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Effect of Packaging Materials and Storage on Physicochemical Properties and Anthocyanin Contents of Red Cabbage-Roselle Blended Drink

Nur Farah Hani Muhamad^{1,2}, Faridah Hussin² and Norlelawati Arifin¹

¹Faculty of Science and Technology, Universiti Sains Islam Malaysia, Bandar Baru Nilai, 71800 Nilai, Negeri Sembilan, Malaysia.

²Food Science and Technology Research Centre, Malaysian Agricultural Research and Development Institute, Persiaran MARDI-UPM, 43400 Serdang, Selangor, Malaysia.

Correspondence should be addressed to:

Norlelawati Arifin; norlela@usim.edu.my

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Abstract — This study aimed to determine the effect of different packaging materials on physicochemical properties and anthocyanins content of red cabbage-roselle blended drink during 6-month storage. The pasteurised blended drink was hot-filled into three different packaging materials: glass bottle, beverage can, and polypropylene (PP) bottle, then stored in a dark place at ambient temperature (29 ± 2 °C). Packaging materials and storage time were found to affect physicochemical properties significantly and anthocyanins content of red cabbage-roselle blended drink packed in a glass bottle, can, and PP bottle during storage. Results show that pH, total soluble solids, colour L^* (lightness), and b^* (yellowness) of red cabbage-roselle blended drink increase significantly ($p < 0.05$) during storage at the ambient temperature, whereas titratable acidity, colour a^* (redness) and total anthocyanins content decrease significantly irrespective to packaging materials used. Blended drink packed in glass bottles significantly ($p < 0.05$) has the highest red colour retention and anthocyanins content, followed by blended drink stored in cans and PP bottles. Hence, glass bottles should be preferred as the most appropriate packaging material to store the blended drink, followed by cans and PP bottles as glass bottles provide better anthocyanins stability for a red cabbage-roselle blended drink.

Keywords — red cabbage-roselle blended drink; packaging materials; storage; anthocyanin

I. INTRODUCTION

Red cabbage and roselle possess health-promoting compounds, including phytochemicals, which are secondary metabolites, and natural antioxidants such as anthocyanins [1,2]. Red Cabbage (*Brassica oleracea*) is a herbaceous, biennial, and dicotyledonous flowering plant. Red cabbage is red or purple and is usually eaten as coleslaw, salad, and drink [3]. The main anthocyanins in red cabbage are cyanidin,

which occurs as cyanidin 3-sophoroside-5-glucoside and cyanidin 3,5-diglucoside acylated with sinapic, ferulic, malonic, and p-coumaric acids [4]. Roselle (*Hibiscus sabdariffa*) belongs to Malvaceae's family and is mainly cultivated in tropical and subtropical nations such as Indonesia and Malaysia [5, 6]. There are two main anthocyanins in the roselle calyx, specifically delphinidin-3-saubioside and cyanidin-3-sambubioside, besides two minor anthocyanins, delphinidin-3-glucoside and cyanidin-3-

glucoside [7, 8]. Roselle calyces have been used in various food products, including syrup, drinks, jams, sweet pickles, tea, and food colourants [9-11].

Blending the juices is one of the best ways to improve a beverage's nutritional quality as it usually offers a better quality juice nutritionally and organoleptically. Juice blends can be made from various fruits and vegetables to combine all the essential nutrients. Furthermore, blending various fruit and vegetable juices enhances storage stability and hinders microbial activity in juice. A previous study done by Chauhan *et al.* [12] noticed that juice blends that contained sugarcane (55%), lemon (2.58%) and ginger (2.0%) juices received the highest sensory score. Moreover, the blended juices can be stored for more than six months at ambient temperature (18 °C to 30 °C) with insignificant variations in composition and flavour. Similarly, Bhardwaj and Mukherjee [13] observed the kinnow (87%), pomegranate (10%), and ginger (3%) juice blends packed in glass bottles along with processing temperature (75 °C for 15 minutes) and potassium metabisulphite (750 ppm) exhibited better physicochemical properties and sensory characteristics in term of total soluble solids, acidity, ascorbic acid, total sugars, limonin, flavour, colour and bitterness during six months of storage at ambient condition (28 ± 4 °C). Thirukkumar *et al.* [14] reported blending the noni juice (80%) with amla juice (20%) improved the sensory qualities and preserved the highest level of ascorbic acid and total antioxidant activity during six-month of storage at chill (4 ± 1 °C) and ambient (32 ± 2 °C) conditions.

Amongst the most crucial quality attributes of an anthocyanin-rich product is its anthocyanin content's stability during processing and storage. Anthocyanins, on the other hand, are unsteady and easily destroyed during processing and storage. Many factors influence anthocyanin stability, which includes pH, processing and storage temperatures, light, the anthocyanins' chemical characteristics (acylation or glucosylation), enzymes, ingredient interactions, and copigmentation [15, 16]. In addition, the monomeric anthocyanins degradation, polymerisation of anthocyanins, and brown colour formation result from the colour degradation of juice containing anthocyanins [17, 18].

Packaging materials also play a key role in preserving juice's pigment stability. Packaging is an essential part of the food processing industry because packaging protects products from chemical and physical damage while also delivering information on product characteristics, nutritional facts, and ingredients [19]. Numerous packaging materials such as polypropylene (PP) bottles, cans, and glass bottles are the popular packaging materials used for fruit juice in Malaysia. Each packaging material will affect the stability of anthocyanin of juice differently. Thus, this study aimed to investigate the influences of packaging materials on the anthocyanin and physicochemical properties of red cabbage-roselle blended drink during six months of storage.

