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Co-pigmentation of sweet potato and greater yam anthocyanins with selected phenolic acids and its effect on *in vitro* antioxidant activity

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Abstract

The effect of co-pigmentation on the antioxidant capacity of anthocyanins isolated from purple colored root tubers of greater yam (*Dioscorea alata*, Acc. Da-340) and sweet potato (*Ipomoea batatas*, *cv* Bhu Krishna) with caffeic, ferulic and p-coumaric acids was investigated in this study. The DPPH radical scavenging activity of both the anthocyanins enhanced significantly after co-pigmentation. However, phenolic acids behaved differently with different concentrations of anthocyanins. Except at very low anthocyanin concentrations, caffeic acid and ferulic acid served as effective co-pigments for greater yam and sweet potato anthocyanins respectively, leading to an increase in antioxidant potential. At very low concentrations of the pigment and co-pigment used, the effect was reverse. The highest % radical scavenging activity was observed for greater yam anthocyanins at a concentration of 6 μgml^{-1} and 26 μg ml^{-1} of ferulic acid as the co-pigment, followed by the same concentration of anthocyanins and 24 μg ml^{-1} of caffeic acid. P-coumaric acid was not as effective as caffeic and ferulic acids. This study indicated the existence of some distinct intermolecular interactions that ensue in the complex framework of natural colors and the results could beuseful in designing bioactive food colorants.

Keywords: Greater yam, Sweet potato, Anthocyanins, Phenolic acids, Co-pigmentation, Radical scavenging activity

Introduction

Anthocyanins are naturally occurring plant phenolics consisting of an aglycone linked to one or more sugar moieties that can be further acylated by aromatic or aliphatic organic acids. Anthocyanins are potential anticancer agents and have the capacity to scavenge active radicals to prevent carcinogenesis (Lila, 2004; Wang & Stoner, 2008). These are also capable of improving visual functions (Khoo, Azlan, Tang, & Lim, 2017; Shim, Kim, Choi, Kim, & Park, 2012) and inhibiting platelet aggregation (Song et al., 2014; Yang et al., 2010). Anthocyanins of purple sweet potato are noted for their stability and physiological functions. Anthocyanins of a purple sweet potato cultivar named Bhu Krishna contain peonidin and cyanidin derivatives, which have anti-proliferate effects on breast, colon and cervical cancer cells (Vishnu et al., 2019). Greater yam (*Dioscorea alata*) tuber anthocyanins were found to be rich in cyanidin derivatives and exhibit high antioxidant activity (Moriya et al., 2015).

Co-pigmentation is the formation of non-covalent complexes due to the interaction between anthocyanins/ anthocyanin derived pigment with a co-pigment, which also changes the optical properties of the pigment (Trouillas et al., 2016). A co-pigment is a compound which can enhance the color of anthocyanin solution

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due to the intermolecular interactions between them (Welch, Wu, & Simon, 2008). Generally, co-pigments have extended π-conjugated systems that favour π-π stacking interaction and have hydrogen bond donors or acceptors like hydroxyl or carbonyl groups. Phenolic acids can serve as co-pigments due to the presence of aromatic rings and hydroxyl groups. Intermolecular co-pigmentation is possible between anthocyanin and phenolic acids, polyphenolic compounds, metal ions, etc. The vertical hydrophobic stacking of the aromatic nuclei is possible between anthocyanins and polyphenolic compounds. The co-pigmentation of anthocyanins with other compounds is responsible for the final colour of anthocyanins.

The factors that affect the co-pigmentation are the nature of aglycone and co-pigment, anthocyanin concentration, the molar ratio between anthocyanin and co-pigment, pH, type of solvent used and temperature (Trouillas et al., 2016). Several models have been proposed for the intermolecular co-pigmentation between differently acylated anthocyanins and other aromatic molecules (Gauche, Malagoli, Terezinha, & Luiz, 2010; Kopjar and Piližota, 2009; Trouillas et al., 2016). Anthocyanins themselves can act as co-pigment due to self-association, but it is less efficient than co-pigmentation with phenolic acids and their derivatives. The force acting behind self-association was the hydrophobic interaction between the aromatic nuclei, which are stacked parallel to each other. The previous reports said that vertical stacking by π-π interactions is more prominent than horizontal stacking by hydrogen bonds (Trouillas et al., 2016). However, there is little information available on how co-pigmentation with phenolic acids leads to changes in the antioxidant activity of anthocyanins. Therefore, this study was undertaken to understand the effect of intermolecular co-pigmentation of anthocyanins isolated from the purple flesh tubers of sweet potato and greater yam with some selected phenolic acids on their antioxidant capacity. The root tubers of anthocyanin rich promising sweet potato cultivar Bhu Krishna and a greater yam accession, Da-340, which are available in the collection of ICAR-Central Tuber Crops Research Institute (ICAR-CTCRI), Thiruvananthapuram, Kerala, India were used as anthocyanins sources.

