

THE APPLICATION OF CARRAGEENAN AND NON-ALCOHOLIC ANTHOCYANIN EXTRACTION OF *ROSA* SP. ON THE PHYSIC-CHEMICAL CHARACTERISTICS OF *ANNONA MURICATA* L. FRUIT LEATHER

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(Received: 9th Nov. 2020, Accepted: 25th May. 2021, Published on-line: 26th Jul. 2021)

ABSTRACT: Fruit leather is a healthy food with higher nutrition, but it has low plasticity. *Annona muricata* L. has potential as raw material due to high fibre and low pectin. *Rosa* sp. acts as the antioxidant and anthocyanin sources, while carrageenan as plasticity improvement. This research aimed to analyse the interaction of anthocyanin (10%; 15%; 20%) and carrageenan (0.1%; 0.2%; 0.3%) to the fruit leather physico-chemical characteristics. The research consisted of 2 main steps: (i) anthocyanin extraction from *Rosa* sp. used aquadest: citric acid (98:2) at 85°C for 30 mins. (ii) Application of anthocyanin on fruit leather. Randomised completed block design factorial was applied. The best fruit leather resulted by combination of anthocyanin 15% and carrageenan 0.3% with pH 3.65, water content 13.08%, antioxidant activity 87.61%, TDS 26.03°Brix, total acid titrated 4.23%, lightness (L) 41.07, redness (a+) 7.03, and yellowness (b+) 2.63.

KEYWORDS: Antioxidant, Halal extraction, Natural Pigment, Soursop

1. INTRODUCTION

Fruit leather production is a solution to increase the shelf life and preserve fruit. It produces from one or a mixture of fresh fruit. The first step was producing crushed fruit (puree) and followed the drying process of the puree in an oven or dehydrator. Fruit leather is in the form of thin sheets that have consistency and taste. It is usually consumed as a snack, a filling in pies, and a topping for desserts. The standard criteria of fruit leather are an attractive colour, a slightly tough and compact texture, good plasticity, can be rolled or not easily broken, (Histoarsih, 2010). Fruit leather has a shelf life of up to 12 months when stored in good packaging at room temperature, around 25°C – 30°C, (Safitri, 2012).

The Carbohydrate type found in soursop (*Annona muricata* L.) is reducing sugar (glucose and fructose) with levels of 81.9% – 93.6% of the total sugar. Soursop fruit contains very little fat (0.3

g per 100 g), (Suranto, 2011) and pectin content of 0.91%, (Bueso, 1980), resulting in it is very good for health. The high fibre content of *A. muricata* L. is about 3.3 g in 100 g of flesh; it facilitates digestion, especially constipation. *A. muricata* L. fruit consists of 67.5% edible pulp, 20% skin, 8.5% seeds, and 0.04% fruit core. The pH of *A. muricata* L. fruit decreased from 5.8 to 3.6, increasing acidity the 3-day ripening period.

Rose petals are rich in anthocyanin red pigments, antioxidants and essential fragrances, (Saati et al., 2020). Rosewater contains essential oil (0.04% – 0.07%), glucosides, bitter resin compounds, rubicansantin (carotenoids), catechinic tannins (up to 20%), anthocyanin (cyanine and flavanate glucosides, i.e., quercetin and quercitrin), saccharides, waxes, oils, gallic acid, minerals, and vitamins, i.e., Vit. C, B1, B2, and carotene. Anthocyanins are natural dyes that act as antioxidants found in plants, (Shamina et al., 2007).

The previous research showed that soursop (*A. muricata* L.) fruit leather with seaweed addition that contains carrageenan at 0.8% was the best treatment. The carrageenan, which acts as a gelling agent able to improve the texture of the fruit leather, resulted in better physical, chemical, and organoleptic characteristics. Commonly carrageenan is used at a concentration ranging from 0.005% to 3% in a wide variety of products. Many types of carrageenan have been made, some of which have been standardised for use as a gelling agent in water or milk, (Putri et al., 2013). Carrageenan comprises D-galactose and 3,6-anhydro-D-galactose units with β -1,3 and α -1,4 bonds in its hexose polymer. At the hydroxyl atom, the sulfate group is bonded with an ester bond. Therefore, the objectives of this research were to analyse the effect of anthocyanin concentration and carrageenan on the chemical, physical and organoleptic characteristics of fruit leather.

