The Antioxidants Improvement of Yogurt with Three Sources of Non-Alcohol Anthocyanin Extract and *Metroxylon sagu* as a Natural-Based Thickener

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Abstract. This study aimed to determine the interaction of water-soluble pigment sources and sago starch concentration on the yogurt quality, especially in increasing the antioxidant and stability. This research applied extraction using aquades: citric acid (95:5) at 10 °C to 12 °C for 120 min. The red rose extract was then analyzed using FTIR and LC-MS measurements. A randomized completed block design factorial was applied with three replications. This research consisted of two factors; the first was adding different sources of pigment (anthocyanin) with four levels (control, rose petal, mulberry, and sappan wood). The second factor was sago starch concentration (2 %, 4 %, and 6 %). The results represent the interaction of pigment sources and sago starch concentration on physicochemical and sensorial properties. The best treatment was A3G3 (rose pigment and 6 % sago starch) with a viscosity of 40.0 d-Pass, pH 4.20, total dissolved solid 8.33 °Brix, total titrated acid 0.60 %, protein 2.19 %, fat content 1.92 %, total anthocyanin 47.94 mg g^{-1}, antioxidants 80.10 % (increase 92.3 %). Furthermore, the organoleptic test resulted in appearance, tends to be attractive, aroma was quite like, viscosity tended to be thick, and good enough taste.

Keywords: Healthy food, lactic acid bacteria, rose pigment, quality improvement, sago palms

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1 Introduction

Nowadays, global consumers are more likely to be interested in preventing various health problems due to their better understanding of food and ingredients. The shifting demand for food products can boost physicochemical function and provide high nutrition [1]. Yogurt is a functional dairy product widely recognized and accepted by global consumers. This product results from the fermentation of lactose in milk into lactic acid by Lactic Acid Bacteria (LAB), usually *Lactobacillus delbrueckii* subsp. *bulgaricus* [(Orla-Jensen 1919) Rogosa & Hansen 1971 Weiss et al. 1984] and *Streptococcus salivarius* subsp. *thermophilus* [(Orla-Jensen, 1919) Farrow et Collins 1984], with the ability to increase shelf life. It also provides high nutritional value of calcium, phosphorus, potassium, vitamin A, vitamin B2, vitamin B12, proteins, and essential fatty acids. This definition makes yogurt a nutrient-dense product and an effective probiotic carrier to maintain human health [2].

Textural properties of yogurt, such as viscosity, smoothness, thickness, and physical resistance to push, are fundamental traits to decide its customer awareness, and these properties these days are attached by certain benefits. Numerous strategies have been utilized to level up the quality of the yogurt, such as expanding the solids in milk as the raw material (including fat, proteins, or sugars such as sucrose and fructose) and the expansion of stabilizers (pectin, starch, alginate, and gelatin). In some cases, these approaches did not fulfill the customer request for items with as few additive ingredients added as conceivable [3]. Compared to conventional yogurt, the added compound's effect on enriching yogurt's physicochemical, textural, sensory, and microbial properties has been commonly evaluated [4]. However, the number of studies examining the effect of antioxidant compound addition from plant extract to increase the nutritional value of yogurts is very limited.

Antioxidants are a group of chemical compounds correlated with significant health benefits and reduce the risk of numerous age-related diseases. Its ability to stop the damaging process of Reactive Oxygen Species (ROS) is involved in several medical conditions, including cancer, heart disease, and premature aging [5]. Antioxidants are widely found in plants, *i.e.*, edible vegetables, fruits, spices, and herbs, known for their rich nutritional value [6]. Antioxidants as additives in food production can inhibit oxidation damage, such as changes in taste, color, and nutritional value. Plant parts can be used as antioxidants directly or after extraction and purification [7]. Some natural antioxidants, including ascorbic acid, tocopherols, lactic acid, and citric acid, are naturally provided in food. On the other hand, synthetical antioxidants like butylated hydroxyanisole, butylated hydroxytoluene, ethoxyquin, tert-butylhydroquinone, and propyl gallat are commonly added in food products [8]. Nevertheless, the utilization of antioxidants in the form of phenolic compounds like flavonoids as food additives in yogurt has not been explored yet.

Anthocyanins, a phenolic compound, are nature's essential water-soluble pigments commonly applied in foods due to their attractive color and health-promising benefits. It is widely found in various parts of plants, including flowers, fruits, stems, leaves, and root organs. It is responsible for fruits and flowers' red, purple, or blue color [9]. Rose petals [10], mulberry fruit [11], and sappan wood [12] have promising levels of anthocyanin content. Anthocyanin can be a natural colorant that is safe to consume even at higher doses than synthetic colorants. Anthocyanins, as natural colorants, have value-added properties. These properties are antioxidants as nutraceuticals and have many health benefits, such as an antimicrobial effect to increase the shelf life of food products and prevent chronic diseases [13–15].

Therefore, this work aims to know the impact of adding different sources of non-alcoholic anthocyanin and combining it with sago (*Metroxylon sagu* Rottb.) starch as a stabilizer to enhance the quality of yogurt based on physicochemical and sensorial evaluation. On the other hand, the anthocyanin from rose petal extract was previously evaluated by Fourier

Transform Infrared (FTIR) and Liquid Chromatography Coupled with a Mass Spectrometer (LC-MS) due to its high potency of anthocyanin content. Furthermore, this work describes the possibility of enhancing the nutritional content of yogurt compared to ordinary yogurt products. As a result, this work can potentially be an essential contribution to dairy products to develop healthier food and beverages for both food scientists and industry practitioners.

