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FULL PAPER

Effect of Gum Arabic (*Acacia senegal* and *Acacia seyal*) addition on antioxidant properties and oxidative stability of roselle juice

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Abstract

Gum Arabic (GA) is the dried exudate obtained from the tree of *Acacia senegal* and *Acacia seyal* that possess a prebiotic effect on humans. Meanwhile, roselle is a popular juice that is rich in antioxidants. However, as a complex polysaccharide, the addition of GA in roselle juice may affect the antioxidant properties of the final product. Therefore, this study aimed to determine the effect of different types of GA (*Acacia senegal* or *Acacia seyal*) addition, at different concentrations (0%, 2%, 4% and 6%) on the antioxidant properties and antioxidant stability of roselle juice during storage. The most accepted formulation of juice for each GA type along with the control sample (0% GA) was analysed for their oxidative stability during five weeks of chill storage (4±1°C). Results showed that the addition of GA increased or at least maintained the vitamin C and total phenolic content (TPC) in the range of 11.35±1.26-25.18±1.70 mg/100 mL and 21.04±1.55-45.08±4.76 mg GAE/100 mL, respectively. Nevertheless, the addition of more than 2% of GA significantly reduced ($p<0.05$) about 7% to 25% of total anthocyanin content. Variation of results was shown by ferric reducing antioxidant power (FRAP) and 2,2-diphenyl-1-picrylhydrazyl free radical-scavenging activity (DPPH) assay. For storage study, all juices (0% GA, 4% *A. senegal* and 2% *A. seyal)* exhibited fluctuation in trend for all the antioxidant properties except for TPC which remained stable. In conclusion, overall findings showed the addition of GA may increase or at least maintain the antioxidant properties of the juices except for anthocyanin content and antioxidant activity (DPPH assay). Meanwhile, the addition of GA had a variation that resulted in the oxidative stability of juices during storage.

1. Introduction

Antioxidants play a crucial role in inhibiting or delaying the oxidation of cellular components and are thus capable of protecting against the oxidative damage of cells as well as preventing degenerative diseases related to ageing such as cardiovascular disease, diabetes, cancer and neurodegenerative diseases (Muniandy *et al.*, 2016). It interacts and stabilises the free radical molecules in the body by donating the electron to free radicals and thus prevents cell damage by free radicals (Engwa, 2018). The powerful effect of antioxidants suggests that adequate intake or incorporation of the compounds in daily life is beneficial to humans which can result in a healthy body (Muniandy, 2016). One of the potent sources of antioxidants is fruit and its derived products for instance fruit juice, jams and nectars. Fruit and its derived products contain many active dietary antioxidant compounds such as polyphenols, anthocyanin, carotenoids and vitamins (Hidalgo and Almajano, 2017; Engwa, 2018).

Nowadays, roselle and its juice have been popular among the consumer since it is rich in antioxidant content which may possess health benefits to the consumers (Islam, 2019). Roselle or *Hibiscus sabdariffa* Linn. is a plant originally from Asia (India to Malaysia) or Tropical Africa, which has been widely cultivated in Sudan, Taiwan, India and Malaysia (Puro *et al.,* 2017; Shruthi and Ramachandra, 2020). Roselle also was reported to contain many other chemical compositions such as bioactive compounds and organic acids. It is also well known for its high vitamin C content, polyphenols,

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phenolic acids, anthocyanins and flavonoids that enhanced the nutritive value of roselle (Solangi *et al.,* 2017; Tajudin *et al.,* 2019; Riaz and Chopra, 2018). Nowadays, roselle has gained prominence due to its health benefits as an anticancer and is capable of reducing chronic diseases such as hypertension and diabetes mellitus (McKay *et al.,* 2010; Mardiah *et al.,* 2014; Formagio *et al.,* 2015; Shruthi and Ramachandra, 2020).

However, due to the high demand for healthy foods and beverages, the manufacturing companies were making efforts to improve the quality of fruit juice with functional ingredients, making it a functional product (Gomes *et al.,* 2017). Among the functional ingredients, the consumer currently had more concern towards prebiotics due to their benefits for the gut (Quigley, 2019). Prebiotic is the substance that may promote the growth and proliferation of beneficial microorganisms in the intestines, improving gut health, and leading to health benefits (Gibson *et al.,* 2017). One of the prebiotic sources is Gum Arabic. Intake of Gum Arabic (GA) at an optimal dose of 10 g daily was capable of providing the prebiotic effect to consumers (Calame *et al.,* 2008; Cherbut *et al.*, 2003). GA is defined as the edible dried gummy exudate obtained from stems and branches of *Acacia senegal* (L.) Willdenow or *Acacia seyal* (fam. Leguminosae) (Mariod, 2018). Generally, the term GA is usually used to refer to the gum exudate from the tree of *Acacia senegal* and/or *Acacia seyal*. Meanwhile, when only *A. senegal* or *A. seyal* is mentioned, it is to refer to the source from that species only.

