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ABSTRACT:

Davidson's plum (*Davidsonia pruriens,* F. Muell.), a native to Australian rainforests, large, crimson-red fruit, which superficially resembles plum, has been commercially cultivated in Australia since 1990's. The current production volume exceeds market demands therefore this study was designed to evaluate the suitability of Davidson's plum extract as a source of anthocyanin-based food colorant. The stability of the Davidson's plum extract towards heat treatment at 95 °C was higher than that of commercial mulberry colorant, but inferior to colorants derived from red cabbage and purple sweetpotato. An addition of a variety of phenolic acids significantly increased color intensity indicating the formation of copigmentation complexes. Commercial chlorogenic acid as well as extract from a native Australian herb rich in chlorogenic acid, Tasmannia pepper leaf (*Tasmannia lanceolata,* R. Br.), were both tested in model soft drink solutions subjected to light irradiation and heat treatment. In both cases the addition of the copigment resulted in a lasting increase in color intensity. In conclusion, Davidson's plum extract can successfully be utilized as a source of natural food color. Extract from Tasmania pepper leaf can be used as a co-pigment for Davidson's plum anthocyanins.

Keywords: Davidson's plum, Tasmannia pepper leaf, copigmentation, anthocyanin, phenolic acid

Practical Application:

The color properties of an anthocyanin colorant derived from the native Australian fruit Davidson's plum are comparable to those of commercial mulberry food colorant, which is currently applied as a food colorant in Australian food products. Utilization of Davidson's plum fruit as a source of natural color will allow the industry to increase the range of natural pigments and will create new opportunities for the emerging native food industry.

Introduction

Davidson's plum (*Davidsonia pruriens,* F. Muell., Cunoniaceae) is a large (5 cm diameter) plum-like fruit native to the Australian rainforests, which has been commercially cultivated in Australia since 1990's (Ahmed and Johnson 2000). As many other fruits, Davidson's plum owes the crimson color of the flesh and skin to anthocyanins. The main anthocyanins of Davidson's plum are the 3-sambubiosides of cyanidin, delphinidin, peonidin and petunidin (Konczak and others 2010a) (Figure 1). Increasing interest has been placed on anthocyanins applied as natural colorants in processed food products substituting synthetic colorants. In addition to having good properties as food colorants research have shown that anthocyanins may be beneficial to human health (Stintzing and Carle 2004). However compared to synthetic colorants anthocyanins have much lower stability toward heat and storage under light (Castañeda-Ovando and others 2009). The stability of anthocyanins depends on many parameters

such as temperature, light, pH and interaction with other compounds in the surrounding media.

The presence of some compounds increases anthocyanin stability through the formation of complexes. This phenomenon, in which pigments and other noncolored organic components form molecular associations or complexes, is known as copigmentation and often results in a color enhancement. Copigmentation of anthocyanins has been shown to occur by formation of intra- or intermolecular complexes (Goto and Kondo 1991). The ability of anthocyanins to form intramolecular complexes depends on the presence of phenolic acid residues covalently bonded to the sugar moieties of the anthocyanins (Giusti and Wrolstad 2003). As none of the anthocyanins present in Davidson's plum include such groups (Figure 1) stabilization by intramolecular copigmentation is not possible. ~~Due to the structure of the anthocyanins present in Davidson's plum (Figure 1) these anthocyanins are incapable of intramolecular copigmentation.~~ Thus stabilization of these compounds has to rely on intermolecular copigmentation alone. Several studies have been performed emphasizing on increasing the stability of anthocyanins by addition of different copigments such as flavonols (Malien-Aubert and others 2001, Pissarra and others 2003), flavones (Ellestad 2006; Pacheco-Palencia and Talcott 2010) and flavanols (Malien-Aubert and others 2002). Phenolic acids have been investigated as copigments either in pure form (Eiro and Heinonen 2002; Wilska-Jeszka and Korzuchowska 1996; Bąkowska and others 2003; Gradinaru and others 2003) or as herb extracts (Del Pozo-Insfran and others 2007; Pacheco-Palencia and Talcott 2010). In recent studies extract of leaves from Tasmannia pepper (*Tasmannia lanceolata,* R. Br.), a native Australian shrub, has been found to have a large content of chlorogenic acid (Konczak and others 2010b).

Research has shown that chlorogenic acid gives a large increase in color intensity when added to an anthocyanin solution (Brouillard and others 1989) and thus addition of the Tasmannia pepper extract may improve anthocyanin stability. As the current production of Davidson's plums exceeds consumers demand (Salvin and others 2004) the potential of using the oversupply as a source of natural food colorants is investigated. The objective of the present study is to compare the stability and color characteristics of Davidson's plum extract with other commercially applied anthocyanin-based natural food colorants. Potential enhancement of the color intensity by the addition of phenolic acids as well as natural extracts from native Australian herb, Tasmannia pepper, is also investigated.

