Jurnal Teknologi Hasil Peternakan, 6(1): 67-80. Maret 2025
http://journal.unpad.ac.id/jthp/index
ISSN: 2722-4783
DOI: 10.24198/jthp.v6i1.61947

SIFAT ANTIOKSIDAN DAN KARAKTERISTIK FISIK SUSU KAMBING PASTEURISASI DENGAN PENAMBAHAN MADU KELULUT

ANTIOXIDANT PROPERTIES AND PHYSICAL CHARACTERISTICS OF PASTEURIZED GOAT MILK WITH KELULUT HONEY ADDITION

Received : Feb 26th 2025
Accepted : Mar 15th 2025

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Abstract. *This study evaluates the effects of kelulut honey (Heterotrigona itama) on the antioxidant activity and physicochemical properties of pasteurized goat milk. Using a completely randomized design (CRD), five treatments (0%, 5%, 10%, 15%, 20% honey) were analyzed via one-way ANOVA (α = 0.05). Antioxidant activity (DPPH assay), viscosity, pH, and color (L*, a*, b*) were assessed. Results revealed a concentration-dependent increase in antioxidant capacity, peaking at 77.92% with 20% honey, attributed to the honey's flavonoids and phenolics. Viscosity rose proportionally with honey concentration, linked to polysaccharide-water interactions, while pH declined due to organic acids (e.g., gluconic acid). Colorimetry showed elevated a* (redness) and b* (yellowness) values, reflecting honey-derived pigments like melanoidins and carotenoids. These findings position kelulut honey as a multifunctional additive, enhancing both the functional (antioxidant) and sensory (color, texture) profiles of goat milk. The study highlights its potential in developing functional dairy beverages; however, further optimization is required to balance pH fluctuations and ensure consumer acceptability.*

Keywords : *Antioxidant, Kelulut Honey, Pasteurized Goat Milk, Physical Properties*

Sitasi :
Sulistyowati, A. N., Wibowo, A., Nurmasytah, A., Suhardi, & Ismanto, A. (2025). Antioxidant Properties and Physical Characteristics of Pasteurized Goat Milk with Kelulut Honey Addition. *Jurnal Teknologi Hasil Peternakan*, 6(1): 67-80.

INTRODUCTION

Milk is universally recognized as a vital source of nutrition, providing essential compounds such as proteins, fats, vitamins, and minerals that are critical for human health and development (Sulmiyati & Najma 2016). Among the various types of milk, goat milk has gained increasing attention as a nutritious alternative to cow milk due to its superior digestibility and lower allergenicity. These properties are attributed to its unique composition, including smaller fat globules, a higher proportion of medium chain triglycerides (MCTs), and a lower concentration of αs1-casein, a major allergenic protein found in cow milk (Pradini *et al.*, 2021). As a result, goat milk is particularly suitable for individuals with lactose intolerance or cow milk protein allergies, making it a valuable component of functional food development.

In recent years, the demand for functional foods products that provide health benefits beyond basic nutrition has grown significantly. Functional foods often incorporate natural additives with bioactive properties, such as antibacterial and antioxidant activities, to enhance their health promoting potential (Maryana *et al.*, 2022). Among these natural additives, honey, particularly Kelulut honey derived from stingless bees (genus Trigona), has emerged as a promising ingredient due to its rich content of bioactive compounds. Kelulut honey contains phenolic acids, flavonoids, and other phytochemicals that contribute to its potent antioxidant, anti inflammatory, and antibacterial properties (Fitriani *et al.*, 2023; Rahmiati *et al.*, 2023). The composition of kelulut honey such as sugar contents, acidity, antioxidants, water content, minerals and Hydroxymethylfurfural (HMF) play an important role in determining the quality of kelulut honey (Jamzuri *et al.*, 2023). These characteristics make Kelulut honey an ideal candidate for incorporation into functional dairy products.