GA is also a complex polysaccharide composed of a few monosaccharides such as D-galactose, L-rhamnose, L-arabinose and D-Glucuronic acid as well as mineral components such as potassium, magnesium, calcium and tannin (Idris, 2017; Mariod, 2018). Thus, due to its complex polysaccharide structure, GA has been extensively used in the food industry as an emulsifier, stabiliser and microencapsulation (Mohammed, 2015; Idris, 2017; BeMiller, 2019). In addition, GA also has been reported to have antioxidant properties, making it a good source of antioxidants (Mirghani *et al.,* 2018). In a previous study, Ali *et al.* (2013) reported that tomato fruits coated with GA had maintained their lycopene content, total phenolic and total antioxidant capacity during storage at 20°C for 20 days. Besides, a recent study by da Silva *et al.* (2019) also found that the treatment of cupuaçu juice with 1% of GA had higher retention of phenolic compounds and vitamin C during storage. Therefore, these previous studies showed that the applications of GA in foods were capable of maintaining or retaining the antioxidant potential. Nevertheless, none of the studies determines the antioxidant properties in roselle juice with GA addition. Besides, there are limited studies found to utilise individual GA specifically *A. senegal* and *A. seyal* as functional ingredients for prebiotic effects in the juice.

Therefore, the objective of the current study is to determine the effect of different types and concentrations of Gum Arabic addition on antioxidant properties and oxidative stability of roselle juice during storage.

2. Materials and methods

2.1 Materials and preparation of roselle juice

Roselle (*Hibiscus sabdariffa* Linn.) of UMKL variety was used as a raw material to prepare the roselle juice. Fresh roselle calyxes were obtained from a local producer in Terengganu and were air-dried at room temperature (25°C) before storage at frozen temperature (-20°C) until further usage. Roselle juice was prepared according to the method described by Tuan Azlan *et al.,* (2020). The steps involved extracting the roselle calyces by heating at 60°C for 15 mins (with roselle calyx to water ratio of 1: 20), mixing with sweeteners (4% xylitol and 0.03% stevia) and GA powder before further pasteurising at 90°C for 30 s. The GA (*Acacia senegal* and the *Acacia seyal*) was added at different concentrations of 0% (control), 2%, 4% and 6% prior to the pasteurisation process. The pasteurised juice was hot-filled into an amber glass bottle, cooled rapidly and stored at 4±1°C until further analysed for its antioxidant properties (total phenolic content, vitamin C content, anthocyanin content, DPPH free radical scavenging ability assay and ferric reducing antioxidant power (FRAP) assay. Then, the oxidative stability of selected juices was analysed at a one-week interval during five weeks of chill storage. All the juices have a Brix value in the range of 5.23±0.25 to 10.47±0.25°Bx.

2.2 Determination of vitamin C content

The vitamin C content in roselle juice was determined by using the colourimetric technique as described by Jagota and Dani (1982) and Mgaya-Kilima *et al.* (2014) with slight modifications. Briefly, 2 mL of each sample was added with 0.2 mL of 10% Trichloroacetic acid (TCA), diluted to 10 mL with distilled water and mixed thoroughly. The mixture was allowed to stand for 1 min and was filtered with filter paper thereafter. The filtered sample (2 mL) was added with 6 mL of distilled water, 0.8 mL Folin-Ciocalteu reagent and was incubated in the dark at room temperature for 10 min. Absorbance was measured at 760 nm using a UV-Vis spectrophotometer (Merck Spectroquant Pharo 300 Spectrophotometer, Germany) against distilled water as blank. A standard curve was plotted using an ascorbic acid standard solution of 0-25

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mg/100 mL and the result was expressed as mg/100 mL sample.

2.3 Determination of total phenolic content

A modified Folin-Ciocalteu assay as reported by Singleton and Rossi (1965) and Wern *et al.* (2016) was used to determine the total phenolic content (TPC) in roselle juice. Prior to the analysis, the sample was centrifuged (Hettich Universal 32 Centrifuge, UK) at 3000 rpm for 10 mins. The supernatant was collected and diluted at factor five for analysis. Approximately 0.4 mL of diluted sample was mixed with 3.6 mL distilled water and 0.4 mL of Folin-Ciocalteu reagent. The mixture was left for 5 mins before 4 mL of 7% sodium carbonate was added to the mixture and was made up to 10 mL with distilled water. The solution was vortexed at 2000 rpm for 1 min and was incubated at room temperature in the dark for 90 min. Then, the absorbance was measured at 765 nm using a UV-Vis spectrophotometer (Merck Spectroquant Pharo 300 Spectrophotometer, Germany) against distilled water as blank. A calibration curve was prepared with Gallic acid standard solution of 0 to 25 mg/100 mL concentration and the result was expressed as Gallic acid equivalent (mg GAE/100 mL).