II. THE MATERIAL AND METHOD

A. Materials

Red cabbage was bought from a local vendor in Kompleks Pasar Borong Seri Kembangan in Selangor, Malaysia, and dried roselle calyces from MARDI Kuala Terengganu (Terengganu, Malaysia). Honey was obtained from Trigona Bee (Serdang, Selangor, Malaysia). The solvents and chemicals were supplied by Merck, which were of analytical grade (Darmstadt, Germany).

B. Methods

1) *Roselle and Red Cabbage Extraction*: The roselle was extracted using the method described by Chumsri *et al.* [20]. Dried roselle calyces were soaked in filtered tap water at a 1:10 ratio and boiled in a stainless-steel pot for 30 minutes at an extraction temperature of 50 °C. A muslin cloth was used to remove the roselle extract. A juice extractor (Model #28, Santos, France) was used to extract red cabbage juice, which was later filtered through a muslin cloth. The roselle and red cabbage juice extracts were maintained frozen at -18 °C, in high-density polyethylene (HDPE) bottles before the blended drink preparation.

2) *Preparation of Red Cabbage-Roselle Blended Drink*: The blended drink was prepared according to Nur Farah Hani *et al.* [21], by mixing and pasteurising the extracts with other ingredients, namely honey, sugar, Arabic gum, citric acid, water, and sodium benzoate at pasteurisation temperature of 70.01 °C and 284.07 s of pasteurisation time (Table 1). The pasteurised blended drink was then hot-filled at a temperature above 65 °C into three different packaging materials, namely glass bottle (250 ml), beverage can (250 ml), and polypropylene (PP) (250 ml) bottle. The packaging materials were then quickly cooled to ambient temperature by submerging the bottles in the iced water (8 ± 2 °C). Finally, the bottles were stored in a dark place at ambient temperature (29 ± 2 °C) for evaluation. For up to six months, three samples of each packaging material were removed for monthly analysis. According to Phimolsiripol and Suppakul [22], food products that have received a thermal treatment and or remained in specific conditions are considered highly stable food and have a medium to long shelf life. So, the red cabbage-roselle blended drink was regarded as a highly stable food. Therefore, measurements are made weekly or monthly to determine shelf life.

Table 1: Formulation of Red Cabbage-Roselle Blended drink

Ingredients	%
Red cabbage: roselle extracts (40:10)	70.00
Water	23.40
Honey	1.00
Sucrose	5.00
Arabic gum	0.50
Citric acid	0.10
Sodium benzoate	0.03

3) *Determination of Total Soluble Solids, pH and titratable acidity*: Total soluble solids (TSS) was determined using a pocket refractometer (Atago, Tokyo, Japan) on a scale of 0 – 53 °Brix. A pH meter was used to determine the pH of the samples (FE20, Mettler Toledo, Switzerland). Titratable acidity was determined by titrating 20 mL samples with 0.1 M NaOH till pH 8.1, the titratable acidity was calculated, and the results were expressed as milliequivalent/litre (mEq/L).

4) *Determination of Colour Intensity*: The colour intensity of the blended drink samples' was measured by placing 40 ml samples within a 20 mm glass cell and analysed using Chroma Meter Minolta CR-400/410 (Konica Minolta Sensing Inc., Osaka, Japan) based on the $L^* a^* b^*$ colour system. L^* denotes lightness on a 0 – 100 scale from black to white while a^* and b^* denote the hues, which represent two colour axes with a^* denoting redness (+) or greenness (–) and b^* denoting yellowness (+) or blueness (–). The instrument was calibrated with a white tile as described by the manufacturer.

5) *Determination of Total Anthocyanin Contents*: The total anthocyanin content was determined by the pH-differential method described by Guisti and Wrolstad [23], using potassium chloride buffer (pH 1.0) and sodium acetate buffer (pH 4.5). The mixed drink samples were diluted with buffer (pH 1.0) to obtain an absorbance reading of less than 1.0 at 520 nm. The dilution strength for the diluted sample should be the same for both pH 1 and pH 4.5. Absorbance was recorded using a UV-Vis spectrophotometer (Perkin Elmer, United Kingdom) at 520 nm and 700 nm against water as a blank. Before taking measurements, the measured samples should be clear and free of haze or sediments. The concentration of anthocyanin pigment was estimated and represented as cyanidin-3-glucoside equivalents as follows:

$$\text{Anthocyanin pigment (mg/L)} = \frac{A \times MW \times DF \times 10^3}{\epsilon \times l}$$

where $A = (A_{520} - A_{700})_{\text{pH 1.0}} - (A_{520} - A_{700})_{\text{pH 4.5}}$; MW (molecular weight) = 449.2 g/mol for cyanidin-3-glucoside (cyd-3-glu); DF = dilution factor; l = pathlength in cm; ϵ (molar absorptivity) = 26,900 molar extinction coefficients; in $L \times \text{mol}^{-1} \times \text{cm}^{-1}$, for cyd-3-glu; and 10^3 = factor conversion from g to mg.

6) *Statistical Analysis*: All analyses were done in triplicate. Experimental data were subjected to the analysis of variance (ANOVA), and The Least Significant Difference (LSD) was applied to determine the significance of variations between means at $p \leq 0.05$ using SAS software (Ver. 9.4., SAS Institute, Cary, NC, USA).

