p-ISSN 2615-787X e-ISSN 2615-790X
Accredited by Directorate General of Higher Education, Research, and Technology, Republic of Indonesia, No. 225/E/KPT/2022

Tropical Animal Science Journal, June 2024, 47(2):235-241
DOI: https://doi.org/10.5398/tasj.2024.47.2.235
Available online at https://journal.ipb.ac.id/index.php/tasj

Characteristics, Antioxidant, and Antihypertensive Activities of Probiotic Greek Yogurt with Roselle Extract Addition

P. A. Juandini[a], I. I. Arief[a,*], Z. Wulandari[a], & Z. Abidin[b]
[a]Department of Animal Production and Technology, Faculty of Animal Science, IPB University
[b]Department of Chemistry, Faculty of Mathematics and Natural Sciences, IPB University
Kampus IPB Dramaga, Bogor 16680, West Java, Indonesia
*Corresponding author: isnafia@apps.ipb.ac.id
(Received 29-08-2023; Revised 02-01-2024; Accepted 03-01-2024)

ABSTRACT

Yogurt is one example of a functional food that can improve health and reduce the risk of diseases. Certain ingredients, such as probiotic bacteria and roselle extract, are added to yogurt to enhance its benefits. The utilized probiotic, *Lactobacillus plantarum IIA-1A5*, provides notable antimicrobial benefits. Additionally, the incorporated roselle extract is established to possess antioxidant and antihypertensive properties. This study aims to analyze the characteristics, antioxidant, and antihypertensive activities of probiotic Greek yogurt (PGY) and probiotic Greek yogurt with roselle extract (PGYR). The roselle extract added to the Greek probiotic yogurt was 3%, and the bacteria used in the fermentation process for both treatments were *S. thermophilus* IFO 13957, *L. bulgaricus* IFO 13953, and *L. plantarum IIA-1A5*. Each treatment was replicated three times in duplicate. The results showed that the addition of roselle extract to probiotic Greek yogurt significantly influenced ($p<0.05$) the antioxidant and antihypertensive activity, water activity (a_w), pH, total acidity (TAT), and the color aspect in the hedonic test and the flavor, color, and aroma aspects in the hedonic quality test. The antioxidant activity and antihypertensive activity tests yielded significantly different results. The antioxidant activity increased from 29.32% (PGY) to 44.93% (PGYR), while the antihypertensive activity increased from 35.68% (PGY) to 81.36% (PGYR). This study concluded that the characteristics of PGYR have met the SNI standards and have a higher antioxidant and antihypertensive activity value than PGY. PGYR also has a promising potential for commercial development due to its health benefits.

Keywords: antihypertensive; antioxidant; characteristics; roselle; yogurt

INTRODUCTION

Yogurt is a globally popular functional food (Kumar *et al.*, 2015). Greek yogurt is known for its thick and smooth texture and often contains higher counts of probiotic bacteria than traditional yogurt (Moineau *et al.*, 2020). In response to the increasing demand for healthier food, modifications include beneficial ingredients. Probiotics like *Lactobacillus plantarum IIA-1A5* offer health benefits, enhancing immunity and regulating antimicrobial and cholesterol levels (Arief *et al.*, 2013; Arief *et al.*, 2015). Roselle is added to yogurt to boost its bioactive components, with studies confirming its antidiabetic, antioxidant, and antihypertensive properties (Wihansah *et al.*, 2018; Suharto *et al.*, 2016; Arief *et al.*, 2016).

Functional foods with antioxidant and antihypertensive activities are sought after by consumers due to their potential health benefits. Antioxidants protect cells from damage caused by free radicals, slowing aging, and preventing degenerative diseases (Tayel & El-Tras, 2012). Furthermore, the symbiotic relationship between foods with antioxidant properties and antihypertensive drugs can effectively manage hypertension by influencing vascular function and oxidative stress regulation (Sorriento *et al.*, 2018). Based on the bioactive components in roselle extract and the proteins in *Lactobacillus plantarum*, this mixture has excellent antioxidant activity potential (Wu *et al.*, 2018; Suharto *et al.*, 2016). Besides that, the advantage of Greek yogurt is that it has a higher nutritional content than regular yogurt (Bridge *et al.*, 2019). However, there has never been an analysis of antioxidant and antihypertensive activity in probiotic Greek yogurt with probiotic *L. plantarum IIA-1A5* and roselle extract. This study is the first research to analyze the potential antihypertensive and antioxidant activities of probiotic Greek yogurt with probiotic *L. plantarum IIA-1A5* and rosella extract. It aims to analyze the characteristics, antioxidant and antihypertensive activities of probiotic Greek yogurt (PGY) and probiotic Greek yogurt with rosella extract (PGYR).