The integration of natural ingredients into dairy processing has been explo5red in various studies, demonstrating the potential to enhance both the functional and sensory properties of milk based products. For instance, matoa leaves (*Pometia pinnata*) and lontar fruit (*Borassus flabellifer*) have been successfully incorporated into dairy formulations, improving their antioxidant activity and overall quality (Triana *et al.,* 2023; Maruddin *et al.*, 2023). Building on these findings, the combination of goat milk and Kelulut honey presents a unique opportunity to develop a functional dairy product with enhanced nutritional and health benefits. Goat milk provides a rich source of essential nutrients, while Kelulut honey contributes bioactive compounds that may help reduce oxidative stress, support immune function, and inhibit the growth of pathogenic bacteria. Oxidative stress, caused by an imbalance between free radicals and antioxidants in the body, is associated with various chronic diseases, including cardiovascular disorders, cancer, and neurodegenerative conditions (Rahmiati *et al.*, 2023). Antioxidants play a crucial role in neutralizing free radicals and mitigating oxidative damage, highlighting the importance of incorporating antioxidant rich ingredients into the diet. Kelulut honey, with its high antioxidant capacity, offers a natural solution to this challenge. Furthermore, its antibacterial properties, attributed to factors such as low pH, high osmolarity, and the presence of hydrogen peroxide, make it an effective natural preservative, extending the shelf life of dairy products while maintaining their safety and quality (Maryana *et al.*, 2022).

Despite the growing interest in functional foods, there is limited research on the combination of goat milk and Kelulut honey, particularly in terms of their synergistic effects on antioxidant activity and physical characteristics. However, there is one example of a yoghurt product with the addition of local honey (Hardiansyah & Kusuma, 2022). Understanding these effects is essential for optimizing the formulation of functional dairy products and ensuring their acceptability among consumers. Therefore, this study aims to investigate the antioxidant activity and physical properties of pasteurized goat milk enriched with Kelulut honey. By evaluating parameters such as free radical scavenging capacity, viscosity, color, and pH, this research seeks to provide valuable insights into the potential of Kelulut honey as a natural additive for enhancing the functional and sensory qualities of goat milk.

MATERIALS AND METHODS

1. Materials

This study was conducted from January to February 2025. The experimental procedures were carried out across multiple laboratories at Mulawarman University. Specifically, the color analysis was performed at the Animal Production and Technology Laboratory, viscosity measurements were conducted at the Chemistry and Biochemistry Laboratory, and the assessment of antioxidant activity was carried out at the Post-Harvest and Packaging of Agricultural Products Laboratory. The primary materials utilized in this research included fresh goat milk and Kelulut honey. A total of 5 liters of fresh goat milk was procured from a supplier located on Jalan Merdeka, Lempake, North Samarinda. Kelulut honey was obtained from a local farmer in Bangun Rejo village. Additional materials required for testing the physical characteristics and antioxidant activity included distilled water, pH 7 and pH 4 buffer solutions, sulfuric acid (H_2SO_4), 2,2-diphenyl-1-picrylhydrazyl (DPPH) solvent, ethanol, and DPPH powder. The equipment employed in this study consisted of a Hunter Lab ColorFlex EZ spectrophotometer equipped with a CIE Lab* color meter for color analysis, a UV-Vis spectrophotometer for antioxidant activity measurements, a pH meter for determining acidity levels, and an NDJ 8S viscometer for viscosity analysis. These instruments were selected to ensure accurate and reliable measurements of the physical and chemical properties of the samples.