2 Materials and methods

2.1 Chemicals and reagents

Methanol p.a, ethanol p.a, butanol p.a, hexane, acetone, petroleum ether, 2,2-diphenyl-1-picrylhydrazyl (DPPH), phenolphthalein (HIn), sodium hydroxide (NaOH), sodium chloride (NaCl), potassium nitrate (KNO_3) hydrogen chloride (HCl), sodium sulfate (Na_2SO_4), sodium acetate ($NaCO_2H$), acetic acid, citric acid, sulfuric acid (H_2SO_4), lactic acid, ascorbic acid, and buffer solution (pH 4) were purchased from Merck KGaA (Darmstadt, Germany).

2.2 Materials

The raw materials for yogurt production were fresh milk from the Jabung Agro Niaga Cooperative in Malang, Indonesia. Sago starch, sappan wood (*Caesalpinia sappan* L.), mulberry (*Morus* sp.) fruit (perfectly ripe, blackish purple) obtained from Sidomulyo, Batu, Indonesia. Fresh rose (*Rosa* sp.) crowns with dark red color were purchased from a local farmer in Batu, Indonesia, and Bangil, Indonesia. Rose petals and mulberry fruits were collected at the optimum maturity stage.

2.3 Experimental design

This work used a Randomized Completely Block Design (RCBD) factorial with two factors and three replications applied. The first factor was pigment sources, which consisted of four levels (without pigment extract or control [A0], rose [A1], mulberry [A2], and sappanwood [A3]). The second factor was sago starch concentration, consisting of three levels (G1, low: 2 %; G2, medium: 4 %; and G3, high: 6 %). This research consisted of several stages: preparation of pigment extracts without alcohol; analysis of pH, total dissolved solids (TDS), antioxidants, and anthocyanins; yogurt production with pigments application; and yogurt physicochemical parameter test including pH, viscosity, total dissolved solids, total titrated acid, fat, protein, antioxidant activity, total anthocyanins, color test, and sensory test (aroma, taste, viscosity, and appearance).

A sensorial test was conducted by taking samples from each final product of all treatments. The test was performed by a group of trained 18 participants [16] consisting of nine males and nine females aged 20 to 30. The participants were asked to analyze the parameters of appearance, viscosity, taste, and aroma of each sample. Each participant was given sheets with a five-point scale for each parameter with unnumbered options to avoid bias in scoring. The parameters of samples were rated between 1 and 5, where 1 means minimum intensity and 5 means maximum intensity. The given score from each participant was then averaged to give a specific score in each parameter for consideration.

2.4 Antioxidant activity analysis

Antioxidant Activity (AA) of three pigment sources (mulberry, sappan, and rose) was determined using the stable DPPH as the radical agent according to the method of Aktumsek

et al. [17] with several modifications. Antioxidant activity using DPPH assay was conducted by placing 1 mL of samples, blanks, or standard into a microplate and then adding 1 mL of DPPH· solution. The reaction was performed at 37 °C for 20 min in the dark. DPPH· was measured using a spectrophotometer (Spectronic Thermo Genesys 20, USA) at 517 nm and expressed as % of absorbance. All measurements were performed in triplicates. Antioxidant activity was calculated using the following formula in the Equation (1).

$$\text{AA}\ (\%) = \frac{(A_0 - A_1)}{A_1} \times 100 \quad (1)$$

Where,
A_0 = the absorbance of the control
A_1 = the absorbance of the extract/standard

2.5 Identification of anthocyanin composition

This research only focused on the anthocyanin content in the red rose petals due to the highest prediction of the anthocyanin content value. The red rose crown was weighed 15 g and crushed into small pieces. Then, the sample was extracted using a mixture of distilled water and four levels of organic acid (acetic acid, lactic acid, sulfuric acid, and methanol-HCl) with a volume of 100 mL. The extraction process was conducted using the maceration method at a cold temperature (10 °C to 12 °C) for 12 h. The extract was then filtered using Whatman No. 1 filter paper and centrifugated at 5 000 rad s^{-1} for 5 min. Adding 1 % to 2 % of petroleum ether was done to separate non-polar compounds. A rotary evaporator (Heidolph W2000, USA) then evaporated the filtrate at 50 °C to get the red rose concentrate. The concentrated extract was then analyzed using Shimadzu IRSpirit (Shimadzu, Kyoto, Japan) to determine the functional group of anthocyanin extracted from the sample.