III. RESULTS AND DISCUSSIONS

A. Effect of Packaging Materials and Time of Storage on Total Soluble Solids

The amount of total solids dissolved in water is measured by the total soluble solids content [24]. Soluble solids are

made up of sugars and acids and small amounts of dissolved vitamins, fructans, proteins, pigments, phenolics, and minerals [25, 26]. Total soluble solids content for red cabbage-roselle blended drink stored in glass bottles, can, and PP plastic bottles ranged from 11.02 to 11.32 °Brix during the six months of storage (Table 2). According to Kamarul Zaman *et al.* [27], fruit juice's total solids content is specifically correlated to sugar and acids because juice with a low pH is comparatively rich in organic acids, leading to low total soluble solids content and vice versa. The results show that the total soluble solids content of the blended drink increases during the storage period. Generally, the total soluble solids content increase is significant ($p < 0.05$) with storage time regardless of the packaging material used. This increment could be attributed to the hydrolysis of polysaccharides like pectin, cellulose, and starch into simple sugars (monosaccharides), a rise in juice concentration triggered by dehydration, and the breakdown of pectic properties in juice to soluble solids [28, 29]. Bhardwaj and Nandal [30] found that the total soluble solids content of Kinnow juice was directly associated with storage duration. The rise in total soluble solids was higher at ambient conditions caused by the high solubilisation rate or hydrolysis of acid into sugars. Similarly, the increase in total soluble solids content throughout the storage period was also noticed in roselle-mango juice blends stored for six months [31], mango-sea buckthorn blended juice stored for 90 days [32], and Kinnow juice stored for six months [13].

It was evident that red cabbage-roselle blended drink packed in glass bottles significantly had the slightest chance of TSS value compared to other blended drinks. The TSS value of blended drinks packed in glass bottles, cans, and PP bottles increased by 1.45%, 2.63 % and 2.36 %, respectively, throughout the 6-month storage period (Table 2). It may be due to its inert nature and impermeable odours and vapours that can prevent certain reactions compared to cans and PP bottles [33].

Table 2: Total Soluble Solids (TSS) of Red Cabbage-Roselle Blended drinks in Different Packaging Materials

Storage time (month)	TSS (° brix)		
	Glass bottle	Can	PP bottle
0	11.02±0.04A ^d	11.03±0.05A ^d	11.02±0.04A ^d
1	11.03±0.05B ^{cd}	11.10±0.00A ^c	11.00±0.05C ^d
2	11.07±0.05B ^{bc}	11.12±0.04A ^c	11.00±0.00C ^d
3	11.08±0.04B ^{bc}	11.13±0.0A ^c	11.00±0.00C ^d
4	11.10±0.00B ^b	11.15±0.05A ^c	11.10±0.00B ^c
5	11.17±0.05B ^a	11.22±0.04A ^b	11.22±0.04A ^b
6	11.18±0.04B ^a	11.32±0.04A ^a	11.28±0.04A ^a

Notes: Means in the same row with the same upper-case letters are not significantly different at $p > 0.05$; Means in the same column with the same superscript lower-case letters are not significantly different at $p > 0.05$.

B. Effect of Packaging Materials and Time of Storage on pH

Table 3 presents the pH value of red cabbage-roselle blended drink stored in three types of packaging materials (glass bottle, can, and PP bottle) ranging from 3.58 to 3.79

during six months of storage at ambient temperature. It can be seen the pH value of the blended drinks increased gradually throughout the storage period. There was no significant difference in the pH of the drinks after 0 months of storage due to packaging materials or storage time. However, the pH of the blended drinks increased significantly ($p < 0.05$) beginning from the first month of storage for every packaging material. The increasing pH value trend was observed with a prolonged storage duration of every single packaging material.

The increment in pH value is followed by the decline in the titratable acidity of juice throughout the storage as they are inversely proportional to each other [8]. Similar findings were also reported in mango juice stored for eight weeks [35]; orange drink stored for seven weeks [19]; cashew apple juice stored for four months [36]; and roselle-mango juice blends stored for six months [31]. As mentioned above, findings also stated that the increasing trend of pH value is observed during storage regardless of packaging materials used. Furthermore, Mgaya-Kilima *et al.* [31] found that the pH value for roselle-mango juice blends was influenced by storage time and storage temperature.

The increment in pH value may be due to acid hydrolysis of some polysaccharides such as starch, cellulose, and pectin into disaccharides and monosaccharides, which are accountable for the increase in sweetness and decrease in sourness [28]. According to Mahadevan *et al.* [37], three water-soluble polysaccharides have been identified from the roselle calyx, which comprises arabinans and arabinogalactans. Moreover, red cabbage contains 15.48 % dietary fibre and 9.98 % cellulose, as reported by Komolka *et al.* [38], while roselle calyx contains 33.9 % dietary fibre as the largest component in the plant [39]. Dietary fibres include cellulose, pectins, gums, oligosaccharides, hemicelluloses, and several lignified substances, which are indigestible plant polysaccharides [40].

Table 3: pH of Red Cabbage-Roselle Blended drinks in Different Packaging Materials For 6 Months Storage

Storage time (month)	pH		
	Glass bottle	Can	PP bottle
0	3.59±0.01A ^g	3.58±0.01A ^g	3.58±0.01A ^g
1	3.64±0.01A ^f	3.65±0.00A ^f	3.63±0.01B ^f
2	3.68±0.01A ^e	3.67±0.01A ^e	3.66±0.01B ^e
3	3.71±0.01A ^d	3.71±0.01A ^d	3.67±0.01B ^d
4	3.72±0.01A ^c	3.72±0.01A ^c	3.70±0.01B ^c
5	3.75±0.01A ^b	3.74±0.01B ^b	3.72±0.00C ^b
6	3.79±0.01A ^a	3.76±0.01B ^a	3.76±0.01B ^a

Notes: Means in the same row with the same upper-case letters are not significantly different at $p > 0.05$; Means in the same column with the same superscript lower-case letters are not significantly different at $p > 0.05$.