2.4 Determination of total anthocyanin content

The protocol of the pH differential method was used to measure the total anthocyanin content in roselle juice (Lee, 2005). Prior to the experiment, two pH buffers, which are potassium chloride buffer (pH 1.0) and sodium acetate buffer (pH 4.5) were prepared. Approximately 3 mL of sample was placed in a test tube and made up to 15 mL with the corresponding buffer. Then, the solution was vortexed at 2000 rpm for 1 min and equilibrated in the dark for 20 mins. The absorbance was measured at wavelengths of 520 nm and 700 nm by UV-Vis spectrophotometer (Merck Spectroquant Pharo 300 Spectrophotometer, Germany) with distilled water as blank. The anthocyanin content in the sample was expressed as mg cyanidin-3-glucoside equivalent/100 mL sample (mg/100 mL).

$$\text{Total anthocyanin content} = \frac{A \times MW \times DF \times 1000}{\in L}$$

Where, A = the difference of sample absorbance between pH 1.0 and 4.5 calculated with formula A = $(A520 - A700)_{pH1.0} - (A520 - A700)_{pH4.5}$; MW = molecular weight (MW) of cyanidin-3-glucoside (449.2 g); DF = the dilution factor; $\in$ = is the molar extinction coefficient for cyanidin-3-glucoside (26,900 L/mol/cm) and L = the path length of the spectrophotometer cell (1.0 cm)

2.5 Determination of antioxidant activity

The antioxidant activity of roselle juice was determined by the Ferric Reducing Antioxidant Power (FRAP) assay and DPPH Free Radical Scavenging Activity Assay. Prior to the analysis, the sample was centrifuged (Hettich Universal 32 Centrifuge, UK) at 3000 rpm for 10 mins. The supernatant was collected and diluted at factor five for analysis.

2.5.1 Ferric Reducing Antioxidant Power assay

The protocol of the Ferric Reducing Antioxidant Power (FRAP) assay was conducted as described by Wern *et al.* (2016) with slight modifications. Stock solutions of 300 mM acetate buffer (pH 3.6), 10 mM TPTZ solution and 20 mM ferric chloride hexahydrate solution were prepared and stored at 4°C prior to the analysis. Briefly, a fresh FRAP reagent was prepared by mixing acetate buffer, TPTZ solution and ferric chloride hexahydrate solution at a ratio of 10: 1: 1, respectively. The FRAP solution was warmed at 37°C in a water bath until further used. An aliquot of 0.1 mL of sample was mixed with 4 mL of warmed distilled water and 4 mL of FRAP reagent in a test tube. The mixture was incubated at 37°C in a water bath for 4 min in the dark. The absorbance of the sample was measured at 593 nm using a UV-Vis spectrophotometer (Merck Spectroquant Pharo 300 Spectrophotometer, Germany) with FRAP reagent as a blank. The calibration curve of the Trolox solution of 0 to 1.0mmol/100 mL was plotted and the result was expressed as mmol TE/100 mL.

2.5.2 DPPH free radical scavenging activity

The antioxidant scavenging free radical activity of roselle juice was measured spectrophotometrically using DPPH free radical scavenging activity according to the protocol as reported by Wern *et al.* (2016) with slight modifications. Briefly, 0.4 mL of diluted sample was mixed with 5.6 mL of methanolic DPPH. The solution was vortexed at 2000 rpm for 1 min and was incubated in a water bath at 37°C for 30 mins. The absorbance was taken at 517 nm using a UV-Vis spectrophotometer (Merck Spectroquant Pharo 300 Spectrophotometer, Germany) and methanol was used as blank. A calibration curve was plotted with Trolox standard solution of 0 to 1.0 mmol concentrations and the result was expressed as mmol TE/100 mL.

2.6 Determination of oxidative stability during storage

For the determination of oxidative stability during the storage study, the study was conducted for three samples of the juice including (1) the most accepted juice with *A. senegal*, (2) the most accepted juice with *A. seyal* and (3) control juice throughout five weeks of

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storage. The most accepted formulation with GA was selected based on their sensory acceptability (result not included) and antioxidant properties such as vitamin C content, total phenolic content and total anthocyanin content. In each GA type (*A. senegal* or *A. seyal*), the juice with high sensory acceptability during sensory evaluation and had reasonable antioxidant properties was selected as the most accepted formulation. Then, all the chosen juices (most accepted formulation juice with *A. senegal,* most accepted formulation juice with *A. seyal,* control juice) were stored at refrigeration temperatures (4±1°C) and further analysed for their oxidative stability at the one-week interval for five weeks of storage. The properties evaluated for oxidative stability included the vitamin C content, TPC, total anthocyanin content and antioxidant activity (FRAP and DPPH assay).

2.7 Experimental design and statistical analysis

Determination of antioxidant properties and oxidative stability of juices with different types and concentrations of GA were performed in triplicate and the results were expressed as mean values and standard deviation (SD). The triplicate data were analysed using one-way ANOVA at a 95% confidence level and the significant differences between means were analysed by Fisher's Least Significance Difference Test. All the statistical analysis was performed by using Minitab 19.0 software (Minitab Inc., USA).

3. Results and discussion

3.1 Antioxidant properties of roselle juice

3.1.1 Vitamin C content

The effect of GA addition on the vitamin C content of roselle juices is shown in Table 1.

