



ORIGINAL ARTICLE

Physicochemical Properties and Sensory Characteristic of Isotonic Drink Incorporated with Roselle (*Hibiscus sabdariffa*) Extract

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Abstract

Roselle (*Hibiscus sabdariffa*) extract is a rich nutrient containing vitamins and antioxidants, but it remains underutilized in the food and beverage industries. This study aimed to explore the potential of roselle extract as a main ingredient in isotonic drinks, a commercially viable product. Four roselle-isotonic drink formulations were prepared, varying in types and concentration of sugar (sucrose, glucose and fructose) and salt (sodium citrate, sodium chloride and potassium phosphate), and labelled as F1, F2, F3 and F4. Then, each formulation was further processed into carbonated and non-carbonated samples. Physicochemical parameters (osmolality, pH, titratable acidity, total soluble solid and colour) and sensory characteristics were analyzed. There was significant difference in the values for osmolality, pH, titratable acidity and total soluble solid, while no significant difference was observed in colour. The samples containing different types of sugars and salts had pH values ranging from 2.48 to 2.59 and total soluble solid ranging from 6.25 to 6.70 °Brix. Results showed that simple sugars like glucose and fructose led to higher osmolality values, while carbonation was associated with increased acidity and lower pH. Sensory evaluation revealed that carbonated roselle isotonic drinks were generally preferred, with carbonated sucrose-sweetened formulation F3 (C) receiving the highest overall ratings for colour (5.70), aroma (4.74), sweetness (5.08), taste (5.02), aftertaste (5.12) and overall acceptability (5.16). These findings show that roselle extract has potential as a main ingredient, serving as both a natural flavor and color agent in isotonic drinks.

Keywords: Isotonic drink; physicochemical properties; roselle extract; sensory evaluation

Introduction

Isotonic drinks, also categorized as sport drinks are formulated to prevent dehydration and glycogen depletion in the liver and muscles. These drinks are designed to quickly replace fluids, electrolytes and carbohydrate, providing fuel for working muscles and giving instant energy. The drinks are suitable for consumption before, during and after exercise (Ruiz & Garcia, 2022). It is essential for isotonic drinks to taste good while simultaneously providing the necessary nutrients,

electrolytes and fluid lost during or after physical activities (Guo, 2009). The isotonic characteristic of these drinks is achieved through the appropriate amounts of ingredients, particularly carbohydrates and minerals. This isotonicity is beneficial as it ensures the rapid absorption of nutrients and water, helping to maintain hydration and promote faster recovery after sports activities or heavy work.

Roselle (*Hibiscus sabdariffa*) plants typically grow in tropical and subtropical regions, and it is underutilised in the food and beverage industries (Cod-Ortega & Guerrero-Beltran, 2015). Warm countries such as Saudi Arabia, Malaysia, India and Thailand are known for actively cultivating roselle plants. The primary producers of roselle flowers are Egypt, Sudan, Mexico, Thailand and China. The most marketed part of the roselle plant is its fleshy petals called sepals, which surround the fruit capsules (Al-Sayed et al., 2020). In Malaysia, the roselle plant is a relatively new crop, having been first introduced in the early 1990s. The Department of Agriculture in Terengganu was the first agency to promote the commercial planting of roselle in 1993, and since then, its cultivation has expanded to other states as well (Eslaminejad & Zakaria, 2011).

Isotonic drinks are important for individuals who lead an active lifestyle, as they are specially designed to promote rehydration during or after physical activities. However, most isotonic beverages contain flavorings and synthetic dyes. Nowadays, consumers are increasingly demanding healthier products derived from natural sources (Nazir et al., 2019). Therefore, natural sources such as roselle extract is seen as suitable natural ingredients for isotonic drinks due to its desirable nutrient profile. It is high in calcium, iron, niacin, riboflavin and rich with antioxidants such as anthocyanin, which act as free radical scavengers and inhibit lipid peroxidation in the human body (Hapsari et al., 2021; Shruthi & Ramachandra, 2019). Additionally, the anthocyanin compounds in roselle provide a natural red pigment, enhancing the visual appeal of isotonic drinks without the need for synthetic coloring agents. Given that roselle is rich in nutrients, particularly anthocyanin; hence this study aims to investigate the physicochemical properties (osmolarity, pH, titratable acidity, total soluble solid and colour) and sensory characteristics of roselle isotonic drink prepared using different sugar and salt types.