MATERIALS AND METHODS

The Procedure of Making Probiotic Greek Yogurt with *Roselle* Extract Addition

Yogurt production begins with the culturing of starter bacteria, which include *S. thermophilus* IFO 13957, *L. bulgaricus* IFO 13953 (Stock Culture from Gadjah Mada University) and *L. plantarum* IIA-1A5 (Accession Number: OR47328-1; Irma Isnafia Arief's collection culture), to activate dormant bacteria for reuse. The extraction of roselle flowers is made by soaking dried roselle flowers in hot water (63-65 ^{o}C) for 30 minutes, with a ratio of 20g:100 mL (Suharto *et al.,* 2016), and evaporating the extract to 15% of the initial volume. Evaporation is done so that the extract becomes thick, does not contain solvent liquid, and does not change the texture of the Greek yogurt. Greek yogurt is made by mixing milk, water, pectin, skim milk, and starch at 55 °C for 30 minutes, followed by pasteurization at 90 °C for 3 minutes, then poured with NDC (Non Dairy Creamer). Next, inoculate with starter bacteria in a ratio of 1:1:1 and incubate for 18 hours until it has a solid texture, then mix with 3% roselle extract and 10% sugar solution.

Sample Analysis

Yogurt characterization analysis. This study analyzed yogurt samples for physicochemical characteristics: water activity (aw), pH, viscosity, and TAT (total acid titrate). Proximate analysis determined protein, fat, ash, and moisture content, following AOAC (2005) methods. Viscosity was measured with a rational viscometer, water activity with an Aw meter, and TAT was assessed using AOAC (2007) guidelines. Total lactic acid bacteria (LAB) were determined using the pour plate method by Fardiaz (1993).

Organoleptic analysis. This analysis was conducted by a panel of semi-trained panelists based on the method proposed by Badan Standardisasi Nasional (2006), with a panel of 35 individuals, and all participating panelists already have filled out the informed consent form. Organoleptic samples were tested hedonically, which shows the level of favorability of the panelists, and hedonic quality, which shows the physical attributes of the samples. Sample attributes that panelists have tested are taste, aroma, color, and texture of PGY and PGYR.

Antioxidant assay. The DPPH antioxidant activity test has been a well-established method for over 50 years (Molyneux, 2004). Notably, researchers such as Tangkanakul *et al.* (2009), Almey *et al.* (2010), Dinçoğlu *et al.* (2023), and Baliyan *et al.* (2022) have conducted general procedures of DPPH for measuring antioxidant properties. This research adopted Tangkanakul *et al.* (2009) modified method based on Ohnishi *et al.* (1994) approach. The DPPH test added 0.15 mL of sample into 0.9 mL of DPPH solution. The mixture was incubated in a water bath in the dark at 37 °C for 20 minutes. Next, the absorbance was measured using a spectrophotometer at a wavelength (λ) of 517 nm until a constant reading was obtained. A standard curve was prepared using the same procedure, replacing the samples with vitamin C (ascorbic acid) at concentrations of 0.0, 0.5, 1.0, 1.5, 2.0, and 2.5 mg per 100 mL. Antioxidant activity was calculated using the following formula:

% Antioxidant activity= [(A Blanko - A Sample)/ A Blanko] x 100%

Calculation of antioxidant capacity was conducted using the vitamin C equivalent antioxidant capacity (VCEAC) method using the following formula (Zang *et al.,* 2017):

VCEAC = [(I - b)/a] x V x (100/W) x n

Where: I is inhibition of sample, b is the intercept of the standard curve, a is the slope of the standard curve, V is sample volume, W is the weight of the sample, and n is dilution time.

Antihypertensive assay. The antihypertensive activity was tested using the method described by Hayes *et al.* (2007) with the HHL (hippuryl-histidyl-L-leucine) substrate enzyme. The first step is sample extraction, where the prepared sample was centrifuged at 6000 g for 10 minutes at 4 °C, and the resulting supernatant was collected. Ultrafiltration will then be performed using a 10 kDa filter tube (Amicon Ultrafilter Brand) to separate proteins below 10 kDa. This filtration is necessary to isolate the target proteins with a molecular weight below 10 kDa. Subsequently, ultrafiltration was conducted at 4000 xg for 30 minutes at 4 °C, and the resulting fraction was used in the ACE (Angiotensin Converting Enzyme) inhibitor or antihypertensive assay (Chusman & Cheung, 1971).

The assay involved HHL (hippuryl-histidyl-L-leucine) and ACE solution. An ACE solution (25 mU) was prepared in sodium borate buffer. Then, 50 µL of the ACE solution and 50 µL of the sample were mixed and pre-incubated at 37 °C in a water bath for 10 minutes. Next, 150 µL of the HHL substrate solution (prepared by mixing 8.3 mM HHL in 50 mM sodium borate buffer containing 0.5 M NaCl at pH 8.3) was added, and the mixture was incubated at 37 °C in a water bath for 30 minutes. The reaction was stopped by adding 250 µL of 1 M HCl. After that, 0.5 mL of ethyl acetate was added, vortexed, and centrifuged at 800 g for 15 minutes at 4 °C. The resulting solution have two layers, and the ethyl acetate layer (top layer) was collected (0.8 µL) and transferred to a clean tube. It was evaporated at 85 °C for 60 minutes, and the obtained residue was dissolved in 1 mL of distilled water. The solution was cooled to room temperature, and the same procedure was performed for the control. The absorbance was measured using a spectrophotometer at a wavelength of 228 nm (Saputri *et al.,* 2015). The ACE inhibition activity was calculated using the following formula:

% ACE Inhibitor activity = [(C - A)/(C - B)[x 100%

Where A is sample absorbance, B is blank absorbance, and C is control absorbance (Captopril).