Compared with the control sample, the addition of GA except for the addition of 2% *A. senegal*, had significantly ($p<0.05$) increased the vitamin C content of roselle juices. These results were in agreement with Nwaokoro and Akanbi (2015) as well as da Silva *et al.* (2019) who found an increase in vitamin C content with the addition of carboxymethyl cellulose (CMC) and xanthan gum into tomato-carrot juice and GA in cupuaçu juice, respectively. Additionally, at similar concentrations of 2% and 6%, the addition of *A. seyal* resulted in higher content of vitamin C than juices with *A. senegal.*

Vitamin C was found to be easily oxidised and degraded when being exposed to light, oxygen and temperature during processing (Al-Ismail *et al.,* 2016). However, in the current finding, the increase in vitamin C content of the juice with GA addition could be attributed to the characteristic of GA that is capable of preventing oxidation of vitamin C in the juice (Nwaokoro and Akanbi, 2015). According to Suhag *et al.* (2016), this might be due to its functional property that possesses an excellent emulsifying effect (Suhag *et al.,* 2016). GA has a protein-polysaccharide complex structure such as the arabinogalactan-protein complex that is responsible for emulsifying properties of the substance (BeMiller, 2019). Thus, as GA was added to the juice, the vitamin C might undergo physical entrapment, encapsulation, a hydrogen bond or another specific or non-specific binding by GA which then helps to retain vitamin C compounds (Mirhosseini *et al.,* 2008; da Silva *et al.*, 2019).

3.1.2 Total phenolic content

Additional effects of different types and concentrations of GA on the TPC of roselle juice are also shown in Table 1. Results showed that the TPC of roselle juice with *A. senegal* or *A. seyal* addition ranged between 21.04±1.55 mg GAE/100 mL to 45.08±4.76 mg GAE/100 mL. The addition of 2 to 6% of *A. senegal* had a minimum effect ($p\geq0.05$) on the TPC of the juice when compared to the control juice. This result was in

Table 1. Antioxidant properties of roselle juice with Gum Arabic (*A. senegal* or *A. seyal*) addition at different concentrations

Sample	A (Control)	B (2% *A. senegal*)	C (4% *A. senegal*)	D (6% *A. senegal*)	E (2% *A. seyal*)	F (4% *A. seyal*)	G (6% *A. seyal*)
Vitamin C (mg/100 mL)	11.66±0.62^{c}	11.35±1.26^{c}	15.95±2.69^{b}	15.34±2.99^{b}	16.08±1.31^{b}	17.51±1.54^{b}	25.18±1.7^{a}
Total Phenolic Content (mg GAE/100 mL)	24.27±2.37^{cd}	21.04±1.55^{d}	24.96±3.00^{cd}	28.44±5.07^{c}	25.80±1.83^{cd}	38.21±1.92^{b}	45.08±4.76^{a}
Anthocyanin (mg/100 mL)	83.74±2.17^{a}	72.80±1.04^{c}	67.19±1.13^{d}	63.04±2.20^{e}	77.53±1.07^{b}	72.31±2.65^{c}	68.16±2.35^{d}
FRAP (mmol TE/100 mL)	1.521±0.197^{c}	1.319±0.126^{c}	1.747±0.105^{c}	2.408±0.433^{b}	2.805±0.497^{b}	2.605±0.117^{b}	3.347±0.229^{a}
DPPH (mmol TE/100 mL)	1.42±0.09^{a}	0.62±0.08^{d}	0.72±0.12^{cd}	1.41±0.14^{a}	1.02±0.10^{b}	0.83±0.13^{bc}	0.80±0.06^{cd}

Value are presented as mean±standard deviation (n = 3). Values with different superscripts within the same row are significantly different ($p<0.05$).

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accordance with the findings of Yi *et al.* (2015) and Kraithong *et al.* (2019), who also found an insignificant effect of hydrocolloids on the TPC values of grape juice and rice noodles, respectively. However, interesting findings for *A. seyal*, 4% and 6% of its addition significantly influenced ($p<0.05$) the TPC of the roselle juice values with an increase of 57.44% and 85.74%, respectively. Moreover, at their similar concentrations, juices with *A. seyal* also exhibited higher phenolic content than juices with 4% and 6% *A. senegal*. The difference in TPC in juices with *A. senegal* and *A. seyal* might be due to the higher polyphenol compounds like tannin (Dauqan and Abdullah, 2013) in *A. seyal.* This result was in fair agreement with the finding by Mirghani *et al.* (2018), who reported that the commercial *A. seyal* powder had a higher phenolic content than commercial *A. senegal* powder. Thus, its original composition became an important factor affecting the final product composition.

3.1.3 Total anthocyanin

Several findings reported that GA was able to enhance the stability of anthocyanin due to its structure that is highly branched with a heteropolymer of sugar that interacts with anthocyanin by hydrogen bonds and forming non-covalent complexes (Dror *et al.,* 2006; Mahdavi *et al.,* 2016). A previous study also reported that GA has high efficiency as a wall material for encapsulation of anthocyanin, thus making it less vulnerable to degradation and hence increasing its stability (Mahdavi *et al.*, 2016). However, in contrast to the previous findings, the current study found that the addition of GA resulted in the destabilisation of anthocyanin pigments, rendering it loses from roselle juices. The addition of GA significantly decreased ($p<0.05$) the anthocyanin content in roselle juice by approximately 7% to 25% (Table 1) as compared to the control juice. A possible explanation might be due to the ‘‘over-crowding” of GA molecules at their higher concentration. According to Dror *et al.* (2006) and Teleszko *et al.* (2019), anthocyanin was found to interact with glycoprotein in GA by hydrogen bond which is responsible for its stability. However, an increase in the GA concentration might cause a more compact structure of the GA molecules and thus hinder their exposure to anthocyanin (Chung *et al.,* 2016). Hence, the interaction between the anthocyanin and glycoprotein of GA to form non-covalent complexes decreased, which consequently leads to the instability of the pigments (Dror *et al.,* 2006; Chung *et al*., 2016). A similar trend was also observed by Chung *et al.* (2016) who found that purple carrot anthocyanin stability decreased when 2.5% to 5.0% of GA was added to the model beverage. Chung *et al.* (2016) also reported that the addition of GA initially enhanced the stability of anthocyanin when in the concentration of between 0.35 to 1.5% but decreased with a further increase of GA concentration from 2.5% to 5.0%.

