporus*, influenced the decrease in HCN in fermentation. This fungus can utilize the N element instead of protein from HCN itself to reduce HCN levels in fermented jack beans (Wahono et al., 2016).

Jack bean tempeh has mainly been consumed as fresh jack bean tempeh. This is due to the relatively short shelf life of jack bean tempeh. Diversification of jack bean tempeh-based food products is needed to provide added value to jack bean tempeh. One of the results of jack bean tempeh-based food diversification that can be developed is jack bean tempeh milk. By including additional ingredients, a distinct flavor of jack bean tempeh milk, often characterized as "beany" can be eliminated.

In this study, when added to jack bean tempeh milk, it may be expected that Ajwa date is a natural sweetener and flavoring. Dates are a fruit that is high in natural sugars like fructose and glucose. Since fructose does not usually require insulin to be absorbed, it is suitable for those with diabetes (Magsi et al., 2021). Ajwa dates are a soft and fruity smoothie date variety with a fine texture and is also a rich source of antioxidant compounds, such as phenolics and flavonoids. Because dates have a higher number of phytochemicals, they play an essential role in forming antioxidants that scavenge free radicals following oxidative stress (Parvez et al., 2021; Bano et al., 2022). Therefore, the organoleptic and nutritional value of jack bean tempeh milk can be improved by adding Ajwa date, so it is expected to increase interest in consuming this jack bean tempeh milk.

Stabilizers must be added while making jack bean tempeh milk to improve quality. The purpose of stabilizers added during the manufacturing of jack bean tempeh milk is to prevent precipitation and maintain its stability (Simanungkalit & Nasution, 2023). The stabilizer has two primary functions, which are to bind water (water binding capacity) and to increase the viscosity of the solution (Juniawati et al., 2022). The polar groups of stabilizers will bind to water and maintain the consistency of the product. The addition of stabilizers also aims to keep the particles evenly dispersed throughout the dispersing medium (Lantika et al., 2020).

Based on research findings of Santoso & Moulina (2017), the results showed the quality of tempeh milk using soybean, kidney bean, and mung bean with CMC and gum Arabic as stabilizers. The results showed that 0.25% CMC was the best stabilizer type and concentration compared to others. Based on the description above, in the making of jack bean tempeh milk, it was necessary to add stabilizers, which were expected to produce high-quality jack bean tempeh milk. The study contributed to determining the effect of various stabilizer types added, such as iota carrageenan, gum Arabic, pectin, and CMC, on the quality characteristics of jack

bean tempeh milk.

## 2. MATERIALS AND METHODS

#### 2.1. Materials

The primary materials used for the production of jack bean tempeh milk were jack bean tempeh obtained from BUMR Paramasera, Bogor, West Java; stabilizers such as pectin obtained from Pharmapreneur Store, Depok, West Java; iota carrageenan obtained from Cargill, France; gum Arabic obtained from TIC GUMS, USA; and CMC (Koepoe Koepoe, Tangerang), Ajwa dates (Safiya, Medina), honey (Tropicana Slim, Bogor), mineral water (AQUA, Klaten), non-dairy creamer (RICH'S, Serang), pandan flavor essence (Red Bell, Tangerang), and ginger powder (Koepoe Koepoe, Tangerang). The materials used for chemical analyses were aquadest, 2,2-Diphenyl-1-Picrylhydrazyl (DPPH), ethanol absolute, 70% ethanol, HgO.K<sub>2</sub>SO<sub>4</sub>, NaOH-Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>, H<sub>2</sub>SO<sub>4</sub>, HCl, boric acid, bromocresol green and methyl red (BCG-MR) indicators, and petroleum ether solvent.

The tools used for the production of jack bean tempeh milk were a blender (Philips series 5000-HR2221/30), liquid strainer 120 mesh, digital kitchen scale (HAN RIVER SF-400), mini hand mixer (HAN RIVER HRDDQ07), and digital food thermometer (Taffware TP101). Tools used for physical measurement and chemical analysis were a soxhlet set, Kjeldahl set, drying oven (Labtech LDO-060E), desiccator, analytical balance (Fujitsu FS-AR 210), chromameter (Konica Minolta CR-400), viscometer (Brookfield DV-II+Pro), hand refractometer (Nohawk BM-03), pH meter (AMTAST AMT20), centrifuge (Oregon LC 04-S), and UV-Vis spectrophotometer (Thermo Scientific Genesys).