Statistical Analysis

The data generated were analyzed using a randomized block design with 2 treatments and 3 replicates in duplicate. The treatments are as follows: PGY= probiotic Greek yogurt with bacteria *Lactobacillus plantarum* IIA-1A5, *S. thermophilus* IFO 13957, and *L. bulgaricus* IFO 13953 without roselle extract, and PGYR= probiotic Greek yogurt with bacteria *Lactobacillus plantarum* IIA-1A5, *S. thermophilus* IFO 13957, and *L. bulgaricus* IFO 13953 with roselle extract.

The analysis tests conducted were independent T-Test and Kruskal-Wallis test as a non-parametric test for organoleptic analysis.

RESULTS

Physicochemical Characteristics of Yogurt

Table 1 displays results for pH, TAT, Aw values, indicating significant differences ($p<0.05$). Therefore, it is concluded that roselle extract significantly affects these aspects. However, viscosity remains unaffected by roselle extract. In Table 1, pH and TAT values correlate: the lowest pH is seen in the second replication of PGYR, coinciding with the highest TAT percentage in the same PGYR replication. Viscosity is consistently high and satisfactory in both treatments, as shown in the average listed in Table 1.

Proximate Characteristics of Yogurt

Table 1 presents average moisture, ash, fat, and protein content values. Statistical analysis found no significant differences, indicating that adding roselle to probiotic Greek yogurt did not notably impact these aspects. The ash, fat, and protein content values of both treatments align with SNI standards. Even the values of protein and fat contents are very good because these contents are higher than SNI standards. The water content value is below the SNI standard; the high viscosity of Greek yogurt causes this.

Microbiological Characteristics of Yogurt

Table 1 presents the average of total LAB values in PGY and PGYR. Statistical analysis showed no significant differences. Therefore, it can be concluded that adding roselle to probiotic Greek yogurt does not notably impact the total LAB count. This is because the bacteria used in both treatments are the same.

Organoleptic Characteristics

Hedonic test. Based on the Kruskal-Wallis test results for the hedonic test (Table 2), it was found that the addition of *roselle* extract had a significant effect on the color aspect ($p<0.05$). However, it did not significantly affect the aroma, texture, and taste.

Hedonic quality test. Based on the Kruskal-Wallis test results for the hedonic quality test (Table 2), it was found that the addition of *roselle* extract had a significant effect on the color, aroma, and taste aspects ($p<0.05$). In contrast, it did not significantly affect the texture aspect.

Antioxidant Characteristics of Yogurt

Table 3 presents the average antioxidant activity and antioxidant capacity for both treatments. Statistical analysis confirms significant differences ($p<0.05$), showing that adding roselle extract notably enhances antioxidant activity and capacity. The high value is caused by the anthocyanin content in roselle extract.

Antihypertensive Activity of Yogurt

Table 3 presents the average of ACE inhibition activity for both treatments. Statistical analysis confirms significant distinctions ($p<0.05$), implying that the inclusion of roselle extract notably impacts ACE inhibition activity in the yogurt samples. The high ACE inhibitor

Table 1. Characteristics of probiotic Greek yogurt and probiotic Greek yogurt with roselle extract

Variables	Treatments	
	PGY	PGYR
Physicochemical		
pH	4.33 ± 0.03^{a}	3.75 ± 0.06^{b}
TTA (%)	0.63 ± 0.10^{a}	1.14 ± 0.07^{b}
a_w	0.87 ± 0.01^{a}	0.83 ± 0.01^{b}
Viscosity (dPa.s)	2.92 ± 0.43	2.80 ± 0.33
Proximate		
Moisture content (%)	77.36 ± 0.42	76.83 ± 0.56
Ash content (%)	0.81 ± 0.09	0.98 ± 0.09
Fat content (%)	4.17 ± 0.39	4.26 ± 0.09
Protein content (%)	4.17 ± 0.60	3.90 ± 0.20
Microbiological		
Total of lactic acid bacteria (Log CFU/mL)	8.24 ± 0.07	8.40 ± 0.06

Note: Means in the same row with different superscripts differ significantly ($p<0.05$). PGY= Probiotic Greek yogurt, PGYR= Probiotic Greek yogurt with roselle extract.