2. MATERIALS AND METHODS

This research consisted of 2 main steps; the first step was anthocyanin extraction from red roses (*Rosa* sp.) and formulation of fruit leather using different concentrations. Randomised completed block design factorial (RCBD) with 2 factors and 3 replications was applied. The first factor was anthocyanin concentration (10%, 15%, and 20%) and the second factor was carrageenan (0.1%; 0.2%, and 0.3%). The parameters analysis of raw material consisted of water content, total dissolved solids (TDS), pH, and total acid of soursop (*A. muricata* L.). The red roses pigment extract analyses were water content, pH, TDS, total anthocyanin, and antioxidant activity. While fruit leather analysed for its antioxidant activity, water content, total acid titrated, pH, colour intensity (L, a+, and b+), TDS, total anthocyanin, and organoleptic (taste, texture, appearance, and preferences).

2.1 Anthocyanin Extraction

According to Saati et al. (2011), anthocyanin pigments from red roses were extracted with a modification. The petals of the red rose were sorted, washed and weighed (90 g). The petals were extracted using the maceration method (with a ratio of 90 g petals:300 mL solvent) in a solution (with ration 98 distilled water:2 citric acids) at 85°C for 30 mins. Then the extracted sample was filtrated used Whatman filter paper 41.

2.2 Fruit leather Production

The fruit leather formulation was conducted according to Ramadhani (2016) method. The soursop (*A. muricata* L.) fruit was peeled, the pulp was taken, the seeds were removed, and the pulp was blended with water (1:1). The composition for fruit leather was 400 g of fruit pulp, red rose pigment extract (with a concentration of 10%, 15%, and 20%) then homogenized with carrageenan (0.1%; 0.2% and 0.3%), 20% sugar, and 0.1% citric acid. The mixture was heated with stirring at 70°C for 2 mins, followed by moulding in a baking sheet and dried using a cabinet dryer at 50°C for 24 hours.

3. RESULTS AND DISCUSSION

3.1 Puree *A. muricata* L.

The water content and pH of soursop (*A. muricata* L.) were higher than stated in the literature, as shown in Table 1. This is due to moisture content, the method used, analysis and storage processes, and the storage area temperatures. The addition of water during the processing of soursop (*A. muricata* L.) juice can also affect the analysis results obtained. Sudarmadji et al. (2003) stated that a higher maturity level leads to water content, total dissolved solids, and increased colour value. Furthermore, vitamin C, total acid, and hardness values decreased along with the maturity of fruits. The vitamin C in *A. muricata* L. is relatively high, about 20 mg in 100 g of puree. Vitamin C is water-soluble and prone to damage by stripping or cutting; thus, decreasing vitamin C affected the total acid content of raw material. The total dissolved solid was lower (13.6 °Brix) compared to the data stated in the literature (Table 1). Due to the addition of water during puree preparation, TDS consists of inorganic and organic water-soluble components such as sugar, protein, and minerals dissolved in water.

Table 1. Chemical composition of puree *A. muricata* L.

No.	Chemical-Composition	Total	
		Analysis	Literature
1.	Water Content (%)	87.32	82.674 ^a
2.	Total Dissolved Solid (TDS) (°Brix)	13.6	15.0 ^b
3.	pH	3.88	3.85 ^b
4.	Total Acid (%)	0.74	0.74 ^a

Reference: (a) Sukara, 2007; (b) Abbo, et al., 2006.

3.2 The Properties of *Rosa* sp. Extract

The analyses of water content, pH, total anthocyanin, and the *Rosa* sp. extract antioxidant activity were higher than the literature (Table 2). Saati (2016) stated that the addition of citric acid at the extraction process, which is a weak organic acid with a constant pKa of 3.14, affected the condition of the water become more acidic, anthocyanin content increased, and decreasing TDS. It also due to the anthocyanin pigments hydrolysis become anthocyanidins which are formed from glycone and aglycone, which are components of the total sugar, (Chan et al., 2009). Based on Leong and Shui (2002) experiment, acid extraction could increase the anthocyanin, becoming more stable. It is well known that anthocyanin consisted of a high-water content of 92.14%, which affects the [H +] ion that derived from organic acids that are diluted resulted [H +] ion that forms acids decrease.

