

Effect of packaging materials and storage time on anthocyanins stability of red cabbage-roselle mixed drink

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Abstract

This study aimed to investigate the effect of different packaging materials on anthocyanins' stability of red cabbage-roselle mixed drink during 6-month storage. The mixed drink was pasteurised and hot-filled into three different packaging materials, i.e., transparent glass bottle, beverage metal can, and polypropylene (PP) bottle, then stored at ambient temperature ($29\pm 2^\circ\text{C}$). During storage, packaging materials and storage time significantly affect the anthocyanin content, colour density, and polymeric colour percentage of red cabbage-roselle mixed drink packed in a glass bottle, can, and PP bottle. Polymeric colour percentage and degradation index of red cabbage-roselle mixed drink were found to increase significantly during storage at the ambient temperature, whereas total anthocyanin content and colour density decreased significantly irrespective of packaging materials used. The correlation analysis reveals that total anthocyanin content has a significant positive correlation with colour density but negatively correlates with polymeric colour percentage and degradation index regardless of packaging materials used. The total anthocyanin content decreases significantly irrespective of the packaging materials used. The mixed drink in glass bottles significantly ($p < 0.05$) has the highest retention of anthocyanins content, followed by mixed drinks stored in cans and PP bottles. Therefore, glass bottles should be chosen as the most suitable packaging material to store the mixed drink, followed by cans and PP bottles as glass bottles provide better stability of anthocyanins for a red cabbage-roselle mixed drink.

1. Introduction

Nowadays, changes in lifestyle and awareness regarding a healthy and balanced diet have directed the growth of the world juice market. Juice produced using more than one kind of fruit or vegetable is also known as a juice blend. Recently, fruit juice blends have been widely marketed because of their distinctive and pleasant flavours (Jan and Masih, 2012). A juice blend is one of the best methods to improve the juice's nutritional quality. A combination of fruits and vegetables can provide basic nutrients and give better quality juice, nutritionally and organoleptically (Akusu *et al.*, 2016).

Roselle juice is a popular juice among Malaysians. On the other hand, pure roselle juice has a sour and bitter taste (Mgaya-Kilima *et al.*, 2015). Juice blends' aroma, taste, nutritional, and antioxidant properties can be improved by combining roselle extract with other anthocyanin-rich plants such as red cabbage. Red

cabbage is underutilised in Malaysia, where it is commonly used in coleslaw and salad. The use of red cabbage in the food industry can be varied because it contains many health benefits, such as anthocyanins. According to Nur Farah Hani *et al.* (2019), the mixed drink containing roselle and red cabbage extracts had significantly better anthocyanin content than the drink containing only roselle juice extract or red cabbage juice extract.

Anthocyanins are water-soluble plant pigments frequently found in numerous fruits and vegetables. Anthocyanins have beautiful colouration on food products and exhibit antioxidant properties and health benefits such as improving sight acuteness, antioxidant capacity, control of Type II diabetes, reduction of coronary heart disease, and cancer prevention (Peleg *et al.*, 2015). *Hibiscus sabdariffa*, also known as roselle, is widely grown in Malaysia and other countries such as

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Indonesia, Africa, and America (Aishah *et al.*, 2013). Roselle's calyces are bright red due to the presence of anthocyanin compounds. The calyx consists of two main anthocyanins, namely delphinidin-3-sambubioside and cyanidin-3-sambubioside.

In comparison, red cabbage (*Brassica oleracea*) belongs to the Brassicaceae family, a native vegetable of the Mediterranean region and southwestern Europe (Arapitsas *et al.*, 2008). Red cabbage has twenty-four anthocyanins, which had been separated and identified. Its anthocyanins are unique, and can exhibit colour over an extensive pH range. According to Mazza and Miniati (1993), the colours of anthocyanins from red cabbage vary from red at low pH (acidic condition) to blue and green at high pH (alkaline condition). There are many usages of red cabbage anthocyanins such as colouring in various beverages, candies, dry mixed concentrates, chewing gums, yoghurts, and sauces (Dorota and Janusz, 2007).

