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The chemical properties of healthy drink from Pedada fruit (Sonneratia caseolaris) with natural dyes of Roselle (Hibiscus sadbariffa L.) and Beetroot (Beta vulgaris L.)

Meyci Trisna[1], Edi Kurniawan[2], Nina Hartati[3], Rajiyatul Muzni[4], Nagita Audya Agnes S[5], Tiara Andriani[6]

[1-6] State Polytechnic of Sriwijaya, Indonesia
* Correspondence e-mail; meyci.trisna@polsri.ac.id

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Abstract Mangrove plants play an important role in maintaining the balance of coastal ecosystems and also produce pedada fruit (Sonneratia caseolaris), which has the potential as a functional food ingredient. However, the use of pedada fruit as a healthy beverage product is still relatively limited. This study aims to develop a healthy beverage based on pedada fruit and analyze the chemical characteristics and safety of the product. The innovation process was carried out by making pedada fruit juice with the addition of roselle flowers and beetroot to improve taste and functional value. Parameters analyzed included temperature, pH, formole number, and arsenic (As) contamination content. The results showed that the resulting drink had a temperature of 33.5°C and a pH of 4.1. The addition of roselle flowers and beetroot contributed to a decrease in pH so that it was within the appropriate acidic range for fruit juice drinks, which is below 4.5, which supports the microbiological safety of the product. The formole number analysis showed an average value of 154, well above the minimum SNI standard of 15, indicating adequate free amino acid content and product quality that was maintained during the production and storage process. Testing for arsenic contamination yielded a concentration of 0.018 mg/kg, lower than the Indonesian National Standard (SNI) maximum limit of 0.1 mg/L. Therefore, the developed pedada fruit juice has good chemical quality, adequate nutritional value, and is safe for consumption.

Keywords Arsenic (As); Formol Index; Healthy Drinks; Peda Fruit; pH; Temperature

INTRODUCTION

Pedada (Sonneratia caseolaris) is a fruit from a mangrove plant, which has the characteristics of its base wrapped in a ball-shaped flower petal, and the tip of the fruit has a stalk (Rajis et al., 2017). In 100 g of pedada fruit flesh (Sonneratia sp.) there is a content of vitamin A of 221.97 IU, vitamin B 5.04 mg, vitamin B2 7.65 mg and vitamin C content of 56.74 mg (Manalu, 2011). Pedada (Sonneratia caseolaris) is a type of mangrove plant included in the Kingdom Plantae or plant group. Taxonomically, pedada is in the Subkingdom Tracheobionta, a group of vascular plants that have water and nutrient transport tissues. This plant is included in the Division Spermatophyta, namely seed plants, and the Class Magnoliopsida which is a group of dicotyledonous plants. At a more specific level, pedada belongs to the Rosidae Subclass and the Myrtales Order, which includes various types of flowering plants with certain characteristics. Furthermore, pedada belongs to the Lythraceae Family, a family of plants commonly found in tropical and subtropical regions. In the genus classification, pedada is in the Sonneratia Genus, which is known as a group of mangrove plants that are able to adapt to coastal environments and brackish waters. The species name is Sonneratia caseolaris, which is one of the mangrove species that produces fruit that can be used as a functional food and beverage ingredient. This taxonomic classification shows the scientific position of pedada in the plant grouping system and shows its closeness to other types of mangroves that have an important role in maintaining the balance of coastal ecosystems (Spalding et al., 2010).