Besides, this current study also revealed that the juice with *A. senegal* had lower anthocyanin content compared to juices with *A. seyal* at similar concentrations. This result may be explained by the fact that *A. senegal* had higher protein content (2.0%) than *A. seyal* (0.8%) (Idris, 2017). Thus, as there was overcrowding of proteins in the juice with *A. senegal*, the formation of non-covalent bonds of the gum molecules with anthocyanin pigments was possibly hindered, causing the anthocyanin pigments to become less stable and thus resulting in a lower anthocyanin content being evaluated as compared to the juice with *A. seyal* (Chung *et al.,* 2016). Therefore, it can be concluded that both the concentration and type of GA have their role in affecting the anthocyanin’s stability in roselle juice.

3.1.4 Antioxidant activity

3.1.4.1 Ferric Reducing Antioxidant Power assay

The effect of GA addition on the antioxidant activity of roselle juice was measured by Ferric Reducing Antioxidant Power (FRAP) assay (Table 1). The antioxidant activity was in the range of between 1.319±0.126 mmol TE/100 mL to 3.347±0.229 mmol TE/100 mL where the highest value was observed in roselle juice with 6% of *A. seyal,* significantly different from others. Between both GA types, roselle juice with 2%, 4% and 6% *A. seyal* possessed higher antioxidant activity than roselle juice with 2%, 4% and 6% *A. senegal*, respectively. A possible explanation for this current result may be due to the fact that *A. seyal* itself has a better antioxidant activity compared to *A. senegal*. Mirghani *et al.* (2018) reported that the gum extract from *A. seyal* had higher antioxidant activity than extract from *A. senegal* when assessed by FRAP assay. The authors also present the parallel result when evaluating the commercial source of *A. senegal* and *A. seyal*. Thus, the addition of *A. seyal* undoubtedly contributed to the increase of antioxidant activity even at low concentrations as compared to *A. senegal* addition. As discussed in the previous results on vitamin C content and total phenolic content, the previous results revealed that *A. seyal* seems to have more phenolic and vitamin C content than juices with *A. senegal*. Thus, the higher content of these antioxidant compounds seems to contribute to the antioxidant activity in the roselle juice of the current study. Hence, it can be concluded that *A. seyal* and *A. senegal* addition is capable of preventing oxidation of vitamin C in the juice and at the same time may also enhance their activity in roselle juice.

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3.1.4.2 DPPH Free Radical Scavenging activity

The effect of GA addition on the antioxidant activity assessed by the DPPH assay is presented in Table 1. Results showed that DPPH free radical scavenging activity of roselle juices ranged from 0.62±0.08 mmol TE/100 mL to 1.42±0.09 mmol TE/100 mL with the highest value reported in control juice (1.42±0.09 mmol TE/100 mL). It is interesting to note that this result had no significant difference ($p \geq 0.05$) with the juice of 6% *A. senegal* addition (1.41±9.89 mmol TE/100 mL). Moreover, the addition of different types and concentrations of GA significantly reduced ($p < 0.05$) the antioxidant activity by approximately 28% to 43% of all juices except for the juice with 6% *A. senegal*. This result was in accordance with a previous study by Ahmad *et al.* (2018) who found that the free radical scavenging ability of roselle-pineapple leather was decreased with hydrocolloids (mixed 0.2% xanthan gum, 0.2% locust bean gum and 1.0% maltodextrin) addition. Similarly, Yi *et al.* (2015) also reported that the scavenging ability of grape juice added with 1% of locust bean gum was decreased by 54.4% compared to the control juice (0%). The decrease in antioxidant activity with hydrocolloid addition may be due to the interaction of the hydrocolloid with antioxidant compounds by a hydrogen bond (Kim *et al.,* 2019; Kraithong *et al.,* 2019). The interactions of hydrocolloids and antioxidants probably decrease the diffusibility of antioxidants towards free radicals, thus decreasing their scavenging ability (Shahidi and Zhong, 2011). However, the current study also reported that the addition of 6% of *A. senegal* into roselle juice retained antioxidant activity and had no significant difference ($p \geq 0.05$) with the control juice. It might be due to the potential of the hydrocolloid that is capable of stabilising the bioactive compounds at higher concentrations (Krumreich *et al.,* 2018). At higher concentrations, the hydrocolloid may trap and protect the antioxidant compounds, thus improving their scavenging activity (Rashima *et al.,* 2017; Shahidi and Zhong, 2011). Nevertheless, the result differed when adding 6% of *A. seyal* showed a significant reduction ($p < 0.05$) of antioxidant activity. The variation in a trend of the antioxidant activity assessed by the DPPH assay on *A. senegal* and *A. seyal* addition may be attributed to the difference in antioxidant compounds that are present in the juice. As the juice may contain various antioxidant compounds, the antioxidant may react differently when different hydrocolloids are added, causing a variation in the antioxidant activity's results.