Materials and Methods

Chemicals

All solvents (methanol, ethanol and acetic acid) were HPLC grade and water was purified in the Milli-Q system (Millipore, Bedford, MA). Analytical grade hydrochloric acid, trifluoroacetic acid, sodium phosphate, citric acid, benzoic acid, gallic acid, ferulic acid, p-coumaric acid, caffeic acid and chlorogenic acid as well as Amberlite XAD-16 column material were acquired from Sigma-Aldrich (Castle Hill, NSW, Australia). C18 SPE columns (C18, 600 mg, Maxi-Clean) were acquired from Alltech Associates (NSW, Australia). Commercial anthocyanin colorants derived from purple sweetpotato (YM), red cabbage and mulberry were provided by Dr Osamu Yamakawa of the Kyushu Okinawa National Agricultural Research Station, Japan.

Preparation of plant extracts

Fresh Davidson's plums were acquired from a local orchard (Ballina, NSW, Australia) and kept frozen at -20 °C until used. The plums were deseeded after thawing, homogenized and extracted with 80% (vv^{-1}) methanol, acidified with 1% (vv^{-1}) acetic acid. The extraction was carried out over 5 min at 20 °C with agitation by magnetic stirrer using 1 L solvent per kg fresh fruit. The suspension was centrifuged (10,000 rpm, 5 min) and the supernatant was removed. Two sequential extractions were performed on the pellet using the same solvent and conditions. The supernatants were combined and concentrated using a rotary evaporator. The extract was dissolved in 1% (vv^{-1}) acetic acid and applied to a XAD-16-2X column. Impurities were eluted with 1% (vv^{-1}) acetic acid and the anthocyanin rich fraction was subsequently eluted by 80% (vv^{-1}) acidified [1% (vv^{-1}) acetic acid] methanol. The purification process was repeated and the anthocyanin fraction was eluted by 80% (vv^{-1}) acidified [1% (vv^{-1}) trifluoroacetic acid] methanol and subsequently concentrated using a rotary evaporator. The resulting fraction was re-dissolved in a minimum amount of distilled water, placed at – 80 °C for 2 h and lyophilized. The lyophilized powder was kept at – 20 °C until used. According to HPLC analysis the anthocyanin content in destoned Davidson's plum was 0.3 mg g^{-1} on dry weight basis. Dried Tasmannia pepper (*Tasmannia lanceolata*, R. Br.) leaves were supplied from a commercial producer (Birch Bay, Tasmania, Australia). The leaves were finely ground upon arrival and 0.2 g of this powder was extracted with 10 ml of distilled water (2 h, 70°C, agitation by magnetic stirrer). Subsequently the suspension was centrifuged (10,000 rpm, 5 min.) and the supernatant was removed and loaded on a C18

SPE column. The column was washed with distilled water in order to elute polygodial which is responsible for the pungent taste of Tasmannia pepper leaves (Drager and others 1998). The remaining fraction was eluted with 20% (vv^{-1}) ethanol. HPLC analysis showed that the content of chlorogenic acid in Tasmannia pepper leaves was 25 mg g^{-1} on dry weight basis.

Copigmentation experiments

Anthocyanin solutions from lyophilized Davidson's plum extract were prepared by initially dissolving it in a small amount of methanol and subsequently diluting with McIlvaine's buffer (pH 3.0) (McIlvaine 1921). Copigment stock solutions (0.1 M) of benzoic acid, gallic acid, ferulic acid, p-coumaric acid, caffeic acid and 0.5 M solution of chlorogenic acid (Figure 2) were prepared by dissolving appropriate amounts of standard compounds in 70% (vv^{1}) aqueous methanol. Model solutions were prepared from equal volumes of anthocyanin solution initially adjusted to an absorbance of 0.2 (520nm) and each phenolic acid stock solution corresponding to a final copigment concentration of 0.050 M and were left for equilibration for 15 minutes at room temperature. In addition, a series of chlorogenic acid solutions in concentrations ranging from 0.025 M to 0.20 M were prepared from diluted chlorogenic acid stock solution and mixed with equal volumes of anthocyanin solution initially adjusted to an absorbance of 0.5 (520nm). The color enhancement due to addition of copigments was calculated as the ratio between the difference in color intensity of the sample with and without copigment and the color intensity of the sample without copigment, following formula:

CE (%) = [(AC_{520} – A_{520}) / A_{520}] x100, where CE stands for Colour Enhancement, AC_{520} – absorbance (520nm) of the sample with copigment, A_{520} – absorbance (520nm) of the sample without copigment.