This underutilized fruit is processed into a nutrient-rich fruit juice drink, with natural preservatives and coloring, resulting in a healthy drink free of harmful chemicals. Fruit juice drinks are a type of fruit processing that has gone through or without a filtering process after pressing, extracting, or crushing fresh fruit (Apriliani & Tamnrin, 2020). Meanwhile, beetroot and rosella flowers are used as natural coloring. Besides providing color, beetroot also has a varied nutritional content and is very beneficial for body health. Beetroot, a type of tuber plant, contains a number of important nutrients such as iron, vitamin C, potassium, phosphorus, magnesium, folic acid, and fiber. According to Wirakusumah, as quoted by Lenni (2015), beetroot contains carbohydrates, protein, fiber, various minerals, and has a high water content. Beetroot is rich in vitamins A, C, calcium, iron, phosphorus, protein, carbohydrates, folate, and betacyanin (Mulyani, 2015). Citing data from the Ministry of Agriculture (2012), beetroot contains 14.8% potassium, 13.6% fiber, 10.2% vitamin C, 9.8% magnesium, 1.4% tryptophan, 7.4% iron, 6.5% copper, 6.5% phosphorus, and coumarin. The chemical content in 100 grams of beetroot can be seen in Table 1.

Table 1. Nutritional content of red beetroot (per 100 g of ingredient)

Nutrition	Amount
Water (g)	87.58
Energy (kcal)	43.00

Protein (g)	1.61
Total lipid/fat (g)	0.17
Carbohydrates (g)	9.56
Fiber, total fiber (g)	2.80
Total sugar (g)	6.76
Calcium, Ca (mg)	16.00
Iron, Fe (mg)	0.80
Magnesium, Mg (mg)	23.00
Phosphorus, P (mg)	40.00
Potassium, K (mg)	325.00
Sodium, Na (mg)	78.00
Vitamin C, total ascorbic acid (mg)	4.90
Thiamin (mg)	0.03
Riboflavin (mg)	0.04
Niacin (mg)	0.33
Vitamin B6 (mg)	0.07
Vitamin E (mg)	0.04
Vitamin K (mg)	0.20
Saturated fatty acids	0.03

Apart from beetroot, other natural dyes are also used, namely rosella flowers.Roselle plants grow as tall shrubs ranging from 0.5-5 meters, have cylindrical, woody stems, and have many branches. When young, the stems are green. After reaching maturity and flowering, the roselle stems will turn reddish brown. Roselle leaves attached to the stems are oval with a green color, serrated edges, and palmate veins. The leaf tips are pointed and the leaf veins are red. Roselle leaves can be 6-15 cm long and 5-8 cm wide. The roots that support the stems are taproots. Roselle flowers have a funnel formed from five petals (Widyanto and Nelistya, 2009).

Table 2. Nutritional content of rosella flowers (Maryani and Kristina)

Nutrition	Amount
Calories, Calories	44
Water, %	86.2
Protein, g	1.6
Fat, g	0.1
Carbohydrates, g	11.1
Fiber, g	2.5
Abu, g	1.0
Calcium, mg	160

Phosphorus, mg	60
Iron, mg	3.8
Beta-carotene, mg	285
Vitamin C, mg	14
Thiamine, mg	0.04
Riboflavin, mg	0.6
Niacin, mg	0.5

METHODS

The pH and temperature values were determined to determine the physical characteristics and acidity level of the resulting fruit juice beverage. A quantity of fruit juice extract was placed into a beaker according to the specified formulation and ratio. The sample temperature was then measured using a thermometer, followed by a previously calibrated pH meter. Measurements were performed on each sample to obtain accurate data on the product's acidity level. The temperature and pH measurements were recorded and analyzed to determine whether they met fruit juice beverage quality standards.

The determination of the formol number was carried out to determine the content of free amino acids contained in fruit juice. A 25 mL sample of fruit juice was pipetted into a titration container, then neutralized using 0.25 N NaOH solution until it reached pH 8.1. After neutral conditions were achieved, 10 mL of formaldehyde solution was added to the sample. The solution was then titrated again using 0.25 N NaOH until it reached pH 8.1. If the volume of 0.25 N NaOH used in the titration exceeded 20 mL, the test was repeated by adding 15 mL of formaldehyde to the 25 mL sample that had been previously neutralized. The volume of NaOH used during the titration process was recorded as the basis for calculating the formol number. The value of the formol number was calculated using the equation:

Formol Number (mL 0.1 N alkali/100 mL)=a×(b/0.1)c×100

With the following information: a is the volume of 0.25 N NaOH used in the titration (mL), b is the normality of the NaOH used, and c is the volume of the sample analyzed (mL). The obtained formol number value is used as an indicator of the free amino acid content and the quality of the resulting fruit juice.

