

## Hydrocolloid-Based Stabilization of Parijoto Ready-to-Drink Beverages and Its Effect on Antioxidant Properties

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### ABSTRACT

*Parijoto fruit contains bioactive components that are beneficial for health, but it has a short shelf life. This study developed a ready-to-drink parijoto fruit drink as an alternative to diversify processed parijoto and increase shelf life. Ready-to-drink beverages are drinks that are made with stabilizing ingredients to ensure product consistency. The study aimed to determine the effect of adding various types and concentrations of stabilizing ingredients on the antioxidant characteristics of ready-to-drink parijoto fruits. The types of stabilizers used were CMC, pectin, and CMC-pectin mixtures (3:1) with stabilizer concentrations of 0.25%, 0.5%, and 0.75%. The ready-to-drink antioxidant characteristics of parijoto fruit were evaluated by measuring of vitamin C levels, total phenols, and DPPH (2,2-diphenyl-1-picrylhydrazyl) activity. The results demonstrated that the vitamin C content across the different stabilizer types ranged from 47.40 to 50.84 mg/100g. Variations in the stabilizers did not affect vitamin C levels. Stabilizer concentrations of 0.5% and 0.75% yielded significantly higher vitamin C levels (52.80 and 53.29 mg/100 g, respectively) compared to the 0.25% concentration (40.58 mg/100 mL). Total phenols range from 7.63 to 10.35 mg GAE/g. Using pectin as a stabilizer, the highest phenol levels (10.35 mg GAE/g) were obtained. The highest Radical Scavenging Activity (RSA) of DPPH (70.89%) was obtained using 0.5% pectin. The results show that formulated drinks are rich in antioxidant agents.*

**Keywords:** Antioxidant; DPPH; Phenol, Parijoto, Vitamin C

### INTRODUCTION

Parijoto fruit (*Medinilla speciosa*) contains antioxidant compounds, including phenols, flavonoids (Damayanti et al., 2023), and tannins (Artanti et al., 2022). In addition to functioning as an antidote to free radicals, antioxidants contribute to body health, especially in preventing and combating degenerative diseases (Hasbullah et al., 2018). Antioxidants can inhibit, delay, and prevent the oxidation of fats and other easily oxidized compounds (Pratiwi et al., 2023). Antioxidants are bioactive compounds that play a crucial role in preventing oxidative damage. Due to their generally lower toxicity compared to synthetic counterparts, natural antioxidants have attracted increasing scientific interest as potential alternatives (Kumalaningrum & Lestari, 2024). The body urgently needs antioxidants to cope with and prevent oxidative stress. Vitamin C can inhibit oxidative reactions in macromolecules such as lipids, DNA, and proteins (Lung and Destiani, 2017). Additionally, phenol compounds can act as antioxidants by reducing free radicals. The free radical reduction ability of phenol compounds depends on their hydroxy group.

Unfortunately, the shelf life of parijoto fruit is relatively short, lasting only 3-4 days after harvesting, which limits its direct consumption and marketability. One of the alternative uses of parijoto is indeed its development into a functional RTD beverage, which not only preserves the fruit's bioactive compounds, such as vitamin C and phenolic antioxidants, but also meets consumer demand for convenient, health-oriented drinks. Fruit drinks are typically soft drinks composed of fruit juice diluted with water, and may include sugar and other approved food additives to improve taste, stability, and nutritional value (SNI, 1995). Ready-to-drink (RTD) formulations refer to beverages that are packaged and marketable in a form suitable for

immediate consumption without further preparation (Nuraini, 2021). The stability of the juice mixture with water and additives is one of the criteria that determines the quality of the juice. One of the alternative uses of parijoto is indeed its development into a functional RTD beverage, which not only preserves the fruit's bioactive compounds, such as vitamin C and phenolic antioxidants, but also meets consumer demand for convenient, health-oriented drinks. However, the stability of the mixture, including the uniform dispersion of juice with water and additives, remains a key quality determinant for such products.