#### 2.2. Research Methods

#### 2.2.1. Preparation of Ajwa Date Puree

The method of making date puree was based on Arifan et al. (2021) with modifications. The dates and seeds were first separated. The dates were then weighed. The dates were blanched for 5 minutes at 90°C. Mineral water was added to the ratio of dates: mineral water (2:1) and then blended until smooth. After that, the dates puree was filtered to separate the puree of the dates from the pulp using a filter cloth and liquid strainer 120 mesh.

#### 2.2.2. Preparation of Jack Bean Tempeh Milk

The method of making jack bean tempeh milk is based on Wusono et al. (2023) with modifications (Table 1). The jack bean tempeh was cut into cubes. The jack bean tempeh cubes were blanched at 80°C for 5 minutes. Mineral water was added to the blender with a ratio of jack bean tempeh: mineral water (2:1) and then blended until sufficiently smooth. The slurry was filtered twice through a liquid strainer 120 mesh and a filter cloth. Jack bean tempeh milk was boiled to a temperature of 95°C. Then, 0.1% stabilizers (iota carrageenan, gum arab, pectin, and CMC) were mixed with 0.5% ginger powder using a mini hand mixer. After that, jack bean tempeh milk was mixed with 25% Ajwa date puree, 10% liquid non-dairy creamer, 8% honey, and 1% pandan flavor essence. Then, heated for 5 minutes at 90°C while stirring. After that, the jack bean tempeh milk was put into a sterile plastic bottle.

## 2.2.3. Organoleptic Test

The organoleptic test was a hedonic quality test with 35 semi-trained panelists aged between 18 and 35 years old, male and female. The samples (30 mL) were served at room temperature ( $25 \pm 2^{\circ}$ C) in 50 mL plastic cups codified with three-digit random numbers. Mineral water at room temperature was served to allow the panelists to cleanse their palates between samples (Terhaag et al., 2013). It was most commonly used for evaluating preference for color, aroma, taste, and texture with a rating scale consisting of color: 1 (dark brown), 2 (brown), 3 (light brown), 4 (white-brown), 5 (white); date aroma: 1 (very weak), 2 (weak), 3 (moderate), 4 (strong), 5 (very strong); texture: 1 (very thin) 2 (thin) 3 (a little bit thick) 4 (thick) 5 (very thick); taste: 1 (very not sweet) 2 (not sweet) 3 (slightly sweet) 4 (sweet) 5 (very sweet).

	. Formulation	on of jack be	an tempeh m	nılk.	
Ingredients %	F0	F1	F2	F3	F4
Jack bean tempeh milk	55.5	55.4	55.4	55.4	55.4
Ajwa date puree	25	25	25	25	25
Iota carrageenan	-	0.1	-	-	-
Gum Arabic	-	-	0.1	-	-
Pectin	-	-	-	0.1	-
CMC	-	-	-	-	0.1
Non-dairy creamer	10	10	10	10	10
Ginger powder	0.5	0.5	0.5	0.5	0.5
Honey	8	8	8	8	8
Pandan flavor essence	1	1	1	1	1

Table 1. Formulation of jack bean tempeh milk.

# 2.2.4. Chemical Analysis

## 2.2.4.1. Protein Content of Jack Bean Tempeh Milk

Protein content was determined according to the method described by Simanungkalit & Nasution (2023) with modifications. One gram of jack bean tempeh milk into the Kjeldahl flask, 2 g of K<sub>2</sub>SO<sub>4</sub> and CuSO<sub>4</sub> mix, and 15 mL of H<sub>2</sub>SO<sub>4</sub>, and heated it over the Kjeldahl apparatus until the solution was clear. Cooled and put into the bottom flask, 100 mL of distilled water, 30% NaOH (w/v) was added and distilled for a few minutes. The distillate results were collected in an Erlenmeyer containing 4% (v/v) H<sub>3</sub>BO<sub>3</sub> and the BCG-MR (bromocresol green and methyl red) indicator until the color changed to green. The distillate results were then titrated with standard 0.02 N HCl solution until the solution changed color from green to purple, the volume of titrant used was recorded, and the protein content was calculated.