Table 2. Organoleptic characteristics of probiotic Greek yogurt and probiotic Greek yogurt with roselle extract

Treatments	Hedonic attributes			
	Color	Scent	Texture	Taste
PGY	1.90 ± 0.70^{a}	2.02 ± 0.83	1.70 ± 0.61	1.72 ± 0.68
PGYR	1.87 ± 0.79^{b}	1.95 ± 0.75	2.10 ± 0.93	1.50 ± 0.72
	Hedonic quality attributes			
	Color	Scent	Texture	Taste
PGY	4.00 ± 0.01^{a}	3.27 ± 0.51^{a}	1.85 ± 0.42	2.37 ± 0.49^{a}
PGYR	2.32 ± 0.52^{b}	1.97 ± 0.48^{b}	1.90 ± 0.54	1.80 ± 0.52^{b}

Note: Means in the same column with different superscripts differ significantly ($p<0.05$). PGY= Probiotic Greek yogurt, PGYR= Probiotic Greek yogurt with roselle extract. Hedonic scale: 1 (Strongly like), 2 (Like), 3 (Neutral), 4 (Dislike), 5 (Strongly dislike); Hedonic quality scale: Taste: 1 (Very sour), 2 (Sour), 3 (Sweet), 4 (Very sweet); Texture: 1 (Very thick), 2 (Thick), 3 (Liquid), 4 (Very liquid); Aroma: 1 (Very roselle scented), 2 (Roselle scented), 3 (Yogurt scented), 4 (Very yogurt scented); Color: 1 (Very purple), 2 (Light purple), 3 (Purplish white), 4 (White).

Table 3. Antioxidant and antihypertensive activities of probiotic Greek yogurt and probiotic Greek yogurt with roselle extract

Treatment	Antioxidant activity (%)	Antioxidant capacity (mg VCE/100g)	Antihypertensive activity (%)
PGY	29.32 ± 0.80[a]	62.06 ± 3.79[a]	35.68 ± 2.13[a]
PGYR	44.93 ± 0.72[b]	135.90 ± 3.41[b]	81.36 ± 5.34[b]

Note: Means in the same column with different superscripts differ significantly (p<0.05). PGY= Probiotic Greek yogurt, PGYR= Probiotic Greek yogurt with roselle extract, VCE= vitamin C equivalent.

value is caused by the anthocyanin content in roselle extract and the protein content in *L. plantarum IIA-1A5.*

DISCUSSION

Physicochemical Characteristics of Yogurt

The addition of roselle flower extract causes a decrease in pH, which follows previous research, indicating that roselle flower extract has a low pH of around two (Purbowati *et al.*, 2018). The roselle extract used in this study had a pH of 1.61 ± 0.02. This study shows that the pH value and TAT are interconnected; the lower the pH value of a substance or solution, the higher the TAT value produced. This is supported by the statement of Toffanin *et al.* (2015), which states that there is a negative and strong correlation between TAT and pH. Both treatments yield favorable a_w values, indicating the ability to inhibit yeast and certain bacteria such as Salmonella, Escherichia, and Clostridia, which require a minimum a_w value of 0.90 for growth. However, the treatments are still unable to inhibit the growth of yeast, which can survive at an a_w value of 0.61 (Tapia *et al.*, 2020).

Both samples display notably high viscosity values, with the PGY sample demonstrating the highest viscosity at 3.4 dPa. This value exceeds the viscosity range reported by Meilanie *et al.* (2018), which examines the characteristics of probiotic yogurt with rosella flower extract, which ranged from 1.27 to 3.07 dPa. The data suggests that both samples exhibit excellent viscosity. The elevated viscosity can be attributed to the presence of specific additives. Protein affects viscosity by binding water, creating a soft and thick texture, as observed in treatments with high protein content (Fatmawati & Prasetyo, 2013).

Proximate Characteristics of Yogurt

The water content in this study is lower (76%-77%) compared to SNI: 2981 (Badan Standardisasi Nasional, 2009) (83%-84%), attributed to added ingredients. Kim *et al.* (2023) found 79.51%-80.06% water content in Greek yogurt after 12 hours of fermentation. This finding is supported by Khusnaini (2014), who stated that low moisture content results in a dense texture in yogurt, which is consistent with the results obtained in this study. The ash content in this study aligns with SNI standards, but treatments with roselle extract show higher values due to the extract's elevated mineral content, including copper, iron, and zinc (Samadi & Fard, 2020). Based on Baralabe (2019), the percentage of water and ash content in dried roselle flowers is 10.50% and 11.67%.

The protein and fat content obtained in the study exceed the SNI standard, which can be attributed to various factors. One of the factors is the selection of the dairy base and the addition of ingredients with high protein and fat content. One of the additives used is non-dairy creamer (NDC), which contains 33.75% fat (Ga Young *et al*., 2019), and skim milk, which contains 35% protein. The value of protein content owned by roselle calyces is relatively high at 4.10%, while fat content is 1% (Baralabe, 2019). The high number of LAB in yogurt also influences the protein content because the constituent components of LAB are proteins (Setyoningsih, 2004).