Anthocyanin stability is affected by many factors, including light, pH, temperature, oxygen, ingredient interactions, and co-pigmentation (Bordenave *et al.*, 2014). Undeniably, elevated temperatures affect anthocyanin stability, which can cause monomeric anthocyanins to become polymerised, thus subsequent in browning, which is undesirable in fruit juices since consumers notice it as an indication of inferior quality (Kirca and Cemeroglu, 2003). However, anthocyanins are unstable and degrade during processing and storage, resulting in colourless or brown-coloured products, further losing most of their beneficial health properties (Pérez-Ramírez *et al.*, 2015). Although storage may further degrade anthocyanins, the anthocyanin stability in food products can be significantly affected by storage conditions (Hellstrom *et al.*, 2013). Food packaging can delay the degradation of products, preserve the beneficial effects of processing, prolong shelf-life, and sustain or enhance food quality and safety (Ramos *et al.*, 2015). Therefore, packaging can play a vital function in protecting anthocyanin stability after processing and during the storage of anthocyanin-rich products.

Anin *et al.* (2010) asserted that packaging in the food processing industry is a significant factor in protecting products from chemical and physical damage, providing information on product characteristics, nutrition status, and the information of ingredients. Packaging materials also have a role in preserving the pigment stability of the juice drink. In Malaysia, the common packaging materials for fruit juices are polypropylene (PP), glass, an aluminium can, and a laminated beverage pouch. Each packaging material has various impacts on the stability of the juice drink with anthocyanin. Thus, this study aimed to investigate the

effect of packaging materials on the anthocyanin stability of a red cabbage-roselle mixed drink throughout six months of storage.

2. Materials and methods

2.1 Materials

Red cabbage was procured from a local supplier at Kompleks Pasar Borong Seri Kembangan (Selangor, Malaysia). Dried roselle calyces were purchased from MARDI Kuala Terengganu (Terengganu, Malaysia). Honey was purchased from Trigona Bee (Serdang, Selangor, Malaysia). Potassium chloride, sodium acetate, and potassium metabisulfite were of analytical grade and purchased from Merck (Darmstadt, Germany). Citric acid, Arabic gum, and sodium benzoate were purchased from Meilun Food Chemical Sdn. Bhd. (Klang, Selangor, Malaysia). Packaging materials were purchased from Plashouse Sdn. Bhd. (Kajang, Selangor, Malaysia).

2.2 Extraction of roselle and red cabbage

Extraction of roselle was done according to the method described by Chumsri *et al.* (2008). Dried roselle calyces were soaked in filtered tap water at a ratio of 1:10 and heated at the extraction temperature of 50°C for 30 mins using a stainless-steel pot. The roselle extract was filtered through a muslin cloth. Red cabbage juice was extracted using a juice extractor (Santos, France) and filtered through a muslin cloth. Both extracted roselle and red cabbage were stored in high-density polyethylene (HDPE) bottles and were kept frozen (-18°C) before the mixed drink preparation.

2.3 Preparation of mixed drink of red cabbage and roselle extracts

The mixed drink was prepared according to Nur Farah Hani *et al.* (2020) 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 mixed drink was then hot-filled into three different packaging materials, namely transparent glass bottle (250 mL), metal beverage can (250 mL), and polypropylene (PP) bottle (250 mL). The packaging materials were then cooled rapidly at room temperature by immersing the bottles in the chilled water (8±2°C). The bottles were stored in a dark place at ambient temperature (29±2°C) for analysis. These mixed drinks had a pH of 3.58 and a total soluble solid of 11°Brix. Three samples of each packaging material were withdrawn for monthly evaluation for up to six months.

Table 1. Formulation of red cabbage-roselle mixed 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

2.4 Determination of total anthocyanin content

The total anthocyanin content was determined by the pH-differential method (Guisti and Wrolstad, 2001) using potassium chloride buffer (pH 1.0) and sodium acetate buffer (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. The measured samples should be clear and contain no haze or sediments before readings are taken. Anthocyanin pigment concentration was calculated and expressed 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 $A = (A_{520\text{nm}} - A_{700\text{nm}})_{\text{pH } 1.0} - (A_{520\text{nm}} - A_{700\text{nm}})_{\text{pH } 4.5}$; 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 $\text{L} \times \text{mol}^{-1} \times \text{cm}^{-1}$, for cyd-3-glu; and 10^3 = factor conversion from g to mg.

2.5 Determination of colour density and polymeric colour percentage

Colour density and percentage of polymeric colour were determined using the method described by Giusti and Wrolstad (2005). Sample extracts were diluted with water to have an absorbance reading between 0.5 and 1.0 at 512 nm when evaluated by a UV-Vis spectrophotometer (Perkin Elmer, United Kingdom). For analysis, 0.2 mL of 0.90 M potassium metabisulfite was added to 2.8 mL diluted sample (bisulfite bleached sample), and 0.2 mL of deionised water was added to 2.8 mL of diluted sample (nonbleached, control sample). After equilibrating for 15 mins, samples were evaluated at $\lambda = 700, 520,$ and 420 nm within 1 hr.