2. Methods

2.1 Sample preparation

This study employed a completely randomized design (CRD) with five experimental treatments and four replications. The treatments included: P0 (pasteurized goat milk without Kelulut honey, serving as the control), P1 (pasteurized goat milk with 5% Kelulut honey), P2 (pasteurized goat milk with 10% Kelulut honey), P3 (pasteurized goat milk with 15% Kelulut honey), and P4 (pasteurized goat milk with 20% Kelulut honey). The Kelulut honey (*Heterotrigona itama*) used in this research was sourced from the Kelulut Bee Farm located in RT 07, Bangun Rejo Village, Tenggarong Seberang District, Kutai Kartanegara Regency, East Kalimantan. The honey was harvested using a suction device connected to a storage container to ensure its purity and quality. The pasteurization process involved heating the goat milk to a temperature of 75°C while continuously stirring to ensure uniform heat distribution. This temperature was maintained for 15 seconds, as recommended by Park *et al.* (2007), to achieve effective microbial inactivation while preserving the nutritional quality of the milk. Following pasteurization, Kelulut honey was added to the goat milk according to the specified treatment concentrations. The mixtures were then subjected to analyses of antioxidant activity and physical characteristics, following the methodology outlined by Maruddin *et al.* (2023).

2.2 Antioxidant Activity

The antioxidant activity of the pasteurized goat milk enriched with Kelulut honey was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method, as described by Widianingsih (2016). Briefly, 1 mL of each sample was mixed with 1 mL of 0.1 mM DPPH solution and incubated at 37°C for 30 minutes. Vitamin C was used as the positive control, while methanol and DPPH solution served as the negative control and blank, respectively. The absorbance of the mixtures was measured at a wavelength of 517 nm using a UV-Vis spectrophotometer. The percentage of DPPH radical scavenging activity, indicative of antioxidant capacity, was calculated using the following formula:

$$\text{Inhibition (\%)} = \frac{(A517\ Control - A517\ Extract)}{A517\ Control}\ x\ 100\%$$

2.3 pH Measurement

The pH of the samples was determined using a calibrated digital pH meter. Before analysis, the instrument was calibrated using standard buffer solutions at pH 4 and pH 7. For each measurement, 30 mL of pasteurized goat milk enriched with Kelulut honey was transferred into a beaker, and the pH electrode was immersed into the sample until the reading stabilized. Between measurements, the electrode was thoroughly rinsed with distilled water and gently dried with laboratory-grade tissue to prevent cross contamination (Maruddin *et al.*, 2023).

2.4 Viscosity Analysis

Viscosity was measured using a rotational viscometer (NDJ 8S). The viscometer spindle was submerged into a beaker containing the sample until the liquid reached the designated immersion level. Measurements were conducted at a rotational speed of 60 rpm for 1 minute, and viscosity values were recorded in millipascal seconds (mPa·s) (Permadi *et al.,* 2021).

2.5 Color Evaluation

Color parameters were assessed using a Hunter Lab ColorFlex EZ spectrophotometer based on the CIE Lab* color space system. The device was calibrated using black and white reference standards before analysis. Each milk sample was placed under the spectrophotometer's aperture, and the following parameters were measured: L (lightness, ranging from 0 [black] to 100 [white]), a (red-green axis, from -80 [green] to +80 [red]), and b (yellow-blue axis, from -70 [blue] to +70 [yellow]) (Widodo *et al.*, 2015).

3. Statistical Analysis

All experimental data were analyzed using one-way Analysis of Variance (ANOVA) at a 5% significance level ($\alpha = 0.05$). In cases where ANOVA indicated significant differences among treatments, Duncan's Multiple Range Test (DMRT) was applied post hoc to identify statistically distinct treatment groups at the same significance level ($\alpha = 0.05$). This approach ensured robust comparisons of the effects of varying Kelulut honey concentrations on the measured parameters.