Microbiological Characteristics of Yogurt

Lactic acid bacteria take on an important role in the yogurt fermentation process as they are able to acidify milk and produce flavor and texture by converting milk proteins due to their proteolytic activity (Widyastuti & Febrisiantosa, 2014). The standard LAB count specified by the SNI is 7 Log cfu/mL, and both treatments are observed to exceed this standard. Table 1 shows no significant difference between the two treatments. This may be attributed to using the same type of bacteria during the fermentation process in both treatments. *L. bulgaricus* and *S. thermophilus* support each other's growth. *S. thermophilus* produces beneficial compounds that stimulate *L. bulgaricus* growth, while *L. bulgaricus* releases amino acids that enhance *S. thermophilus* growth (Chen *et al*., 2018). Additionally, *L. plantarum*, a probiotic bacterium known for its acid tolerance and high antioxidant activity (Arief *et al*., 2013), was also used in both treatments. This is further supported by the high protein content observed in both treatments.

Organoleptic Characteristics of Yogurt

Two tests assessed organoleptics, a hedonic test and a hedonic quality test, which are related. The hedonic quality test showed that adding roselle extract noticeably changed the yogurt's appearance, giving it a light purple color. Additionally, the aroma and taste profiles differed significantly, with a distinct roselle aroma and a tangier taste compared to the sample without roselle extract. However, there were no significant changes in texture after incorporating the extract. In the hedonic test, consumers preferred the pale purple color of the yogurt product with roselle extract. The color is the effect of anthocyanins because anthocyanins are the main flavonoid group responsible for the diversity of colors in plants (Grajeda *et al*., 2016). Anthocyanins also act as natural antioxidants by donating electrons or transferring hydrogen atoms to free radicals (Suharto *et al.*, 2016). Previous studies have demonstrated that anthocyanins can reduce the synthesis of vasoconstricting molecules through ACE inhibition (Amin *et al.*, 2020; Vendrame & Klimis, 2019). However, regarding aroma and taste, the liking level remained similar between the sample with roselle extract and the sample without it, despite visual changes. Additionally, the panelists expressed the same preference for both treatments in terms of texture due to their equal thickness.

Antioxidant Characteristics of Yogurt

DPPH (2,2-diphenyl-1-picrylhydrazyl) is a stable free radical compound that resists dimerization. Antioxidant compounds induce reduction reactions by donating hydrogen atoms (Francenia *et al.*, 2019). Roselle's high antioxidant value is attributed to anthocyanins, which act as natural antioxidants, providing electrons or hydrogen atoms to free radicals (Suharto *et al*., 2016). With hydroxyl groups (-OH) and conjugated double bonds, anthocyanins effectively donate electrons to radicals. The para- and ortho-phenolic groups in anthocyanins enhance electron stability (Mattioli *et al*., 2020). Earlier research noted that 250 µg/mL roselle extract inhibited 86% of DPPH free radicals. The test also assessed roselle flower extract, yielding a total phenolic content of 41.07 mg gallic acid/g (Sirag *et al.*, 2014). Rosella extract concentration of as much as 7.5 mg/mL has antioxidant activity above 80% (Wu *et al*., 2018). The sample not given the addition of roselle extract also has antioxidant activity even though it is low. Some previous studies have found that dairy products, such as yoghurt, fermented milk, and cheese have antioxidant activity due to the functional activity of the protein components, such as casein and whey protein (Aloglu, 2013). Jamil *et al.* (2016) stated that the hydrolysis process in yoghurt fermentation will increase hydrophobic amino acids such as valine, isoleucine, phenylalanine, and methionine, which contribute to the antioxidant activity of protein hydrosilicates. Another study also stated that Ala-Tyr peptide has the greatest antioxidant activity with a low molecular weight (253.2 Da) (Ngo *et al*., 2014).

Antihypertensive Activity of Yogurt

Hypertension can be caused by various mechanisms, including the renin-angiotensin system. This system, centered on the kidneys, regulates blood pressure. When blood pressure drops, the body releases renin, which combines with angiotensinogen to form angiotensin I. Angiotensin I is inactive until ACE (Angiotensin Converting Enzyme) in the lungs converts it to angiotensin II, an active enzyme (Patel *et al.*, 2017). Utilizing roselle extract to inhibit ACE in probiotic yogurt intensifies this effect due to ACE-inhibiting flavonoids like anthocyanins. Notably, anthocyanins serve as ACE inhibitors (Ojeda *et al.*, 2010), working as vasodilators that relax blood vessels through endothelium-derived relaxing factor (EDRF), thus aiding blood pressure reduction (Farida & Farasari, 2020). Earlier research outlined anthocyanin's role in curtailing the synthesis of blood vessel-constricting molecules via ACE inhibition (Amin *et al.*, 2020; Vendrame & Klimis, 2019). Several recent studies have found a significant inverse association between anthocyanin intake levels and blood pressure in adults aged 50 years and older (Igwe *et al.*, 2019). Ndanuko *et al.* (2016) state that dietary patterns with food intake containing natural antioxidants such as anthocyanins are proven to reduce the risk of high blood pressure. In addition to anthocyanins, yogurt peptides can also inhibit ACE. Well-known peptides are Gly-Thr-Trp and Gly-Val-Trp, with IC50 values of 0.0884 mg/mL and 0.185 mg/mL, respectively (Chen *et al.*, 2007).