C. Effect of Packaging Materials and Time of Storage on Titratable Acidity

Titratable acidity is a measure of the product's shelf life and protector against the attack of microorganisms. Titratable acidity value of red cabbage-roselle blended drink stored in a

glass bottle, can, and PP bottle ranged from 67.43 mEq/L to 57.28 mEq/L for six months of storage (Table 4). Table 4 shows that red cabbage-roselle blended drink shows a similar declining trend of titratable acidity value irrespective of packaging materials. The titratable acidity value gradually decreased during storage, probably due to converting acids into sugar and salt by invertase enzymes [34]. Mgaya-Kilima *et al.* [8] reported that titratable acidity value in roselle-mango juice blends in various packaging materials was significantly decreased during six months of storage. Besides that, Bhardwaj and Nandal [30] stated a decline in Kinnow juice's acidity as a prolonged storage period. Several factors influence the changes in the acidity of the beverages, such as storage temperature, storage period, and packaging materials [41].

Table 4: Titratable Acidity (mEq/L) of Red Cabbage-Roselle Blended drinks in Different Packaging Materials

Storage time (month)	Titratable Acidity (mEq/L)		
	Glass bottle	Can	PP bottle
0	65.43±0.31B ^a	65.27±0.20B ^a	67.43±0.40A ^a
1	63.33±0.14B ^b	64.13±0.83B ^b	66.93±1.58A ^{ab}
2	63.10±0.36B ^b	63.38±0.78B ^b	66.00±0.65A ^b
3	63.02±0.72B ^b	63.32±0.79A ^b	63.13±0.20A ^c
4	62.27±1.45A ^b	62.85±0.95A ^b	62.42±0.20A ^c
5	60.43±0.47A ^c	59.80±0.66A ^c	60.02±0.43A ^d
6	57.28±0.18B ^d	58.17±0.40A ^d	57.92±0.28A ^e

Notes: Means in the same row with the same upper-case letters are not significantly different at $p > 0.05$; Means in the same column with the same superscript lower-case letters are not significantly different at $p > 0.05$.

D. Effect of Packaging Materials and Time of Storage on Colour

Colour is an essential characteristic of food as consumers perceive colour as a preference of acceptability [27]. Lightness (L^*) for red cabbage-roselle blended drink stored in various packaging materials ranged from 22.13 to 27.87 after storage for six months at ambient temperature (Table 5). The L^* value increased with the storage period, indicating that the blended drinks were lighter in colour during storage.

Table 5: Colour L^* of Red Cabbage-Roselle Blended drinks in Different Packaging Materials

Storage time (month)	Colour L^*		
	Glass bottle	Can	PP bottle
0	22.16±0.05A ^f	22.15±0.07A ^f	22.13±0.02A ^f
1	22.05±0.12A ^f	21.98±0.01A ^g	21.97±0.10A ^g
2	22.76±0.03A ^e	22.62±0.10B ^e	22.83±0.09A ^e
3	23.11±0.13B ^d	22.87±0.04C ^d	23.97±0.10A ^d
4	25.49±0.32B ^c	25.92±0.05A ^c	25.81±0.15A ^c
5	25.87±0.09C ^b	26.98±0.22B ^b	26.68±0.13A ^b
6	26.90±0.05C ^a	27.15±0.03B ^a	27.87±0.14A ^a

Notes: Means in the same row with the same upper-case letters are not significantly different at $p > 0.05$; Means in the same column with the same superscript lower-case letters are not significantly different at $p > 0.05$.

A similar trend of increased L^* value (lightness) during storage was also observed in maqui berry juice stored for 70 days [42], coloured model juices made from anthocyanin-rich fruit and vegetable extracts (eggplant peel, strawberry, grape, bilberry, red raspberry, and plum peel) stored for 17 weeks [43] and sour cherry cloudy juices stored for 180 days [44]. Previous studies by Amperawati *et al.* [45] stated that the increment in L^* values during storage might be linked to the establishment of translucent extracts due to colour disappearance.

For six months of storage at ambient temperature, the redness (a^*) of red cabbage-roselle blended drink stored in cans, glasses, and PP bottles varied from 8.43 to 3.67 (Table 6). The anthocyanins in red cabbage and roselle extract contributed to the red colour of the blended drink. The a^* value decreased with longer storage duration regardless of the packaging material used. It can be seen that the blended drink stored in glass bottles significantly had the highest a^* value, while blended drink in PP bottles had the lowest a^* value after six months of storage. The red colour degradation can be visually observed in the blended drink packed in different packaging materials. It was proven by the similar decreasing trend of a^* values during six months of storage. The previous study by Amperawati *et al.* [45] explained that the decrease in a^* value (redness) could be associated with anthocyanin's degradation. When cloudy, sour cherry juices were stored for 180 days, there was a rise in lightness (L^* value) and a loss in redness (a^* value) [44]. The colour of anthocyanins depends on the pH of the red cabbage-roselle blended drink, as pH is among the significant factors that affect the pigment colour variations and stability. The pH of red cabbage-roselle blended drink during six months of storage in different packaging materials ranged between 3.58 and 3.79 (Table 3). Anthocyanin is more stable in acidic (low pH) conditions than in neutral or alkaline (high pH) conditions [46]. Generally, anthocyanins occur in the structure of four species in equilibrium depending on the pH, namely flavylium cation, quinoidal base, carbinol or pseudobase, and chalcone in aqueous solutions. The flavylium cation (red colour) is the dominant species at pH 1 and is responsible for purple and red. At pH values between 2 and 4, the quinoidal blue is the dominant species. Two colourless species, a carbinol pseudobase and a chalcone, can be noticed at pH levels between 5 and 6. Anthocyanins are degraded based on their substituent groups at pH values higher than 7.