Arsenic (As) metal contamination testing was conducted using the Atomic Absorption Spectrophotometry (AAS) method to determine the safety level of fruit juice beverage products against heavy metal contamination. A total of 5–10 grams of sample was weighed and placed into a 250 mL Kjeldahl flask. Next, 5–10 mL of concentrated nitric acid (HNO_3) and 4–8 mL of concentrated sulfuric acid (H_2SO_4)

were added. After the initial reaction was complete, the mixture was heated and concentrated HNO_3 was added gradually until the sample turned brown or blackish. Then, 2 mL of 70% perchloric acid ($HClO_4$) was added little by little while continuing to heat until the solution became clear or yellow. If charring still occurred after the addition of $HClO_4$, then more concentrated HNO_3 was added as needed. The clear solution was cooled, then 15 mL of distilled water and 5 mL of saturated ammonium oxalate solution [$(NH_4)_2C_2O_4$] were added. The mixture is heated again until SO_3 vapor appears at the neck of the flask.

FINDINGSAND DISCUSSION

Results of pH and temperature measurements

The acidity level, or pH, is a crucial parameter in fruit juice formulation, playing a significant role in determining the product's taste, stability, and safety. Various studies have shown that the ideal pH for fruit juice beverages ranges from 3.0 to 4.5. This range is considered optimal because it meets several crucial criteria, including sensory balance, microbiological stability, and food safety.Previous research has demonstrated that low pH plays a crucial role in natural preservation. Guerrero-Beltrán and Barbosa-Cánovas (2004), in their study on the stabilization of fruit juices using high-pressure processing, found that a pH below 4.0 suppressed the growth of pathogenic microorganisms without the need for additional chemical preservatives. This suggests that proper pH control can extend the shelf life of products without compromising the nutritional or sensory quality of beverages.

González-Molina et al. (2008) also confirmed that a fruit juice pH within the acidic range (3.0 to 4.5) provides a fresh and natural flavor. In their study of orange juice, they found that a balanced sweet-sour flavor is produced at a pH of approximately 3.5 to 4.2. Juices with a higher pH tend to lose their characteristic freshness, while juices with a lower pH produce an overly sharp, acidic flavor that is undesirable to consumers. In addition to affecting flavor, low pH also serves as a key factor in microbiological preservation. According to Jay et al. (2005), pathogenic bacteria such as Clostridium botulinum cannot grow at a pH below 4.6. This study emphasizes the importance of maintaining the pH of fruit juices within the acidic range to prevent potentially harmful microbiological contamination. Therefore, industry standards stipulate that the pH of fruit juice beverages should be below 4.5 to ensure product safety.

However, other studies have also shown that too low a pH can affect the sensory quality of beverages. Sharma et al. (2012) found that apple juice with a pH below 3.0 experienced a significant decrease in taste and aroma perceived by a test panel. Too strong an acid creates a sharp sensation that is less pleasant for consumers, so very low pH should be avoided if flavor balance is a priority. Research by Kabasakalis et al. (2000) also highlighted that in addition to safety and taste, pH also affects the vitamin C content of fruit juices. This study showed that juices with a lower pH had more stable vitamin C levels during storage, but a decrease in pH below 3.0 could affect the content of other compounds that may decrease during storage.

Figure 1. Pedada fruit juice drink

The pH and temperature of pedada juice drinks in this study were measured using a pH meter. Based on the measurement results as shown in Table 3, the addition of rosella flower extract and beetroot causes the fruit juice drink to be more acidic than pure pedada extract.