RESULTS AND DISCUSSION

1. pH Value

The pH of a substance serves as a critical indicator of its acidity, reflecting the equilibrium between hydrogen (H^+) and hydroxide (OH^-) ions in a solution (Putri & Anggraini, 2021). In dairy systems, pH variations significantly influence physicochemical properties. Elevated pH levels can enhance milk viscosity through the disintegration of casein micelles, while abrupt pH reductions may induce protein aggregation, further altering viscosity. These effects are compounded by homogenization processes, which modulate the structural stability of milk components (Candelas *et al.,* 2022). Notably, in goat milk with a baseline pH of 6.9, the surface hydrophobicity of milk proteins reaches its peak at 85°C, highlighting the temperature dependent nature of protein interactions (Li *et al.*, 2020). The pH of pasteurized goat milk supplemented with Kelulut honey exhibited a progressive decline across treatments, ranging from 6.14 (control, P0) to 4.71 (20% honey, P4). Statistical analysis confirmed significant differences ($P<0.05$) among treatments, with higher honey concentrations correlating strongly with reduced pH. This trend aligns with the inherent acidity of Kelulut honey (pH ≈ 3.07) (Nurlaila *et al.*, 2022), which introduces hydrogen ions into the milk matrix. The inverse relationship between honey concentration and pH is further attributed to enhanced lactic acid bacterial activity, as honey's fermentable sugars serve as substrates for microbial metabolism.

This metabolic activity generates organic acids, amplifying H^+ ion concentration and driving pH below the isoelectric point of casein (pH≈4.6) (Purbasari *et al.*, 2014). At this threshold, casein proteins lose colloidal stability, leading to aggregation and potential textural modifications. Consistent with honey's low pH and microbial acidogenesis. This pH reduction has dual implications: (1) it enhances antimicrobial efficacy, as many pathogens exhibit diminished viability in acidic environments, and (2) it modifies protein interactions, potentially affecting product stability and sensory attributes.

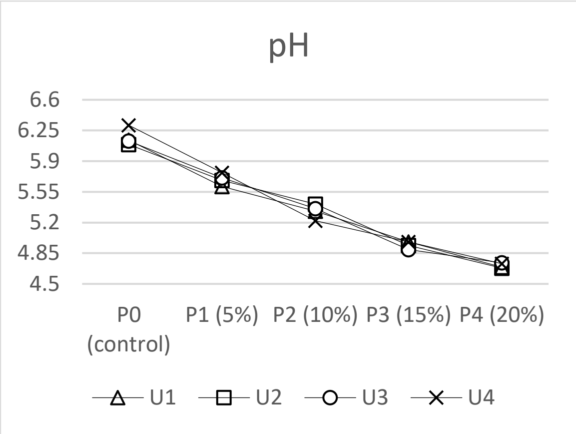

Figure 1. Average pH value of pasteurized goat's milk with the addition of kelulut honey

As illustrated in Figure 1. the pH of pasteurized goat milk supplemented with Kelulut honey exhibited a progressive decline across treatments, ranging from 6.14 (control, P0) to 4.71 (20% honey, P4). Statistical analysis confirmed significant differences ($P < 0.05$) among treatments, with higher honey concentrations correlating strongly with reduced pH. This trend aligns with the inherent acidity of Kelulut honey (pH ≈ 3.07) (Nurlaila *et al.*, 2022), which introduces hydrogen ions into the milk matrix. The inverse relationship between honey concentration and pH is further attributed to enhanced lactic acid bacterial activity, as honey's fermentable sugars serve as substrates for microbial metabolism. This metabolic activity generates organic acids, amplifying H^+ ion concentration and driving pH below the isoelectric point of casein (pH ≈ 4.6) (Purbasari *et al.*, 2014). At this threshold, casein proteins lose colloidal stability, leading to aggregation and potential textural modifications.

The observed pH values (4.71–6.14) deviate from the optimal range for fresh milk (6.3–6.8) (Mutaqin *et al.*, 2021), reflecting the pronounced acidic contribution of Kelulut honey. While the control (P0) remained within the typical pH range for goat milk, treatments P1–P4 transitioned into acidic territory, consistent with honey's low pH and microbial acidogenesis. This pH reduction has dual implications: (1) it enhances antimicrobial efficacy, as many pathogens exhibit diminished viability in acidic environments, and (2) it modifies protein interactions, potentially affecting product stability and sensory attributes. The pH dependent viscosity changes described in the literature where in alkaline conditions disrupt casein micelles and acidic conditions promote aggregation (Candelas *et al.*, 2022).