Table 6: Colour a^* of Red Cabbage-Roselle Blended drinks in Different Packaging Materials

Storage time (month)	Colour a^*		
	Glass bottle	Can	PP bottle
0	8.45±0.06A ^a	8.43±0.05A ^a	8.48±0.13A ^a
1	6.81±0.03A ^b	6.13±0.21B ^b	6.16±0.06B ^b
2	5.71±0.13A ^c	5.16±0.18B ^c	4.64±0.11C ^c
3	5.41±0.13A ^d	4.92±0.15B ^d	4.02±0.05C ^d
4	5.28±0.13A ^e	4.86±0.19B ^d	3.98±0.14C ^{de}
5	5.28±0.13A ^e	4.81±0.09B ^d	3.88±0.04C ^e
6	5.17±0.08A ^e	4.81±0.17B ^d	3.67±0.03C ^f

Notes: Means in the same row with the same upper-case letters are not significantly different at $p>0.05$; Means in the same column with the same superscript lower-case letters are not significantly different at $p>0.05$.

Yellowness (b^*) for red cabbage-roselle blended drink stored in different packaging materials ranged from 0.05 to 3.22 after storage for six months (Table 7). The b^* value of the blended drink increased significantly ($p<0.05$) in all packaging materials. Blended drinks stored in PP bottles showed the highest b^* value, while blended drinks stored in glass bottles had the lowest b^* value after six months of storage. Pilando *et al.* [47] define that the increase of L^* and b^* values are due to anthocyanin loss. It has a strong inversely correlation of L^* and b^* values with total anthocyanins content. All samples of red cabbage-roselle blended drinks stored in different packaging materials had decreased total anthocyanin content, and the colours noticeably changed and receded along with longer storage time. This was due to the instability of anthocyanin in blended drinks. Furthermore, the increased L^* value could be linked to the establishment of translucent extracts due to colour fading. The decreased values of a^* may be due to the depletion of monomeric anthocyanin. In contrast, changes in b^* values, on the other hand, would be linked to the emergence of yellow chalcone species.

Table 7: Colour b^* of Red Cabbage-Roselle Blended drinks in Different Packaging Materials

Storage time (month)	Colour b^*		
	Glass bottle	Can	PP bottle
0	0.05±0.02A ^g	0.05±0.02A ^f	0.06±0.02A ^g
1	0.50±0.02C ^f	0.71±0.03B ^e	0.87±0.02A ^f
2	0.70±0.11C ^e	0.98±0.07B ^d	1.76±0.01A ^e
3	0.82±0.05C ^d	0.94±0.10B ^d	1.84±0.03A ^d
4	1.19±0.02B ^c	1.26±0.07B ^c	2.46±0.14A ^c
5	1.20±0.08C ^b	1.39±0.03B ^b	2.85±0.03A ^b
6	1.68±0.10C ^a	1.94±0.04B ^a	3.22±0.02A ^a

Notes: Means in the same row with the same upper-case letters are not significantly different at $p>0.05$; Means in the same column with the same superscript lower-case letters are not significantly different at $p>0.05$.

E. Effect of Packaging Materials and Time of Storage on Total Anthocyanins Content

Table 8 shows the total anthocyanins content of red cabbage-roselle blended drink in three types of packaging materials over six months of storage at ambient temperature. The total anthocyanin content of red cabbage-roselle blended drink at 0 months was similar to other studies [21]. The anthocyanin degradation was significant ($p<0.05$) during storage, regardless of the packaging materials used. The total anthocyanin content of the packed blended drink declined significantly after the first month, as shown in the table. More than half of the total anthocyanins content in every blended drink was lost after one month of storage in various packaging materials. The blended drink in PP bottles significantly showed the lowest reading of total anthocyanin content at 96.88 mg cyd-3-glu/L.

In contrast, the highest reading of total anthocyanin content was observed in glass bottles (132.10 mg cyd-3-glu/L). At 2-month storage, the total anthocyanin content of blended drinks in glass bottles, cans, and PP bottles decreased by 67 %, 64 %, and 66 %, respectively, compared to the

blended drinks at 0-month storage, but did not have any significant difference between packaging materials. At 3-month storage, the total anthocyanin content of blended drinks in glass bottles, cans, and PP bottles decreased by 72 %, 73 %, and 85 %, respectively. The blended drink stored in cans significantly showed the lowest total anthocyanin content value among all packaging materials used. There were significant differences ($p < 0.05$) for all blended drinks stored in different packaging materials for 4-, 5- and 6-month storage as the blended drink stored in glass-bottled recorded the highest total anthocyanin content remaining, followed by blended drinks stored in cans and PP bottles for the same period of storage.

The characteristics of the packaging materials used may have enlightened this observation. As mentioned before, several factors affect the stability of anthocyanins, such as light and oxygen. Among the three types of packaging materials, PP bottles are neither oxygen- nor light-proof. According to Ramos *et al.* [48], the variable permeability of plastic to light, gases (oxygen), and vapours are its significant drawbacks. Therefore, the total anthocyanin content values of blended drinks in PP bottles were the lowest compared to those packed in cans and glass bottles. Although cans are light and airtight, the decrease in total anthocyanin content in canned blended drinks may be due to the reaction of the cans' coating and the compound inside the blended drinks [49]. On the other hand, after six months of storage, the blended drink stored in the glass bottle showed the highest total anthocyanin content value. According to Franco and Falqué [50], glass is impermeable to oxygen and vapours and inert to chemical, biological, and physical effects. Furthermore, Pérez-Vicente *et al.* [51] discovered that during the storage of pomegranate juice, the types of glass (green or transparent) did not affect anthocyanin diminishment.