Materials and Methods

Roselle Extract Preparation

The calyces of roselle were harvested from the farm at UniSZA Kampus Besut, then unseeded and washed before proceeding to the hot water extraction process. The calyces were mixed with purified water in a ratio of 1:2, and the mixture was boiled for 30 minutes, and then filtered to obtain the roselle extract.

Roselle Isotonic Drink Preparation

Four roselle isotonic drink formulations, labelled F1, F2, F3 and F4 were prepared using the same amount of roselle extract (10%) and water, but with different types of sugars and salts, as shown in Table 1. F1 contains only sucrose (6%) with sodium salts (sodium citrate and sodium chloride), while F2 contains of sucrose (2%), glucose (2%) and fructose (2%) with the same sodium salts. F3 is similar with F1 in sugar type but include potassium phosphate, while F4 combines mixed sugars (sucrose, glucose and fructose) and includes both sodium and potassium salts. All the sugar and salt were mixed with purified water and roselle extract, and pasteurized at 85°C for 15 minutes. Then, each formulation was divided into two batches i.e. carbonated and non-carbonated samples.

The carbonated samples were labeled as F1 (C), F2 (C), F3 (C) and F4 (C), while the non-carbonated samples were labeled as F1 (NC), F2 (NC), F3 (NC) and F4 (NC). The carbonated

samples were gone through carbonation process by using sparkling water express (Soda Xpress Pinnacle, Malaysia). All samples were filled into the sterilized plastic bottles and stored at cool and ambient temperature for further studies.

Osmolarity Analysis

The osmolality of roselle isotonic drinks were determined by measuring the freezing point of the drinks. 10 mL of each sample was poured into boiling tubes. Then, the boiling tubes filled with sample were placed in the center of a 500 mL beaker filled with ice. Salt was added to the ice to lower its freezing point, which kept the ice cooler for a longer period. A digital thermometer was inserted into the boiling tube until the temperature dropped to 10°C. Afterward, the temperature was recorded every 30 seconds until crystals began to form. The freezing point was measured as the temperature at which crystal first appeared. The osmolality of the roselle isotonic drinks was calculated using Equation 1 based on the method described by Porfírio et al. (2020).

$$\text{Osmolarity (mOsm. kg}^{-1}\text{)} = \frac{T_c}{K_c} \times 1000 \quad (1)$$

Where $K_c = 1.86 \text{ }^{\circ}\text{C mol}^{-1} \text{ kg}^{-1}$ (cryoscopic water constant); T_c = freezing point temperature of the beverage samples in degrees Celsius.

Table 1. Formulations of roselle isotonic drinks

	Formulation			
	F1	F2	F3	F4
Extract roselle (%)	10	10	10	10
Purified water (%)	83.950	83.950	83.935	83.935
Sucrose (%)	6	2	6	2
Glucose (%)	-	2	-	2
Fructose (%)	-	2	-	2
Sodium citrate (%)	0.025	0.025	0.025	0.025
Sodium chloride (%)	0.025	0.025	0.025	0.025
Potassium phosphate (%)	-	-	0.015	0.015

pH Analysis

The pH measurement procedure was based on the AOAC (2005) method. A Thermo Scientific pH electrode (Orion Star A211, United States) was used for the pH analysis. The pH meter was first calibrated using phosphate buffers with pH 4.0 and 7.0 to standardize the instrument. Then, 10 ml of each sample formulation was transferred into a 20 mL beaker. The electrode probe was submerged into the sample while stirring it gently for a few seconds. The pH readings for each sample were recorded in triplicate to ensure precise measurements.