Ready-to-drink fruit juices are often hampered by the formation of sediment during storage. The sediments consist of soluble solid particles that are not fully suspended in the liquid medium. To prevent sedimentation and ensure a homogeneous distribution of solid particles throughout the beverage, stabilizing agents are commonly added during processing. These stabilizers help maintain the uniform dispersion of particles and prevent their aggregation over time (Sakinah et al., 2021; Masna et al., 2022). Stabilizing agents enhance the texture and viscosity of food products by promoting interaction between food components and water molecules, thereby improving product consistency and stability (Sutrisno et al., 2019; Gao et al., 2024). Commonly used food-grade stabilizers in the beverage industry include carboxymethyl cellulose (CMC), xanthan gum, gum arabic, and pectin.

Carboxymethyl cellulose (CMC) offers several functional advantages as a food stabilizer, including being colorless, odorless, highly soluble in both hot and cold water, and more economically viable compared to other common hydrocolloids, such as carrageenan and xanthan gum (Rahmawati, 2017). Similarly, pectin has been widely recognized for its gelling ability in beverage formulations, where its concentration markedly influences the physicochemical properties of citrus-based drinks, particularly affecting viscosity, turbidity, and stability (Cahyanto, 2017). Moreover, Agustina et al. (2019) demonstrated that increasing the concentration of stabilizing agents beyond 1% significantly enhances the viscosity and induces the formation of gel-like textures in liquid food systems. Ikarini et al. (2020) also revealed that both the type (CMC, xanthan gum, gum arabic) and concentration (ranging from 0.025% to 0.1%) of stabilizers substantially affect key quality parameters such as vitamin C retention, total soluble solids, viscosity, visual appearance, and sensory properties, including mouthfeel in tangerine-based beverages. The present study explores the novel effects of stabilizer type (CMC, pectin, and their 3:1 combination) and concentration levels (0.25%, 0.5%, and 0.75%) on the antioxidant potential and overall quality of ready-to-drink parijoto (*Medinilla speciosa*) fruit beverages, offering new perspectives for the development of functional drink formulations with enhanced health benefits.

## **METHODS**

### ***Material***

The primary materials used in this study included parijoto fruit sourced from Banyumas, granulated sugar (Gulaku), carboxymethyl cellulose (CMC), pectin, and citric acid. Reagents for chemical analyses comprised distilled water (aqua dest), anhydrous glucose (Merck), Nelson reagent A and B (Merck), arsenomolybdate solution (Merck), and sodium hydroxide (NaOH, Merck).

### ***Tools***

The main equipment employed included an analytical balance (Sartorius TE 150 2S), pH meter, hand refractometer, Lovibond Brookfield viscometer, UV-Vis spectrophotometer (Hitachi U-2001), vortex mixer, general-purpose mixer, and standard laboratory glassware. used include

analytical scales (Sartorius TE 150 2S), pH meter, hand refractometers, Lovibond Brookfield viscometers, spectrophotometers (UV-Vis Hitachi U-2001), vortexes, mixers, and glassware.

### **Research Design**

The experiment was designed as a completely randomized factorial design consisting of two factors with three replications. The first factor was the type of stabilizing agent at three levels: carboxymethyl cellulose (CMC), pectin, and a 3:1 mixture of CMC and pectin. The second factor was the concentration of the stabilizing agent at three levels: 0.25%, 0.5%, and 0.75%. The parameters measured included vitamin C content, determined by the titration method described by Jacobs (1973); total phenolic content, assessed using the Folin-Ciocalteu method (Andriani & Murtisiwi, 2018); and antioxidant activity, evaluated by the DPPH radical scavenging assay (Huda & Widyaningsih, 2015).