Materials and Methods

Materials

Freshly harvested tubers of greater yam (Acc. Da-340) and sweet potato (*cv* Bhu Krishna) were collected from the ICAR-CTCRI experimental farm. The tubers were washed thoroughly, sliced and subjected to solvent extraction for isolating the anthocyanins. The phenolic acids used were hydroxy cinnamic acids *viz*., caffeic, ferulic and p-coumaric acids.Amberlite XAD-7, 1,1-Diphenyl-2-picryl-hydrazil (DPPH),ethyl acetate, trifluoroacetic acidand methanol were purchased from Merck India Ltd. Citric acid, sodium citrate, ethanol, hydrochloric acid, sodium hydroxide and potassium hydroxide were of highest analytical grade. Caffeic acid, ferulic acid and p-coumaric acid were purchased from Sigma Aldrich (St. Louis, USA).

Isolation and purification of anthocyanins

Anthocyanins were extracted from a weighed quantity (100g) of the fresh tubers using methanol, acidified with 0.5% triflouroacetic acid (TFA). The extraction was continued until the residue was colourless. The filtrates were combined and concentrated in a rotary flash evaporator (Buchi Multivapor P-6) at 30°C under reduced pressure. The crude anthocyanin pigment was then dissolved in distilled water and partitioned with ethyl acetate to remove other non-polar compounds. The remaining aqueous layer was collected and subjected to column chromatography using Amberlite XAD-7 resin. The anthocyanins adsorbed on Amberlite were eluted using methanol acidified with 0.5% TFA. The eluent was collected, pooled and concentrated using a rotary flash evaporator at 30°C under reduced pressure and then lyophilized to obtain purified anthocyanins.

Co-pigmentation of anthocyanins

The anthocyanins were prepared at a concentration of 2.23×10^{-3} mmol ml^{-1} of cyanidin-3-O-glycoside equivalent. The concentration of the co-pigments, *viz.,* caffeic, ferulic and p-coumaric acids were made to millimolar equivalent or proportional to the fixed concentration of anthocyanins.The citrate buffer at pH 3.5 was used as a solvent for all the co-pigmentation studies. The preliminary screening for determining the extent of co-pigmentation was done at different molar ratio of anthocyanins with selected phenolic acids (1:1, 1:5, 1:10, 1:20, 1:30 and 1:40). The shift in λ_{max} and increase in absorbnce was analyzed in each case and the ratio at which large increase in absorbance was considered as the effective co-pigmentation ratio and these concentrations were selected for further studies.

UV-Visible spectroscopy

Spectroscopic evaluation of purified anthocyanins and co-pigmented anthocyanins was done by using a UV-Vis spectrophotometer (Perkin Elmer, Lambda 25, Switzerland). The samples were scanned in a wavelength region of 400-700 nm and the absorbance was also recorded at the maximum wavelength (λ_{max}). The difference in absorbance at λ_{max} of anthocyanins before and after co-pigmentation was expressed as ΔA.

Evaluation of antioxidant activity by DPPH assay

A known weight of anthocyanins was dissolved in citrate buffer solution (pH 3.5) and used for preparing the test solution. Free radical scavenging activity of the anthocyanins of greater yam and sweet potato was

measured by 2, 2- diphenyl-1-picryl hydrazyl (DPPH) assay. Briefly, 0.2 mM solution of DPPH in methanol (1 ml) was added to anthocyanin solution of different concentrations. The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. Then, absorbance was measured at 517nm using a spectrophotometer (UV-Vis Perkin Elmer, Lambda 25, Switzerland). The IC_{50} value of anthocyanins, which is the concentration of sample required to inhibit 50% of the DPPH free radicals, was calculated using the Log dose inhibition curve. The percent DPPH scavenging effect was calculated by using the following equation:

$$\text{DPPH scavenging effect (\%)} = \frac{A0\text{-}A1}{A0} \times 100$$

Where, A_0 was the absorbance of the control, which contains DPPH alone, and A1 was the absorbance of anthocyanins.