Rose flower is also known to contain anthocyanin pigments classified as flavonoids, and the types are pelargonidin and cyanidine, which can function as free radical scavengers or antioxidants. Dark red roses contained cyanidine, and pink roses had pelargonidin, (Saati, 2016). Antioxidant activity is related to the flavonoid components present in food materials. In addition, there is an anthocyanin content that increases the value of the antioxidant activity. There is a high number of phytochemical components contained in roses, which can act as antioxidants, including tannins, geraniol, nerol, citronellol, geranic acid, terpenes, flavonoid, pectin, polyphenols, vanillin, carotenoids, stearopten, farnesyl, eugenol, phenyl ethyl alcohol, vitamins B, C, E, and K.

Table 2. Chemical properties of *Rosa* sp. extract.

No.	Properties	Total	
		Analysis	Literature
1.	Water Content (%)	92.14	90.02 ^a
2.	pH	2.54	2.15 ^b
3.	Total Dissolved Solid (TDS) (°Brix)	9.6	12.45 ^c
4.	Total Anthocyanin (mg L ⁻¹)	18.6	14.28 ^d
5.	Antioxidant Activity (%)	81.14	79.22 ^d

Reference: (a) Rahardjo et al., 2012; (b) Saati et al., 2014; (c) Saati, 2012; (d) Putri and Nisa, 2015.

3.3 Fruit Leather Analysis

3.3.1 Water Content of Fruit Leather

The anthocyanin concentration and carrageenan had a very significant effect on the fruit leather water content. The highest water content (14.57%) was in treating anthocyanin 20% and carrageenan 0.1%. While the lowest water content (12.15%) obtained 10% anthocyanin and 0.3% carrageenan. This value still met the Indonesian National Standard about dried fruit, (SNI 01-1718-1996); the maximum was 25%. The water content of fruit leathers decreased by increasing the concentration of carrageenan that acts as a gelling agent. Gel formation is a phenomenon of cross-linking polymer chains to form a mesh with a continuous two-dimensional structure.

Furthermore, this mesh immobilises the water in it and forms a solid and rigid structure. However, increasing carrageenan concentration caused free water and adsorbed water in the fruit

leather to decrease. Fruit leather is a processed food product derived from dried pulp until the water content ranges from 10% to 15%.

3.3.2 Total Dissolved Solid of Fruit Leather

Based on the analysis of variance, there was the interaction between anthocyanin concentrations and carrageenan on the total dissolved solids (TDS) of fruit leather (Table 3.). The highest yield (26.03 °Brix) was at the 15% anthocyanin and 0.3% carrageenan. At the same time, the lowest yield (21.43 °Brix) was shown by the treatment of 10% anthocyanin and 0.1% carrageenan. This result was lower than SNI No. 1718 requirement of about min. 40%. The low value of TDS caused by low pectin content in the *A. muricata* L. fruit used about 0.91%, (Bueso, 1980).

Table 3. Fruit leather water content and total dissolved solids.

Pigment (%)	Carrageenan (%)	Water Content (%)	TDS (°Brix)
0	0.1	12.68 ab	21.40 a
	0.2	12.23 a	22.80 ab
	0.3	12.43 a	24.60 cd
10	0.1	12.34 a	21.43 a
	0.2	12.29 a	21.80 a
	0.3	12.15 a	23.40 bc
15	0.1	12.59 ab	24.73 cd
	0.2	13.06 b	21.93 ab
	0.3	13.08 b	26.03 d
20	0.1	14.57 c	21.80 ab
	0.2	13.15 b	21.93 ab
	0.3	12.38 a	23.10 bc

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

The pectin formed a colloidal solution in water during the ripening process of the fruit. The pectin hydrolysis becomes dissolved components and increases the water-soluble components. It is known that soursop (*A. muricata* L.) fruit has a high carbohydrate content of 4% as glucose and fructose with levels of 81.9% – 93.6% of the total sugar content, (Suranto, 2011). Carrageenan as a gelling agent could bind water into a double helix structure, where this binding makes water-soluble components such as sugar be retained and read in total dissolved solids. The added pigment affects the pH to become acid, resulting in the covalent bond between the sugar and the anthocyanidin components.