Anthocyanin was isolated using silica gel as a stationary phase, sodium sulfate, alumina, and glasswoll. Fractionation of pigment was carried out using methanol-HCl (1 %) with hexane (ratio 6:4) as mobile phase and followed by elution using methanol-HCl (1 %) with ethyl acetate (ratio 6:4). The column used in this study was Shim-pack GIST C18 (Shimadzu, Kyoto, Japan). Elution was performed at a solvent flow rate of 1 mL min^{-1}. The method of LC-MS analysis using Shimadzu HP Mariner 5972 (Shimadzu, Kyoto, Japan) was conducted according to the procedure described by Trikas *et al.* [18] with a slight modification on the flow rate 1 mL min^{-1} with a 30 min gradient elution program. The sample volume injected was 10 µL, and the UV-visible spectra were scanned from 200 nm to 600 nm. The mass spectrometer analysis was carried out for the full scan over m z^{-1} 0 to 1 000 in both positive and negative ionization modes. MS sample analysis followed the previous study conducted by Grace *et al.* [19]. The anthocyanin and flavonol glycosides were quantified as their extracted-ion chromatogram in the positive ion mode with suitable internal standard [19].

2.6 Statistical analysis

All experiments were carried out at least three times (n = 3), and the data are calculated and presented as mean values ± standard deviations (SD). The statistical software package SPSS 24.0 version (SPSS Inc., Chicago, IL, USA) was used to analyze the effect of the concentration of anthocyanin pigments added to the produced fruit leather on the physicochemical and sensorial quality parameters. Statistical significance was measured through a one-way Analysis of Variance (ANOVA) followed by a Least Significance Different (LSD) post hoc test. Consideration of statistical significance was determined if the p-value result was less than 0.05 ($P < 0.05$) [20, 21].

3 Results and discussion

3.1 Fresh milk analysis

The raw material properties used for this work were fresh milk with the appropiate standard. The grade A specification was selected as the fresh milk standard for yogurt production. Other chemical analyses showed that the alcohol and antibiotic tests were negative, and the minimum density was 1.0240 g mL^{-1}. The organoleptic test consisted of color, hygiene, aroma, taste, and viscosity parameters which showed normal results.

3.2 Physicochemical parameters of pigment

Pigment sources used to produce natural dyes were red rose petals, sappan wood, and mulberry fruit. Table 1 describes the analysis result of pH, total dissolved solids, antioxidants, glycon peak, and anthocyanidins. Based on the data, it is known that sappan wood, rose, and mulberry fruit had significantly different pH values. Sappan wood had a more alkaline pH value, while mulberry and rose had a more acidic pH. According to Vij *et al.* [12], the sappan wood extraction using diluted water as the solvent showed the range of the pH value of 6.2 to 7.0 [12]. According to Saati *et al.* [10], the difference in pH in raw materials was due to anthocyanins being more stable at acidic pH, namely 2 to 5 and brazilein was stable at neutral pH 6 to 7 [10]. Anthocyanin pigment from mulberry has a more acidic pH than sappan wood brazilein. The total dissolved solid of mulberry was also the highest (5 °Brix) compared to another. The Antioxidant Activity (AA) of rose petals was the highest at about 80.10 %, in contrast with sappan wood, with the lowest value of about 55.05 %. The red rose has functioned as an antibacterial agent and has high potency to improve its shelf life [22].

The glycon peak test results for the three pigment extracts showed that in the 250 nm to 600 nm, the rose extract had the highest value at the glycon and aglycon peaks. Anthocyanin pigments were stable at acidic pH (1 to 4) and appeared in pink, red, purple, and blue colors [23]. Both mulberry and red roses showed the peak of anthocyanin compounds, while sappan showed brazilein compounds. According to Saati [24], the results of absorption of pigment extracts (distilled water and citric acid) showed that the peak distribution was around 239.5 nm to 241 nm (band I), band II at 513 nm to 518 nm as a glycon (cyanidin), referring to the absorption range area of the two bands. Band I often appeared at 230 nm to 280 nm, while band II ranged between 465 nm to 535 nm as anthocyanin pigments, not betacyanin, which was also red in polar. However, both never existed in one plant [24].

Table 1. Characteristics of pigment extract from three different sources.

Sample	pH	TDS (°Brix)	AA (%)	Glycon peak (~250 nm)	Aglycon peak (~600 nm)
Sappan wood	6.23	1.5	55.05	λ 283.20 (1.693 A)	λ 573.00 (0.013 A)
Rose	3.25	4.7	80.10	λ 280.80 (2.307 A)	λ 511.80 (1.497 A)
Mulberry	3.65	5.0	62.54	λ 258.80 (0.436 A)	λ 526.00 (0.049 A)

3.3 Identification of anthocyanin contents of red rose extract

The FTIR spectra of concentrated red rose petals extract was analyzed using a Shimadzu IRSpirit with the KBR pellet technique. This measurement was carried out to identify the functional group correlated to bioactive compounds contained in *Rose* sp. petal extract. Concentrated red rose petal extract before FTIR measurement was freeze-dried to obtain the red rose petal powder. The color of the red rose petal powder was identified as a dark reddish powder. In the FTIR spectrum shown in Figure 1, the vibration received at the peak positions

at 3 372.12 cm^{-1} was associated with the stretching vibrations of O-H bonds, probably due to the presence of sugar vibrations and phenol group. The band at 2 989.46 cm^{-1} is a characteristic band for C-H stretching vibration [25]. A band at 1 635.52 cm^{-1} corresponds to C-C core aromatic rings for stretching vibration of the benzene rings [26]. In addition, bands correspond to the skeletal stretching vibration of the aromatic rings and =C-O-C group of flavonoids (1 456.16 cm^{-1}, 1 220.86 cm^{-1}, and 1 097.42 cm^{-1}). The band near 1 300 cm^{-1} may be assigned to the O-H plane deformation in polyphenols [27]. The bands between 1 128 cm^{-1} and 900 cm^{-1} are characteristic of C-O and C-C bonds, and the stretching C-O-C bonds indicate the existence of glycosidic bonds [28].