Light and heat stability experiments

Model drink solutions consisting of Davidson's plum lyophilized extract dissolved in McIlvaine's buffer (pH 3.0) were added different amounts of chlorogenic acid either in pure form or as extract from Tasmannia pepper leaves dissolved in 20% (vv^{-1}) ethanol. The samples were filtered through 0.45 µm Millipore membranes before storage in sterilized 20 ml capped vials. Heat stability experiments were performed by placing the samples at 98 °C in a water bath for 15 min followed by cooling to 25 °C over 45 min before analysis in order to mimic a commercial pasteurization process. Light stability experiments were performed by placing the samples in front of a fluorescent light source (3000 lux, 4 cm from the light source) at 25 °C. Model drink solutions with extract from Tasmannia pepper leaves had an additional content of 70 g/l sucrose and 0.25 g/l ascorbic acid, which are common additives in commercially available fruit juices. For comparative purposes heat stability experiments were conducted on model drink solutions of extracts from Davidson's plum and three commercial pigments derived from purple sweetpotato, red cabbage and mulberry. Samples of each pigment (n = 15), prepared in McIlvaine's buffer (pH 3.0) with initial absorbance of 2.0 (520nm) were exposed to 95 °C (water bath) over 8 hours. The color changes of evaluated samples (n = 3) were assessed after 30, 60, 120, 240 and 480 min of treatment.

The color retention of a sample subjected to light or heat treatment was calculated as the ratio between the color intensity of a sample after treatment and the initial color intensity, following formula: CR (%) = [CF_{520} / CI_{520}] x 100, where CR stands for Colour Retention, CF_{520} – final absorbance (520nm), and CI_{520} – initial absorbance (520nm).

HPLC analysis

Prior to analysis all samples and solvents were filtered through 0.45 µm Millipore membranes. Quantification of chlorogenic acid and anthocyanins in solution was performed by a HPLC system (Shimadzu Co., Kyoto, Japan) consisting of a binary pump system (LC-10AD), a diode array detector (SPD-M10A), a column oven set at 25 °C (CTO-10AS), a degasser (DGU-12A), an autoinjector (SIL-10AD) and a system controller (SCL-10A). The column used was a Luna C18(2) (150 x 4.6 mm i.d., 5µm, Phenomenex, NSW, Australia). The mobile phase consisted of 0.5% (vv^{-1}) triflouroacetic acid in water (solvent A) and 95% (vv^{-1}) acetonitrile in solvent A (solvent B). The gradient program consisted of a linear gradient from 10% (vv^{-1}) B to 70% (vv-1) B over 40 min, then to 100% (vv-1) B over 2 min, maintained at 100% (vv-1) B for 8 min, further decreased to 10% (vv-1) over 1 min and finally kept at 10% (vv-1) B for 4 min. Chlorogenic acid was detected at 326 nm and quantified by calibration using chlorogenic acid standards whereas the anthocyanins were detected at 520 nm and quantified as cyanidin-3-glucoside equivalents.

Color measurements

The changes in color properties of the copigmented samples were monitored using a Shimadzu UV-1601 UV-visible spectrophotometer (Shimadzu Co., Kyoto, Japan). The absorption profile in the range of 400 nm to 800 nm was measured using the software UVPC Personal Spectroscopy Software v. 391 (1995) and the CIELAB parameters were measured using the UVPC Optional Color Analysis software v.2.7 (1995). The color coordinates were determined using illuminant D_{65} and 10^{o} observation angle.

Results and Discussion

Heat stability of Davidson's plum as compared to commercial colorants

An exposure of Davidson's plum anthocyanins to a heat treatment (95 C, 8 hours) resulted in a decrease of color intensity over time (Figure 3). The same trend was observed for all commercial pigments evaluated in this study. The thermal degradation is often found to follow first order kinetics (Gizir and others 2008) but in this case data was better described by second order degradation kinetics (Figure 4).

The colorant derived from Davidson's plum exhibited a lower heat stability ($t_{½}$ = 2 h) than the purple sweetpotato ($t_{½}$ = 8 h) and red cabbage ($t_{½}$ = 5 h) colorants. This is consistent with findings of other studies which show that pigments rich in acylated anthocyanins are more stable than pigments primarily consisting of nonacylated anthocyanins (Cevallos-Casals and Cisneros-Zevallos 2004, Sadilova and others 2006). The half-life of Davidson's plum anthocyanins was 2-fold of that of a commercially applied mulberry colorant ($t_{½}$ = 1 h). Cyanidin 3-glucoside is the main anthocyanin of a mulberry extract (ca. 71% of the total) (Hassimotto and others, 2007). In case of

Davidson's plum anthocyanins, each molecule comprises a sambubioside moiety (Figure 1). Sambubioside, a disaccharide of the β-D-xylosyl-(1→2)-β-D-glucose, offers a higher protection to the aglycone against hydrophilic attack of water molecules than the moiety of glucose present in mulberry anthocyanin, which enhances the stability of Davidson's plum colorant.

Copigmentation with phenolic acids

An immediate color enhancement, measured as an increase in the intensity of absorption in the visible range (hyperchromism) was observed after the addition of phenolic acids for all copigments tested in the experiment (Table 1). The highest effect was observed for chlorogenic acid, followed by ferulic acid, coumaric acid and caffeic acid. Gallic acid and benzoic acid had only minor influence on color intensity. The effect on color enhancement by the different copigments was strongly correlated with a bathochromic shift of the maximum absorption wavelength and decrease in lightness (L*). In each case a significant increase in colour saturation (chroma, C*) was observed with the greatest effect after the addition of chlorogenic acid (Table 1).