3.3.3 pH of Fruit Lather

Based on the analysis of variance, there was no interaction between the concentration of anthocyanin and carrageenan to the pH value of fruit leather (Table 4.). Separately, anthocyanin concentrations had a very significant effect and the addition of carrageenan on the pH value of the fruit leather. The decreasing pH value was in line with the increase in the concentration of anthocyanin pigments, (Moldovan et al., 2012). Soursop (*A. muricata* L.) fruit used and water utilisation in extracting pigments and producing puree affected the pH value. It is known that anthocyanin pigments have a high water content of 92.14%, which affects the [H +] ion. The [H +] ion derived from organic acids is also diluted so that the [H +] ion that forms acids decreases and the material's pH will increase. This is following Winarno (2002), who stated that an acidic substance added to water causes an increase in hydrogen ion [H +] in water and a reduced hydroxide ion [OH-]; as a result, the pH of a substance will decrease and vice versa.

More carrageenan was added, resulting in a higher pH value of the fruit leather. The highest pH value was 3.70 at 0.3% carrageenan, while the lowest pH value was 3.61 at 0.1 % carrageenan. It was seaweed sap extracted with an alkaline solution; therefore, it tends to have alkaline and increase pH, (Prasetyaningrum et al., 2019). The pH value was in the range of 3 because the anthocyanin pigments used were acid (pH value 2.54) and the addition of citric acid in making fruit leather, so even though carrageenan can neutralise acid, it is low because acidity is more dominant. The addition of citric acid was to create stable conditions for anthocyanin pigment extracts, prevent degradation, and helps in the formation of gel (texture) fruit leather.

Table 4. Fruit leather pH and total acid titrated.

Treatment	pH	Total Acid Titrated (%)
Pigment (%) (v/b)		
0	2.88 c	2.69 a
10	2.79 b	3.05 b
15	2.72 b	3.29 bc
20	2.58 a	3.49 c
Carrageenan (%)		
0.1	3.61 aa	4.46 c
0.2	3.65 ab	4.14 b
0.3	3.70bb	3.91 a

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

3.3.4 Total Acid Titrated of Fruit Leather

Table 4. showed that total acid titration increased as anthocyanin concentration but decreased with the addition of carrageenan. The lowest total acid was found in the treatment with 0% anthocyanin concentration and the highest total acid at 20% pigment extract concentration. This was due to the anthocyanin extract's pH, so it is suspected that organic acids in rose flowers were also significant.

3.3.5 Colour of Fruit Leather

Based on analysis of variance, there was no interaction between the concentration of anthocyanin and carrageenan to lightness, redness, and yellowness (Table 5). But the anthocyanin concentrations separately had a very significant effect on the lightness, redness, and yellowness of fruit leather. The highest lightness was 32.08 at 0% pigment extract (control), and the lowest was 30.30 at 20% anthocyanin concentration. Anthocyanin pigments gave fruit leather a red colour; the higher concentration applied resulted in darker product colour and decreased lightness intensity. According to Rachmayati et al. (2017), the red colour of anthocyanin pigment tends to be thicker when applied in a high concentration. The maturity of raw materials used to affect lightness decreased due to the change in the material's colour becoming more concentrated.

Table 5. Fruit leather colour and antioxidant analysis.

Pigment (%) (v/b)	Lightness (L)	Redness (a⁺)	Yellowness (b⁺)	Antioxidant (%)
0	32.08 d	2.74 a	2.54 c	58.01 a
10	31.19 c	4.63 b	1.76 a	65.28 b
15	30.91 b	5.24 c	1.97 b	65.59 bc
20	30.30 a	5.91 d	1.70 a	66.04 c

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

The results showed that with a higher concentration of anthocyanin pigments added, the level of redness in the fruit leather was increasing. The redness value was affected by the anthocyanin concentration. This anthocyanin pigment has a red colour from the red rose used with acid pH. Anthocyanin pigments will stabilise at acidic conditions, despite heating and drying in making fruit leather. Saati (2012) identified that at high anthocyanin concentrations, resulting in redness intensity was also high and accompanied by decreasing in the lightness value. The higher level of redness of the product is also influenced by higher TDS in the material. It was due to higher pigment content bound to TDS, especially sugar, that resulted in a higher intensity of redness, (Saati et al., 2014).