CONCLUSION

Probiotic Greek yogurt with the addition of roselle flower extract has a value that is in line with the standards listed in SNI, such as pH, TAT, a_w, viscosity, ash, fat, protein content, and a total of LAB. It also has a higher antioxidant and antihypertensive activity value compared to probiotic Greek yogurt without the addition of roselle flower extract. The color of probiotic Greek yogurt with roselle flower extract is preferred by panellists over probiotic Greek yogurt without the addition of roselle flower extract. At the same time, other parameters have similar liking values.

CONFLICT OF INTEREST

Irma Isnafia Arief serves as an editor of the Tropical Animal Science Journal but has no role in the decision to publish this article. We also declare no conflict of interest to any financial, personal, or other relationships with other people or organizational matters related to the material discussed in the manuscript.

ACKNOWLEDGEMENT

This publication was financially supported by the Indonesian Collaborative Research PTNBH activities with contract number 17187/IT3.D10/PT.01.03/P/T/2023.

REFERENCES

Almey, A., A. J. Khan, S. Zahir, M. Suleiman, & K. Aisyah Rahim. 2010. Total phenolic content and primary antioxidant activity of methanolic and ethanolic extract of aromatic plants' leaves. Int. Food Res. J. 17:1077-1088.

Aloglu, H. S. 2013. The effect of various heat treatments on the antioxidant capacity of milk before and after simulated gastrointestinal digestion. Int. J. Dairy Technol. 66:170–174. https://doi.org/10.1111/1471-0307.12021

Amin, A. R., R. B. Kassab, A. E. A. Moneim, & H. K. Amin. 2020. Comparison among garlic, berberine, resveratrol, *Hibiscus sabdariffa*, genus *Zizyphus*, hesperidin, red beetroot, *Catha edulis*, *Portulaca oleracea*, and mulberry leaves in the treatment of hypertension and type 2 DM: A comprehensive review. Nat. Prod Commun. 15:1-24. https://doi.org/10.1177/1934578X20921623

AOAC. 2005. Official Methods of Analysis of AOAC International. Analysis. Ass of Anal. Chem., USA.

AOAC. 2007. Official Methods of Analysis. Ass of Anal. Chem., USA.

Arief, I. I., B. S. L. Jenie, M. Astawan, K. Fujiyama, & A. B. Witarto. 2015. Identification and probiotic characteristics of lactic acid bacteria isolated from Indonesian local beef. Asian J. Anim. Sci. 9:25-36. https://doi.org/10.3923/ajas.2015.25.36

Arief, I. I., C. Budiman, R. Hanifah, & M. S. Soenarno. 2016. Antihypertensive potency of goat milk yogurt supplemented by probiotic and roselle extract. Int. J. Sci. Basic Appl. Res. 30:207-214.

Arief, I. I., T. Suryati, Z. Wulandari, & E. Andreas. 2013. Isolation and characterization of plantaricin produced by *Lactobacillus plantarum* strains *(IIA-1A5, IIA-1B1, IIA- 2B2)*. Med. Pet. 36:91-100. https://doi.org/10.5398/medpet.2013.36.2.91

Badan Standardisasi Nasional. 2006. Petunjuk Pengujian Organoleptik atau Sensori. SNI 01 2346-2006. BSNI, Jakarta.

Badan Standardisasi Nasional. 2009. Syarat Mutu Yoghurt. SNI: 2981-2009. BSNI, Jakarta.

Baliyan, S., R. Mukherjee, A. Priyadarshini, A. Vibhuti, A. Gupta, R. P. Pandey, & C. M. Chang. 2022. Determination of antioxidants by dpph radical scavenging activity and quantitative phytochemical analysis of *Ficus religiosa*. Molecules 27:1326. https://doi.org/10.3390/molecules27041326

Bridge, A., J. Brown, H. Snider, M. Nasato, W. E. Ward, B. D. Roy, & A. R. Josse. 2019. Greek yogurt and 12 weeks of exercise training on strength, muscle thickness and body composition in lean, untrained, university-aged males. Front. Nutr. 6:55. https://doi.org/10.3389/fnut.2019.00055

Chen, G. W., J. S. Tsai, & B. S. Pan. 2007. Purification of angiotensin I-converting enzyme inhibitory peptides and antihypertensive effect of milk produced by protease-facilitated lactic fermentation. Int. Dairy J. 17:641-647. https://doi.org/10.1016/j.idairyj.2006.07.004

Chen, W., A. Narbad, W. Wu, & H. Li. 2018. Metabolites of Lactic Acid Bacteria. Lactic Acid Bacteria in Foodborne Hazards Reduction: Physiology to Practice. Springer, Singapore. https://doi.org/10.1007/978-981-13-1559-6

Chusman, D. W. & H. S Cheung. 1971. Spectrophotometric assay and properties of the angiotensin-converting enzyme of rabbit lung. Biochem. Pharmacol. 20:1637-1648. https://doi.org/10.1016/0006-2952(71)90292-9

Dinçoğlu, A. H., A. Ileri, & J. Rugji. 2023. Determination of bioactive properties of *Capparis spinosa* fruits and use in production of Tulum cheese. Emir. J. Food Agric. 35:1-12. https://doi.org/10.9755/ejfa.2023.3179

Fardiaz, S. 1993. Analisis Mikrobiologi Pangan. Raja Grafindo Persada, Jakarta.