### **Ready-To-Drink Parijoto Beverages Preparation**

The preparation of ready-to-drink parijoto fruit as beverages began with raw material selection and extraction. For each 250 mL portion of the final product (1 unit of treatment), 25 g of fresh parijoto fruit was utilized. The fruits were thoroughly washed and subjected to steam blanching for 3 minutes to inactivate enzymatic activity. Following blanching, the fruits were homogenized using a blender with the addition of 125 mL of distilled water, maintaining a fruit-to-water ratio of 1:5 (w/v). The homogenization process was conducted for 2 minutes to achieve a uniform puree. The resulting mixture was then filtered through a muslin cloth to separate the juice, yielding parijoto fruit extract. A stabilizer solution was prepared separately by dissolving the designated stabilizer in 50 mL of hot water. The stabilizers tested included carboxymethyl cellulose (CMC), pectin, and a CMC–pectin blend in a 3:1 ratio. These were incorporated at concentrations of 0.25% (0.625 g), 0.5% (1.25 g), and 0.75% (1.875 g) w/v.

To formulate the ready-to-drink beverage, 125 mL of distilled water was heated, followed by the addition of 37.5 g of granulated sugar (15%). After complete dissolution of the sugar, the pre-prepared stabilizer solution was incorporated with continuous stirring to ensure homogeneity. After complete dissolution of the sugar, the pre-prepared stabilizer solution was incorporated with continuous stirring to ensure homogeneity. Subsequently, 125 mL of parijoto juice extract was added to the mixture, and then the mixture was heated to boiling. Upon reaching the boiling point, the heating intensity was reduced, and the stabilizer was once again added to the beverage mixture to enhance consistency and stability. Stirring continued until all components were fully homogenized, after which the heating process was terminated.

### **Test Parameters:**

#### **1. Vitamin C Iodine titration (Jacobs, 1973)**

The vitamin C (ascorbic acid) content was determined using the iodine titration method. A 10 g sample of slurry was accurately weighed and transferred into a 100 ml volumetric flask, followed by dilution to volume with aquadest. The mixture was thoroughly homogenized. An aliquot of 5-25 mL of the filtrate was pipetted into a 125 mL erlenmeyer flask. Subsequently, 2 mL of 1% amylum solution (serving as the indicator) was added to the ingredient. The sample was titrated against a standardized 0.01 N iodine solution until a persistent blue endpoint appeared, indicating the completion of the reaction between the iodine and ascorbic acid. The vitamin C concentration (expressed as mg of ascorbic acid per 100 g of sample) was calculated using the following equation:

$$\text{Vitamin C (mg/100g)} = \frac{\text{mL of iod} \times 0.88 \times \text{dilution factor}}{\text{sample weigh}} \times 100 \quad (1)$$

## 2. Total phenol (Andriani and Murtisiwi, 2018)

### a. Gallic acid standard curve creation

To create a phenol standard curve, a gallic acid stock solution was made by dissolving 50 mg of gallic acid with 0.5 mL of ethanol and diluted with aquadest up to 100 mL. Then, a concentration series of 0, 10, 20, 30, 40, and 50 ppm of a pre-made gallic acid stock solution was made. Each dilution series was taken 0.3 mL, added with 1.5 mL of Folin-Ciocalteu 10%, vortexed until homogeneous, and then incubated in a dark room for 3 minutes. After that, 1.2 mL of 7.5% Na<sub>2</sub>CO<sub>3</sub> solution was added and then vortexed until homogeneous and re-incubated in a dark room for 60 minutes. The absorption value was measured at a wavelength of 765 nm.

### b. Total phenol analysis in samples

1g of the sample was dissolved in 100 mL of aquadest. A sample solution was taken, 0.3 mL, and then 1.5 mL of 10% Folin reagent was added, further vortexed in a 30-second vortex. The solution is incubated for 8 minutes in a closed (dark) room. Na<sub>2</sub>CO<sub>3</sub> 7.5% as much as 1.2 mL is added to the solution, dissolved for 1 minute, followed by incubation for 1 hour in a closed (dark) room. The sample solution was measured for absorbance at a wavelength of 765 nm. The sample solution was measured for absorbance at a wavelength of 765 nm.

$$\text{Total Phenol (mg GAE/g)} = \frac{\text{absorbance} \times \text{dilution factor}}{\text{sample weigh}} \quad (2)$$

## 3. Antioxidant activity of DPPH (Huda and Widyaningsih, 2015)

### a. Preparation of DPPH reagents

0.00393 g of DPPH powder was weighed and dissolved in 100 mL of methanol. After the DPPH powder was dissolved with methanol, the tip of the measuring flask was closed using aluminum foil, then whisked so that the DPPH powder and methanol dissolved. The solution was then stored in the refrigerator by covering all surfaces using aluminum foil.