## 2.2.4.2. Fat Content of Jack Bean Tempeh Milk

Fat content was determined according to the method described by Simanungkalit & Nasution (2023) with modifications. Two grams of jack bean tempeh milk were weighed and placed into a beaker glass, and 30 mL of 25% HCl (aq) and 20 mL of distilled water were boiled for 15 minutes, then filtered and washed with hot distilled water. The filter paper and its contents are dried at 100-105°C. The filter paper was wrapped, put into the soxhlet apparatus, and extracted with petroleum ether solution for 2-3 hours at 80°C. Distilled petroleum ether solution from fat extract at 100-105°C. Cooled in a desiccator and weighed to constant weight.

## 2.2.4.3. Antioxidant Activity of Jack Bean Tempeh Milk

Antioxidant activity expressed in terms of DPPH assay was determined according to the method described by Nurhayati et al. (2022) with modifications. A total of 0.5 g of sample was dissolved with 10 mL ethanol 70%. 0.001 g of DPPH (2,2-diphenyl-1-picrylhydrazyl) was dissolved in 100 mL of ethanol and shaken well till solubility, and then 4 mL of DPPH solution was added to different concentrations of samples as 0.001, 0.003, 0.006, 0.008, and 0.01 g/mL sample. The mixture was then incubated at room temperature in the dark for 30 min. The absorbance of the mixture was measured by UV-Vis spectrophotometer at a wavelength of 517 nm. The amount of % DPPH inhibition was calculated with equation (1).

$$Inhibition (\%) = \frac{Abs \text{ control} - Abs \text{ sample}}{Abs \text{ control}} \ge 100$$
(1)

The sample concentration and % inhibition was plotted on the sample absorbance curve and plotted on the x and y axes of the linear regression equation, respectively. Sample concentration is the x-axis, and % inhibition is the y-axis. Each sample's IC50 (50% inhibitor concentration) is expressed by the y value of 50 and the x value obtained as IC<sub>50</sub>.

#### 2.2.5. Physical Test

## 2.2.5.1. pH Value of Jack Bean Tempeh Milk

The pH value of 10 mL of homogenized jack bean tempeh milk was measured by using a pH meter (AMTAST AMT20, Indonesia), which was calibrated with standard buffers of pH 4.0, pH 7.0, pH 10.0 before the measurement (Saruan et al., 2018).

#### 2.2.5.2. Total Dissolved Solids Content of Jack Bean Tempeh Milk

The total dissolved solids content of jack bean tempeh milk was measured using a hand refractometer (Nohawk BM-03, China) and calibrated using distilled water. The surface of the lens was washed with distilled water and dried with a paper towel to remove excess water. Then, it was tested with jack bean tempeh milk. A scale will appear on the refractometer, which shows total solids in units of °Brix (Saruan et al., 2018).

#### 2.2.5.3. The viscosity of Jack Bean Tempeh Milk

The viscosity of jack bean tempeh milk was measured using a viscometer (Brookfield, model DV-II+Pro, United Kingdom). A sample of 300 mL was placed into a beaker glass of 500 mL, then a #4 spindle dipped into the sample at 100 rpm and 17°C (Terhaag et al., 2013). After 3 minutes, a viscosity value would appear in centipoise (cP) units.

#### 2.2.5.4. Colors of Jack Bean Tempeh Milk

The colors of jack bean tempeh milk were determined using a chromameter (Konica Minolta CR-400, Japan). A cleaned petri dish containing the jack bean tempeh milk was placed below the light source, and postprocessing L, a, and b values were recorded. The color was expressed in CIE Lab system L\*, a\*, and b\* values, where L\* indicates lightness on a 0-100 scale from black to white, a\* red (+) and green (-), and b\* yellow (+) and blue (-) (Saruan et al., 2018).

#### 2.2.6. Research Design

This study used a one factorial Completely Randomised Design (CRD) consisting of 4 treatments. The treatments were (F0) without stabilizer, (F1) addition of iota carrageenan (0.1%), (F2) addition of gum Arabic (0.1%), (F3) addition of pectin (0.1%), and (F4) addition of CMC (0.1%). The results of the organoleptic test were then analyzed for variance or One-way analysis of Variance (ANOVA) with a significance level of p-value 0.05 using SPSS Statistics V27.0. If there is a significant difference, continue with further tests using Duncan's Multiple Range Test (DMRT).