Evaluation of type of interaction

The type of interaction between the anthocyanins and the co-pigments was evaluated by comparing the theoretical and experimental (real) radical scavenging activities. The theoretical radical scavenging activity (RSA) was calculated by adding the individual radical scavenging activities of pigment and co-pigment at a particular concentration. Real RSA was experimentally determined as per the protocol mentioned in the previous section by using the same combination of pigment and co-pigment which were taken for calculating theoretical radical scavenging activity. If real RSA was greater than that of theoretical RSA, a positive effect occurred and otherwise the effect was considered as negative.

Statistical analysis

Single-factor analysis of variance (ANOVA) of data was performed using the package SAS 9.3. Duncan Multiple Range Test (DMRT) was done for the pair-wise comparison of the mean values.

Results and Discussion

Effect of co-pigmentation on UV-Vis spectra of anthocyanins

Co-pigmentation can be identified by analyzing the change in colour intensity and spectral shifts observed by UV-visible spectroscopy, which is used widely for identifying the co-pigmentation because only low pigment concentration is needed for it (Trouillas et al., 2016). It is possible to evaluate co-pigmentation interms of bathochromic or hyperchromic shifts. The UV-vis spectra of co-pigmented greater yam and sweet potato anthocyanins are shown in Fig. 2A and Fig. 2B respectively. The greater yam and sweet potato anthocyanins showed wavelength of the maximum absorbance (λ_{max}) at 528.97 nm and 527.43 nm, respectively at a concentration of 2.23×10^{-3} mmolml^{-1} (Table 1). These peaks in the visible region are considered the characteristic peaks of anthocyanins and are responsible for their deep red colour. Anthocyanin color may vary according to the number and position of hydroxyl groups. The distinctive peak of anthocyanins generally ranges from 450-560 nm and is due to the B-ring of anthocyanins (Figure 1). Another characteristic band in the UV-visible spectrum at 240-280 nm is due to the A-ring, called the benzoyl system. The evidence of positive co-pigmentation can be identified by evaluating the increase in absorbance (ΔA) and the shift in wavelength (λ_{max}). A significant change in absorbance (hyperchromic shift) was observed after co-pigmentation of greater yam and sweet potato anthocyanins with caffeic acid and ferulic acid at a pigment/co-pigment ratio of 1:10. However, in the case of p-coumaric acid, the hyperchromic shift was observed at the pigment/co-pigment ratio of 1:20 (Table 1). Co-pigmentation interaction of anthocyanins with phenolic acids resulted in the presence of hydroxyl group in the aromatic ring.

Co-pigmentation of purple yam anthocyanins with caffeic acid resulted in an increase in absorbance of the former by 56.10 % ($\Delta A = 0.087$) (Table 1), which was observed as the most effective co-pigmentation in greater yam tuber anthocyanins in the present study. However, p-coumaric acid was found to be a less effective co-pigment with greater yam anthocyanins by virtue of the minimal increase in absorbance ($\Delta A = 0.046$) after co-pigmentation with it. Ferulic acid showed about a 45.80% increase in absorbance with an ΔA value of 0.071. The two important factors that are expected behind these observations are π-π stacking and intermolecular hydrogen bonding. Cyanidin and peonidin are reported to be the common aglycones present in greater yam anthocyanins and among these, cyanidin was the major one (Moriya et al., 2015; Shoyama et al., 1990). Cyanidin contains two hydroxyl groups in the B ring (Figure 1) and can effectively participate in intermolecular hydrogen bonding and π-π stacking with the co-pigment. Caffeic acid, which also contains two hydroxyl groups was able to participate in co-pigmentation interaction with cyanidin-rich greater yam anthocyanins with identical symmetry, more effectively than ferulic and p-coumaric acids, both of which contain only one hydroxyl group attached to the aromatic ring.

Co-pigmentation of sweet potato anthocyanins with ferulic acid resulted in an increase in absorption intensity with a A value of 0.103, which was observed as the most effective co-pigmentation among the three selected phenolic acids (Table 1). This increase in absorbance was 84.4% greater than that of individual sweet potato anthocyanins. Here, unlike the case with greater yam anthocyanins, caffeic acid was the least effective co-pigment ($\Delta A = 0.046$). Co-pigmentation of anthocyanins with p-coumaric acid showed an increase in absorbance value by 57.37% at a molar ratio of 1:20.