Table 3. pH Value of Pedada Fruit Juice Drink with Rosella Flowers and Beetroot

Sample Code	Pedada Extract (mL)	Roselle Flower Extract (mL)	Beetroot Extract (mL)	Temperature (°C)	pH
A	20	-	-	33	4.77
B	20	-	10	33.3	4.51
C	20	2	10	33.3	4.22
D	20	4	8	33.5	4.04
E	20	6	6	33.4	3.69
F	20	8	4	33.5	3.8
G	20	10	2	33.6	3.79
H	20	10	0	34.2	3.6

The addition of rosella flowers and beetroot lowers the pH of pedada juice drinks. This is because rosella flowers contain organic acids, especially citric acid, malic acid and hibiscus acid. These organic acids are strong acids. Like rosella flowers, beets also contain oxalic acid, citric acid and malic acid which are naturally found in them. When rosella flowers and beetroot are added to fruit juice drinks, these acids will release hydrogen ions (H^+) into the solution, thereby lowering the pH of the drink and making it more acidic. The higher the concentration of acid contained in the rosella flowers and beetroot that is added, the greater the pH decrease that occurs in the fruit extract. Furthermore, the anthocyanin content in roselle flowers and beetroot makes them a safe source of food coloring. Anthocyanins are stable at pH 3–5 (Hidayah et al., 2014). Dewi et al. (2017) stated that adding roselle flower extract to Kalamansi orange powdered drink resulted in a decrease in pH, making it more acidic.

Results of Determination of Formol Number

The formol number is a number used to determine the amino acid content in fruit juice. This parameter is also influenced by compounds that can react with formaldehyde and at the same time increase the acidity of the fruit extract (Giuffre et.al, 2017). The results of measuring the formol number in fruit juice beverage samples show an important value in determining the content of free amino acids. The formol number is a test method used to measure amino acid content through reaction with formaldehyde, which can then be calculated based on the titration volume required to reach the equivalence point. This measurement produces a formole number, which reflects the amount of free amino acids in the sample. The higher the formole number, the greater the amount of free amino acids in the beverage. Free amino acids are important because they contribute to the nutritional value and flavor of fruit juice, as well as being an indicator of product quality and freshness.

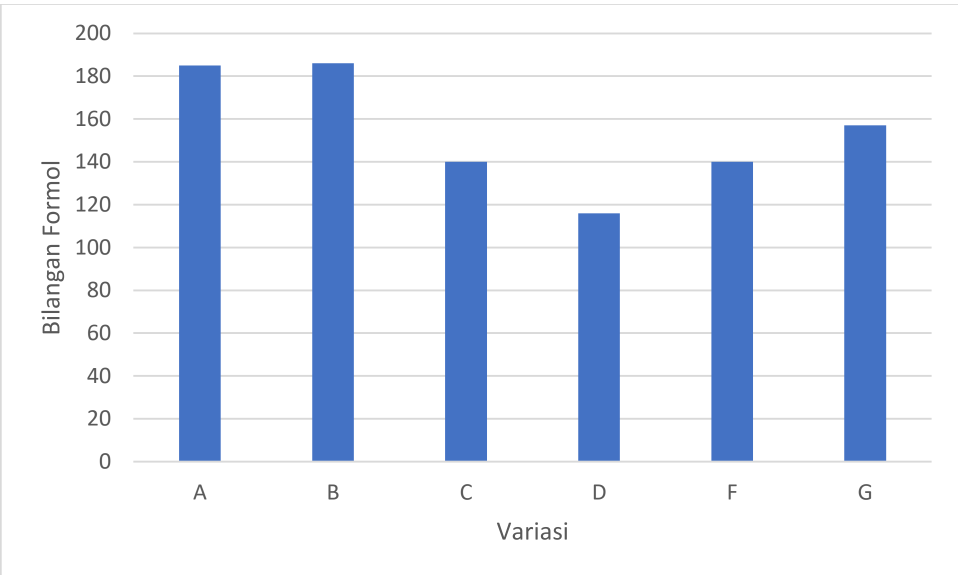

Figure 2. Graph of Formol Number Values of Fruit Juice Extracts with Various Variations