Table 8: Total Anthocyanins Content (mg cyd-3-glu/ L) of Red Cabbage-Roselle Blended drinks in Different Packaging Materials

Storage time (month)	Total Anthocyanins Content (mg cyd-3-glu/ L)		
	Glass bottle	Can	PP bottle
0	263.40 ± 1.67A ^a	262.66±0.87A ^a	262.17 ± 1.04A ^a
1	132.10 ± 3.60A ^b	113.20±3.96B ^b	96.88 ± 0.71C ^b
2	87.75±16.60A ^c	94.54±23.17A ^c	88.21±18.55A ^b
3	74.38±2.21A ^d	71.80±7.35A ^d	40.42±0.49B ^c
4	55.60±0.87A ^e	49.27±1.93B ^e	21.09±1.31C ^d
5	48.29±0.96A ^e	39.85±0.85B ^{ef}	14.38±0.17C ^e
6	34.36±1.83A ^f	30.50±0.17B ^f	10.44±1.42C ^e

Notes: Means in the same row with the same upper-case letters are not significantly different at $p > 0.05$; Means in the same column with the same superscript lower-case letters are not significantly different at $p > 0.05$.

IV. CONCLUSIONS

Packaging materials and storage time significantly affect the physicochemical and anthocyanins stability of blended drinks stored in glass bottles, cans, and PP bottles during six months of storage. Acid hydrolysis of some polysaccharides into simple sugars (monosaccharides) justifies the increase in

sweetness and decrease in sourness for pH, titratable acidity. Therefore, total soluble solids values during storage at ambient temperature, irrespective of packaging materials used. Meanwhile, the increase of colour L^* (lightness) and b^* (yellowness) values along with the decrease of colour a^* (redness) value in the red cabbage-roselle blended drink are associated with colour fading and loss of total anthocyanin content. Concerning packaging materials, blended drink packed in glass bottles has the highest retention of red colour and anthocyanins content, followed by blended drink stored in cans and PP bottles. As a result, glass bottles should be recommended as the ideal packaging materials for storing the blended drink, followed by cans and PP bottles, as glass bottles provide greater anthocyanin protection. Future studies can investigate the effect of various storage temperatures of red cabbage-roselle blended drink on physicochemical properties and anthocyanin content.

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REFERENCES

- [1] Bechoff, Aurélie, et al. Relationships between anthocyanins and other compounds and sensory acceptability of Hibiscus drinks. *Food Chemistry* 148 (2014): 112-119. doi: 10.1016/j.foodchem.2013.09.132
- [2] Podszędek, Anna, et al. Matrix effects on the stability and antioxidant activity of red cabbage anthocyanins under simulated gastrointestinal digestion. *BioMed research international* 2014 (2014). doi: 10.1155/2014/365738
- [3] E. S. Chauhan, A. Tiwari, and A. Singh, "Phytochemical screening of red cabbage (*Brassica oleracea*) powder and juice - A comparative study," *Journal of Medicinal Plants Studies*, vol. 4, (5), pp. 196-199, 2016.
- [4] Arapitsas, Panagiotis, Per JR Sjöberg, and Charlotta Turner. Characterisation of anthocyanins in red cabbage using high resolution liquid chromatography coupled with photodiode array detection and electrospray ionization-linear ion trap mass spectrometry. *Food Chemistry* 109.1 (2008): 219-226. doi: 10.1016/j.foodchem.2007.12.030
- [5] Dhar, Priyanka, et al. Chemistry, phytotechnology, pharmacology and nutraceutical functions of kenaf (*Hibiscus cannabinus* L.) and roselle (*Hibiscus sabdariffa* L.) seed oil: An overview. *Industrial Crops and Products* 77 (2015): 323-332. doi: 10.1016/j.indcrop.2015.08.064
- [6] Wu, Hai-Yao, Kai-Min Yang, and Po-Yuan Chiang. Roselle anthocyanins: Antioxidant properties and stability to heat and pH. *Molecules* 23.6 (2018): 1357. doi: 10.3390/molecules23061357
- [7] Cisse, Mady, et al. Impact of the extraction procedure on the kinetics of anthocyanin and colour degradation of roselle extracts during storage. *Journal of the Science of Food and Agriculture* 92.6 (2012): 1214-1221. doi: 10.1002/jsfa.4685
- [8] Mgaya-Kilima, Beatrice, et al. Influence of storage temperature and time on the physicochemical and bioactive