Titrateable Acidity (TA)

The method for titrateable acidity measurement was adapted from Nielsen (2017). 5 ml of the roselle isotonic drinks was homogenized in 30 mL of distilled water. Then, 3 drops of

phenolphthalein were added as an indicator. The homogenized sample was titrated against 0.05 M sodium hydroxide (NaOH) until the solution turned a pale pink colour. The amount of sodium hydroxide used was recorded, and the titratable acidity was calculated in percentage.

Total Soluble Solids Analysis

The total soluble solids of the roselle isotonic drink sample were determined using a digital refractometer (Atago PAL-1, Japan) (Cavalcanti et al., 2006). A few drops of each sample were placed on the measuring prism, and the measurement process was initiated. The °Brix reading, which represents the percentage of TSS in samples, was recorded. The °Brix reading for each sample was repeated three times to ensure accuracy.

Colour Measurement

The colour of the roselle isotonic drink samples was determined using the CieLab colour system (HunterLab MiniScan, United States) which measures the colour components (L^* , a^* and b^*) (AOAC, 2005). The L^* , a^* and b^* values for each formulation were recorded. The reading was taken in triplicate to ensure precise results.

Sensory Analysis

The sensory evaluation was approved by the UniSZA Human Research Ethics Committee (UHREC) and assigned the study protocol code (UniSZA/UHREC/2021/336). Fifty untrained panelists were randomly selected for this evaluation. The evaluation was conducted by using 7-point hedonic scale to determine consumer acceptance of the product samples. Samples of each roselle isotonic drink formulation were prepared in glass and coded with random three-digit number, with each sample assigned a different number. Randomization and permutation techniques were applied throughout the preparation of the sensory analysis. Each panelist was provided with eight different formulations of roselle isotonic drinks labelled with random three-digit number, a cup of drinking water and an informed consent form. They evaluated the coded samples for attributes including colour, aroma, sweetness, taste, aftertaste and overall acceptability. Panelists marked their evaluation on the scale for each attribute based on their degree of liking and the score were recorded for analysis.

Statistical Analysis

The statistical analysis was performed by using SPSS software. All the data were reported as mean and standard deviation using One-Way ANOVA method. The significance difference among the sample was compared by using significant level ($p < 0.05$).

Results and Discussion

Osmolarity and pH of Roselle Isotonic Drink

Osmolarity is one of the crucial parameters that need to be analyzed in the production of isotonic drinks. According to Malaysia Food Regulation 1985, isotonic drinks should have an osmolarity between 250 and 340 mOsm/kg. Fig. 1 shows the result of osmolarity and pH analysis for different formulations of roselle isotonic drinks. An osmolarity below 250 mOsm/kg is considered hypotonic, while above 340 mOsm/kg is considered hypertonic solution (Stasiuk & Przybyłowski, 2017).

Based on Fig. 1, the osmolality of samples F4 (NC), F2 (C) and F4 (C) exceeded 340 mOsm/kg, thus these formulations were not considered isotonic drinks. The osmolality of isotonic drinks is influenced by the type of sugar and salt used. As seen in Fig. 1, the addition of simple sugars such as glucose and fructose in formulations F2 and F4 increased the osmolality. This result aligns with the study by Campos-Pérez & Cámara-Martos (2019), which found that glucose contributes to a higher osmolality compared to sucrose and maltodextrin. Their findings also reported that beverages containing simple sugars can improve sports performance compared to use of others sugars such as maltodextrin and sucrose. Meanwhile, the higher osmolality in formulation F4 for both non-carbonated and carbonated samples may be due to the higher salt concentration. This is consistent with the report by Valenta and Dorofeeva (2018), which states that salt concentrations is directly proportional to osmolality.