Subsequently, the effect of chlorogenic acid concentration on color intensity was investigated. An increase of chlorogenic acid concentration enhanced the intensity of color (Figure 5). This enhancement was concomitant with the increase of bathochromic shift (Table 2) as well as decrease of the lightness (L*) and hue (H^{o}). The observed decrease of hue indicated color shift from red towards purple and blue. A significant increase of color saturation (C*) was observed until a level of 0.050 M of copigment was reached and remained stable at higher concentrations of chlorogenic acid. This result is in

agreement Brouillard and others (1989) who observed a significant increase of color intensity when chlorogenic acid was added to a solution of malvidin-3-glucoside.

Shelf life of anthocyanin-containing product can determine its commercial application. The effect of concentrations of both, anthocyanin and chlorogenic acid, on the stability of the Davidson's plum – chlorogenic acid complex was evaluated in an accelerated shelf life study (continuous illumination of 3000 lux, 20 days). Model drink solutions, containing two levels of Davidson's plum anthocyanins (0.02 and 0.05 mM) were enriched with various concentrations of chlorogenic acid. The addition of chlorogenic acid resulted in an immediate color enhancement (Table 3). A larger color enhancement was obtained when higher concentration of chlorogenic acid was applied. Moreover, color enhancement was significantly affected by the initial concentration of anthocyanin: a larger color enhancement was observed at the higher concentration of anthocyanin (0.05mM), indicating that the degree of copigmentation was a function of the concentration of both, chlorogenic acid and anthocyanin. Concentrations of anthocyanins and chlorogenic acid in the model drink solution were monitored over time. Degradation of both, anthocyanins and chlorogenic acid, was observed. The results indicate that degradation of anthocyanins and chlorogenic acid was adversely proportional to their concentration (Table 3). The addition of chlorogenic acid significantly reduced the degradation of anthocyanins over 20 days time from 56% (initial concentration of anthocyanins 0.02 mM) or 46% (initial concentration of anthocyanins 0.05 mM) to, respectively, 25% and 23%, when the highest concentration of chlorogenic acid was added. Subsequently, the largest color enhancement after 20 days was achieved, when the highest concentrations of both, anthocyanin and chlorogenic acid, were applied. These

results clearly suggest that both anthocyanins and chlorogenic acid are stabilized at increasing concentrations. The results are in agreement with earlier observations of Brouillard and others (1989) as well as Eiro and Heinonen (2002) on storage of cyanidin-3-glucoside model solutions in the presence of chlorogenic acid.

At the evaluated concentrations of Davidson's plum anthocyanins and chlorogenic acid the color retention over storage period was not significantly influenced (Table 3) and hence the copigmentation of anthocyanins with chlorogenic acid did not contribute to a significant increase in color stability. It can be expected that this effect would be more pronounced, if higher concentrations of anthocyanins and copigment were used.

~~These results clearly suggest that both anthocyanins and chlorogenic acid are stabilized at increasing concentrations. The reason that the color stability does not increase may be that the decrease in color contribution from the copigment complex is affected by the decrease in anthocyanin concentration as well as the decrease in chlorogenic acid concentration.~~ Over the course of this experiment the lightness (L*) and hue (H^{o}) parameters increased due to degradation of anthocyanins causing color fading and formation of yellow chalcone degradation products (data not presented). The degradation products contributed also towards an increase in color saturation (C*), which is in agreement with earlier observation by Reyes and Cisneros-Zevallos (2007).

Copigmentation with aqueous extract of Tasmannia pepper leaf

A native Australian herb, Tasmannia pepper leaf, is a rich source of chlorogenic acid, and therefore has been selected as a potential additive that could enhance the intensity of Davidson's plum color. A model drink solution containing an aqueous extract of Tasmannia pepper leaf (0.000; 0.044; 0.088 or 0.123 mM chlorogenic acid) added to Davidson's plum anthocyanin solution with an initial absorbance of 1.5 (520nm) was exposed to 98 °C over 15 min, followed by cooling to 25 °C over 45 min. This treatment represents a common commercial approach to pasteurize beverages. Moreover, the stability of intermolecular copigment complex between anthocyanins and phenolic acids is highly dependent on temperature (Brouillard and others 1989; Wilska-Jeszka and Korzuchowska 1996) with heat treatment significantly reducing the copigmentation effect (Gradinaru and others 2003; Pachenco-Palencia and Talcott 2010) and therefore this approach is suitable for accelerated stability studies.

The addition of aqueous extract of Tasmannia pepper leaf resulted in an immediate color enhancement of up to 52% at the highest concentration of chlorogenic acid (Table 4).

The color intensity of all samples decreased during heat treatment, with the largest degradation observed for Davidson's plum anthocyanins without Tasmannia pepper leaf extract. Subsequently, the color enhancement for each sample containing aqueous extract of Tasmannia pepper leaf after the heat treatment increased (Table 4). Color retention was improved with the increased concentration of Tasmannia pepper leaves extract (Table 4).