### b. Antioxidant testing (Inhibition)

0.4 g of samples was weighed, dissolved in 10 mL of methanol, and vortexed. Next, a sample of 0.2 mL and a DPPH solution of 2.8 mL were taken and then vortexed and incubated for 30 minutes in a dark place. The absorbance of the extract was read on a UV-Vis spectrophotometer with a wavelength of 517 nm. Then the % inhibition calculation was carried out

$$\% \text{ Inhibition} = 100\% \times \frac{\text{blank absorbance} - \text{sample absorbance}}{\text{blank absorbance}} \quad (3)$$

## Data Analysis

The data were analyzed using analysis of variance (ANOVA), and if significant differences were found between treatments, the Least Significant Difference Test (LSDT) was performed at the 5% level.

## RESULT AND DISCUSSIONS

### 1. Vitamin C

The result of this study demonstrated that the interaction between stabilizer type and concentration significantly influences the vitamin C content of the ready-to-drink parijoto. Among all treatments, the addition of 0.25% CMC resulted in the highest vitamin C (Figure 1), while other stabilizer types and concentrations did not exhibit statistically significant differences in ascorbic acid levels. The highest vitamin C level observed with 0.25% CMC may be attributed to

its ability to enhance the colloidal stability of the beverage by reducing particle aggregation and limiting oxygen diffusion, thereby slowing down oxidative degradation processes (Rahman et al., 2021; Staubmann et al., 2023). Vitamin C functions as a potent antioxidant by donating electrons to neutralize reactive oxygen species, forming ascorbyl radicals that are substantially less reactive compared to other free radicals (Zheng et al., 2024). CMC acts as a barrier against oxygen diffusion, reducing the rate of free radical formation that could degrade vitamin C (Sahraee et al., 2019). In addition, the relatively low concentration (0.25%) might have maintained a balance between matrix viscosity and molecular mobility, preventing excessive entrapment of oxygen within the beverage system. In contrast, higher CMC concentrations (0.5% and 0.75%) and the use of pectin or CMC–pectin mixtures appeared less effective in retaining vitamin C. It is possible that increasing hydrocolloid concentration results in excessive matrix thickening, leading to microenvironments where oxygen may become trapped, thereby promoting localized oxidation reactions despite the general barrier effect. Hydrocolloid prevent vitamin C against oxydation (Mousa, 2020). Maintaining higher levels of vitamin C not only reflects reduced oxidation within the beverage matrix but also contributes to the antioxidant capacity and functional quality of the final product.