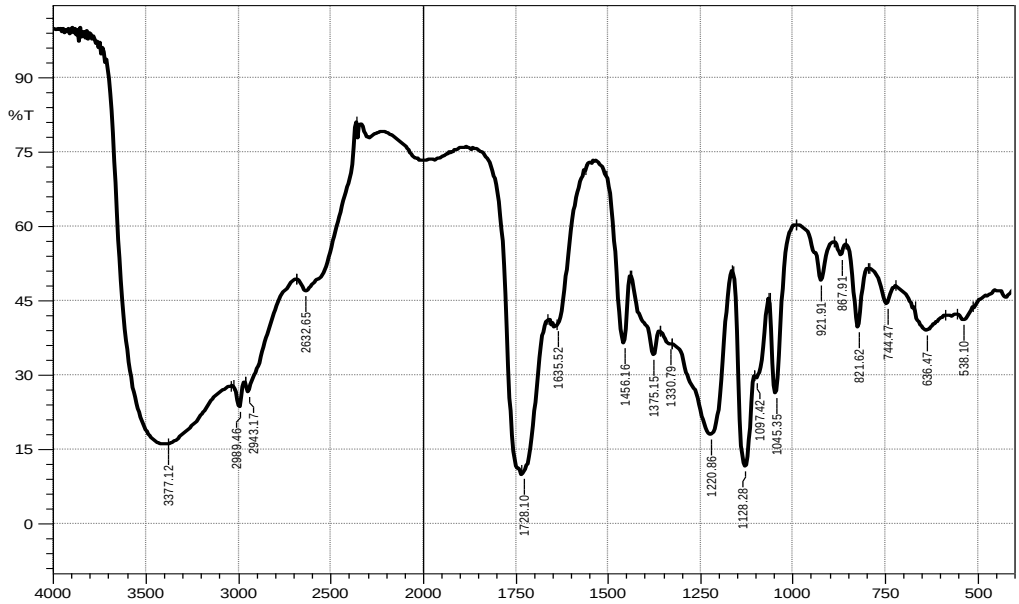

Fig. 1. Infrared spectrum of red rose petals extract.

LCMS analysis observations show that the distribution of ion fragments in bands 331/493/655 m z^{-1} and 331/493/639 m z^{-1} shows that the peak distribution of m z^{-1} malvidin (aglycon) ion fragments is at 331, plus peak fragments of glycon ions at 146 m z^{-1}, one peak of 162 m z^{-1} and 146 m z^{-1}, namely malvidin compounds, one molecule of ramnose, two molecules of glucose and one molecule of coumaric acid [29, 30, 19]. Thus, it can be known that the dominant type of anthocyanin pigment in red roses is malvidin-3,5-diglucoside with a mass weight of 655 or malvidin 3-p-coumarioil-glucoside (MW 713) or malvidin 3-p-coumarioil-rutinosida-glucoside (MW 801) according to the previous study of Saha *et al.* [31].

Based on Table 2, it is known from the peak ion molecular weight of LCMS fraction, red rose petals pigment contains cyanidin-3-6'malonyl)-glucoside, cyanidin-3-(3'xylosil, 6'malonyl)-glucoside, cyanidin-3-(3'firusyl, 6'malonyl)-glucoside and cyanidin 3-rutinose-5-glucoside (42.21 %), malvidin-3,5-diglucoside and malvidin 3-(coumaril)-5-glucoside (32.16 %), pelargonidin-3-rutinose (6-O-α-L-ramnosyl-D-glucose, and pelargonidin-3-oxalosyl-5-ramnoside (18.03 %). LCMS analysis of malvidin-3-glucoside isolates in rose extract pigments is shown in Figure 2, with molecular structure predictions in Figure 3.

Table 2. Molecular weight distribution of anthocyanin pigment analysis from LC-MS.

Retention time (min)	Ion distribution (m z^{-1})	Types of anthocyanidin	MW	Anthocyanin compound estimation	Molecular formula estimation	Ratio (%)
3.41	535/449/284	Cyanidin-glucoside	535	Cyanidin-3(6'malonyl)-glucoside	$C_{24}H_{23}O_{14}$	10.25
3.41	668/535/449/287	Cyanidin-glucoside	668	Cyanidin-3(3'xilosyl, 6'malonyl)-glucoside	$C_{30}H_{36}O_{17}$	10.25
3.44	713/535/449/287	Cyanidin-glucoside	713	Cyanidin-3(3'firusyl,6'malonyl)-glucoside	$C_{35}H_{37}O_{16}$	10.25
3.44	757/449/287	Cyanidin-glucoside	757	Cyanidin-3-O-(2"O-glucosyl) rutinose	$C_{33}H_{41}O20$	7.45
3.89	655/493/331	Malvidin-glucoside	655	Malvidin-3,5-diglucoside	$C_{29}H_{35}O_{17}$	32.15
5.67	491/419/271	Pelargonidin-glucoside	491	Pelargonidin-3-oxalosyl-5-ramnocide	$C_{22}H_{19}O_{13}$	2.60
10.00	579/433/71	Pelargonidin-glucoside	579	Pelargonidin-3-rutinose-(6-O-L-ramnosyl-D-glucoside)	$C_{27}H_{31}O_{14}$	9.02