Although the stability of intermolecular copigment complex between anthocyanins and phenolic acids is negatively affected by high temperature (Brouillard and others 1989), our study demonstrates a significant color enhancement accompanied with an increase of

color retention after heat treatment when Davidson's plum extract is enriched by a natural extract of Tasmannia pepper leaf.

Conclusion

With a red color and better heat stability than commercially applied food colorant based on mulberry, the anthocyanins derived from Davidson's plum have a potential to be utilized as a food colorant. The results of this study show that extract from Tasmania pepper leave, due to a large content of chlorogenic acid, has the ability to increase the color intensity of anthocyanins derived from Davidson's plum. The increase in color intensity clearly indicated the formation of a copigmentation complex between chlorogenic acid and anthocyanins. The ability to increase the color intensity combined with high antioxidant capacity of Tasmannia pepper leave found in previous studies may result in an industrial application of extract from Tasmannia pepper leaves as color enhancing additive for anthocyanin colorants.

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Table 1. Immediate change in color of Davidson's plum anthocyanin solutions with an initial absorbance of 0.5 at 520 nm caused by addition of phenolic acids at a concentration of 0.05 M. Letters given in parenthesis indicate that results are not significantly different at a 95 % confidence level between samples denoted with the same letter. Mean of 2 or 3 samples ± SD.

Copigment	CE* [%]	Wavelength[#] (nm)	L*	H°	C*
Anthocyanin alone	0 ± 16 (d)	523 ± 1 (d)	90 ± 4 (a)	-5 ± 5 (a,b)	21 ± 1 (c)
Benzoic acid	42 ± 25 (c)	530 ± 1(c)	87 ± 3 (a,b)	-3 ± 5 (a,b)	29 ± 6 (a)
Coumaric acid	100 ± 25 (a,b)	534 ± 1 (b)	80 ± 3 (c)	-9 ± 2 (a)	36 ± 3 (a,b)
Gallic acid	61 ± 11 (c)	530 ± 1 (c)	85 ±3 (a,b)	-6 ± 3 (a,b)	34 ± 2 (a)
Caffeic acid	92 ± 19 (b)	535 ± 1 (a,b)	80 ± 3 (b,c)	-2 ± 2 (b)	35 ± 1 (a)
Chlorogenic acid	132 ± 10 (a)	534 ± 1 (b)	76 ± 2 (c)	-5 ± 3 (a,b)	40 ± 2 (b)
Ferulic acid	103 ± 7 (b)	537 ± 1 (a)	79 ± 1 (c)	-9 ± 2 (a)	37 ± 2 (a,b)

*CE: Colour enhancement; [#] Wavelength: Maximum absorption wavelength

Table 2. Immediate changes in color of Davidson's plum anthocyanin solution with an initial absorbance of 0.2 at 520 nm caused by addition of different levels of chlorogenic acid. Letters given in parenthesis indicate that results are not significantly different at a 95% confidence level between samples denoted with the same letter. Mean of 2 or 3 samples ± SD.

Chlorogenic acid (M)	CE (%)	Wavelength[#] (nm)	L*	H°	C*
0.000	0 ± 5 (e)	524 ± 1 (d)	78 ± 1 (a)	-1 ± 1 (c)	34 ± 1 (c)
0.025	68 ± 31 (d)	532 ± 1 (c)	66 ± 2 (b)	-3 ± 3 (b,c)	48 ± 8 (b)
0.050	164 ± 6 (c)	535 ± 1 (b)	57 ± 1 (c)	-2 ± 1 (b,c)	62 ± 2 (a)
0.100	196 ± 6 (b)	537 ± 1 (a,b)	52 ± 1 (d)	-4 ± 1 (b)	63 ± 1 (a)
0.200	252 ± 6 (a)	539 ± 1 (a)	48 ± 1 (e)	-8 ± 1 (a)	65 ± 1 (a)

CE: Colour enhancement; [#] Wavelength: Maximum absorption wavelength; L: Lightness; H°: Hue; C*: Chroma

Table 3. Effect on color retention of Davidson's plum anthocyanin model drink solution with an initial absorbance of 1.5 at 520 nm added different amounts of extract from Tasmannia pepper leaves and subjected to heat treatment (15 min. at 98 °C). Half-lives are based on measurements taken after 0, 7, 14, 20 and 30 days (data not shown) assuming second order degradation kinetics. Letters given in parenthesis indicate that results are not significantly different at a 95 % confidence level between samples denoted with the same letter. Mean of 2 or 3 samples ± SD.