3.2 Oxidative stability of roselle juice during storage

For the determination of oxidative stability during storage, only the control juice and the most accepted roselle juice from each GA type (*A. senegal* or *A. seyal*) were evaluated. The most accepted formulation was selected based on the sensory acceptability test (result not shown) and antioxidant properties, mainly vitamin C, TPC and total anthocyanin content. For the juice with *A. senegal* addition, no significant difference ($p \geq 0.05$) was shown for most of the sensory attributes. Therefore, the most accepted formulation was chosen based on the antioxidant properties where the juice with 4% *A. senegal* was chosen due to its satisfactory vitamin C content, TPC and anthocyanin content. Meanwhile, for the roselle juice with *A. seyal* addition, juice with 2% *A. seyal* was the most accepted by panellists compared to the juice with 4% and 6% *A. seyal* (result not shown). Concerning the antioxidant properties, juice with 2% *A. seyal* also had reasonable vitamin C content, TPC as well as anthocyanin content, strengthening the suitability of its selection. Thus, roselle juice with GA (4% *A. senegal* and 2% *A. seyal*) along with control juice (0% GA) were further investigated for the storage stability study.

3.3 Oxidative stability during storage

3.3.1 Stability of vitamin C content during storage

Results on the stability of vitamin C content in roselle juices during storage are presented in Figure 1. Roselle juice with 2% *A. seyal* and 4% *A. senegal* had significantly higher ($p < 0.05$) vitamin C compared to control juice at the beginning of storage (week 0). At the end of storage (week 5), the juice with *A. seyal* remained the highest as compared to others. There were small fluctuations of vitamin C content in all samples between weeks of the storage period. Nevertheless, when comparing the vitamin C values of the juices at weeks 0 and 5 of the same sample, there was no significant difference ($p \geq 0.05$) observed for all samples showing that the vitamin C had recovered and was stable at the end of the storage. The addition of GA is capable of preserving approximately 92% to 100% of vitamin C content in the juices during chill storage. A similar result was observed by Krumreich *et al.* (2018) who reported that the vitamin C content has remained stable in guava nectar with xanthan or guar gum addition at the beginning of storage at 22°C for 180 days. The study also conveyed that there was more excellent protection on vitamin C content in nectars with gum as compared to the nectars without the gum or with enzymes. In this current study, the possible explanation for the protection of vitamin C by GA might be due to the hydrocolloid that is capable of preventing the oxidation of vitamin C during storage (Nwaokoro and Akanbi, 2015). The fluctuation of vitamin C values can be explained by

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several reasons. According to Kaur *et al.* (2019), the reduction of vitamin C content might be due to the oxidation of the compounds by residual oxygen, which might be enhanced by the prolonged storage period. Besides, vitamin C may also be utilised as a response to the oxidative stress caused by low-temperature storage (Hubert *et al.,* 2017). Meanwhile, the increase in vitamin C content of the juice might be explained by the enzyme activity in the pathway of vitamin C metabolism during storage. When there was stress by storage temperature, the enzyme in the juice might promote the synthesis of vitamin C, causing an increase in vitamin C content (Hubert *et al.,* 2017).

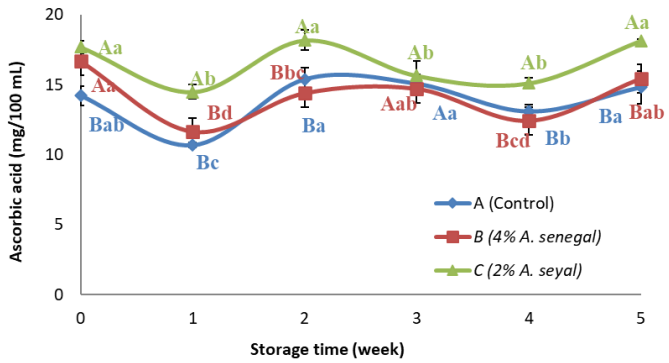

Figure 1. Vitamin C content of roselle juices with *A. senegal* (4%), *A. seyal* (2%) and juice without GA addition (control) during storage. Lines with different uppercase alphabets are significantly different within the storage time ($p<0.05$) while lines with different lowercase alphabets are significantly different between the same sample ($p<0.05$).

3.3.2 Stability of total phenolic content

Figure 2 demonstrates the stability of the total phenolic content (TPC) of roselle juices during storage. The result depicts that there was no significant ($p\geq0.05$) difference observed in TPC for all tested juices at the beginning of storage (week 0). However, at the end of storage (week 5), both roselle juices with 2% *A. seyal* and 4% *A. senegal* addition were found to have higher TPC as compared to the control juice. The higher phenolic content in roselle juices with GA addition may be due to the contribution of phenolic compounds by GA. Mirghani *et al.* (2018) reported the presence of phenolic compounds in GA extract as well as the commercial products of GA. Thus, during storage, the increase of phenolic content in roselle juices may be due to the release of polyphenols from the GA. Moreover, environmental stress such as low-temperature storage also might enhance the generation of phenolic compounds via the phenylpropanoid pathway by activation of phenylalanine ammonia-lyase (PAL) (Külen *et al.,* 2013). Besides, the current result also showed that the phenolic content in all juices was similar in values ($p \geq 0.05$) within 5 weeks indicating their stability during chill storage.