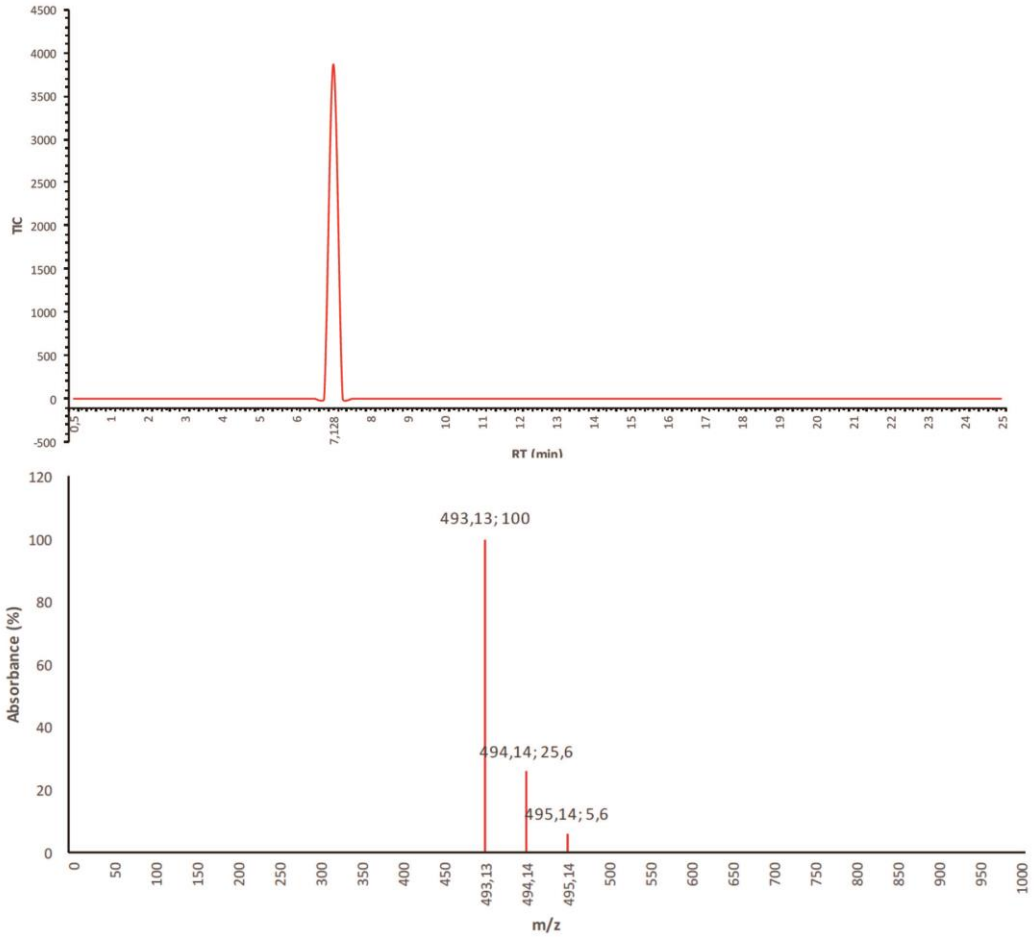

Fig. 2. LC-MS identification of malvidin-3-glucoside isolated from *Rose sp* petals.

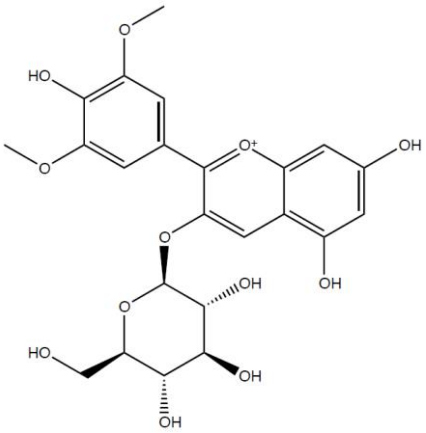

Fig. 3. Anthocyanin isolated from *Rose* sp. petals that predicted as malvidin-3-glucoside.

3.4 Yogurt physicochemical properties

The highest viscosity was found in A2G3, as shown in Table 3, with a viscosity of 40.7 d-Pass, and the lowest viscosity was A1G1, with a viscosity of 16.3 d-Pass. Increasing sago starch concentration in line with improving viscosity value [32]. The addition of sago starch caused gelatinization of the starch granules during the pasteurization process, resulting in the water contained in the milk shrinkage and the milk viscosity increased. According to Liu *et al.* [33], gelatinization causes the starch in granules to absorb water. Starch grains swell and cannot return to their original position [33]. Swelling of the starch grains occurs when the kinetic energy of water molecules becomes stronger than the attraction between starch molecules [34]. As a result, the water enters the starch granules and automatically increases the yogurt's viscosity. On the other hand, the sago starch gelatinization process occurred at 72 °C to 76 °C, while the pasteurization temperature used was 80 °C.