2. Color Evaluation

2.1 Lightness (L*)

Lightness (L*), a parameter in the CIE Lab* color space, measures material brightness on a scale from 0 (black) to 100 (white), reflecting reflected light intensity (Arifin et al., 2020). In this study, the L* values of pasteurized goat milk supplemented with Kelulut honey ranged from 82.35 to 91.44 (Table 1). Statistical analysis showed a significant decrease in L* values with increasing honey concentration ($P < 0.05$). The control (0% honey) exhibited the highest lightness (91.44), while the 20% honey treatment

Table 1. Average color value of pasteurized goat's milk with the addition of kelulut honey

Treatment	Atribute		
	L*	a*	b*
P0	91.44±0.16[a]	-3,81±0,06[a]	12,69±0,21[a]
P1	90.41±1.20[b]	-1.68±0.33[b]	13.66±0.10[b]
P2	87.41±0.67[c]	-0.96±0.41[b]	13.79±0.65[b]
P3	85.25±1.18[c]	0.70±0.50[c]	15.24±1.55[c]
P4	82.35±0.40[c]	1.16±0.35[c]	15.58±0.64[c]

Description: Mean values in columns followed by different notations indicate significant differences (P<0.05).

resulted in the lowest (82.35). This inverse relationship underscores the impact of Kelulut honey on goat milk's optical properties. The decline in lightness aligns with studies showing that pigmented additives alter dairy reflectance. For example, Evadewi & Tjahjani (2021) reported similar L* reductions in goat milk yogurt with black rice extract, attributed to its dark pigmentation. The dark amber hue and colloidal components (e.g., polyphenols, proteins) of Kelulut honey likely reduced light reflection, lowering L* values. Goat milk's inherent whiteness, influenced by fat globule size, protein dispersion, and lack of pigments, is sensitive to compositional changes (Chudy *et al.*, 2020). Kelulut honey introduces chromophores like melanoidins and flavonoids, which absorb specific light wavelengths, reducing brightness. Increased viscosity in honey enriched milk may also enhance light scattering, further affecting perceived lightness.

The reduction in L* values has implications for product acceptability, as color influences consumer perception. While a darker hue may align with expectations for "natural" or "fortified" products, significant deviations from milk's characteristic white could deter traditional consumers. Future studies should correlate instrumental color measurements with sensory evaluations to assess preferences. In conclusion, Kelulut honey significantly reduces goat milk lightness in a concentration dependent manner, driven by its pigmentation and colloidal constituents.

2.2 Redness (a*)

The redness (a*) value, a parameter in the CIE Lab* color space, quantifies the red (positive values) or green (negative values) intensity in a material, ranging from +80 (red) to -80 (green) (Arifin *et al.*, 2020). In this study, the a* values of pasteurized goat milk supplemented with Kelulut honey ranged from -3.81 to 1.16 (Table 1). Statistical analysis revealed that Kelulut honey significantly influenced the a* values (P < 0.05). The control (0% honey) exhibited the highest green intensity (-3.81), while increasing honey concentrations progressively shifted the color toward red, peaking at

1.16 in the 20% honey treatment. This shift indicates that Kelulut honey imparts a reddish hue to goat milk, altering its chromatic properties.

The observed color transition is attributed to the flavonoid content of Kelulut honey, which ranges from 519.180 to 1,342.593 mg/kg (Jayadi & Susandarini, 2020). Flavonoids, known for their pigmentation and antioxidant properties, contribute to the reddish-brown coloration of honey. As honey concentration increases, these pigments enhance the red intensity of the milk. This aligns with Jaya (2016), who reported increased redness in honey enriched whey kefir, with a peak a* value of 1.20 at 40% honey concentration.