Table 1. UV-visible absorbance* and λ_{max} of anthocyanins before and after co-pigmentation with phenolic acids

Anthocyanin source	Co-pigment	Molar ratio of anthocyanins/ Co-pigment	λ_{max} (nm)	Absorbance at λ_{max}	ΔA	$\Delta\lambda_{max}$ (nm)
Greater yam tuber	-	-	528.97	0.155 ± 0.03^{f}	-	-
	Ferulic acid	1:10	529.74	0.226 ± 0.05^{b}	0.071	0.77
	Caffeic acid	1:10	529.44	0.242 ± 0.03^{a}	0.087	0.47
	p-Coumaric acid	1:20	529.10	0.201 ± 0.06^{c}	0.046	0.13
Sweet potato tuber	-	-	527.43	0.122 ± 0.04^{g}	-	-
	Ferulic acid	1:10	528.54	0.225 ± 0.07^{b}	0.103	1.11
	Caffeic acid	1:10	528.24	0.168 ± 0.05^{e}	0.046	0.81
	p-Coumaric acid	1:20	528.46	0.192 ± 0.05^{d}	0.070	1.03

*Mean values with similar alphabets in the superscript are not significantly different

Sweet potato tubers contain acylated anthocyanins with peonidin and cyanidin aglycones, and among these peonidin was reported as the major aglycone (Cuevas et al., 2011; Montilla et al., 2010; Sun et al., 2014; Vishnu et al., 2019). A previous study revealed that the major compound present in the tuber anthocyanins of sweet potato cultivar Bhu Krishna are caffeoyl derivatives of peonidin (Vishnu et al., 2019). The substituents attached to the B ring of aglycone is responsible for the extent of stacking with co-pigments, which leads to the intermolecular co-pigmentation and increase in color intensity of anthocyanins (Boulton, 2001b; Eiro and Heinonen, 2002; Kammerer, 2016; Trouillas et al., 2016; Welch et al., 2008). The comparatively lower co-pigmentation efficiency of caffeic acid towards sweet potato anthocyanins could be explained as follows. Instead of stacking with the B-ring of the aglycone, the stacking of caffeic acid takes place with the structurally similar caffeoyil group attached to the sugar moiety present in the sweet potato anthocyanins. However, the aforementioned stacking was irrelevant tothe increase in color intensity and stability of anthocyanins.Peonidin contains one each of the -OH and -OCH_3 groups in the B-ring. Similar groups are present in ferulic acid and hence it can serve as an effective co-pigment through π-π stacking with sweet potato anthocyanins. This result is also in agreement with those of the previous studies, which reported that hydroxycinnamic acids and its derivatives are more effective co-pigments than benzoic acid and its derivatives (Marković, et al., 2000; Malaj et al., 2013; Trouillas et al., 2016). P-coumaric acid was observed as a more effective co-pigment for sweet potato tuber anthocyanins than that for greater yam anthocyanins.

The co-pigmentation effects of greater yam and sweet potato anthocyanins with the three selected phenolic acids were found distinctive. Based on change in absorbance, caffeic acid was found to be the best co-pigment for cyanidin-rich greater yam anthocyanins, but the same was identified as the least potent co-pigment with sweet potato anthocyanins with peonidin as the major aglycone. The results of the study revealed that the structure of aglycone and the acyl group attached to anthocyanins plays an important role in the co-pigmentation reaction.

DPPH assay of anthocyanins

The antioxidant activity of a sample is due to its hydrogen-donating ability. When chemical substances or biological structures interact with each other, it results in an overall effect that is greater than the sum of the individual effects of any of them and is termed as synergistic effect (Boulton, 2001a; Palafox-Carlos et al., 2012). When co-pigmentation results in a radical scavenging activity (real or experimental), which is greater than theoretical activity, it is termed as positive co-pigmentation.

The individual antioxidant activity of anthocyanins in the tubers of greater yam and sweet potato was determined and the results are presented as percentage radical scavenging capacity (% RSA) in Table 2. Greater yam anthocyanins were found to have the greater antioxidant capacity with an IC_{50} value of 8.72 $\mu g ml^{-1}$, than sweet potato anthocyanins, which have an IC_{50} value of 11.58 $\mu g ml^{-1}$ (Table 2). This could be attributed to the presence of higher levels of potentially bioactive cyanidin content in greater yam anthocyanins when compared to that in sweet potato anthocyanins (Cuevas et al., 2011; Moriya et al., 2015; Shoyama et al., 1990; Vishnu et al., 2019).