Colour density was calculated using the control sample (nonbleached) according to the following formula:

$$\text{Colour density} = [(A_{420\text{nm}} - A_{700\text{nm}}) + (A_{520\text{nm}} - A_{700\text{nm}})] \times \text{dilution factor}$$

Polymeric colour was determined using the bisulfite-

bleached sample using the following formula:

$$\text{Polymeric colour} = [(A_{420\text{nm}} - A_{700\text{nm}}) + (A_{520\text{nm}} - A_{700\text{nm}})] \times \text{dilution factor}$$

Percentage of polymeric colour was calculated using the formula:

$$\% \text{ Polymeric colour} = \text{Polymeric Colour/Colour Density} \times 100$$

2.6 Determination of degradation index

The degradation index (DI) was determined using the method by Fuleki and Francis (1968). The degradation index is the ratio between total anthocyanins and monomeric anthocyanins content in the sample calculated at absorption (A) 520 nm and 700 nm using the following formula:

$$\text{DI} = \frac{(A_{520\text{nm}} - A_{700\text{nm}})_{\text{pH } 1.0}}{[(A_{520\text{nm}} - A_{700\text{nm}})_{\text{pH } 1.0} - (A_{520\text{nm}} - A_{700\text{nm}})_{\text{pH } 4.5}]}$$

2.7 Statistical analysis

All analyses were done in triplicate. Experimental data were subjected to the analysis of variance (ANOVA), and the significant differences among means were determined by the Least Significant Difference (LSD) at $p \leq 0.05$ using SAS software (Ver. 9.4., SAS Institute, Cary, NC, USA).

3. Results and discussion

3.1 Effect of packaging materials and time of storage on total anthocyanins content

The stability of anthocyanin is influenced by many factors such as oxygen, pH, the temperature of processing and storage, light, the chemical nature of anthocyanins (acylation or glycosylation), enzymes, ingredient interactions, and co-pigmentation (West and Mauer, 2013; Bordenave *et al.*, 2014). Table 2 shows the total anthocyanins content of a red cabbage-roselle mixed drink in three types of packaging materials over six months of storage at ambient temperature. During storage, the anthocyanin degradation was significant ($p < 0.05$), irrespective of the packaging materials used. The total anthocyanin content of the mixed drinks in different packaging materials declined significantly from the first month onwards (Table 2). At the 1-month of storage, more than 50% of the total anthocyanin content was lost from all the mixed drinks in different packaging materials, with the mixed drink in PP bottles significantly showing the lowest reading of total anthocyanin content at 96.88 mg cyd-3-glu/L, while 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 mixed

Table 2. Total anthocyanins content of red cabbage-roselle mixed drinks in different packaging materials

Storage time (month)	Total Anthocyanins Content (mg cyd-3-glu/ L)		
	Glass bottle	Can	PP bottle
0	263.40±1.67 ^{Aa}	262.66±0.87 ^{Aa}	262.17±1.04 ^{Aa}
1	132.10±3.60 ^{Ab}	113.20±3.96 ^{Bb}	96.88±0.71 ^{Cb}
2	87.75±16.60 ^{Ac}	94.54±23.17 ^{Ac}	88.21±18.55 ^{Ab}
3	74.38±2.21 ^{Ad}	71.80±7.35 ^{Ad}	40.42±0.49 ^{Bc}
4	55.60±0.87 ^{Ae}	49.27±1.93 ^{Be}	21.09±1.31 ^{Cd}
5	48.29±0.96 ^{Ae}	39.85±0.85 ^{Bef}	14.38±0.17 ^{Ce}
6	34.36±1.83 ^{Af}	30.50±0.17 ^{Bf}	10.44±1.42 ^{Ce}