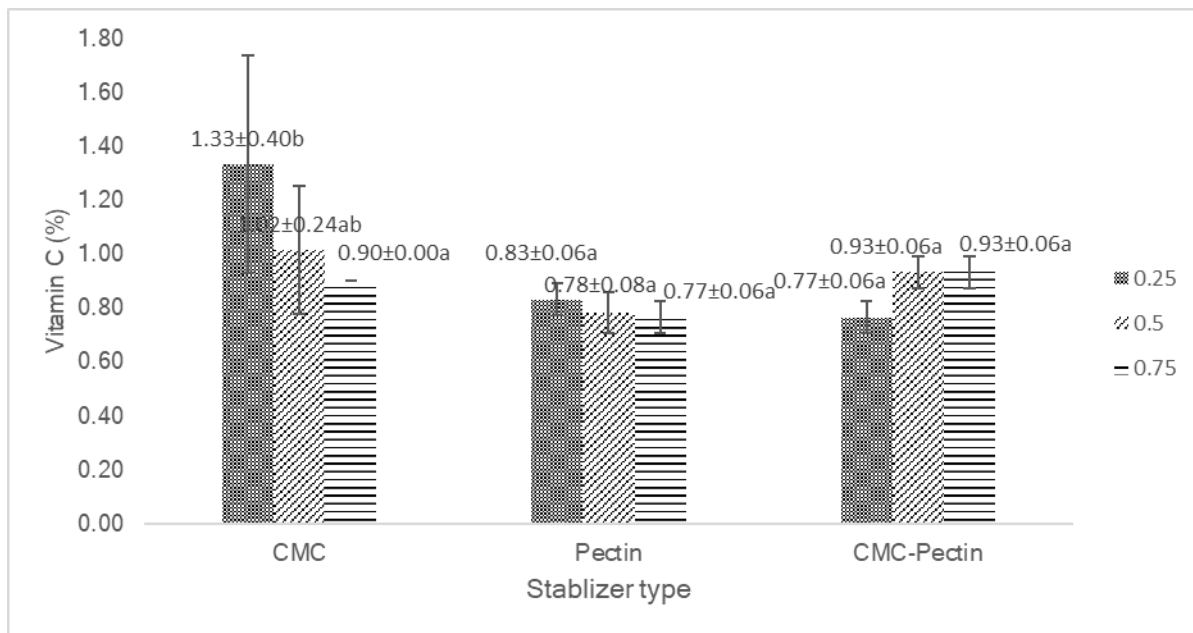


Figure 1. The vitamin C level in the ready-to-drink parijoto beverage varied with different stabilizing agent concentrations.

## 2. Phenol

Phenol compounds represent the largest class of secondary metabolites naturally present in plants (Zagoskina et al., 2023). These compounds are characterized by one or more aromatic rings bearing hydroxyl (-OH) groups, which are responsible for their biological activity. As antioxidants, phenols donate hydrogen atoms from their hydroxyl groups to neutralize free radicals, thereby stabilizing these reactive species and preventing the propagation of oxidative chain reactions (Gonzalez & Idaboy, 2023; Dewi & Sari, 2024). In the present study, the interaction between the type and concentration of stabilizers significantly influenced the total phenolic content of the ready-to-drink parijoto (Figure 2). This indicates that hydrocolloid stabilizers not only affect the physical properties of the beverage but also modulate the retention and stability of phenolic compounds.