Table 2. DPPH radical scavenging activity[1] of sweet potato and greater yam tuber anthocyanins and different phenolic acids[2]

	Concentration ($\mu g\ ml^{-1}$)	% RSA[3]	IC_{50} ($\mu g\ ml^{-1}$)
Anthocyanin source			
Greater yam tuber anthocyanins	1.5	16.5 ± 0.35^{e}	8.92^{b}
	3	25.8 ± 0.56^{d}	
	6	36.8 ± 0.75^{b}	
	12	58.1 ± 0.97^{a}	

Sweet potato tuber anthocyanins	1.5	7.9 ± 0.54[g]	11.58[a]
	3	12.6 ± 0.86[f]	
	6	16.8 ± 1.23[e]	
	12	33.8 ± 1.66[c]	
Phenolic acid			
Caffeic acid	6	6.2±0.39[i]	63.54[c]
	12	11.6±0.78[g]	
	24	19.6±0.72[e]	
	48	38.5±1.13[a]	
Ferulic acid	6.5	8.3 ± 0.55[h]	70.28[b]
	13	14.8 ±0.61[f]	
	26	22.2 ± 0.95[c]	
	52	37.4 ± 1.37[b]	
P-Coumaric acid	11	3.2±0.54[j]	108.75[a]
	22	8.6± 0.66[h]	
	44	20.8± 1.13[d]	
	88	38.9± 1.56[a]	

[1] Mean values with similar alphabets in the superscript are not significant different.

[2] Concentration of phenolic acids used was proportional to 2.22×10^{-3} mmol of cyanidin-3-O-glycoside.

[3] RSA - Radical scavenging activity.

Individual antioxidant potential of caffeic, ferulic and p-coumaric acids were also estimated to understand their biological action. Among these, caffeic acid had comparatively higher DPPH radical scavenging activity (IC_{50}- 63.54 μg ml^{-1}) than the other two (Table 2). The activity was lowest for p-coumaric acid (IC_{50} - 108.75 μg ml^{-1}). The antioxidant activity of greater yam and sweet potato anthocyanins, after co-pigmentation with phenolic acids, is presented in Tables 3 and 4, respectively and the activity was found to be dose-dependent, *i.e.*, the activity increased with increase in concentration.

Co-pigmentation of greater yam anthocyanins with caffeic, ferulic and p-coumaric acids resulted in an increase in experimental values of % RSA in comparison to the theoretical values, except at the highest anthocyanin concentration of 12 μg ml^{-1} (Table 3). At the highest anthocyanin concentration of 12 μg ml^{-1}, a negative effect was observed with a decrease in the experimental % RSA value when compared to the theoretical value. The results also revealed that at higher concentrations of greater yam anthocyanins (3-12 μg ml^{-1}), co-pigmentation with ferulic acid resulted in comparatively higher antioxidant activity than that with caffeic acid and p-coumaric acid. The highest real % RSA was observed for greater yam anthocyanins at a concentration of 6 μg ml^{-1} and 26 μg ml^{-1} of ferulic acid as the co-pigment, followed by the same concentration of anthocyanins and 24 μg ml^{-1} of caffeic acid.

The sweet potato anthocyanins showed positive interaction with caffeic acid and p-coumaric acid at all concentrations with an increase in real % RSA when compared to the theoretical values (Table 4). However, with ferulic acid, the interaction was negative at the highest concentration of anthocyanins (12 μg ml^{-1}). An interesting factor noted was that at the highest concentration of anthocyanins, caffeic acid showed significantly higher antioxidant activity than other phenolic acids. Even though ferulic acid served as an excellent co-pigment for sweet potato anthocyanins, with caffeic acid, especially at higher concentrations, these anthocyanins exhibited

Table 3. The DPPH radical scavenging activity* of greater yam anthocyanins co-pigmented with phenolic acids

Sample	Concentration (μg ml^{-1})		% RSA		Type of interaction
	Anthocyanins	Caffeic acid	Real	Theoretical	
Greater yam anthocyanins + caffeic acid (1:10 mM ratio)	1.5	6	32.3±0.91[j]	22.7±0.74[g]	Positive
	3	12	48.6±1.21[h]	37.4±1.34[d]	Positive
	6	24	71.8±0.83[e]	56.4±1.47[b]	Positive
	12	48	88.4±2.34[c]	96.6±2.10[a]	Negative
Greater yam anthocyanins + ferulic acid (1:10 mM ratio)	1.5	6.5	27.6± 0.98[k]	24.8 ± 0.90[f]	Positive
	3	13	50.4± 1.11[g]	40.6 ± 1.17[c]	Positive
	6	26	77.06±1.33[d]	59.0 ± 1.70[b]	Positive
	12	52	91.98±0.93[a]	95.5 ± 2.34[a]	Negative
Greater yam anthocyanins + p-coumaric acid (1:20 mM ratio)	1.5	11	22.7±0.48[l]	19.7±0.89[h]	Positive
	3	22	35.4±0.81[i]	34.4±1.22[e]	Positive
	6	44	61.8±0.93[f]	57.6±1.88[b]	Positive
	12	88	90.1±1.34[b]	97.0±2.53[a]	Negative

*Mean values with similar alphabets in the superscript are not significantly different.