The highest total titrated acid was in the A1G1 (0.83 %). In comparison, the lowest was A0G1 (0.42 %), as shown in Table 3. According to the Indonesian National Standard for yogurt specification, the acidity of yogurt is in the range of 0.5 % to 2.0 %. The treatment of anthocyanin pigments from rose and mulberry tended to have a higher titrated total acid value than sappan wood and control. According to Wang *et al.* [35], an increase in lactic acid levels is due to Lactic Acid Bacteria (LAB) activity which breaks down lactose and other sugars into lactic acid [35]. LAB activity affects yogurt's acidity level due to its metabolite products in the form of lactic acid during the fermentation process [36].

Adding sago starch increased the TDS, according to Table 3, which was directly in line with the viscosity value. The highest TDS in the A3G3 was 8.33 °Brix, while the lowest was A1G1 (5.67 °Brix). Different extract pigments also affect the yogurt TDS because each raw material has a different TDS. According to Rahayu *et al.* [37], sappan wood extract has relatively low TDS compared to extracts from other pigment sources [37]. According to the previous study by Arganis *et al.* [38], it was known that the enhancing concentration of stabilizers used automatically increased the total dissolved solids [38]. It caused higher stabilizer concentration to improve the binding capacity of the stabilizer to water. Compared to the combination of rose extract and mint leaves drink, with a TDS value of 21.17 °Brix, this research range value was lower [39].

The highest anthocyanin levels were shown in the A3G2 (55.34 mg g^{-1}). The lowest is in the A0G1 (0.28 mg g^{-1}), as shown in Table 3. The difference in anthocyanin levels in each treatment was caused by adding different pigments. Sappan wood has a colored pigment caused by the brazilein compound, which provides dark orange to deep red [12]. Mulberry

fruit contains cyanidin, which acts as anthocyanin, insoquercetin, and vitamin C [40]. Rose petals still contain anthocyanin pigments, malvidin, and cyanidin glycosides [24]. The increase in anthocyanin was in line with the improving antioxidant, and this result was consistent with the analysis of teak leaves [41, 27].

The highest antioxidant was shown in the A3G1 (80.5 %). The lowest was A0G2 (5.4 %), as shown in Table 3. The control without pigment had the lowest antioxidant compared to the sample with pigment added. Anthocyanin was a flavonoid compound that could act as an antioxidant [42]. Sappan wood extract contains five active compounds of flavonoids that function as antioxidants [43]. Mulberry also contained a variety of antioxidant compounds, *i.e.*, quercetin, isoquercetin, vitamin C, and anthocyanins [11]. Arfan *et al.* [44] found that mulberry fruit had an antioxidant activity of 283.3591 μg mL^{-1} [44]. Shahidi and Zhong [45] identified that many factors influenced antioxidant activity, *i.e.*, lipid, antioxidant, temperature, oxygen pressure, and other chemical components [45].

Table 3. Yogurt characteristics based on pigment sources and sago starch concentration.

Treatment (pigment + sago starch)	Viscosity (d-Pass)	TTA (%)	TDS (°Brix)	Anthocyanin (mg g^{-1})	Antioxidant - DPPH (%)
A0G1 (control + 2 %)	24.0 c	0.42 a	6.50 a	0.28 a	7.6 a
A0G2 (control + 4 %)	25.7 c	0.51 a	6.67 a	0.62 b	5.4 a
A0G3 (control + 6 %)	30.3 d	0.45 a	8.17 c	0.51 a	6.1 b
A1G1 (mulberry + 2 %)	16.3 a	0.83 c	5.67 a	46.16 e	70.9 b
A1G2 (mulberry + 4 %)	18.7 b	0.52 a	6.33 b	50.69 f	64.3 b
A1G3 (mulberry + 6 %)	36.7 d	0.60 b	7.17 c	51.38 g	68.0 b
A2G1 (sappan + 2 %)	25.3 c	0.48 a	7.67 c	2.89 c	80.3 c
A2G2 (sappan + 4 %)	34.0 d	0.65 b	7.07 a	2.50 b	63.6 b
A2G3 (sappan + 6 %)	40.7 d	0.61 b	6.67 b	2.35 b	75.4 c
A3G1 (rose + 2 %)	21.7 c	0.71 c	5.83 a	43.55 d	80.5 c
A3G2 (rose + 4 %)	37.3 d	0.63 a	6.83 a	55.34 g	73.6 b
A3G3 (rose + 6 %)	40.0 d	0.60 b	8.33 c	47.94 f	80.0 c

Note: Numbers followed by the same letter in a column were not significantly different on the LSD test of 5 %.

Figure 4(a) shows the pH value in the range 4.08 to 4.47, indicating that the yogurt product has an acidic pH, which means it was stable. The graph showed no significant effect of two factors on the pH of yogurt. It caused the fermentation time to be 24 h and the addition of the same pigment concentration. The optimum acidic conditions of commercial yogurt should be 7.0 mg g^{-1} to 9.0 mg g^{-1} of lactic acid and pH 4.0 to 4.4 to avoid the excessive acidic taste of the product [46]. It can be inferred that the produced yogurt has suitable acidity to consume. Anthocyanin pigments were more stable in an acidic pH range of 1 to 5 [22], such as for several commercial drinks like fruit juice (3.10), yogurt (4.17), and carbonated drinks (2.92) [47].