Anthocyanin				Chlorogenic acid				CE* (%)	
Initial (mM)	20 days (mM)	Decrease (%)	$t_{½}$ (days)	Initial (mM)	20 days (mM)	Decrease (%)	CR[◇] (%)	Initial	20 days
0.019	0.009	56	1 (b)	-	-	-	65 ± 14 (a,b)	-	-
0.047	0.025	46	1 (b)	-	-	-	66 ± 7 (b)	-	-
0.020	0.010	47	1 (b)	0.176	0.103	41	62 ± 3 (b)	3 ± 1	-2 ± 4
0.048	0.032	34	1 (b)	0.425	0.375	12	79 ± 4 (a)	12 ± 1	8 ± 22
0.019	0.015	25	2 (a, b)	1.503	1.389	8	63 ± 13 (a,b)	1 ± 1	19 ± 6
0.048	0.037	23	2 (a,b)	2.175	2.072	5	78 ± 3 (a)	22 ± 1	43 ± 5

[◇] CR: Color retention; *CE: Color enhancement

Table 4. Effect of Tasmannia pepper leaves extract as a source of chlorogenic aicd on colour retention of Davidson's plum anthocyanin model drink solution subjected to heat treatment (15 min. at 98 °C, followed by cooling to 25 °C over 45 min). Letters given in parenthesis indicate that results are not significantly different at a 95 % confidence level between samples denoted with the same letter. Mean of 2 or 3 samples ± SD.

Chlorogenic acid (mM)	CE* (%)		CR◇ (%)
	Initial	After treatment	
0.000	0 ± 0 (d)	0 ± 0 (d)	57 ± 1 (d)
0.044	22 ± 1 (c)	39 ± 1 (c)	66 ± 1 (c)
0.088	39 ± 1 (b)	65 ± 1 (b)	68 ± 1 (b)
0.123	52 ± 1 (a)	86 ± 1 (a)	70 ± 1 (a)

◇ CR: Color retention; *CE: Colour enhancement

Captions

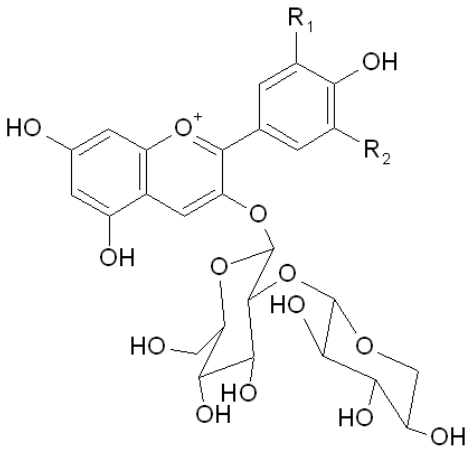
Figure 1: Structure of anthocyanins in Davidson's plum

Figure 2. Structure of phenolic acids included in copigmentation experiment.

Figure 3: Degradation of Davidson's plum anthocyanins during heat treatment at 95 °C.

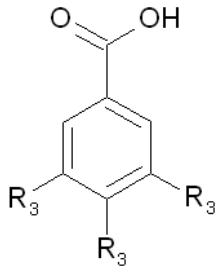
Figure 4. Linear regression on the reciprocal values during heat treatment at 95 °C shows that anthocyanin degradation can be described by second order kinetics.

Figure 5. Colour enhancement in anthocyanin solution as function of increasing chlorogenic acid concentration.

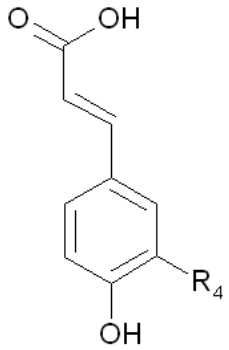


Name	R_1	R_2
Cyanidin-3-sambubioside	OH	H
Delphinidin-3-sambubioside	OH	OH
Peonidin-3-sambubioside	OCH_3	H
Petunidin-3-sambubioside	OH	OCH_3

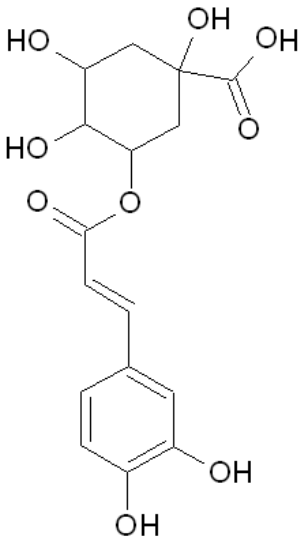
Figure 1: Structure of anthocyanins in Davidson’s plum



Name	R_3
Benzoic acid	H
Gallic acid	OH



Name	R_4
Ferulic acid	OCH_3
Caffeic acid	OH
Coumaric acid	H



Name
Chlorogenic acid

Figure 2. Structure of phenolic acids included in copigmentation experiment.