Farida, F. Y. & P. Farasari. 2020. Effectiveness of roselle tea on decreasing blood pressure in hypertension patients in Tulungagung district. STRADA Jurnal Ilmiah Kesehatan 9:162–169. https://doi.org/10.30994/sjik.v9i1.277

Francenia, S. S. N., R. Salas-Coronado, C. Villanueva-Cañongo, & B. Hernández-Carlos. 2019. Antioxidant Compounds and Their Antioxidant Mechanism. Antioxidants. IntechOpen, London. pp. 1-29.

Ga Young, D., K. E. Young, K. Y. Jin, H. Youngji, K. Seong-Bo, K. Y. Hee, & C. Myung-Sook. 2019. Supplementation of non-dairy creamer-enriched high-fat diet with D-Allulose ameliorated blood glucose and body fat accumulation in C57BL/6J mice. Applied Sciences 9:2750. https://doi.org/10.3390/app9132750

Grajeda, I. C., E. M. C. Figueroa, N. Barouh, B. Baréa, A. Fernandes, F. V. De, & E. Salas. 2016. Isolation and characterization of anthocyanins from *Hibiscus sabdariffa* flowers. J. Nat. Prod. 79:1709-1718. https://doi.org/10.1021/acs.jnatprod.5b00958

Igwe, E. O., K. E. Charlton, & Y. C. Probst. 2019. Usual dietary anthocyaninintake, sources and their association with blood pressure in arepresentative sample of Australian adults. J. Hum. Nutr Diet. 32:578-90. https://doi.org/10.1111/jhn.12647

Jamil, N. H., N. R. A. Halim, & N. M. Sarbon. 2016. A response surface approach on hydrolysis condition of eel (*Monopterus* Sp.) protein hydrolysate with antioxidant activity. Int. Food Res. J. 24:1081-1093.

Khusnaini, M. 2014. Utilization of papaya fruit (*Carica papaya l.*) in the manufacture of fruit yogurt with different amounts of starter concentration and fermentation duration. Jurnal Agrina 1:23-30.

Kim, J., M. Kim, & I. Choi. 2023. Physicochemical characteristics, antioxidant properties and consumer acceptance of greek yogurt fortified with apple pomace syrup. Foods 12:1856. https://doi.org/10.3390/foods12091856

Kumar, B. V., S. V. Vijayendra, & O. V. Reddy. 2015. Trends in dairy and non-dairy probiotic products - A review. J. Food Sci Technol. 52:6112-6124. https://doi.org/10.1007/s13197-015-1795-2

Mattioli, R., A. Francioso, L. Mosca, & P. Silva. 2020. Anthocyanins: A comprehensive review of their chemical properties and health effects on cardiovascular and neurodegenerative diseases. Molecules 25:3809. https://doi.org/10.3390/molecules25173809

Meilanie, R., I. I. Arief, & E. Taufik. 2018. Karakteristik yoghurt probiotik dengan penambahan ekstrak bunga rosella (*Hibiscus sabdariffa* L.) selama penyimpanan suhu dingin. J. I. Produksi Teknol. Hasil Peternakan 6:36-44. https://doi.org/10.29244/jipthp.6.1.36-44

Moineau-Jean, A., Y. Raymond, H. Sabik, N. Graveline, C. P. Champagne, D. Roy, & G. LaPointe. 2020. Effect of manufacturing processes and storage on aroma compounds and sensory properties of yoghurt. Int. J. Dairy Sci. 105:104662. https://doi.org/10.1016/j.idairyj.2020.104662

Molyneux, P. 2004. The use of the stable free radical *diphenylpicrylhydrazyl* (DPPH) for estimating antioxidant activity. Songklanakarin J. 26:211-219.

Ndanuko, R. N., L. C. Tapsell, K. E. Charlton, E. P. Neale, & M. J. Batterham. 2016. Dietary patterns and blood pressure in adults: A systematic review and meta-analysis of randomized controlled trials. Adv. Nutr. 7:76–89. https://doi.org/10.3945/an.115.009753

Ngo, D. H., B. Ryu, & S. K. Kim. 2014. Active peptides from skate (*Okamejei kenojei*) skin gelatin diminish angiotensin-I converting enzyme activity and intracellular free radical-mediated oxidation. Food Chem. 143:246–255. https://doi.org/10.1016/j.foodchem.2013.07.067

Ohnishi, M., H. Morishita, H. Iwahashi, S. Toda, Y. Shirataki, M. Kimura, & R. Kido. 1994. Inhibitory effects of chlorogenic acids on linoleic-acid peroxidation and 33 hemolysis. Phytochemistry 36:579–583. https://doi.org/10.1016/S0031-9422(00)89778-2