Following the Indonesian National Standard — SNI No. 2981:2009 (*Standar Nasional Indonesia*) [48], the protein requirement in yogurt is at least 2.7 % and around 3 % for yogurt enriched with natural functional ingredients, according to Rashwan *et al.* [1]. The result showed that the protein content meets the SNI (1.71 to 2.34), as shown in Figure 4(b). The protein content of yogurt is determined by the quality of fresh milk as the raw material; the higher milk protein content results in better quality of yogurt produced [49]. The more microbes contained in yogurt, the higher protein content is because most of the components of microbes are protein. This phenomenon was due to the decreased protein content due to lactic acid fermentation caused by microorganisms active in fermentation. The protein was

hydrolyzed into dissolved components during fermentation to form microbial cell protein. It was further reported that 20 % of dissolved nitrogen was used for its growth [50].

According to the Indonesian National Standard, the yogurt requirement should contain at least 3.0 % fat and around 3 % to 4 % for yogurt enriched with natural functional ingredients, according to Rashwan *et al.* [1]. The average value of fat content almost meets the standard requirements for yogurt (1.83 to 3.45) according to Figure 4(c). The milk fat levels changed during fermentation, and lactic acid bacteria broke the fat into simpler compounds [51]. The hydrolysis of triglycerides by lipase enzymes will produce fatty acids and glycerol [52]. The hydrolysis of the fat makes a small contribution to yogurt products.

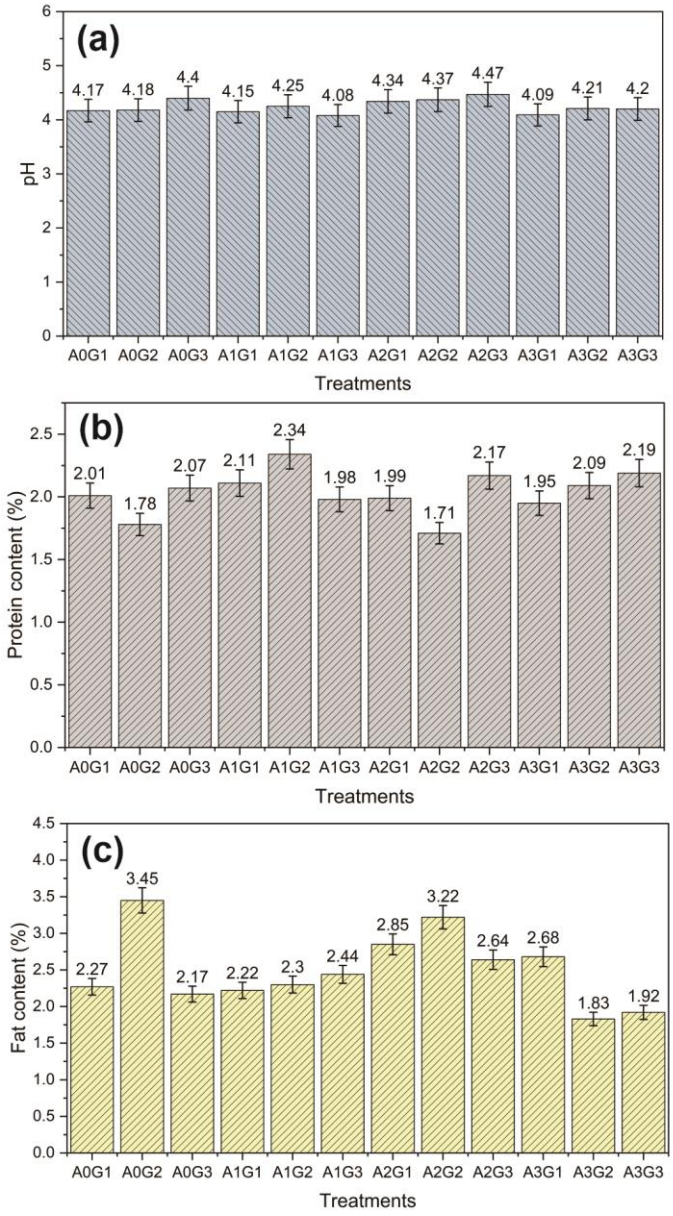

Fig. 4. Chemical properties of produced yogurt. (a) pH, (b) protein content, and (c) fat content.

3.5 Yogurt sensorial analysis

Table 4 shows the sensorial test for appearance in the range of 1.57 to 2.97. The highest score was A1G3 (2.97), described as attractive, while the lowest score was A0G1 (1.57) which means not attractive. In this case, the panelists preferred the appearance of the yogurt added with the natural coloring of the mulberry fruit, which gave a light purple color. Natural pigments from red roses produce a pink color in the product, pigments from mulberry fruit produce a red-purple, and the sappan tend to be yellowish red. The anthocyanin pigments had a lower brightness level than brazilein [53]. The more anthocyanin extract that was added caused the red color of the product to get sharper.