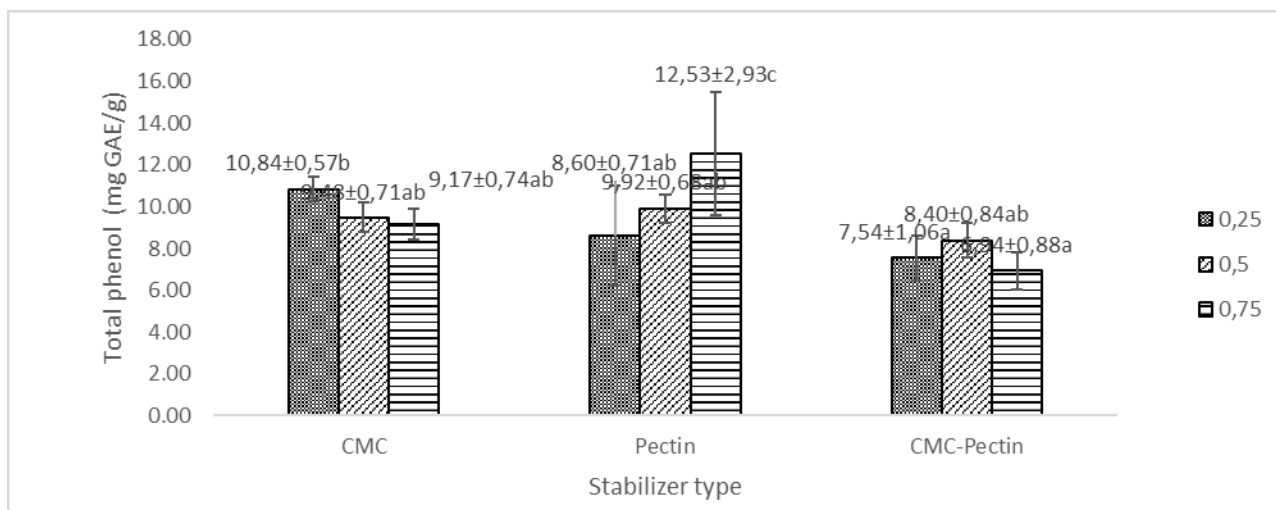


Figure 2. The total phenol level in the ready-to-drink parijoto beverage varied with different stabilizing agent concentrations.

Pectin at a concentration of 0.75% resulted in the highest total phenolic content compared to other stabilizer types and concentrations, indicating its superior capability in preserving or even enhancing the extraction and retention of phenolic compounds. This phenomenon may be attributed to the unique gelling and film-forming properties of pectin, which can create a protective network around phenolic molecules, reducing their exposure to environmental factors such as oxygen, light, and heat that can lead to phenolic degradation. Moreover, pectin's molecular structure, rich in carboxyl and hydroxyl groups, allows for potential interactions such as hydrogen bonding or hydrophobic associations with phenolic compounds, thereby enhancing their stability within the beverage matrix (Buyuk & Yemenicioglu, 2024; Shi et al., 2024). The concentration of pectin also plays a critical role in this stabilization process. At 0.75%, pectin may form an adequately dense matrix capable of entrapping phenolic compounds more effectively than lower concentrations. However, excessive matrix densification was not observed to impair phenolic availability in this case, possibly due to the moderate viscosity levels that still permit sufficient molecular mobility and diffusion of phenolic compounds within the system.

### 3. Free radical scavenging activities of DPPH

Antioxidant activity is described through the free radical binding percentage of DPPH. The DPPH method is an assay used to evaluate antioxidant activity based on the ability of compounds to donate electrons to neutralize free radicals (Rosida et al., 2023). Radical DPPH will receive hydrogen atoms from antioxidant compounds to obtain electron pairs, inhibiting oxidation reactions (Aryanti et al., 2021). The interaction of stabilizer type and concentration significantly affected the free radical scavenging activities of DPPH (Figure 3)