Ojeda, D., E. Jimenez-Ferrer, A. Zamilpa, A. Hererra-Arellano, J. Tortoriello, & L. Alvarez. 2010. Inhibiton of angiotensin converting enzyme activity by the anthocyanins delphinidin and cyanidin-3-O-sambubiosides from *Hibiscus sabdariffa*. J. Ethnopharmacol. 127:7-10. https://doi.org/10.1016/j.jep.2009.09.059

Patel, S., A. Rauf, H. Khan, & T. Abu-Izneid. 2017. Renin-Angiotensin-Aldosterone (RAAS): The ubiquitous system for homeostasis and pathologies. Biomed. Pharmacother 94:317–325. https://doi.org/10.1016/j.biopha.2017.07.091

Purbowati, I. S. M., Sujiman, & A. Maksum. 2018. Antibacterial activity of roselle (*Hibiscus sabdariffa*) extract phenolics compound produced with varying drying methods and duration. Journal Agroindustrial Technology 28:19-27.

Samadi, S. & F. R. Fard. 2020. Phytochemical properties, antioxidant activity and mineral content (Fe, Zn and Cu) in Iranian produced black tea, green tea and roselle calyces. Biocatal. Agric. Biotechnol. 23:101472. https://doi.org/10.1016/j.bcab.2019.101472

Saputri, F. C., D. Mun'im, S. N. Lukmanto, Aisyah, & J. S. Rinandy. 2015. Inhibition of angiotensin converting enzyme (ACE) activity by some Indonesia edible plants. Int. J. Pharm. Sci. Res. 6:1054–1059.

Sirag, N. M. M., Elhadi, A. M. Algaili, H. M. Hassan, & M. Ohaj. 2014. Determination of total phenolic content and antioxidant activity of roselle (*Hibiscus sabdariffa L.*) calyx ethanolic extract. Standard Research Journal Pharmacy Pharmacology 1:34-39.

Sorriento, D., L. N. De, B. Trimarco, & G. Iaccarino. 2018. The antioxidant therapy: New insights in the treatment of hypertension. Front. Physiol. 9:258 https://doi.org/10.3389/fphys.2018.00258

Suharto, E. L. S., I. I. Arief, & E. Taufik. 2016. Quality and antioxidant activity of yogurt supplemented with roselle during cold storage. Med Pet. 39:82-89. https://doi.org/10.5398/medpet.2016.39.2.82

Tangkanakul, P., P. Auttaviboonkul, B. Niyomwit, N. Lowvitoon, P. Charoenthamawat, & G. Trakoontivakorn. 2009. Antioxidant capacity, total phenolic content and nutritional composition of Asian foods after thermal processing. Int. Food Res. J. 16:571-580.

Tapia, M. S., S. M. Alzamora, & J. Chirife. 2020. Effects of Water Activity (a_w) on microbial stability as a hurdle in food preservation. Water Activity Foods Fundamentals Applications. 2nd Ed. Chapter 14. pp. 323-355. https://doi.org/10.1002/9781118765982.ch14

Tayel, A. A. & W. F. El-Tras. 2012. Plant extracts as potent biopreservatives for *Salmonella typhimurium* control and quality enhancement in ground beef. J. Food Saf. 32:115-121. https://doi.org/10.1111/j.1745-4565.2011.00357.x

Toffanin, V., M. De Marchi, N. Lopez-Villalobos, & M. Cassandro. 2015. Effectiveness of mid-infrared spectroscopy for prediction of the contents of calcium and phosphorus, and titratable acidity of milk and their relationship with milk quality and coagulation properties. Int. Dairy J. 41:68-73. https://doi.org/10.1016/j.idairyj.2014.10.002

Vendrame, S. & D. Klimis-Zacas. 2019. Potential factors influencing the effects of anthocyanins on blood pressure regulation in humans: A review. Nutrients 11:1-19. https://doi.org/10.3390/nu11061431

Widyastuti, Y. R. & A. Febrisiantosa. 2014.The role of lactic acid bacteria in milk fermentation. Food Nutr. Sci. 5:435-442. https://doi.org/10.4236/fns.2014.54051

Wihansah, R., I. I. Arief, & B. Irmanida. 2018. Antidiabetic potency and characteristics of probiotic goat-milk yogurt supplemented with roselle extract during cold storage. Trop. Anim. Sci. J. 41:191-9. https://doi.org/10.5398/tasj.2018.41.3.191

Wu, H. Y., K. M. Yang, & P. Y. Chiang. 2018. Roselle anthocyanins: Antioxidant properties and stability to heat and pH. Molecules 23:1357. https://doi.org/10.3390/molecules23061357

Zang, S., S. Tian, J. Jiang, D. Han, X. Yu, K. Wang, D. Li, D. Lu, A. Yu, & Z. Zhang. 2017. Determination of antioxidant capacity of diverse fruits by electron spin resonance (ESR) and UV–vis spectrometries. Food Chem. 221:1221-1225. https://doi.org/10.1016/j.foodchem.2016.11.036