The data in Table 4 showed that the highest viscosity was A0G3 (3.23), which means thick, while the lowest was A1G1 (1.87), described as quite thick. Panelists preferred yogurt with a low thickness, at a concentration sago of 2 % compared to 4 % and 6 %, because it was too thick when consumed. Moreover, a higher increase in sago concentration results in higher viscosity of the yogurt. In addition, the viscosity was also caused by the fermentation process of lactic acid bacteria. The viscosity of milk was contributed by the presence of casein, myelin, and fat globules [54]. Furthermore, the bonds between protein and fat affected viscosity; changes in casein milk with the same hydrophilic properties as other protein types caused increased viscosity [55]. Fermentation of lactose produces lactic acid, which plays a role in milk protein to produce a unique gel-like texture and odor in yogurt [56].

In yogurt products, the lactic acid bacteria *Streptococcus thermophilus* dramatically contributes to the formation of flavors [36]. The highest score on the taste assessment was A3G2 (2.33), which stated that it was pretty good. In contrast, the lowest score was A0G3 (1.53), which stated it was not good based on Table 4. According to the Indonesian National Standard, the requirement for yogurt taste was a sour taste, and the result met that requirement. Most panelists preferred the yogurt taste with rose pigment addition, followed by the mulberry and sappan.

Table 4. Yogurt sensorial test based on pigment sources and sago starch concentration.

Treatment	Appearance	Viscosity	Taste
A0G1 (control + 2 %)	1.57 a	2.53 b	1.57 a
A0G2 (control + 4 %)	1.70 b	2.67 b	1.60 b
A0G3 (control + 6 %)	1.60 a	3.23 c	1.53 a
A1G1 (mulberry + 2 %)	2.68 c	1.87 a	1.87 b
A1G2 (mulberry + 4 %)	2.73 d	2.03 a	1.90 b
A1G3 (mulberry + 6 %)	2.97 d	2.30 b	2.20 c
A2G1 (sappan + 2 %)	2.27 b	1.93 a	2.03 b
A2G2 (sappan + 4 %)	2.10 b	2.47 b	2.07 b
A2G3 (sappan + 6 %)	2.30 c	2.87 c	2.17 b
A3G1 (rose + 2 %)	2.63 c	2.13 b	2.13 b
A3G2 (rose + 4 %)	2.67 c	2.37 b	2.33 c
A3G3 (rose + 6 %)	2.67 c	2.80 b	2.00 b

Note: Numbers followed by the same letter in a column were not significantly different on the LSD test of 5 %.

Yogurt has a distinctive aroma that results from the fermentation of lactic acid bacteria [50]. The highest score on the aroma assessment was A1G2 (2.43), which was quite good. In contrast, the lowest score was A0G1 (1.53), which tended to be unlike based on Figure 5. The aroma of yogurt was caused by *Lactobacillus bulgaricus* bacteria, which had a role in aroma formation. These bacteria remodel glucose in the fermentation process and then produce lactic acid and other compounds in the form of ethanol, acetic acid, and CO_2 [35].

Dairy products with a fermentation process, *i.e.*, yogurt, have the same aroma, which is the distinctive sour aroma of yogurt, even though it is added with flavorings or dyes. *Lactobacillus bulgaricus* plays an essential role in producing aroma through lactic acid and acetaldehyde [57].

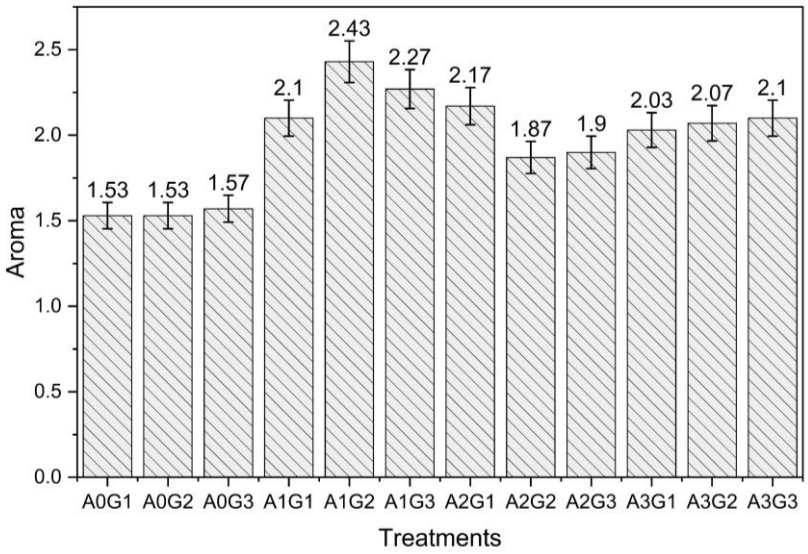

Fig. 5. Yogurt aroma analysis based on pigment and sago concentration.

4 Conclusion

In conclusion, the result of this study represents a valid correlation between three pigment sources (mulberry, sappan, and rose) with concentrations of sago starch on the quality improvement of yogurt based on several physicochemical and sensorial evaluations. Adding natural functional ingredients in yogurt production is suitable to enhance stability, taste, texture, and nutritional content that is beneficial for human health. The experiment showed that rose petal extract with 6 % sago starch addition resulted in the best quality improvement of yogurt compared to other treatments. The outcomes of yogurt production with natural functional ingredients addition are highly dependent on various factors. Another number of experiments must be performed to fully understand the process and easily predict the final product's properties.

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