The highest yellowness (b +) value was 2.54 at 0% anthocyanin (control), and the lowest level was 1.70 at 20% pigment extract. The addition of anthocyanin pigments decreased the yellowish value of the product. It was due to the white colour of soursop (*A. muricata* L.) containing carotenoid pigments and the addition of sugar that interacted during the drying process. Based on Wrolstad (2004) research, red rose flower extract is stable against agar because the intensity of the red colour increases and the yellow colour's intensity decreases with the addition of red rose flower extract. Therefore, red rose flower extract applied to agar showed a red or orange colour (a combination of red and yellow) as a characteristic of anthocyanin pigments.

3.3.6 Antioxidant Activity of Fruit Leather

The anthocyanin concentration had a very significant effect on the antioxidant (Table 5.). The highest anthocyanin pigment extract application (20%) has the highest antioxidant activity (66.04%). This result was higher than the anthocyanin content of blueberry, 28% – 59%, (Brownmiller et al., 2008). This is presumably due to anthocyanin pigments have synergistic properties with citric acid, which has been shown to function as antioxidants, (Saati et al., 2011). Anthocyanin is natural dyes that act as antioxidants and found in plants. It was more than 300 anthocyanin structures had been identified naturally, (Wrolstad, 2004). Roses also contain vitamins C found in hybrid and local varieties. Based on a previous study, it was found that vitamin C in hybrid roses was more significant (0.95 mg) than local roses (0.67 mg). Vitamin C content varies greatly depending on the variety, storage and processing all affect vitamin content. Anthocyanin is glycosides of anthocyanidin that is a phenolic class giving a blue-red-orange-purple colour. Nowadays, more than 540 anthocyanin pigments have been identified, most of the structural variations of substituted glycosides at positions 3 and 5 and the acylation of sugar residues with organic acids, (Navas et al., 2012).

3.4 Fruit Leather Organoleptic Analysis

3.4.1 Fruit Leather Taste

Based on the analysis of variance, anthocyanin pigment concentration and carrageenan had a very significant effect on the taste of the fruit leather (Table 6.). The highest taste value was in the 10% pigment and 0.2% carrageenan (3.56), while the lowest obtained to 0% pigment extract and 0.1% carrageenan (2.96). Based on 25 panellists, it was found that the fruit leather product had a sweet-sour taste and delicious enough. It was due to the pigment used had a sour taste which was indicated by the acidic pH (2.54). This was also related to the pigment extraction process using citric acid, which contributed to sour taste and its ability to prevent crystallisation of sugar in products and acts as a catalyst for hydrolysis of sucrose to invert sugar. Therefore, it can extend the shelf life, (Siskawardani et al., 2017). The soursop (*A. muricata* L.) fruit used also had a characteristic of sour-sweet taste. Carrageenan had not contributed to the taste, but its role in binding water could reduce acidity because water binds organic acids in the product.

3.4.2 Fruit Leather Texture

There was a significant interaction between anthocyanin pigment concentration and carrageenan on fruit leather texture (Table 6.). The highest texture value was in 10% anthocyanin pigment and carrageenan 0.3% (4.00); moreover, the lowest obtained to 10% anthocyanin pigment and 0.1% carrageenan (3.28). This range of 3–4 implied that fruit leather was relatively easy to roll and easy to roll. This is due to the addition of carrageenan, a hydrocolloid that acts as a gelling agent that binds water in the material. According to Gulrez et al. (2011), gel formation was a phenomenon of joining or cross-linking polymer chains to form a continuous three-dimensional mesh that immobilises the water and creates a solid and rigid structure. The thickness and the temperature and drying time also play a role in forming a rolled texture.