The measurement results showed that the formole number was within the range that meets the quality standards for fruit juice drinks, which is at least 15. This indicates that the drink contains an adequate amount of free amino acids, ensuring that its nutritional quality and freshness are maintained. This test is important to verify that the product does not experience any deterioration during the production and storage process. Based on the test results, the more rosella flowers added, the lower the formole number, even in variations E and H, the test could not be carried out. This is likely because phenolic compounds such as anthocyanins, flavonoids, and organic acids found in rosella flowers react with the aldehyde group of formaldehyde, thus interfering with the test results.

Arsenic (As) Metal Contamination Level Testing

Arsenic, especially in the form of inorganic arsenic, is extremely dangerous to human health. Long-term exposure to arsenic can cause various serious health problems, including chronic poisoning, kidney damage, neurological disorders, and even cancer. Global health agencies such as the WHO and FAO have categorized arsenic as one of the most dangerous carcinogenic (cancer-causing) substances. Arsenic can enter plants and fruit through contaminated soil or water. Soil exposed to industrial waste, water pollution, or the use of fertilizers and pesticides containing arsenic can increase the levels of this heavy metal in agricultural produce. Fruit juice derived from plants grown in areas with potential contamination is at risk of containing arsenic.
in certain levels.

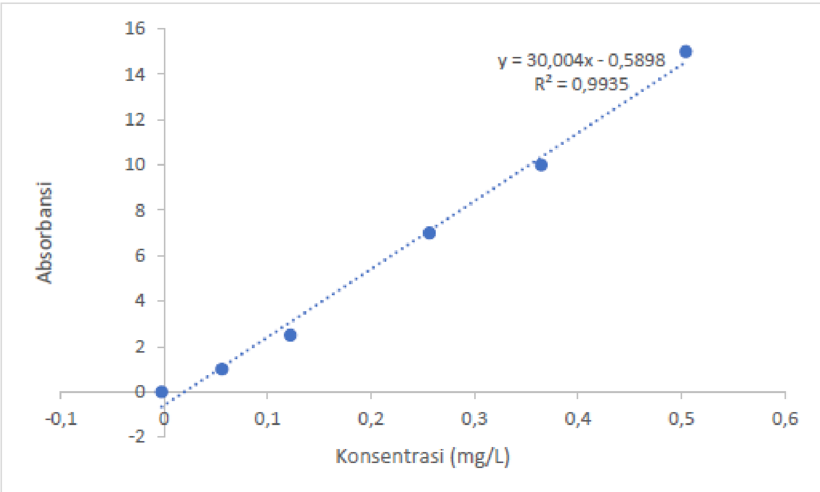

Figure 3. Standard calibration curve of Arsenic (As)

In this study, the levels of heavy metal Arsenic (As) were measured in several fruit juice samples obtained from various manufacturers. The measurements were carried out using the Atomic Absorption Spectroscopy (AAS) method, with the aim of ensuring the safety of the product based on the standards set by the Indonesian National Standard (SNI). Based on the analysis results, the levels of Arsenic (As) detected in all fruit juice samples were below the threshold value set by SNI, which is 0.1 mg/L. The As concentration value in the sample was 0.018 mg/Kg, indicating that the product is safe for consumption and complies with food safety standards.

CONCLUSION

The results of this research not only provide a solution to the Pedada fruit which has not been used properly, but also guarantee the quality of the drinks produced, with sufficient nutritional content and product safety from dangerous ingredients. The pH value, formol number and metal content in the pedada fruit juice drink extract with rosella flowers and beetroot meet the values set in the Indonesian National Standard.

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