## 3. RESULT AND DISCUSSION

## 3.1. Organoleptic Characteristic of Jack Bean Tempeh Milk

The results of the hedonic quality test of jack bean tempeh milk can be seen in Table 2. According to the hedonic quality test, the color of jack bean tempeh milk ranged from 3.54 to 3.86 (light brown). The stabilizers used had no significant effect (p > 0.05) on jack bean tempeh

milk color. When pectin (F3) and CMC (F4) were added, the jack bean tempeh milk's color hedonic quality test value was the highest. This showed that panelists prefer light brown jack bean tempeh milk. According to Nurhayati & Alfian (2017), CMC is a linear polymer, cellulose ethers, and anion form colorless compounds. On the other hand, the color of food products became lighter after adding CMC. The brown color in this study was due to the addition of Ajwa date puree to the jack bean tempeh milk. Similar findings of Paul et al. (2017) showed that added date paste can produce a dark brown color in soy milk.

Table 2. Hedonic quality test of jack bean tempeh milk.				
Treatment	Color	Aroma	Texture	Taste
F1	$3.71^{\text{NS}} \pm 0.789$	$2.20^{c}\pm0.833$	$3.00^{\text{NS}} \pm 1.085$	$2.83^{\text{NS}} \pm 1.043$
F2	$3.54^{NS}\pm0.701$	$2.91^b\pm0.612$	$2.74^{\text{NS}}\pm0.780$	$3.29^{\text{NS}}\pm0.750$
F3	$3.86^{\text{NS}} \pm 0.879$	$3.17^{ab}\pm0.568$	$2.74^{\text{NS}}\pm0.980$	$3.11^{\text{NS}} \pm 0.832$
F4	$3.86^{\text{NS}} \pm 0.845$	$3.43^a\pm0.502$	$2.97^{\text{NS}} \pm 0.822$	$3.26^{\text{NS}} \pm 1.039$

Description: (F1) addition of iota carrageenan stabilizer (0.1%), (F2) addition of gum Arabic stabilizer (0.1%), (F3) addition of pectin stabilizer (0.1%), and (F4) addition of CMC stabilizer (0.1%). Color: 1 (dark brown) 2 (brown) 3 (light brown) 4 (white-brown) 5 (white). Date aroma: 1 (very weak), 2 (weak), 3 (moderate), 4 (strong), 5 (very strong). Texture: 1 (very thin), 2 (thin), 3 (a little bit thick), 4 (thick), 5 (very thick). Taste: 1 (very not sweet) 2 (not sweet) 3 (slightly sweet) 4 (sweet) 5 (very sweet). The values with different superscripts in a column differ significantly (p < 0.05), and NS is not significant.

The jack bean tempeh milk date aroma hedonic quality test ranged from 2.20 to 3.43 (weak and moderate). The F1 with F2, F3, and F4 had a significant effect (p < 0.05) on the aroma of jack bean tempeh milk. Jack bean tempeh milk had the highest aroma hedonic quality test value with the addition of CMC (F4). With the addition of CMC (F4), jack bean tempeh milk had the aroma of dates, but it still had the aroma of jack bean tempeh. Anggrahini & Pratama (2017) explained that CMC was a hydrocolloid that could be used as a binding agent.

The texture of the jack bean tempeh milk hedonic quality test ranged from 2.74 to 3.00 (thin and slightly thick). The stabilizer used had no significant effect (p > 0.05) on the texture of jack bean tempeh milk. Jack bean tempeh milk had the highest texture hedonic quality test value with the addition of iota carrageenan (F1). With the addition of iota carrageenan, jack bean tempeh milk showed the highest texture hedonic quality test value (F1). As linear, water-soluble polymers, Carrageenans typically form highly viscous aqueous solutions (Necas & Bartosikova, 2013).

The jack bean tempeh milk taste hedonic quality test findings ranged from 2.83 to 3.29 (not sweet and slightly sweet). The stabilizer used had no significant effect (p > 0.05) on the taste of jack bean tempeh milk. When gum Arabic was added, the taste hedonic quality test value of the jack bean tempeh milk was highest (F2). According to Fanani et al. (2022), gum Arabic has the ability as a binder to maintain the taste. The sweetness of jack bean tempeh milk comes from the Ajwa date puree and honey used.