Table 4. The DPPH radical scavenging activity* of sweet potato tuber anthocyanins co-pigmented with phenolic acids

Sample	Concentration (μg ml^{-1})		% RSA		Type of interaction
	Anthocyanins	Caffeic acid	Real	Theoretical	
Sweet potato anthocyanins + caffeic acid (1:10 mM ratio)	1.5	6	16.2±0.43^{i}	14.1±0.93^{g}	Positive
	3	12	31.2±0.83^{g}	24.2±1.64^{e}	Positive
	6	24	46.8±1.43^{d}	36.4±1.95^{c}	Positive
	12	48	94.4±2.43^{a}	72.3±2.79^{a}	Positive
Sweet potato anthocyanins + ferulic acid (1:10 mM ratio)	1.5	6.5	22.9±0.78^{h}	16.2±1.09^{g}	Positive
	3	13	31.8±1.11^{g}	27.4±1.47^{d}	Positive
	6	26	44.0±1.39^{e}	39.0±2.18^{b}	Positive
	12	52	70.6±1.53^{c}	71.2±3.03^{a}	Negative
Sweet potato anthocyanins + p-coumaric acid (1:20 mM ratio)	1.5	11	14.2±0.18^{j}	11.1±1.08^{h}	Positive
	3	22	23.1±0.81^{h}	21.2±1.52^{f}	Positive
	6	44	39.0±0.79^{f}	37.6±2.36^{bc}	Positive
	12	88	74.9±0.53^{b}	72.7±3.23^{a}	Positive

*Mean values with similar alphabets in the superscript are not significantly different.

greater antioxidant activity. When comparing the effect of co-pigmentation of caffeic acid with greater yam and sweet potato anthocyanins, at lower concentrations of 1.5, 3 and 6 μg ml^{-1}, the co-pigmentation increased the %RSA of greater yam anthocyanins more than that of sweet potato anthocyanins. Although at the highest concentration of caffeic acid, a positive interaction that leads to an increase in antioxidant activity was observed for sweet potato anthocyanins. However, under the same conditions, a negative interaction that leads to a decrease in real %RSA was observed for greater yam anthocyanins. The effect of co-pigmentation with ferulic acid on the antioxidant activity was higher in sweet potato anthocyanins than that of greater yam anthocyanins at a concentration of 1.5 μg ml^{-1}. However, at all the other selected concentrations of anthocyanins, the activity was more with sweet potato anthocyanins. When comparing all three phenolic acids, p-coumaric acid was the least potent to increase the antioxidant potential of both anthocyanins.

From the above results, it is clear that caffeic acid can serve as an effective co-pigment with cyanidin rich greater yam anthocyanins only at low anthocyanin concentrations. This was attributed to the formation of more stable aglycone after co-pigmentation with caffeic acid due to the increased intermolecular hydrogen bonding and π-π stacking, which enhanced the antioxidant potential. However, at low concentrations of sweet potato anthocyanins, ferulic acid was found to be a more effective co-pigment. The stacking between sweet potato anthocyanins and ferulic acid enhances the stability of B-ring of peonidin-rich anthocyanins which also stabilizes the flavylium cation resulting in an increase in antioxidant activity.

Previous reports revealed that anthocyanins in greater yam were found as mostly acylated with sinapic and ferulic acids (Moriya et al., 2015). Sinapic acid contains two methoxy and one hydroxyl group, while ferulic acid contains one methoxy and one hydroxyl group. At low anthocyanin concentrations, the co-pigment, ferulic acid can stack with the structurally similar acyl moiety present in greater yam anthocyanins, while sufficient co-pigment molecules might not be available to interact with the B-ring of the aglycone. When the concentration of pigment and co-pigment increases, more co-pigment molecules were expected to stack with the B-ring of aglycone. On the other hand, sweet potato anthocyanins are reported to be acylated with caffeic acid (Vishnu et al., 2019). At low anthocyanin concentrations, the caffeic acid co-pigment added can stack with the structurally similar caffeoyl moiety present in the sweet potato anthocyanins. Therefore, here the interaction of co-pigment with the B-ring of aglycone might be minimal. Caffeic acid can stack with the B-ring of sweet potato anthocyanins effectively at still higher anthocyanin and co-pigment concentrations.