Values are presented as mean±SD. Values with different uppercase superscripts within the same row are significantly different at $p<0.05$. While values with different lowercase superscripts within the same column are significantly different at $p<0.05$

drinks in glass bottles, cans, and PP bottles decreased by 67%, 64%, and 66%, respectively, compared to the mixed drinks at 0-month storage, but there was no significant difference between packaging materials. At 3-month storage, the total anthocyanin content of mixed drinks in glass bottles, cans, and PP bottles decreased by 72%, 73%, and 85%, respectively. The mixed 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 mixed drinks stored in different packaging materials for 4-, 5- and 6-month storage as the mixed drink stored in glass-bottled recorded the highest total anthocyanin content remaining, followed by mixed drinks stored in cans and PP bottles for the same period of storage. A previous study by Brownmiller *et al.* (2008) also noticed that the anthocyanin content in the fresh blueberry juice in glass bottles decline during storage at ambient temperature with losses of 48%, 67% and 80% after 1, 3 and 6 months. Numerous factors such as residual enzyme activities or condensation reactions of anthocyanins with other phenolics may contribute to the loss of monomeric anthocyanins and the massive increase in polymeric colour values (Brownmiller *et al.*, 2008). Besides that, past findings also have a similar trend of degradation of anthocyanins in different packaging materials during storage of pomegranate juices packed in transparent and green glass, and paperboard carton with polyethylene layers (Minibrik-200) (Pérez-Vicente *et al.*, 2004), sour cherry nectars stored in two different sizes of carton (200 and 1000 mL) and aluminium cans (330 mL) (Bakan and Eksi, 2014) and roselle-mango juice blend packed in glass and plastic bottles (Mgaya-kilima *et al.*, 2015).

This observation could be explained by the characteristics of the packaging materials used. As mentioned before, the stability of anthocyanins was influenced by many factors, including light and oxygen. Among the three types of packaging materials, PP bottles are neither oxygen- nor light-proof. According to Ramos *et al.* (2015), the major disadvantage of plastic is its variable permeability to light, gases (oxygen), and

vapours. Therefore, the total anthocyanin content values of mixed drinks in PP bottles were the lowest compared to those packed in cans and glass bottles. Even though cans are light and oxygen-proof, the decrease in total anthocyanin content value in the canned mixed drink may be due to the reaction between the coating of the cans and the compound inside the mixed drinks (Siah *et al.*, 2011). After six months of storage, the mixed drink stored in the glass bottle showed the highest total anthocyanin content value. According to Franco and Falque (2016), glass is impermeable to oxygen and vapours as well as inert to chemical, biological, and physical external influences. Furthermore, a study done by Pérez-Vicente *et al.* (2004) found that the type of glass (green or transparent) did not affect the anthocyanin degradation in pomegranate juice during storage.

3.2 Effect of packaging materials and time of storage on colour density

The changes in colour density and polymeric colour percentage of red cabbage-roselle mixed drinks stored in different packaging materials were monitored for six months of storage. Prolonged storage periods resulted in lower total anthocyanin content, including colour density and polymeric colour percentage of mixed drinks stored in cans, glass, and PP bottles.

Colour density is defined as the sum of absorbencies at the maximum wavelength (Aishah *et al.*, 2013). Colour density considers the contribution of the compounds to be able to determine the colour of the juice, such as melanoidin (brown) and anthocyanins (red) (Vegara *et al.*, 2013). Therefore, a low or high value of colour density of red cabbage-roselle mixed drink could be related to its total anthocyanin content, as colour density also faces a decreasing trend in total anthocyanin content. Table 3 shows a significant difference ($p<0.05$) in the colour density in mixed drinks stored in different packaging materials for six months of storage. During storage, all samples' colour density values decreased by

Table 3. Colour density of red cabbage-roselle mixed drinks in different packaging materials

Storage time (month)	Colour Density		
	Glass bottle	Can	PP bottle
0	4.56±0.09 ^{Aa}	4.66±0.12 ^{Aa}	4.74±0.13 ^{Aa}
1	4.05±0.15 ^{Ab}	4.27±0.22 ^{Ab}	4.34±0.14 ^{Ab}
2	3.81±0.15 ^{Ac}	3.71±0.11 ^{Ac}	3.71±0.01 ^{Ac}
3	3.54±0.11 ^{Ad}	3.50±0.08 ^{Ad}	3.10±0.05 ^{Bd}
4	2.86±0.16 ^{ABf}	3.00±0.04 ^{Ac}	2.76±0.08 ^{Bc}
5	3.32±0.08 ^{Ac}	3.35±0.06 ^{Ad}	2.59±0.04 ^{Bef}
6	2.91±0.07 ^{Af}	2.93±0.05 ^{Ac}	2.41±0.03 ^{Bf}