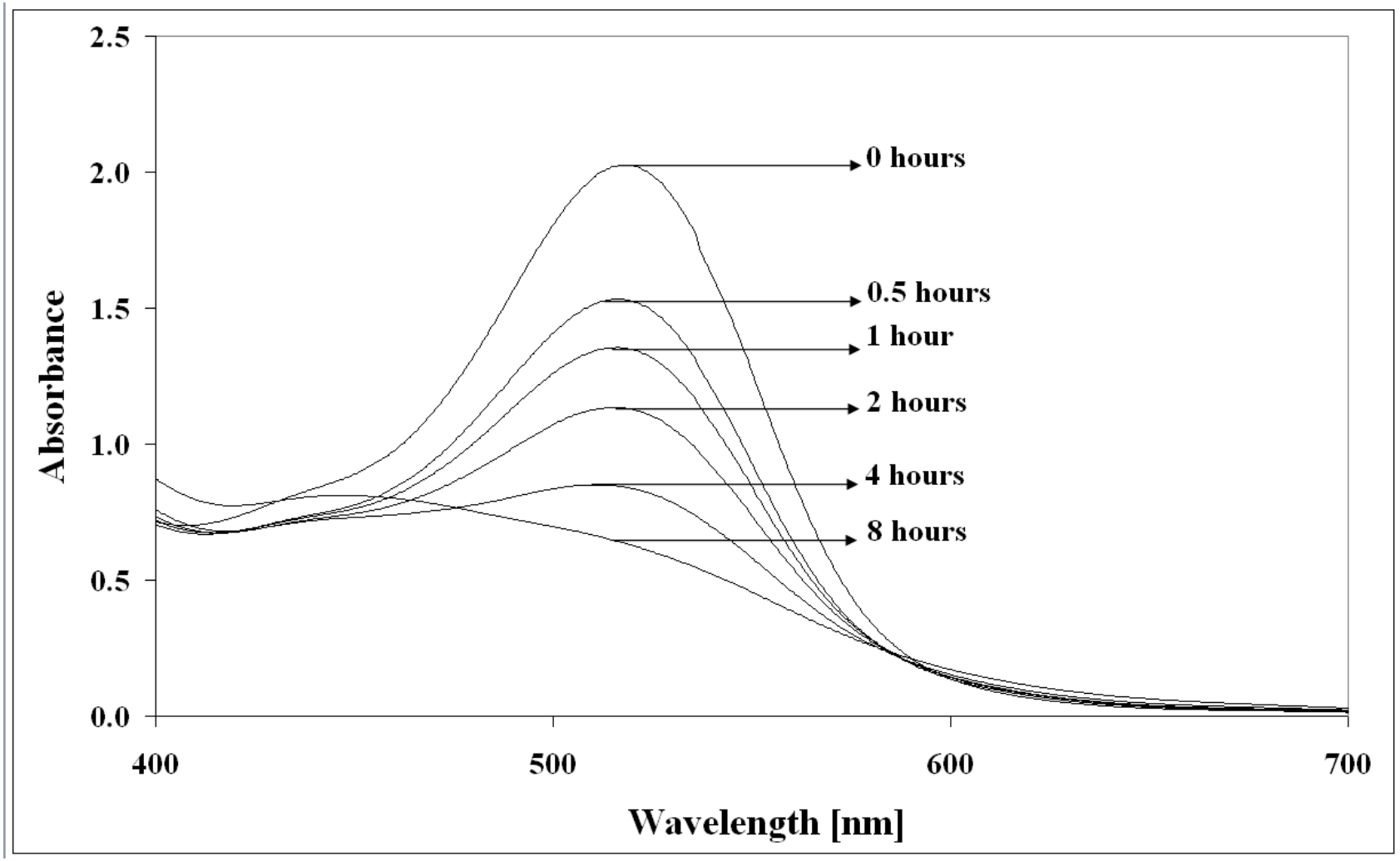


Figure 3: Degradation of Davidson's plum anthocyanins during heat treatment at 95 °C.