Positive interaction which leads to an increase in antioxidant potential may be mainly due to the increase in the availability of the coloured flavylium cation which was converted back from colourless hemi-ketal form during the addition of the co-pigment. However, at higher concentrations, the decrease in experimental radical scavenging activity might be due to the increase in intramolecular co-pigmentation. According to previous

studies, intramolecular co-pigmentation of anthocyanins was less effective than intermolecular co-pigmentation (Trouillas et al., 2016). The structure of anthocyanins present in both the tuber was highly influencing the change in antioxidant potential after co-pigmentation. The co-pigmentation of anthocyanins with phenolic acids is also responsible for the conversion of less active hemiketal structure to bioactive flavylium cation.

Recently, co-pigmentation has a remarkable interest in food industry because of the opportunities to design unique combinations of natural pigment and co-pigment with finely harmonized stable colors coupled with enhanced bioactivity. Anthocyanins and anthocyanidins can be used as a natural food colorants not only due to the high color intensity but also due to their prominent nutraceutical properties which is responsible for potential health benefits (Khoo et al., 2017). Most of the food products such as fruits, vegetables, wine, cocoa and tea contain high concentrations of hydroxy cinnamic acids having potent anti-oxidant, anti-inflammatory, anti-diabetic and anti-hyperlipidemic properties (Alam et al., 2016; El-Seedi et al., 2018). These two classes of compounds, *viz*., the non-toxic, bioactive anthocyanins and phenolic acids (hydroxycinnamic acids) together can contribute to an enhanced color intensity and antioxidant potential than that of their individual contributions. According to the results of the present study, the color intensity and antioxidant potential of greater yam and sweet potato tuber anthocyanins could be enhanced by co-pigmentation with hydroxycinnamic acids such as caffeic and ferulic acids.

Conclusions

Greater yam and sweet potato tuber anthocyanins exhibited a positive co-pigmentation with phenolic acids resulting in an increase in antioxidant activity at lower concentrations. Ferulic acid and caffeic acid served as effective co-pigments and lead to a significant increase in antioxidant potential of both the anthocyanins. The co-pigmentation efficiency of p-coumaric acid was less when compared to the other two phenolic acids. Very high concentration of anthocyanins caused a negative interaction in most cases. This study proved that the structure of aglycone, presence of acylation and concentration of anthocyanins as well as phenolic acids can affect the antioxidant potential of anthocyanins. In the light of these results, the importance of choosing the best combination of anthocyanins and phenolic acid is important in increasing the colour intensity and antioxidant potential of anthocyanins in functional foods.

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References

Alam, M. A., Subhan, N., Hossain, H., Hossain, M., Reza, H. M., Rahman, M. M., & Ullah, M. O. (2016). Hydroxycinnamic acid derivatives: a potential class of natural compounds for the management of lipid metabolism and obesity. *Nutrition & Metabolism*, **13**:27.

Boulton, R. (2001b). The Copigmentation of Anthocyanins and Its Role in the Color of Red Wine: *A Critical Review*. *Am. J. Enol. Vitic* (Vol. 52).

Cuevas, E., Silke, M., & Peter, H. (2011). Anthocyanins in Purple Sweet Potato (*Ipomoea batatas L.*) Varieties. *Fruit, Vegetable and Cereal Science and Biotechnology*, 0–5.

Dimitrić Marković, Petranović, N. A. & Baranac, J. M. (2000). A Spectrophotometric Study of the Copigmentation of Malvin with Caffeic and Ferulic Acids. *Journal of Agricultural and Food Chemistry*, **48**(11):5530–5536.

Eiro, M. J., & Heinonen, M. (2002). Anthocyanin Color Behavior and Stability during Storage: Effect of Intermolecular Copigmentation. *Journal of Agricultural and Food Chemistry*, **50**(25):7461–7466.

El-Seedi, H. R., Taher, E. A., Sheikh, B. Y., Anjum, S., Saeed, A., AlAjmi, M. F., Göransson, U. (2018). Hydroxycinnamic Acids: Natural Sources, Biosynthesis, Possible Biological Activities, and Roles in Islamic Medicine. *Studies in Natural Products Chemistry*, **55**:269–292.

Gauche, C., Malagoli, S., Terezinha, M., & Luiz, B. (2010). Effect of pH on the copigmentation of anthocyanins from Cabernet Sauvignon grape extracts with organic acids Cabernet Sauvignon com ácidos orgânicos, (February), 41–46.

Kammerer, D. R. (2016). Anthocyanins. *Handbook on Natural Pigments in Food and Beverages*, 61–80.