Values are presented as mean±SD. Values with different uppercase superscripts within the same row are significantly different at $p>0.05$. While values with different lowercase superscripts within the same column are significantly different at $p>0.05$

36% for a mixed drink in glass bottles, 37% for a mixed drink in cans, and 49% for a mixed drink in PP bottles. This result indicated that mixed drinks packed in PP bottles had the lowest colour density than mixed drinks packed in glass bottles and cans. Similarly, the colour density of elderberry juice also decreased from 60 to 40 after 140 days of storage at 40°C (Busso Casati *et al.*, 2015). Moreover, colour density for three different roselle beverages showed significantly ($p<0.05$) decreased eventually after 14 weeks of storage at 4°C. These findings indicated a decline in the absorbance at 520 nm, which can be associated with the degradation of anthocyanins (Ramírez-Rodrigues *et al.*, 2012).

3.3 Effect of packaging materials and time of storage on polymeric colour percentage

The polymeric colour percentage is an index of the degree of anthocyanins polymerisation. The polymeric colour percentage showed a significant increase ($p<0.05$) throughout the storage of mixed drink samples stored in three packaging materials (Table 4). The polymeric colour percentage indicates red colour contribution before monomeric pigments polymerise and turn into a brownish colour (Aishah *et al.*, 2013). Thus, a higher percentage of polymeric colour illustrates a higher degradation rate and decline in colour intensity. At six months of storage, the polymeric colour percentage

increased by 55%, 58%, and 78% for mixed drinks packed in glass bottles, cans, and PP bottles, respectively. Mixed drinks stored in glass bottles had the lowest polymeric colour percentage, while those in PP bottles had the highest polymeric colour percentage. This result clearly showed that PP bottles should not be preferred as suitable packaging material to store the red cabbage-roselle mixed drink. Several studies have reported an increase in the polymeric colour percentage of anthocyanin-containing juice and products during storage. For example, Hager *et al.* (2008) and Brownmiller *et al.* (2008) reported that when anthocyanin content decreased, the percentage of polymeric colour increased during six months of storage of processed black raspberry products and blueberry products, respectively.

3.4 Correlation between total anthocyanins content with colour density, polymeric colour percentage and degradation index of different packaging materials

The correlation analyses were carried out to determine the relationship between total anthocyanin content with colour density, polymeric colour percentage, and red cabbage-roselle mixed drink degradation index stored in different packaging materials during storage (Table 5). The mixed drink stored in all packaging materials (glass bottles, cans, and PP bottles)

Table 4. Polymeric colour percentage of red cabbage-roselle mixed drinks in different packaging materials

Storage time (month)	% Polymeric Colour		
	Glass bottle	Can	PP bottle
0	26.02±3.73 ^{Ad}	26.63±1.76 ^{Ac}	26.26±3.27 ^{Af}
1	37.85±1.29 ^{Bc}	42.13±3.76 ^{Bd}	49.72±2.58 ^{Ac}
2	44.97±2.83 ^{Bb}	47.32±1.67 ^{Bc}	59.58±5.18 ^{Ad}
3	45.36±0.73 ^{Bb}	47.75±1.07 ^{Bbc}	63.35±1.7 ^{Ac}
4	48.44±1.66 ^{Cb}	51.42±0.61 ^{Bb}	67.46±1.43 ^{Ac}
5	53.57±2.62 ^{Ca}	57.26±0.42 ^{Ba}	72.71±1.56 ^{Ab}
6	55.37±2.41 ^{Ba}	57.89±3.25 ^{Ba}	77.68±1.38 ^{Aa}

Values are presented as mean±SD. Values with different uppercase superscripts within the same row are significantly different at $p<0.05$. While values with different lowercase superscripts within the same column are significantly different at $p<0.05$

Table 5. Correlation between total anthocyanins content, colour density, polymeric colour percentage, and degradation index of the mixed drinks stored in a) glass bottles b) cans and c) pp bottles