The color shift reflects the interaction between goat milk's inherent whiteness and the chromatic contributions of Kelulut honey. Goat milk, characterized by high reflectance, initially exhibits a slight greenish tint due to shorter wavelength scattering (Getaneh *et al.*, 2020). However, honey introduces chromophores that absorb green light, shifting the perceived color toward red. This aligns with colorimetry principles, where pigmented substances alter spectral reflectance. Additionally, the flavonoid content enhances the product's antioxidant activity, highlighting Kelulut honey's dual role as a functional and aesthetic additive (alkaisy *et al.*, 2023). Thus, Kelulut honey significantly increases the redness of goat milk, enhancing both functional and sensory properties.

2.3 Yellowness (b*)

Yellowness (b*) shows a blue-yellow mixed chromatic color with a positive B value from 0 to +70 for yellow and a negative B value from 0 to -70 for blue (Arifin *et al.*, 2020). Based on statistical analysis that has been done, it is known that the addition of kelulut honey has a significant effect ($P<0.05$) on the yellowish value (b*) of pasteurized goat milk. The results of testing the yellowish value of pasteurized goat milk with the addition of kelulut honey can be seen in Table 1.

The yellowish value of goat milk ranged from 12.69 to 15.58 in P4. Treatment had the highest value among the other values (15.58). The value of Treatment P4 shows pasteurized goat milk with the addition of 20% is yellowish.

The fat pigments dissolved and contained in the milk give the milk a yellowish color (Arifin *et al.*, 2020). The color of carotene varies from yellow to Orange and dark red-orange as the concentration of carotene increases (Hunter *et al.* 2021). The intensity of the color depends on how much pigment is present in the milk.

3. Viscosity

Viscosity refers to the internal resistance of a fluid to flow, representing its thickness or resistance to deformation under shear stress. The viscosity coefficient is a fundamental physical property of fluid materials, serving as a quantitative measure of a substance's viscosity (Mawarni & Subali, 2022). This parameter is critical in characterizing the flow behavior and texture of fluid based products, particularly in food science applications. Higher viscosity values indicate greater resistance to flow, which can influence the sensory properties, stability, and processing requirements of food products. Understanding viscosity is essential for optimizing product formulations, ensuring consistent quality, and meeting consumer expectations in terms of texture and mouthfeel.

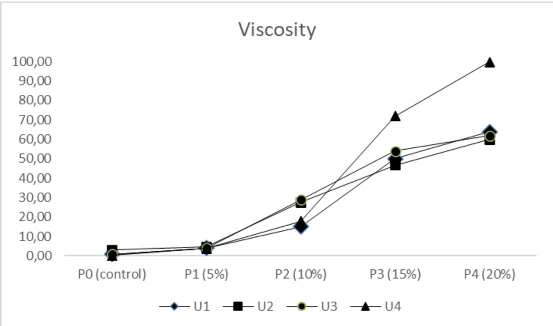

Figure 2. The average value of viscosity of pasteurised goat's milk with the addition of kelulut honey has the highest value in treatment 4 and replicate 4.

Statistical analysis confirmed that Kelulut honey significantly increased the viscosity of pasteurized goat milk ($P < 0.05$, Figure 2). Viscosity ranged from 1.27 mPa·s (control, 0% honey) to 71.43 mPa·s at 20% honey (P4), reflecting a pronounced concentration dependent rheological response. This trend aligns with the hypothesis that honey-derived polysaccharides and high molecular weight compounds, such as fructooligosaccharides, interact with milk proteins (e.g., casein micelles), forming cohesive networks (Divayanti, 2023). The exponential rise in viscosity at 20% honey suggests a critical concentration threshold, where intermolecular interactions dominate consistent with non-linear behavior observed in colloidal systems (Brudzynski, 2017).

Comparatively, Divayanti (2023) reported a smaller viscosity increase (0.95–2.28 cP) in honey-fortified kefir at >5% honey, attributed to honey's hygroscopicity and water binding capacity. The substantially higher viscosity here underscores the influence of the base matrix: goat milk's smaller fat globules and higher β-casein content may amplify interactions with honey components (Park *et al.*, 2007). These findings highlight the dual role of additive concentration and matrix composition in modulating rheological properties. The results suggest Kelulut honey's potential as a functional ingredient to enhance texture in dairy products, though extreme viscosity shifts may require sensory optimization for consumer acceptance.