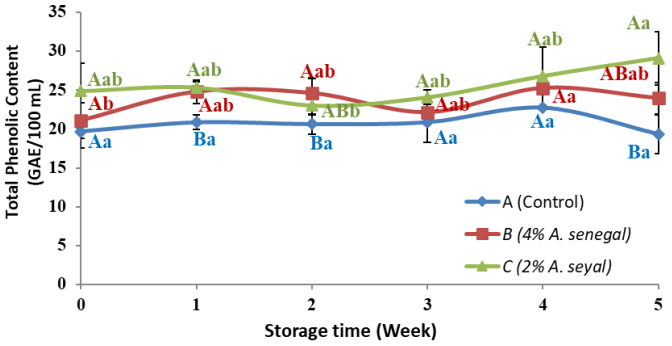

Figure 2. Total phenolic content of roselle juice with *A. senegal* (4%), *A. seyal* (2%) and without GA addition (control) during chill storage. Lines with different uppercase alphabets are significantly different within the storage time ($p<0.05$) while lines with different lowercase alphabets are significantly different between the same sample ($p<0.05$).

3.3.3 Stability of total anthocyanin content

Figure 3 presents the stability of anthocyanin content in roselle juice with 2% *A. seyal*, 4% *A. senegal* and juice without GA addition throughout the storage period. The anthocyanin content is tested juices exhibited significant changes ($p<0.05$) during five weeks of chill storage except for the juice with 2% *A. seyal*. At the beginning of storage (week 0), the control juice showed the highest ($p<0.05$) anthocyanin content followed by juice with 2% *A. seyal* and 4% *A. senegal*. However, changes occurred during storage which then resulted in similar anthocyanin content in all tested juices at the end of the storage period (week 5). Over five weeks of storage, the anthocyanin content in roselle juice without GA addition started to decrease at week 2 and was in decreasing trend until the end of storage ($p\leq0.05$). Meanwhile, the addition of 2% *A. seyal* had no significant effect ($p\geq0.05$) on anthocyanin content in

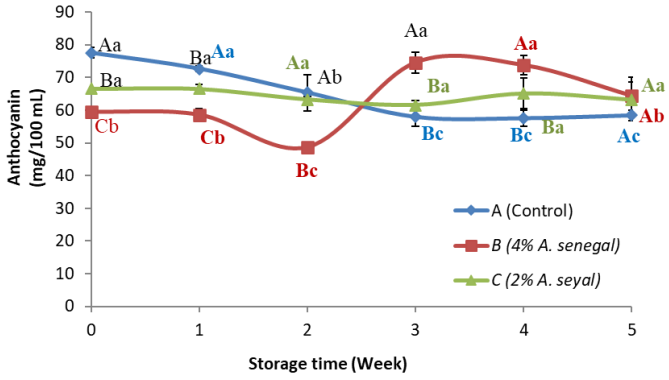

Figure 3. Total anthocyanin content of roselle juice with *A. senegal* (4%), *A. seyal* (2%) and without GA addition (control) during storage. Lines with different uppercase alphabets are significantly different within the storage time ($p<0.05$) while lines with different lowercase alphabets are significantly different between the same sample ($p<0.05$).

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roselle juice throughout storage. In contrast, the addition of 4% *A. senegal* caused a small fluctuation of anthocyanin content in the juice between weeks of storage but had no significant difference ($p \geq 0.05$) between week 0 and week 5. Hence, it can be seen that the juice with 4% *A. senegal* was able to retain its anthocyanin content at the end of storage even with the fluctuation trend.

The decrease in anthocyanin content in roselle juice is expected to be due to the instability of anthocyanin itself during storage. A few factors that usually contributed to the instability of anthocyanin compounds in the juice included the light, oxygen, storage condition, pH value and encapsulating agent used (Patras *et al.,* 2010; Cavalcanti *et al.*, 2011; Idham *et al.,* 2012; Wu *et al.*, 2018). Nevertheless, the addition of 2% *A. seyal* stabilised the anthocyanin content in roselle juice throughout the storage period. GA seems capable of enhancing the stability of anthocyanin during storage. Its complex structure may interact with anthocyanin by hydrogen bonds preventing the anthocyanin from nucleophilic attack by water molecules (Chung *et al.,* 2016; Mahdavi *et al.,* 2016). But again, for 4% of *A. senegal* usage, the "over-crowding" effect of higher GA concentration minimizes its interaction with anthocyanin resulting in instability of this pigment in roselle juice during storage as compared to the one with lower concentration. Though, its presence still was able to retain the anthocyanin content even after a series of fluctuations during storage.