- properties of roselle-fruit juice blends in plastic bottle. *Food science & nutrition* 2.2 (2014): 181-191. doi: 10.1002/fsn3.97
- [9] Da-Costa-Rocha, Inês, et al. Hibiscus sabdariffa L.—A phytochemical and pharmacological review. *Food chemistry* 165 (2014): 424-443. doi: 10.1016/j.foodchem.2014.05.002
- [10] Ifie, Idolo, et al. The effect of ageing temperature on the physicochemical properties, phytochemical profile and α -glucosidase inhibition of Hibiscus sabdariffa (roselle) wine. *Food chemistry* 267 (2018): 263-270. doi: 10.1016/j.foodchem.2017.05.044
- [11] M. Osman, S. Saberi, A. Nezhadahmadi, Z. Hossain, and F. Golam, "Development and evaluation of fruit related morphological and physico-chemical characteristics in three roselle," *Pensee Journal*, vol. 75, (9), pp. 332-339, 2013.
- [12] S. K. Chauhan, V. K. Joshi, and B.B. Lal, "Preparation and evaluation of refreshing sugarcane juice beverages," *Journal of Science and Industrial Research*, vol. 56, (4), pp. 220–223, 1997.
- [13] Bhardwaj, R. L., and S. Mukherjee. Effects of fruit juice blending ratios on kinnow juice preservation at ambient storage condition. *African Journal of Food Science* 5.5 (2011): 281-286. doi: 10.5897/AJFS.9000111
- [14] S. Thirukkumar, P. Vennila and S. Kanchana, "Physico-chemical characteristics of noni fruit juice blended squashers during storage," *International Journal of Chemical Sciences*, vol. 6, (1), pp. 449-455, 2018
- [15] Bordenave, Nicolas, Bruce R. Hamaker, and Mario G. Ferruzzi. Nature and consequences of non-covalent interactions between flavonoids and macronutrients in foods. *Food & function* 5.1 (2014): 18-34. doi: 10.1039/C3FO60263J
- [16] West, Megan E., and Lisa J. Mauer. Color and chemical stability of a variety of anthocyanins and ascorbic acid in solution and powder forms. *Journal of Agricultural and Food Chemistry* 61.17 (2013): 4169-4179. doi: 10.1021/jf400608b
- [17] Turfan, Özge, et al. Anthocyanin and colour changes during processing of pomegranate (*Punica granatum* L., cv. Hicaznar) juice from sacs and whole fruit. *Food Chemistry* 129.4 (2011): 1644-1651. doi: 10.1016/j.foodchem.2011.06.024
- [18] Vegara, Salud, et al. Effect of pasteurization process and storage on color and shelf-life of pomegranate juices. *LWT-Food Science and Technology* 54.2 (2013): 592-596. doi: 10.1016/j.lwt.2013.06.022
- [19] S. K. Anin, W.O. Ellis, and J. Adubofuor, "Effects of two packaging materials and storage conditions on the quality of fresh taste, a natural and locally produced orange drink in Ghana," *African Journal of Food Science and Technology*, vol. 1, (6), pp. 132-138, 2010.
- [20] P. Chumsri, A. Sirichote, and A. Itharat, A. "Studies on the optimum conditions for the extraction and concentration of roselle (*Hibiscus sabdariffa* Linn.) extract," *Songklanakarin Journal Science and Technology*, vol. 30, pp. 133 – 139, April 2008.
- [21] M. Nur Farah Hani, N. Huda-Faujan, Y. Hafiza, H. Faridah, and N. Arifin, "Optimisation of mixed drink made from red cabbage (*Brassica oleracea* L.) and roselle (*Hibiscus sabdariffa* L.) extracts," *Malaysian Applied Biology*, vol. 49, (3), pp. 129–138, 2020.
- [22] Y. Phimolsiripol, and P. Suppakul, "Techniques in shelf life evaluation of food products," *Reference Module in Food Science*, vol. 1, pp. 1–8, 2016.
- [23] Giusti, M. Mónica, and Ronald E. Wrolstad. Characterization and measurement of anthocyanins by UV-visible spectroscopy. *Current protocols in food analytical chemistry* 1 (2001): F1-2. doi: 10.1002/0471142913.faf0102s00
- [24] S. R. Gindi, K. C. Chung, S. C. P. Lun, and H. S. Ling, "Physicochemical characteristics and proximate analysis of fruit jam from *Baccaurea angulata* peel," *Borneo Journal of Sciences and Technology*, vol. 1, (2), pp. 74-77, 2019.
- [25] Chope, Gemma A., Leon A. Terry, and Philip J. White. Effect of controlled atmosphere storage on abscisic acid concentration and other biochemical attributes of onion bulbs. *Postharvest Biology and Technology* 39.3 (2006): 233-242. doi: 10.1016/j.postharvbio.2005.10.010
- [26] Magwaza, Lembe Samukelo, and Umezuruike Linus Opara. Analytical methods for determination of sugars and sweetness of horticultural products—A review. *Scientia Horticulturae* 184 (2015): 179-192. doi: 10.1016/j.scienta.2015.01.001
- [27] A. A. Kamarul Zaman, R. Shamsudin, and N. Mohd Adzahan, "Effect of blending ratio on quality of fresh pineapple (*Ananas Comosus* L.) and mango (*Mangifera Indica* L.) juice blends," *International Food Research Journal*, vol. 23, (Suppl), S101-S106, 2016.
- [28] Pareek, Sunil, Ravinder Paliwal, and Subrata Mukherjee. Effect of juice extraction methods and processing temperature-time on juice quality of Nagpur mandarin (*Citrus reticulata* Blanco) during storage. *Journal of food science and technology* 48.2 (2011): 197-203. doi: 10.1007/s13197-010-0154-6
- [29] M. A. Rehman, M. R. Khan, M. K. Sharif, S. Ahmad, and F. H. Shah, "Study on the storage stability of fruit juice concentrates," *Pakistan Journal of Food Sciences*, vol. 24, (2), pp. 101- 107, 2014.
- [30] Bhardwaj, R. L., and Urvashi Nandal. Effect of storage temperature on physico-chemical and sensory evaluation of kinnow Mandarin juice blends. *Journal of Food Processing & Technology* 5.8 (2014): 1. doi: 10.4172/2157-7110.1000361
- [31] Mgaya-Kilima, Beatrice, et al. Physicochemical and antioxidant properties of roselle-mango juice blends; effects of packaging material, storage temperature and time. *Food science & nutrition* 3.2 (2015): 100-109. doi: 10.1002/fsn3.174
- [32] R. U. Khan, S. R. Afridi, M. Ilyas, H. Abid, M. Sohail, and S. A. Khan, "Effect of different chemical preservatives on the storage stability of Mango Sea Buckthorn blended juice," *Pakistan Journal of Biochemistry and Molecular Biology*, vol. 45, (1), pp. 6–10, 2012.
- [33] Matche, Rajeshwar S. Packaging technologies for fruit juices. *Fruit Juices*. Academic Press, 2018. 637-666. doi: 10.1016/B978-0-12-802230-6.00032-1
- [34] Singh, Shailesh Kumar, and Madhu Sharma. Review on biochemical changes associated with storage of fruit juice. *Int. J. Curr. Microbiol. Appl. Sci* 6.8 (2017): 236-245. doi: 10.20546/ijemas.2016.501.032
- [35] Alaka, O. O., J. O. Aina, and K. O. Falade. Effect of storage conditions on the chemical attributes of Ogbomoso mango juice. *European Food Research and Technology* 218.1 (2003): 79-82. doi: 10.1007/s00217-003-0800-6
- [36] Tamuno, Emelike Nkechi Juliet, and Ebere Caroline Onyedikachi. Effect of packaging materials, storage conditions on the vitamin C and pH value of cashew apple (*Anacardium occidentale* L.) juice. *Journal of Food and Nutrition Sciences* 3.4 (2015): 160-165. doi: 10.11648/j.jfns.20150304.14
- [37] N. Mahadevan, S. Kamboj, and P. Kamboj, "Hibiscus sabdariffa Linn. —An overview," *Natural Product Radiance*, vol. 8, (1), pp. 77-83, 2009.
- [38] P. Komolka, D. Górecka, and K. Dziejczak, "The effect of thermal processing of cruciferous vegetables on their content of dietary fiber and its fractions," *Acta Scientiarum Polonorum, Technologia Alimentaria*, vol. 11, (4), pp. 347-354, 2012
- [39] Sáyago-Ayerdi, Sonia G., et al. Dietary fiber content and associated antioxidant compounds in roselle flower (*Hibiscus sabdariffa* L.) beverage. *J. of Agricultural and Food Chemistry* 55.19 (2007): 7886-7890. doi: 10.1021/jf070485b