4. Antioxidant Activity

Antioxidants neutralize free radicals via electron/hydrogen donation, inhibiting oxidative degradation (Ibroham *et al.*, 2022). Kelulut honey enhances dairy matrices by stabilizing reactive species and chelating pro-oxidant metals through its phenolic-flavonoid constituents, as evidenced by elevated DPPH inhibition rates. This dual mechanism underscores structural stability and antioxidative resilience (Huang & Prior, 2005).

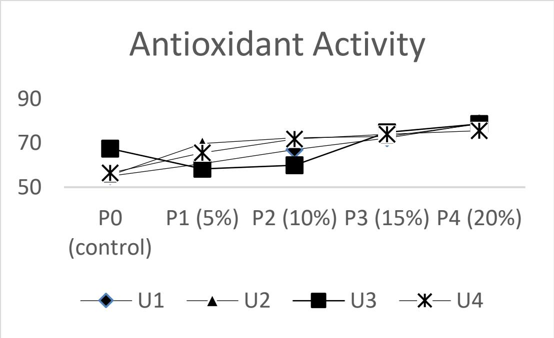

Figure 3. Average antioxidant activity value of pasteurized goat's milk with the addition of kelulut honey

Significantly enhanced antioxidant activity (P < 0.05) was observed in pasteurized goat milk supplemented with kelulut honey, with DPPH radical scavenging activity ranging from 58.56% to 77.92% (Figure 3). The 20% honey treatment (P4) exhibited the highest inhibition (77.92%), reflecting a concentration dependent trend linked to honey derived polyphenols and flavonoids, which neutralize free radicals via electron/hydrogen donation (Ibroham *et al.*, 2022). This efficacy surpasses conventional honey varieties: Chayati & Miladiyah (2014) reported 11.9%–48% DPPH inhibition, while Malaysian Tualang honey showed 41.3% activity (Mohamed *et al.*, 2010). Kelulut honey's superior performance likely arises from its unique phytochemical profile, characterized by elevated gallic acid, quercetin, and hydroxylated phenolics with potent radical scavenging capacity (Khalil *et al.*, 2012).

The antioxidative mechanism involves synergistic interactions among flavonoids, phenolic acids, vitamin E, and ascorbic acid. Flavonoids chelate pro-oxidant metal ions and inhibit lipid peroxidation, while phenolic acids stabilize radicals via proton donation (Bradbear, 2009; Hardiansyah & Kusuma, 2022). This multi-compound synergy disrupts oxidative chain reactions, enhancing the system's antioxidative resilience. The results highlight kelulut honey's potential as a functional additive to fortify dairy products, leveraging its bioactive constituents to mitigate oxidative degradation.

CONCLUSION

This study demonstrates that kelulut honey (*Heterotrigona itama*) significantly enhances the antioxidant capacity and physicochemical properties of pasteurized goat milk in a concentration dependent manner. Antioxidant activity increased from 58.56% (5% honey) to 77.92% (20% honey), driven by phenolic acids and flavonoids that scavenge free radicals via electron transfer and metal chelation. Increasing honey concentration also reduced pH due to organic acids, while viscosity and yellowness (b* values) rose, attributed to honey's polysaccharides and pigments. The 20% honey treatment yielded optimal results but required pH consideration. These findings highlight kelulut honey's potential as a multifunctional additive for innovative dairy products, warranting further research on stability and consumer acceptance.

ACKNOWLEDGMENT

The authors extend their sincere gratitude to PT Indofood Sukses Makmur Tbk for supporting this research through the Indofood Research Nugraha Programme 2024–2025. Additionally, the authors would like to express their appreciation to the Faculty of Agriculture, Mulawarman University, for providing valuable opportunities and essential facilities that contributed to the successful completion of this study.

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