The pH results for each formulation of roselle isotonic drinks are shown in Fig. 1. In general, the carbonated samples show slightly lower pH than non-carbonated samples. For example, the pH value for non-carbonated F4 (NC) is 2.56 ± 0.03 , while the carbonated sample is 2.49 ± 0.04 . This is likely due to the addition of carbon dioxide gas in carbonated samples, which increases the acidity of the drinks. During carbonation process, carbon dioxide is injected and dissolved in roselle isotonic drinks, forming a small amount of carbonic acid. The formation of carbonic acid increases acidity, leading to a decrease in pH value. The benefits of carbon dioxide in carbonated roselle isotonic drinks include removing oxygen from the headspace, thus inhibiting microbial growth and extending the shelf life of products (Newbold & Koppel, 2018). As illustrated in Fig. 1, the pH of roselle isotonic drinks ranged from 2.48 to 2.59 which indicates an acidic condition. According to Reddy et al. (2016), about 93% of sport drinks in the United States have a pH range between 2.5 and 4.0. Beverages with a pH below 4.0 are considered common for sport drinks

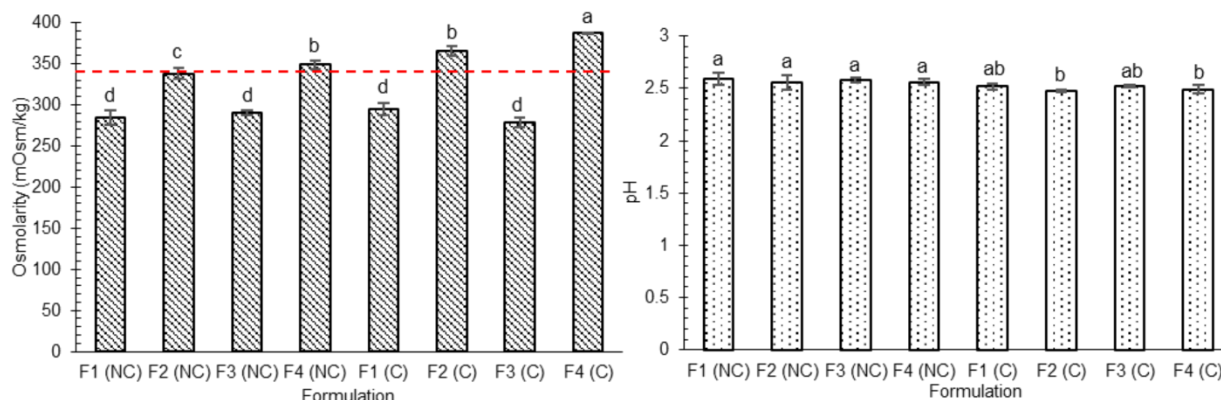


Figure 1. Osmolality and pH values of different formulation of roselle isotonic drinks

Titrateable Acidity and Total Soluble Solid of Roselle Isotonic Drink

Table 2 lists the results of total titratable acidity and total soluble solid for all formulations of isotonic roselle drinks. As we can see, the carbonated isotonic samples consistently exhibit significantly higher values for both titratable acidity and total soluble solid compared to their non-carbonated samples. For example, in the case of Formulation 4, F4, the non-carbonated sample, F4 (NC) shows a titratable acidity of 0.014 ± 0.02 . However, after carbonation, the carbonated formulation 4, F4 (C) displays a marked increase in titratable acidity to 0.044 ± 0.03 . This is because in carbonated samples, carbon dioxide dissolve in water and reacts with water molecules to form carbonic acid, a weak acid. The formation of carbonic acid increases the hydrogen ion concentration in the carbonated samples, which raises the titratable acidity and decreases the pH value (as shown in Fig. 1) (Tyl & Sadler, 2017).