3.3.4 Antioxidant activity stability

In the current study, the stability of the antioxidant activity of roselle juice was evaluated by FRAP and DPPH assay. Figure 4 and Figure 5 show the stability of antioxidant activity of all tested juices measured by FRAP and DPPH assay during five weeks of chill storage, respectively. Results showed that there was a fluctuation in trends of the antioxidant activity evaluated. For FRAP assay, roselle juice with both GA additions showed significant reduction ($p<0.05$) of antioxidant activity after two weeks of storage but was recovered from the 4th week until the end. In contrast, the antioxidant activity of control juice was increased significantly ($p \geq 0.05$) at week 5 as compared to week 0. Besides, for the DPPH assay, it can also be observed that the antioxidant activity of juice with 4% *A. senegal* addition remained stable for the first three weeks of storage. Then, a marked increase of free radical scavenging activity in the juice was observed at week 4 and remained stable until the end of storage. Differing from *A. senegal*, a reduction of antioxidant activity in the juice with 2% *A. seyal* addition was observed after two weeks of storage and only recovered at week 4. Meanwhile, the antioxidant activity of control juice showed no significant difference ($p \geq 0.05$) throughout five weeks of storage except at week 3 which showed a significant reduction ($p<0.05$) compared to the initial value (week 0) of antioxidant activity.

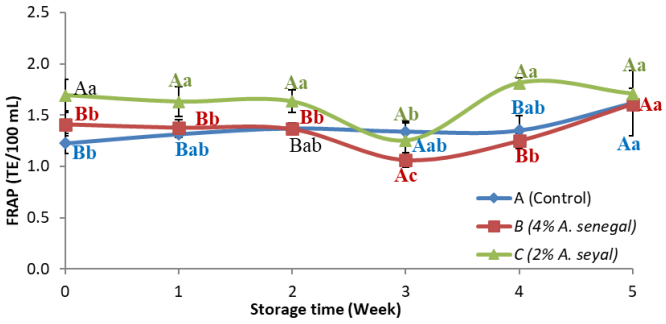

Figure 4. Antioxidant activity of roselle juice with addition of *A. senegal* (4%), *A. seyal* (2%) and without GA addition (control) assessed by Ferric reducing antioxidant power (FRAP) assay during chill storage. Lines with different uppercase alphabets are significantly different within the storage time ($p<0.05$) while lines with different lowercase alphabets are significantly different between the same sample ($p<0.05$).

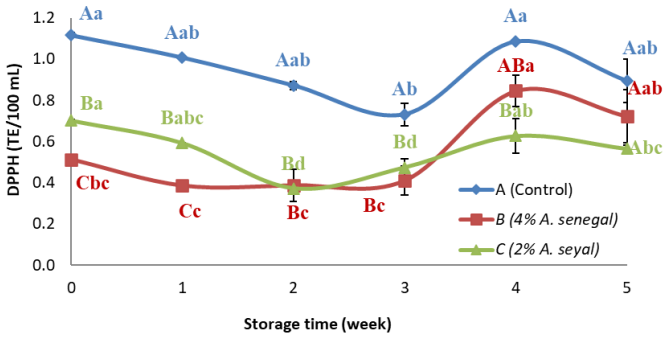

Figure 5. Antioxidant activity of roselle juice with *A. senegal* (4%), *A. seyal* (2%) and without GA addition (control) assessed by DPPH free radical scavenging activity assay during chill storage. Lines with different uppercase alphabets are significantly different within the storage time ($p<0.05$) while lines with different lowercase alphabets are significantly different between the same sample ($p<0.05$).

These current results could be explained by several explanations from other findings. A possible explanation for the reduction of antioxidant activity during storage might be due to the antioxidant antagonism occurring in the juices. According to Castro-López *et al.* (2016), due to the presence of diverse bioactive compounds and interactions in the sample, antioxidant antagonism occurred which then resulted in a decrease in antioxidant activity during storage. Besides, the antagonism among antioxidants might also result from the competitive generation of antioxidant adducts and/or reformation of a less effective antioxidant by a more effective antioxidant in the sample (Olszowy *et al.,* 2019). It is also reported that the increase of antioxidant activity in the sample may be due to the increase of antioxidant compounds

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during the storage period and vice versa (Hubert *et al.,* 2017). While another study reported that the increase in antioxidant activity may also be attributed to the polymerisation of polyphenols in the samples which produced oligomers that had larger areas for charge delocalisation (Castro-López *et al.,* 2016). Thus, as there was a larger area for charge delocalisation, it may reduce and delocalised the free radical effectively, showing an increase in antioxidant activity.

4. Conclusion

In conclusion, the results of the current study revealed that the addition of GA (*A. senegal* or *A. seyal*) had increased or at least maintained the vitamin C content and total phenolic content of the roselle juice. However, the addition of more than 2% GA into roselle juice caused a significant reduction in the total anthocyanin content in the roselle juice. Meanwhile, for the antioxidant activity of the roselle juice, the current study found variation in the results of FRAP and DPPH assay. Besides, the trends for vitamin C content, anthocyanins and antioxidant activity assayed by FRAP and DPPH showed fluctuations although, at the end of the storage time, the vitamin C content and anthocyanin content in the roselle juices with 4% *A. senegal* and 2% *A. seyal* addition was able to be retained until the end of storage. Therefore, this current study indicated that *A. senegal* and *A. seyal* have the potentials to be used as functional ingredients that contributed to stable antioxidant properties in beverages during storage.

Conflict of interest

The authors declare no conflict of interest.

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