- [40] S. Ullah, A. A. Khalil, F. Shaukat, and Y. Song, "Sources, extraction and biomedical properties of polysaccharides," *Foods*, vol. 8, (8), pp. 1-23, 2019.
- [41] S. Thirukkumar, and P. Vennila, "Processing of Blended Beverages and Its Storage Stability" in *Trends & Prospects in Processing of Horticultural Crops*. New Delhi: Today & Tomorrow's Printers and Publishers. pp. 105-120, 2019.
- [42] Gironés-Vilaplana, Amadeo, et al. A novel beverage rich in antioxidant phenolics: Maqui berry (*Aristotelia chilensis*) and lemon juice. *Lwt* 47.2 (2012): 279-286. doi: 10.1016/j.lwt.2012.01.020
- [43] Hernández-Herrero, José Antonio, and María José Frutos. Degradation kinetics of pigment, colour and stability of the antioxidant capacity in juice model systems from six anthocyanin sources. *International journal of food science & technology* 46.12 (2011): 2550-2557. doi: 10.1111/j.1365-2621.2011.02780.x
- [44] Wojdyło, Aneta, Paulina Nowicka, and Mirosława Teleszko. Degradation kinetics of anthocyanins in sour cherry cloudy juices at different storage temperature. *Processes* 7.6 (2019): 367. doi: 10.3390/pr7060367
- [45] S. Amperawati, P. Hastuti, Y. Pranoto, and U. Santoso, "The anthocyanins content, colour changes and thermal stability of roselle (*Hibiscus sabdariffa* L.) petal extract," *International Journal of Science and Research*, vol. 8, (4), pp. 428-435, 2019
- [46] Akkarachaneeyakorn, Suthida, and Sirikhwan Tinrat. Effects of types and amounts of stabilizers on physical and sensory characteristics of cloudy ready-to-drink mulberry fruit juice. *Food science & nutrition* 3.3 (2015): 213-220. doi: 10.1002/fsn3.206
- [47] PILANDO, LETICIA S., RONALD E. WROLSTAD, and DAVID A. HEATHERBELL. Influence of fruit composition, maturity and mold contamination on the color and appearance of strawberry wine. *Journal of Food Science* 50.4 (1985): 1121-1125. doi: 10.1111/j.1365-2621.1985.tb13025.x
- [48] Ramos, Marina, et al. New trends in beverage packaging systems: A review. *Beverages* 1.4 (2015): 248-272. doi: 10.3390/beverages1040248
- [49] W. M. Siah, H. Faridah, M. Z. Rahimah, S. Mohd Tahir, and D. Mohd Zain, "Effects of packaging materials and storage on total phenolic content and antioxidant activity of *Centella asiatica* drinks," *Journal of Tropical Agriculture and Food Science*, vol. 39, (1), pp. 1-7, 2011.
- [50] I. Franco, and E. Falqué, "Effect of different types of glass container on food shelf life," *Reference Module in Food Sciences*, pp. 1-5, 2016
- [51] Pérez-Vicente, A., et al. Influence of packaging material on pomegranate juice colour and bioactive compounds, during storage. *Journal of the Science of Food and Agriculture* 84.7 (2004): 639-644. doi: 10.1002/jsfa.1721