Table 6. Fruit leather organoleptic analysis.

Pigment (%)	Carrageenan (%)	Taste	Texture	Appearance	Preferences
0	0.1	2.96 a	3.64 aa	1.00 aa	2.24 aa
	0.2	3.00 a	3.56 ab	1.08 aa	2.76 bc
	0.3	3.08 a	3.68 cc	1.08 aa	2.60 bb
10	0.1	3.48 b	3.28 ab	2.15 cc	2.44 ab
	0.2	3.56 b	3.56 bc	2.38 bc	2.56 ab
	0.3	3.48 b	4.00 bc	2.62 cc	2.56 bb
15	0.1	3.52 b	3.60 ab	2.92 dd	3.00 cd
	0.2	3.40 b	3.64 bc	3.31 ee	3.36 dd
	0.3	3.48 b	3.68 bc	3.19 ee	3.96 ee
20	0.1	3.44 b	3.60 bc	3.92 ff	3.24 dd
	0.2	3.48 b	3.68 aa	3.92 ff	3.92 ee
	0.3	3.44 b	3.68 bc	4.08 ff	3.76 ee

Note: numbers followed by the same letter in a column were not significantly different on the Duncan Test of 5%.

3.4.3 Fruit Leather Appearance

It was known that with a higher concentration of pigment and carrageenan, the appearance value increased (Table 6.). The anthocyanin pigment concentration and carrageenan had a very significant effect on the appearance of fruit leather. The highest appearance was found in 20% anthocyanin pigment and 0.3% carrageenan (4.08); moreover, the lowest obtained to 0% anthocyanin pigment and 0.1% carrageenan (control) (1.00). Appearance assessment referred to the attractiveness of the colour and smoothness of the fruit leather surface. The effect of carrageenan on appearance due to binding water ability to form a gel with sugar affected the acidity. Anthocyanin pigments were stable at an acidic pH (3–4), resulting in [H +] ion was relatively slightly reduced because it was insoluble in the water of material, (Siskawardani et al., 2017).

3.4.4 Fruit Leather Preferences

The preference value refers to the attractiveness of the rolled colour and texture of the fruit leather. The highest preferences value was found in the 15% anthocyanin pigment and 0.3% carrageenan (3.96). But the lowest was in 0% anthocyanin pigment and 0.1% carrageenan (2.24) (Table 6.). Anthocyanin pigments play an important role in appearance value which affect attractiveness. It gave the product a red colour and improved the appearance of the fruit leather compared to controls (0% pigment extract). At the same time, the role of carrageenan in preference ratings was in the formation of gel (texture) fruit leather that was rigid but elastic. Determination of the best treatment was using the effective index method. The principle of this method was to compare all measured parameters. The best treatment was obtained with 15% anthocyanin extract and 0.3% carrageenan.

4. CONCLUSION

There was an interaction between anthocyanin concentrations and carrageenan to water content, total dissolved solids, and organoleptic (texture, appearance and preferences). The concentration of anthocyanin pigments separately had a significant effect on water content, TDS, pH, antioxidant activity, total titrated acid, colour intensity (L, a+, b+), and organoleptic (taste). Moreover, the addition of carrageenan separately had a very significant effect on water content, TDS, and taste. The best treatment was identified at treatment 15% anthocyanin and 0.3% carrageenan with characteristics consisted of: water content 13.08%; antioxidant activity 87.61%; TDS 26.03 °Brix; pH 3.65; total acid titrated 4.23%; lightness (L) 41.07; redness (a+) 7.03; yellowness (b+) 2.63; the total anthocyanin 28.68 mg L⁻¹. While organoleptic score showed 3.48 (quite tasty) for taste, 3.68 (easy to roll) for texture, 3.19 (interesting enough) for appearance, and 3.96 (quite like) for preference.

ACKNOWLEDGMENT

Thank you very much to the Directorate of Research and Community Service of Higher Education of the Republic of Indonesia, which has provided research grants on the topic of rose flower pigment. Also, gratitude feelings to the Directorate of Research and Community Services, University of Muhammadiyah Malang (DPPM-UMM) for supporting this research.

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