Meanwhile, total soluble solid refers to the total amount of dissolved solids dissolved in a solution, primarily sugars such as sucrose, fructose, and glucose, as well as fruit acids, pectins and minerals (Tuan Azlan et al., 2020). Findings in Table 2 show that the range of total soluble solid is from 6.25 to 6.70 °Brix, which is comparable to most commercial isotonic drinks. According to Setyadjit et al. (2013), most commercial isotonic drinks have a total soluble solid around 6 °Brix. Table 2 also shows that formulations 2 and 4, for both non-carbonated and carbonated samples have higher total soluble solids than formulations 1 and 3. The type of sugar used may be a factor regulating the total soluble solids of the roselle isotonic drinks. Additionally, the total soluble solids may increase due to the structure of the hydrocolloid, which consists of linear and water-soluble polysaccharides, as well as simple sugars such as glucose and fructose (Tuan Azlan et al., 2020).

Table 2: Titratable acidity and total soluble solids of different formulation of roselle isotonic drinks

Formulation	Analysis	
	Total Titratable acidity (%)	Total soluble solid (°Brix)
F1 (NC)	0.015 ± 0.03 ^b	6.25 ± 0.08 ^d
F2 (NC)	0.018 ± 0.08 ^b	6.70 ± 0.01 ^a
F3 (NC)	0.017 ± 0.04 ^b	6.36 ± 0.01 ^c
F4 (NC)	0.014 ± 0.02 ^b	6.52 ± 0.03 ^b
F1 (C)	0.027 ± 0.01 ^a	6.28 ± 0.04 ^{cd}
F2 (C)	0.038 ± 0.07 ^a	6.65 ± 0.04 ^a
F3 (C)	0.041 ± 0.02 ^a	6.32 ± 0.08 ^{cd}
F4 (C)	0.044 ± 0.03 ^a	6.46 ± 0.07 ^b

Table 3. Colour values of different formulation of roselle isotonic drinks

Formulation	Colour values		
	L*	a*	b*
F1 (NC)	41.10 ± 3.57 ^a	29.31 ± 5.33 ^a	8.36 ± 3.31 ^a
F2 (NC)	38.43 ± 1.97 ^a	29.45 ± 1.71 ^a	8.81 ± 1.40 ^a
F3 (NC)	40.48 ± 1.39 ^a	30.49 ± 0.77 ^a	9.31 ± 1.40 ^a
F4 (NC)	39.32 ± 1.79 ^a	29.26 ± 2.65 ^a	7.27 ± 2.61 ^a
F1 (C)	40.63 ± 2.47 ^a	29.91 ± 3.51 ^a	8.30 ± 1.84 ^a
F2 (C)	39.38 ± 3.20 ^a	32.79 ± 3.31 ^a	10.38 ± 2.15 ^a
F3 (C)	37.80 ± 3.16 ^a	31.07 ± 1.53 ^a	10.12 ± 0.90 ^a
F4 (C)	39.35 ± 1.61 ^a	29.94 ± 4.01 ^a	9.53 ± 2.22 ^a

Colour Values of Roselle Isotonic Drink

Colour is one of the key parameters of food products that interacts with consumer perception and quality assessment, as it is the first sensory distribute that significantly influences the perception of freshness and quality (Hani, et al., 2019). Table 3 illustrates the colour values of all roselle

isotonic drinks and in general, there are no significant differences in the L^* , a^* and b^* colour values for all samples. The L^* value represents the lightness, with higher values indicating a lighter colour. The a^* value represents the redness and b^* value represents the yellowness of the sample. The findings show that the L^* , a^* and b^* values of the samples range from 37.80 to 41.10, 29.26 to 32.79 and 7.27 to 10.38, respectively. Overall, the variation in colour between all samples is minimal. The addition of carbon dioxide and the type of sugar and salt did not affect the colour variation of the roselle isotonic drinks. This is likely because the colour variation is primarily due to the roselle extract, which was used in the same amount for all samples.