#### 3.2. Best Formulation of Jack Bean Tempeh Milk

Figure 1 shows the physical appearance of jack bean tempeh milk with the addition of various types of stabilizers. The colors of each jack bean tempeh milk appeared similar. Jack bean tempeh milk with the addition of CMC stabilizer (F4) was selected based on the hedonic quality test in the parameters of color and aroma. The selected jack bean tempeh milk had a light brown color, a slight aroma of dates, a thin texture, and a slight sweetness. CMC is a white ether cellulose molecule, solid and odorless (Sebayang et al., 2019). It is stable at pH 3-7 and very easy to find in various places with relatively low prices (Nurhaliza et al., 2021). CMC is

a thickening and sweet flavor-stabilizing agent (Anggrahini & Pratama, 2017).

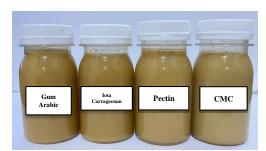


Figure 1. Physical appearance of jack bean tempeh milk.

#### 3.3. Chemical Characteristics of Best Formulation

#### 3.3.1. Protein Content

F0 jack bean tempeh milk had the same protein content as F4 jack bean tempeh milk, which was 0.65% (Table 3). This result was less than that of a study by Ramadhania et al. (2023), which found that the protein content of soy milk with different proportions of soybeans and *lamtoro gung* was 3.304%. As shown in Table 3, the stabilizer did not significantly affect the protein content of jack bean tempeh milk (p > 0.05).

	Table 3. Protein c	ontent of jack bean tempeh	ı milk.	
Variable -	Variable Treatment r value			
v ar lable	F0 (% wb)	F4 (% wb)	p-value	
Protein Content	$0.65\pm0.03$	$0.65\pm0.05$	$0.928^{*}$	

Description: Control (F0): no stabilizer added; Selected formula: CMC stabilizer (F4). \*) *Independent Samples T*-*Test* difference is significant at (p < 0.05).

According to SNI 01-3830-1995, the minimum protein content in soy milk is 2.0%. Jack bean tempeh was made through the fermentation process of jack bean. Many of the nutrients in jack beans had changed to become more soluble in water and easy to digest. As seen in Table 3, the protein content of jack bean tempeh milk was low because about half of the protein content of the jack bean was broken down during fermentation into smaller, water-soluble molecules such as peptides and amino acids (Tukiran et al., 2021). In addition, the protein content of jack bean tempeh milk was low because of the heating process. Excessive heating in more extended periods can damage protein, decreasing protein content. Appropriate heating can produce a highly preferred jack bean tempeh milk taste and aroma for consumers. In contrast, excessive heating can damage protein and change flavor due to overcooking, which consumers do not prefer (Yustina et al., 2020).

#### 3.3.2. Fat Content

F4 jack bean tempeh milk had a fat content of 2.48%, higher than F0 jack bean tempeh milk of 2.14%. Compared to a study by Tukiran et al. (2021), which found that almond-tempeh milk had a protein content of 4.69%, this result was lower. As shown in Table 4, the fat content of the jack bean tempeh milk was not significantly affected by the stabilizer (p > 0.05).

According to SNI 01-3830-1995, the minimum fat content in soy milk is 1.0%, which means that the jack bean tempeh milk in this study was in line with the quality standards of soy milk. According to Rosida et al. (2016), non-dairy creamer uses vegetable oil (palm oil) as a source of fat. The oil content of non-dairy creamer ranged from 16.45% to 34.91%. This might cause increases in the milk fat content in jack bean tempeh milk. Treatment with high temperatures also affects fat content and tends to cause fat damage. Consequently, water and

	Table 4. The fat co	ntent of jack bean tempeh n	uilk.
Variable	Trea	itment	n voluo
Variable — F0	F0 (% wb)	F4 (% wb)	p-value
Fat Content	$2.14\pm0.26$	$2.48\pm0.11$	$0.056^*$

high temperatures can trigger lipolysis or fat hydrolysis reactions, leading to fat loss in jack bean tempeh milk (Kristiningrum et al., 2021).

Description: Control (F0): no stabilizer added; Selected formula: CMC stabilizer (F4). \*) *Independent Samples T*-*Test* difference is significant at (p < 0.05).