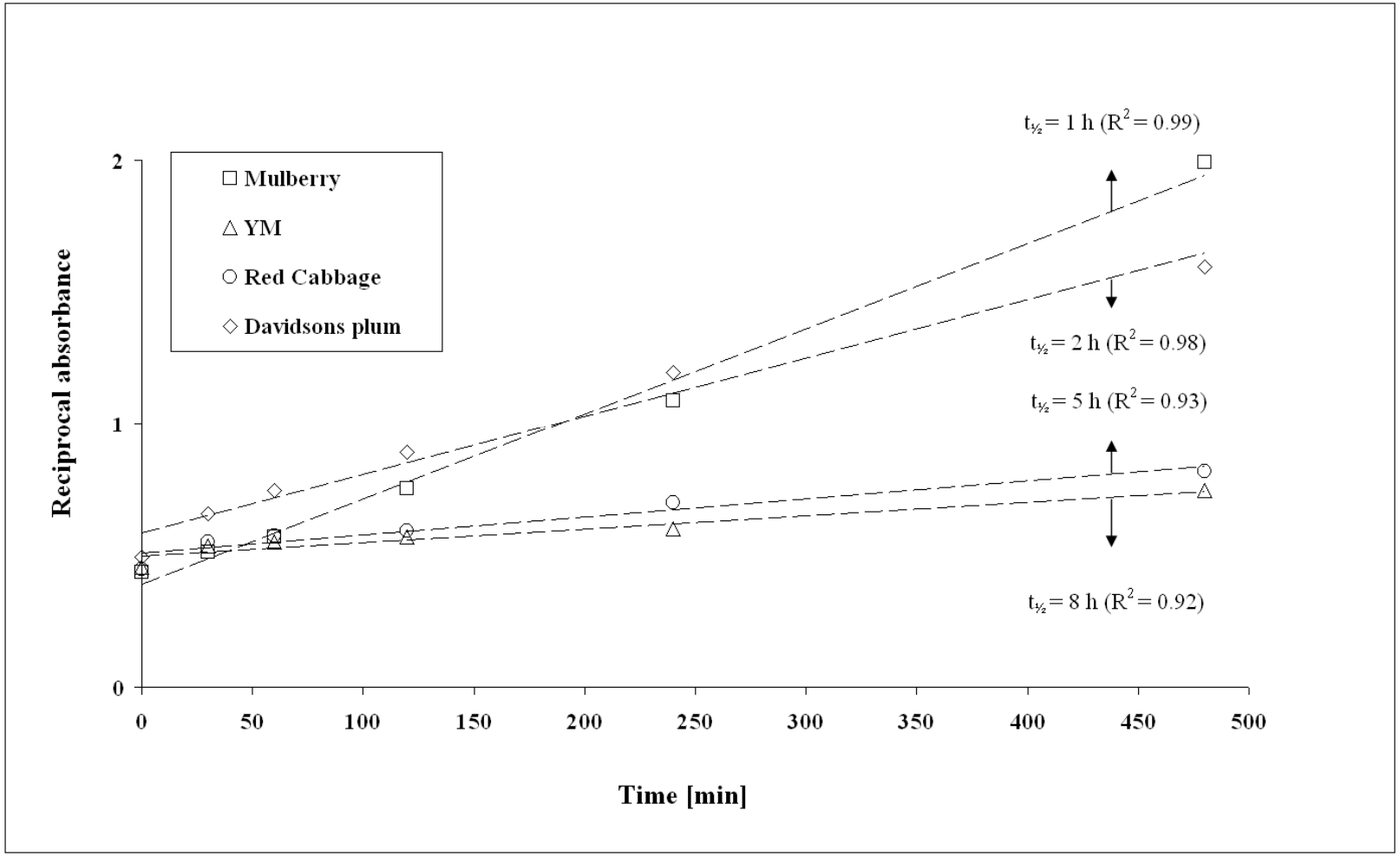


Figure 4. Linear regression on the reciprocal values during heat treatment at 95 °C shows that anthocyanin degradation can be described by second order kinetics.

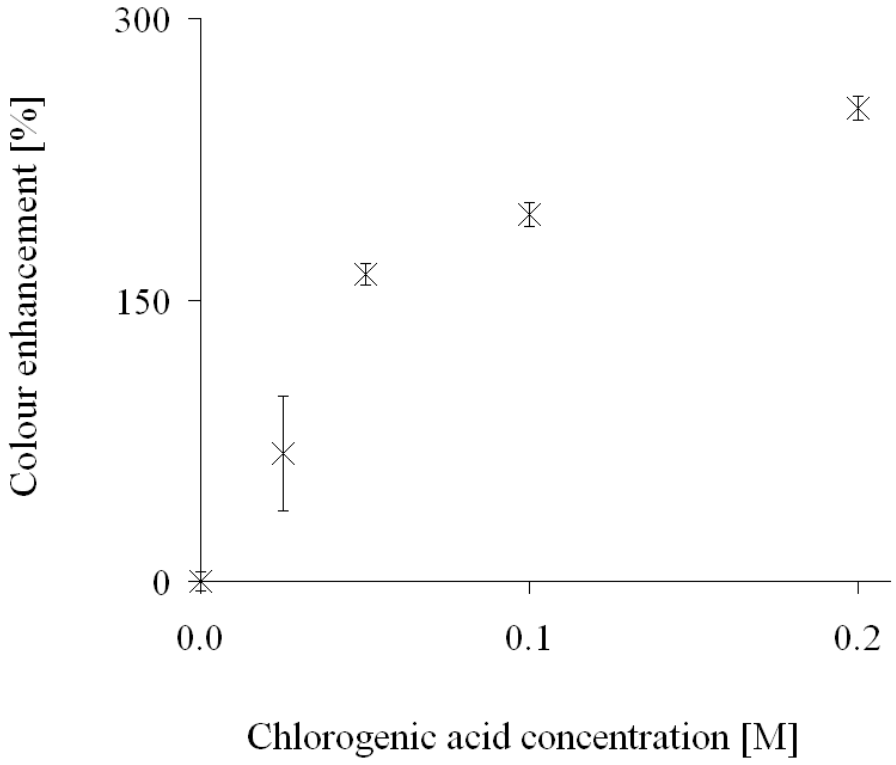


Figure 5. Colour enhancement in anthocyanin solution as function of increasing chlorogenic acid concentration.