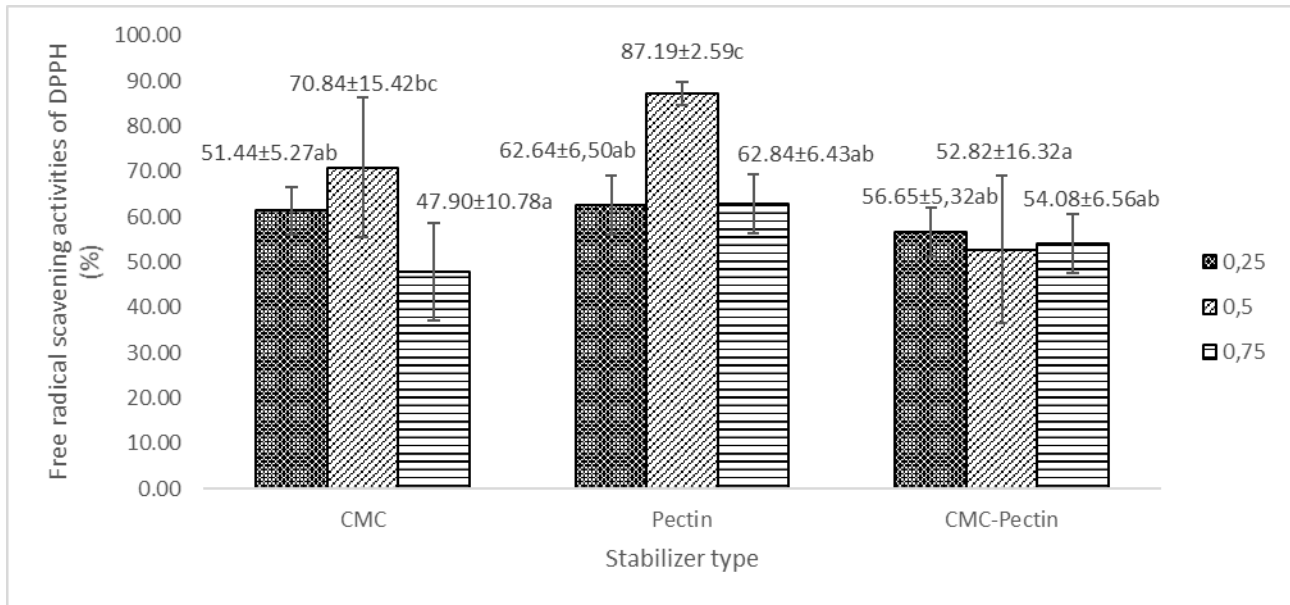


Figure 3. DPPH free radical scavenging activity level in the ready-to-drink parijoto varied with the types and concentrations of stabilizing agents.

The results of this study demonstrated that the type and concentration of hydrocolloid stabilizers significantly influenced the free radical scavenging activity of the ready-to-drink *parijoto*. The highest antioxidant activity, measured by the DPPH radical scavenging assay, was observed in the formulation containing 0.5% pectin, which was not significantly different from the formulation containing 0.5% CMC. In contrast, other treatments exhibited lower radical scavenging activities. The superior radical scavenging activity observed with 0.5% pectin could be attributed to its ability to enhance the solubility, dispersion, and stabilization of phenolic compounds and other antioxidants within the beverage matrix. Pectin is known to form a three-dimensional network that not only improves the physical stability of food products but also encapsulates and enhances the functional properties of bioactive compounds and binds heavy metal (Roman-Benn et al., 2023), effectively scavenging all three free radical species, thereby protecting them from oxidative degradation (Yan et al., 2025). Additionally, the moderate concentration of 0.5% may provide an optimal matrix density that facilitates the release and availability of antioxidant compounds without excessively hindering their diffusion and reactivity with free radicals. Similarly, CMC at 0.5% concentration demonstrated comparable antioxidant activity. CMC is recognized for its ability to improve the dispersion of hydrophilic components such as antioxidant molecules (Rahman et al., 2021) and to reduce oxygen permeability (Sabo et al., 2024), thereby preserving the activity of radical-scavenging compounds such as vitamin C and phenolic constituents. The comparable performance of 0.5% CMC and pectin suggests that both hydrocolloids at this concentration effectively create a protective environment that supports the stability and bioactivity of antioxidant compounds in the beverage. Treatments with higher or lower concentrations, or with different hydrocolloid types, yielded reduced antioxidant activity, potentially due to the formation of overly dense or insufficiently structured matrices that either trap antioxidants or expose them to degradation.

## CONCLUSION

The type and concentration of hydrocolloid stabilizers significantly influenced the retention of bioactive compounds and the antioxidant potential of ready-to-drink parijoto. The highest vitamin C content was observed in the formulation containing 0.25% CMC, indicating its effectiveness in protecting ascorbic acid from oxidative degradation. In contrast, the highest total phenolic content was achieved with 0.75% pectin, suggesting that this concentration and stabilizer type favored the stabilization and retention of phenolic compounds. Furthermore, the greatest free radical scavenging activity, as measured by the DPPH assay, was observed in beverages formulated with 0.5% pectin, followed closely by those formulated with 0.5% CMC. These findings indicated that the selection and optimization of hydrocolloid type and concentration are essential to enhance the functional quality and antioxidant properties of *parijoto*-based beverages. The ability of these formulations to retain high levels of vitamin C, total phenolics, and radical scavenging capacity underscores ready-to-drink *parijoto* as a natural source of antioxidants, offering promising applications in the development of functional drinks with health-promoting benefits.

## ACKNOWLEDGMENT

The study was funded by Universitas Jenderal Soedirman through the Hibah Penerapan Ipteks Program in 2023.

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