Khoo, H. E., Azlan, A., Tang, S. T., & Lim, S. M. (2017). Anthocyanidins and anthocyanins: colored pigments as food, pharmaceutical ingredients, and the potential health benefits. *Food & Nutrition Research*, **61**(1):1361779.

Kopjar, M., & Piližota, V. (2009). Copigmentation effect of phenolic compounds on red currant juice anthocyanins during storage, **1**:16–20.

Lila, M. A. (2004). Anthocyanins and Human Health: An In Vitro Investigative Approach. *Journal of Biomedicine & Biotechnology*, **2004**(5):306–313.

Malaj, N., De Simone, B. C., Quartarolo, A. D., & Russo, N. (2013). Spectrophotometric study of the copigmentation of malvidin 3-O-glucoside with p-coumaric, vanillic and syringic acids. *Food Chemistry*, **141**(4):3614–3620.

Montilla, E. C., Hillebrand, S., Butschbach, D., Baldermann, S., Watanabe, N. & Winterhalter, P. (2010). Preparative isolation of anthocyanins from Japanese purple sweet potato (*Ipomoea batatas L.*) varieties by high-speed counter current chromatography. *Journal of Agricultural and Food Chemistry*, **58**(18):9899–9904.

Moriya, C., Hosoya, T., Agawa, S., Sugiyama, Y., Kozone, I., Shin-ya, K., Kumazawa, S. (2015). New acylated anthocyanins from purple yam and their antioxidant activity. *Bioscience, Biotechnology, and Biochemistry*, **79**(90): 1484–1492.

Palafox-Carlos, H., Gil-Chávez, J., Sotelo-Mundo, R., Namiesnik, J., Gorinstein, S., González-Aguilar, G., González-Aguilar, G. A. (2012). Antioxidant Interactions between Major Phenolic Compounds Found in 'Ataulfo' Mango Pulp: Chlorogenic, Gallic, Protocatechuic and Vanillic Acids. *Molecules*, **17**(11):12657–12664.

Shim, S. H., Kim, J. M., Choi, C. Y., Kim, C. Y. & Park, K. H. (2012). *Ginkgo biloba* Extract and Bilberry Anthocyanins Improve Visual Function in Patients with Normal Tension Glaucoma. *Journal of Medicinal Food*, **15**(9):818–823.

Shoyama, Y., Nishioka, I., Herath, W., Uemoto, S., Fujieda, K. & Okubo, H. (1990). Two acylated anthocyanins from Dioscorea alata. *Phytochemistry*, **29**(9):2999–3001.

Song, F., Zhu, Y., Shi, Z., Tian, J., Deng, X., Ren, J., Yang, Y. (2014). Plant food anthocyanins inhibit platelet granule secretion in hypercholesterolaemia: Involving the signalling pathway of PI3K-Akt. *Thrombosis and Haemostasis*, **112**(5):981–991.

Sun, H., Mu, T., Liu, X., Zhang, M. & Chen, J. (2014). Purple sweet potato (*Ipomoea batatas L.*) anthocyanins: Preventive effect on acute and subacute alcoholic liver damage and dealcoholic effect. *Journal of Agricultural and Food Chemistry*, **62**(11):2364–2373.

Trouillas, P., Sancho-García, J. C., De Freitas, V., Gierschner, J., Otyepka, M., & Dangles, O. (2016). Stabilizing and Modulating Color by Copigmentation: Insights from Theory and Experiment. *Chemical Reviews*.

Vishnu, V. R., Renjith, R. S., Mukherjee, A., Anil, S. R., Sreekumar, J., & Jyothi, A. N. (2019). Comparative Study on the Chemical Structure and *In Vitro* Antiproliferative Activity of Anthocyanins in Purple Root Tubers and Leaves of Sweet Potato (*Ipomoea batatas*). *Journal of Agricultural and Food Chemistry*, **67**(9):2467–2475.

Wang, L.-S. & Stoner, G. D. (2008). Anthocyanins and their role in cancer prevention. *Cancer Letters*, **269**(2):281–290.

Welch, C. R., Wu, Q., & Simon, J. E. (2008). Recent Advances in Anthocyanin Analysis and Characterization. *Current Analytical Chemistry*, **4**(2):75–101.

Yang, Y., Shi, Z., Reheman, A., Jin, W., Li, C., Zhu, G., Ni, H. (2010). Anthocyanins Inhibit Platelet Activation and Attenuate Thrombus Growth In Both Human and Murine Thrombosis Models. *Blood*, **116**(21).