## 3.3.3. Antioxidant Activity

 $IC_{50}$  (Inhibitory concentration) is the concentration of sample solution needed to inhibit 50% of DPPH free radicals (Nurhayati et al., 2022). The results of the antioxidant activity of jack bean tempeh milk (F0) obtained an  $IC_{50}$  value of 4178 ppm. In comparison, the results of the antioxidant activity of jack bean tempeh milk (F4) obtained an  $IC_{50}$  value of 7760 ppm (Table 5).

Table 5. Antioxidant activity of jack bean tempeh milk.VariableTreatmentp-value $IC_{50}$ 4178  $\pm$  0.0017760  $\pm$  0.0000.042\*Description: Control (F0): no stabilizer added: Selected formula: CMC stabilizer (F4) \*) Independent Samples To

Description: Control (F0): no stabilizer added; Selected formula: CMC stabilizer (F4). \*) *Independent Samples T*-*Test* difference is significant at (p < 0.05).

A comparison of concentration with % inhibition will get a linear regression equation (Figure 2 and Figure 3). The higher the sample concentration, the more the percentage of inhibition increases. The inhibition value indicates the inhibitory activity of the sample against DPPH radical expressed in percent (Tang'nga et al., 2019). Then, the IC<sub>50</sub> value is calculated using the linear regression equation. In this study, the antioxidant activity contained in jack bean tempeh milk was classified as very weak. Based on Maryam et al. (2023), having an IC<sub>50</sub> value of less than 50 ppm, strong antioxidants have IC<sub>50</sub> values in the range of 50 ppm to 100 ppm, moderate antioxidants have IC<sub>50</sub> values ranging from 100 ppm to 150 ppm, weak antioxidants have a range of 150 ppm to 200 ppm, and IC<sub>50</sub> values of more than 200 ppm are very weak antioxidants. As shown in Table 5, the stabilizer used had a significant effect (p < 0.05) on the antioxidant activity of jack bean tempeh milk.

Previous research showed that Safawy, the strongest scavenger of DPPH free radicals, was found in the three date cultivars. The activity order is Safawy > Ajwah > Sukkari (IC<sub>50</sub> 104, 125, and 177 µg/mL, respectively). All three extracts show good scavenging activity (IC<sub>50</sub> < 1 mg/mL) when compared to the positive control, i.e., ascorbic acid (IC<sub>50</sub> 12.09 µg/mL) (Zihad et al., 2021). Ajwa dates contains quercetin, gallic acid, p-Coumaric acid, m-Coumaric acid, ferulic acid, and vitamin C. Each of these substances has the potential to function as an antioxidant. The jack bean tempeh milk had previously been cooked and mixed with other nutrients that can inhibit antioxidant activity (Afifah et al., 2022). Furthermore, the heat process applied to the food processing also influenced the stability of the product's antioxidant activity (Damayanti et al., 2021).

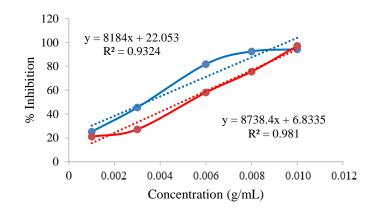
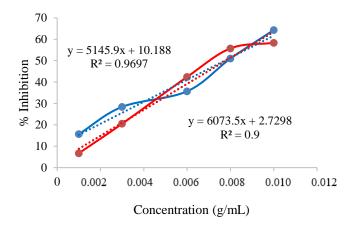


Figure 2. The percentage of DPPH inhibition by F0 jack bean tempeh milk (n = 2).





# **3.4.** Physical Test Results of Selected Formulation Jack Bean Tempeh Milk **3.4.1.** pH Value

Table 6 showed that the pH of the F4 jack bean tempeh milk was 5.8, which was higher than that of the F0 jack bean tempeh milk, which was 5.78. Because of its hydrocolloid characteristics, which include a large number of carboxylic groups and allow it to hydrolyze efficiently, adding CMC to food and beverage products can increase their pH value (Table 6) (Simanungkalit & Nasution, 2023). In comparison, the pH of almond milk was reported to vary from 6.74 to 6.88 in a study by Manzoor (2016). This result was lower. As shown in Table 6, the stabilizer used had no significant effect (p > 0.05) on the pH value of jack bean tempeh milk.