A) Glass bottle				
	Total Anthocyanin Content	Colour Density	% Polymeric Colour	Degradation Index
Total Anthocyanin Content	1	0.974**	-0.951**	-0.849
Colour Density	0.974**	1	-0.923**	-0.748
% Polymeric Colour	-0.951**	-0.923**	1	0.877
Degradation Index	-0.849**	-0.784**	0.877**	1
** significant at $p < 0.01$				
B) Cans				
	Total Anthocyanin Content	Colour Density	% Polymeric Colour	Degradation Index
Total Anthocyanin Content	1	0.976**	-0.962**	-0.839**
Colour Density	0.976**	1	-0.936**	-0.801**
% Polymeric Colour	-0.962**	-0.936**	1	0.881**
Degradation Index	-0.839**	-0.801**	0.881**	1
** significant at $p < 0.01$				
C) PP bottles				
	Total Anthocyanin Content	Colour Density	% Polymeric Colour	Degradation Index
Total Anthocyanin Content	1	0.958**	-0.966**	-0.733**
Colour Density	0.958**	1	-0.973**	-0.771**
% Polymeric Colour	-0.966**	-0.973**	1	0.836**
Degradation Index	-0.733**	-0.771**	0.836**	1
** significant at $p < 0.01$				

showed similar correlation trends between total anthocyanins content and colour density, polymeric colour percentage, and degradation index.

Total anthocyanins content was found to be very strongly correlated with colour density regardless of packaging materials; glass bottles ($r^2 = 0.974$), cans ($r^2 = 0.976$), and PP bottles ($r^2 = 0.958$), indicating that total anthocyanin content played an important part in colour properties of the mixed drink. The increase in polymeric colour percentage during storage demonstrated the polymerisation of red anthocyanins pigment, which was confirmed by the strong negative correlation between total anthocyanin content and polymeric colour percentage for the mixed drink stored in a glass bottle ($r^2 = -0.951$), cans ($r^2 = -0.962$) and PP bottles ($r^2 = -0.966$). Similarly, there was a negative correlation between total anthocyanin content and degradation index in the mixed drink packed in glass bottle ($r^2 = -0.849$), cans ($r^2 = -0.839$) and PP bottles ($r^2 = -0.733$). These results anticipated the loss of total anthocyanin content in mixed drinks stored irrespective of packaging materials during storage, accompanied by increased polymeric colour percentage and degradation index. As observed, the loss of total anthocyanin content in the mixed drink samples was due to both polymerised anthocyanin and anthocyanin degradation. A study done by Muche *et al.* (2018) found that polymeric colour was inversely correlated to total anthocyanin content in grape juices stored at 35°C.

There was a strong negative correlation between colour density and degradation index for the mixed drink stored in glass bottle ($r^2 = -0.784$), cans ($r^2 = -0.801$) and PP bottles ($r^2 = -0.771$) during storage at ambient temperature. The decrease in colour density can be associated with a high degradation index value and vice versa (Tsai and Huang, 2004). Besides that, it can be seen that polymeric colour percentage was strongly correlated with the degradation index for the mixed drink packed in a glass bottle ($r^2 = 0.877$), cans ($r^2 = 0.881$) and PP bottles ($r^2 = 0.836$). The polymeric colour percentage was calculated to determine the level of polymerisation, as it was part of the reactions that contributed to the increase in the degradation index. The polymeric colour percentage would indicate the red colour contribution before the polymerisation of monomeric pigments and finally turn into a brownish colour. Thus, a higher percentage of polymeric colour indicates a higher degradation rate and decreased colour density (Aishah *et al.*, 2013).

4. Conclusion

Packaging materials and storage time significantly affected the physicochemical and anthocyanins stability of mixed drinks stored in glass bottles, cans, and PP bottles. The correlation analysis revealed that total anthocyanin content had a significant positive correlation with colour density but negatively correlated with polymeric colour percentage and degradation index

regardless of packaging materials used. The mixed drinks stored in glass bottles significantly ($p < 0.05$) had the highest retention of anthocyanin content and the lowest percentage of polymeric colour and anthocyanin degradation index, compared to those stored in cans and PP bottles. This result is undoubtedly contributed by features of glass bottles that are impermeable to oxygen, vapours and inert to chemical, biological and physical impacts contrasted to cans and PP bottles. Therefore, glass bottles were the most appropriate packaging material for storing red cabbage-roselle mixed drinks, followed by cans and polypropylene bottles, as glass bottles provided anthocyanin stability in the mixed drink better than cans and PP bottles. For future studies, the effect of non-thermal processing methods such as high-pressure processing (HPP) compared to thermal processing methods on the colour and anthocyanins content of the mixed drink to preserve the nutritional quality should be investigated. Furthermore, the usage of other packaging materials such as cartons and aluminium pouches can be explored in future studies to store the mixed drink to determine which packaging material can sustain the mixed drink's colour and anthocyanins content.

Conflict of interest

The authors declare no conflict of interest.

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