Sensory Evaluation of Roselle Isotonic Drink

The sensory attributes of roselle isotonic drinks from four formulations (both non-carbonated and carbonated) were analyzed for colour, aroma, sweetness, taste, aftertaste and overall acceptability, as presented in Table 4. The results of the colour attribute indicated that there was no significant difference between the formulations, with hedonic scores ranging from 5.24 ± 1.32 to 5.70 ± 1.07 . All formulations exceeded the acceptable score for colour attribution, as all scores were above 5 (indicating a slightly preference). In commercial roselle beverages, the colour intensity is significantly influenced by the quantity and temperature of water used during the hot water extraction process. Anthocyanins are the key component responsible for the colour of roselle drinks (Darnal et al., 2021).

Table 4. Sensory attribute of different formulation of roselle isotonic drinks

Formulation	Sensory attribute					
	Colour	Aroma	Sweetness	Taste	Aftertaste	Overall acceptability
F1 (NC)	5.46 ± 1.15^a	4.42 ± 1.11^a	4.66 ± 1.42^a	4.78 ± 1.28^a	4.90 ± 1.20^a	4.88 ± 1.19^a
F2 (NC)	5.40 ± 1.21^a	4.56 ± 1.01^a	4.62 ± 1.31^a	4.56 ± 1.33^a	4.62 ± 1.34^a	4.72 ± 1.18^a
F3 (NC)	5.34 ± 1.24^a	4.48 ± 1.17^a	4.90 ± 1.27^a	4.88 ± 1.29^a	4.94 ± 1.22^a	4.98 ± 1.21^a
F4 (NC)	5.24 ± 1.32^a	4.56 ± 1.26^a	4.54 ± 1.30^a	4.54 ± 1.30^a	4.66 ± 1.33^a	4.72 ± 1.29^a
F1 (C)	5.56 ± 1.13^a	4.64 ± 1.01^a	4.78 ± 1.38^a	4.94 ± 1.35^a	4.86 ± 1.39^a	4.92 ± 1.18^a
F2 (C)	5.54 ± 1.25^a	4.66 ± 1.02^a	4.72 ± 1.25^a	4.88 ± 1.24^a	4.78 ± 1.36^a	4.96 ± 1.23^a
F3 (C)	5.70 ± 1.07^a	4.74 ± 0.94^a	5.08 ± 1.29^a	5.02 ± 1.13^a	5.12 ± 1.22^a	5.16 ± 1.15^a
F4 (C)	5.50 ± 1.15^a	4.60 ± 0.90^a	4.94 ± 1.25^a	4.98 ± 1.24^a	4.76 ± 1.38^a	4.96 ± 1.15^a

The hedonic scores also indicate no significant difference among the samples in terms of aroma, sweetness, taste and aftertaste. This is because the amount of roselle extract used in each sample was the same, resulting in similar characteristics. Additionally, the different types of sugar did not influence the sensory characteristics of the roselle isotonic drink. However, in general, the panellists preferred carbonated roselle drinks over non-carbonated ones across all formulations. For example, the score rating for F3 (C) was consistently higher than for F3 (NC). This may be because carbonation provides a unique texture and mouthfeel creating a refreshing sensation.

For overall acceptability, the result indicated no significance difference among the formulations. However, the carbonated formulation F3 (C) received the highest score, at 5.16 ± 1.15 . Table 4 shows that F3 (C) was the most preferred formulation in terms of overall acceptability, while the non-carbonated formulation F2 (NC) received the lowest score.

Conclusion

The findings of this study demonstrate the promising potential of roselle (*Hibiscus sabdariffa*) extract as a primary ingredient in isotonic drink formulations. The physicochemical analysis revealed that the sugar type significantly influenced osmolality, with simple sugars (glucose and fructose) resulting in higher values of osmolality.

Meanwhile, carbonation resulted in increased acidity and lower pH, contributing to the refreshing quality of the isotonic drinks. Carbonated roselle isotonic drinks were also preferred overall, particularly those sweetened with sucrose, which received the highest rating in sensory evaluations. These findings suggest that roselle extract can be effectively utilized in isotonic drink formulations, offering a functional and natural alternative in the beverage industry.

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