	Table 6. pH	value of jack bean tempe	h milk.
Variable	Tre	eatment	
variable	F0	0 F4	- p-value
pН	$5.78\pm0.19$	$5.8\pm0.25$	$0.890^{*}$

Description: Control (F0): no stabilizer added; Selected formula: CMC stabilizer (F4). \*) *Independent Samples T*-*Test* difference is significant at (p < 0.05).

According to SNI 01-3830-1995, the pH of soy milk is between 6.5 and 7.0. In this study, the pH of jack bean tempeh milk was less than what the SNI quality requirements specified. A low pH indicates microorganisms or enzyme activity that can cause changes in

food quality (Kristiningrum et al., 2021). This may be due to the soaking process during jack bean tempeh, which allows lactic acid bacteria to grow. The acidification process that occurs due to the activity of lactic acid bacteria in the soaking process causes good conditions for the growth of Rhizopus mould and can also inhibit the growth of pathogenic microbes (Fallo & Sine, 2022). The pH value, which can affect a wide range of chemical and enzymatic reactions and prevent the growth of microorganisms, indicates whether or not there are microorganisms or enzyme activity in food (Kristiningrum et al., 2021). The acidic pH of jack bean tempeh milk can inhibit the growth of microorganisms.

# **3.4.2.** Total Dissolved Solids

Table 7 showed that F4 jack bean tempeh milk had a total dissolved solids of 20.7°Brix, higher than F0 jack bean tempeh milk of 19.8°Brix. This result was higher than that of a study by Pratama et al. (2021), which reported that malted red rice milk had a total dissolved content varying from 8.0 to 9.96°Brix. As shown in Table 7, the stabilizer used had no significant effect (p > 0.05) on the total dissolved solids of jack bean tempeh milk.

|--|

Variable	Treatment			
Variable	F0 (°Brix)	F4 (°Brix)	p-value	
Total Dissolved Solids	$19.8\pm0{,}01$	$20.7\pm0{,}01$	$0.155^{*}$	
$\mathbf{D}$ $\mathbf{i}$ $\mathbf{i}$ $\mathbf{i}$ $\mathbf{i}$ $\mathbf{i}$ $\mathbf{i}$ $\mathbf{i}$ $(\mathbf{D}0)$	1.1.1.1.0.1	16 1 010 111		1 7

Description: Control (F0): no stabilizer added; Selected formula: CMC stabilizer (F4). \*) Independent Samples T-*Test* difference is significant at (p < 0.05).

The total dissolved solids show the presence of dissolved substances in a solution. The high total dissolved solids are due to the increase in the amount of free water that is bound. According to Farikha et al. (2013), the more particles are bound by the stabilizer, the more the total soluble solids will increase, and the precipitate will be reduced. Total dissolved solids can be obtained from the breakdown of carbohydrates, protein breakdown, and fat breakdown (Pratama et al., 2021). Carboxymethyl cellulose is a cellulose derivative included in carbohydrates. Carbohydrates are included in the total solid, so adding CMC also increased the total solid of jack bean tempeh milk (F4) (Manurung et al., 2020).

## 3.4.3. Viscosity

Table 8 showed that jack bean tempeh milk (F4) had a viscosity of 13.43 cP, which means it was higher than jack bean tempeh milk (F0) of 9.66 cP. Because of the absence of the addition of CMC that can bind water, the viscosity of jack bean tempeh milk (F0) was lower. This finding was in line with a study conducted by Sanjana et al. (2022), which found that the viscosity of the blended milk (coconut milk and cashew milk) ranged from 9.93 to 13.4 cP. As shown in Table 8, the viscosity of the jack bean tempeh milk was significantly (p < 0.05) affected by the stabilizer that was used.

	Table 8. The	viscosity of jack bean te	mpeh milk.
Variable Treatment a value			
Variable -	$\frac{1}{\text{FO}(\text{cP})}$	F4 (cP)	p-value
Viscosity	$9.66 \pm 0.11$	$13.43\pm0.25$	$0.000^{*}$

Description: Control (F0): no stabilizer added; Selected formula: CMC stabilizer (F4). \*) Independent Samples T-*Test* difference is significant at (p < 0.05).

According to Anggrahini & Pratama et al. (2017), CMC is hydrophilic and dispersed in water, allowing it to bind water. The structure of CMC is a polysaccharide with a polymer chain composed of cellulose molecular units in the form of linear chains, and it contains many glucose components. By adding CMC, free water molecules were trapped in CMC molecules, increasing the viscosity of jack bean tempeh milk.

In addition, the viscosity of jack bean tempeh milk was also influenced by the amount of total dissolved solids (Table 7). Viscosity has a linear relationship with total dissolved solids. The higher the total value of dissolved solids, the higher the viscosity value (Pratama et al., 2021). When a stabilizer was added to jack bean tempeh milk, the viscosity value increased because the suspended particles, such as water, bind to protein complexes. CMC stabilizer jack bean tempeh milk by forming a CMC-protein complex. The jack bean tempeh milk protein with a positive charge,  $NH_3^+$ , combines with the anionic CMC functional group COO- to create a soluble and stable complex (Sebayang et al., 2019).

#### 3.4.4. Color

Table 9 showed that F4 jack bean tempeh milk had a lightness (L\*) value of 60.61, which means it was higher than F0 jack bean tempeh milk of 59.31. It might be caused by the base color of commercial CMC. The color of commercial CMC was white. As shown in Table 9, the stabilizer used had no significant effect (p > 0.05) on the L\* value of the jack bean tempeh milk. Our results clearly showed that the L\* value of jack bean tempeh milk tended to be slightly dark in color. The L\* value of jack bean tempeh milk was influenced by the addition of Ajwa date puree, which was dark brown. Ajwa dates have a deep black color, so when used as Ajwa date puree, the resulting color tends to be dark brown. Previous research found that the L\* value of probiotic-enriched fermented camel milk with the addition of Sukkari dates of 76.95 (Aljutaily et al., 2022).

Variable	Treatment		
Variable	F0	F4	- p-value
$L^*$	$59.31\pm0.79$	$60.61\pm0.25$	$0.073^{*}$
$a^*$	$-1.16 \pm 5.69$	$-0.12 \pm 1.11$	$0.743^{*}$
$\mathbf{b}^*$	$21.90 \pm 1.11$	$19.56\pm2.06$	$0.092^{*}$

Description: Control (F0): no stabilizer added; Selected formula: CMC stabilizer (F4). \*) *Independent Samples T*-*Test* difference is significant at (p < 0.05).

Jack bean tempeh milk (F4) had a\* (redness) value of -0.12, while jack bean tempeh milk F0 had a\* value of -1.16. As shown in Table 9, the stabilizer unaffected a\* value of the jack bean tempeh milk (p > 0.05). According to Khalid et al. (2020), Ajwa dates are deep black, and some are close to red. F4 jack bean tempeh milk had a yellowness (b\*) value of 19.56, while F0 jack bean tempeh milk had a b\* value of 21.90. As shown in Table 9, the b\* value of the jack bean tempeh milk was not significantly affected by the stabilizer (p > 0.05). The Maillard reaction, which occurs during jack bean tempeh milk preparation, produces melanoidin or brown pigment. Melanoidin led to the color of jack bean tempeh milk changing to brown (Anggrahini & Pratama, 2017). Previous research found that the b\* value of probiotic-enriched fermented camel milk with the addition of Sukkari dates of 26.24 (Aljutaily et al., 2022).

#### 4. CONCLUSIONS

Four stabilizers used, namely iota carrageenan, gum Arabic, pectin, and CMC with a concentration of 0.1% had no significant effect on the organoleptic color, texture, and taste of the jack bean tempeh milk. Based on the hedonic quality test results, the panelist preferred F4 jack bean tempeh milk with the addition of a CMC stabilizer. The selected jack bean tempeh milk had a light brown color, a slight aroma of dates, a thin texture, and a slight sweetness. The

chemical characteristics of the selected jack bean tempeh milk were protein content of 0.65%, fat content of 2.48%, and antioxidant activity (IC50) of 7760 ppm. In contrast, the physical characteristics of the selected jack bean tempeh milk were the pH value of 5.8, total soluble solids of 20.7°Brix, viscosity of 13.43 cP, L\* value of 60.61, a\* value of -0.12, and b\* value of 19.56.

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