Mini-review

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Japanese plums (*Prunus salicina* Lindl.) and phytochemicals – breeding, horticultural practice, postharvest storage, processing and bioactivity

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Abstract

Previous reviews of plum phytochemical content and health benefits have concentrated on the European plum, *Prunus domestica* L. However, the potential bioactivity of red- and dark red-fleshed Japanese plums, *Prunus salicina* Lindl., so-called blood plums, appears to warrant a significant increase in exposure, as indicated in a recent review of the whole *Prunus* genus. Furthermore, Japanese plums are the predominant plum produced on an international basis. In this review the nutrient and phytochemical content, breeding, horticultural practice, postharvest treatment and processing as well as bioactivity (emphasising *in vivo* studies) of Japanese plum are considered, with a focus on the anthocyanin content that distinguishes the blood plums.

Keywords: *Prunus salicina*; Japanese plum; phytochemicals; horticultural practice; processing; bioactivity

JAPANESE PLUMS

Prunus salicina Lindl., the Japanese plum, is considered to have originated in China.[1,2] Although a native of China, the common name Japanese plum is used because the first imports of this fruit tree to the USA were from Japan. Modern Japanese plum cultivars are predominantly *P. salicina* but also include other species due to Luther Burbank's early breeding work and the subsequent use of his cultivars as parents.[3] Other species present in modern Japanese plums are *Prunus simonii* Carr., *Prunus cerasifera* Ehrh., *Prunus americana* Marsh. and *Prunus angustifolia* Marsh.[4] Globally, Japanese plum production is larger than that of European plum, *Prunus domestica* L.[3] Japanese plums are grown mostly in temperate zones, but there are cultivars adapted to the subtropics. China is the largest producer, with significant quantities also in Europe and the USA.[3]

There is great variability in both peel and flesh colour of Japanese plum cultivars (see Table 2). Peel colour may be black, purple, red, green or yellow. Flesh colour can be yellow or red, with many shades of both colours and some cultivars having a combination of both yellow and red flesh. Cultivars with red/black peel and red flesh are commonly called blood plums.

Previous reviews of plum phytochemical content and health benefits have concentrated on the European plum.[5,6] However, the potential health profile of Japanese blood plums appears to warrant a significant increase in exposure, as indicated by Vicente *et al.*[7] in a recent review of the whole *Prunus* genus. Consequently, the present review will concentrate on the contributions of the Japanese plum, in particular the Japanese blood plums and their distinguishing anthocyanin content.

NUTRIENT AND PHYTOCHEMICAL CONTENT

The average nutritional value of commercial Japanese and European plum cultivars is assumed to be similar (Table 1). Nutritionally, Japanese plum is a reasonable source of fibre and a good source of vitamin C, typical of many tree fruits (Table 1). The major phytochemicals in Japanese plum are anthocyanins, carotenoids, flavonols, proanthocyanidins and hydroxycinnamic acid derivatives (Tables 2 and 3), but it is the anthocyanins that are predominantly responsible for the colours in blood plums (Fig. 1). These phytochemicals are more highly concentrated in the peel

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Table 1. Indicative nutrient data for Japanese plum per 100 g fresh weight (USDA nutrient database, online version 26: http://ndb.nal.usda.gov/ndb/foods/show/2428?fg=&man=&lfacet=&format=&count=&max=25&offset=&sort=&qlookup=plum; data are a mix of Japanese and European plums)

Component	Amount
Energy (kJ)	192
Moisture (g)	87.23
Protein (g)	0.7
Fat (g)	0.28
Fructose (g)	3.07
Glucose (g)	5.07
Sucrose (g)	1.57
Total dietary fibre (g)	1.4
Calcium (mg)	6
Iron (mg)	0.17
Magnesium (mg)	7
Phosphorus (mg)	16
Potassium (mg)	157
Sodium (mg)	0
Zinc (mg)	0.1
Copper (mg)	0.057
Manganese (mg)	0.052
Flouride (μg)	2
Thiamin (B1) (mg)	0.028
Riboflavin (B2) (mg)	0.026
Niacin (B3) (mg)	0.417
Pantothenic acid (B5) (mg)	0.135
Vitamin B6 (mg)	0.029
Folate (μg)	5
Vitamin C (mg)	9.5
Vitamin A, RAE (μg)	17
Vitamin E (mg)	0.26
Vitamin K1 (μg)	6.4
Phytosterols (mg)	7

Figure 1. Queen Garnet, a high-anthocyanin blood plum.

than in the flesh, which is the case with most fruits and vegetables that have an edible peel/skin (Table 2).

Anthocyanin content varies considerably between varieties, which is associated with a wide range of colour intensities of both peel and flesh (Table 2). The very dark, black peel of certain yellow-fleshed varieties (e.g. Black Amber) has very high anthocyanin content and can result in whole fruit anthocyanin content of up to about 30 mg per 100 g. This total content is similar to or greater than the values for some of the lighter-coloured, red-fleshed plums. However, the darker-coloured blood plums have levels that far exceed those of other plum varieties, with values ranging from 54 to 272 mg per 100 g.[8–12] For the variety Queen Garnet (Fig. 1), average hue angle of both peel and flesh correlates well with mean total anthocyanin content, showing an inverse relationship between hue angle and anthocyanins (i.e. the darker the colour of the peel and flesh, the higher the anthocyanin content).[8]

The main anthocyanins in Japanese plum are cyanidin-3-glucoside and cyanidin-3-rutinoside (Fig. 2),[9,13,14] but other cyanidin derivatives, including cyanidin 3-(6″-acetoyl) glucoside, cyanidin 3-(6″-malonyl) glucoside and cyanidin-3-galactoside,[15,16] and peonidin derivatives[17] have been quantified. The anthocyanin content of dark-fleshed and dark-peeled varieties presents them as very significant sources of dietary anthocyanins. As mentioned previously, blood plums can have levels approaching 300 mg per 100 g,[8,9] exceeding or comparable to those of berry fruits,[18] and are regarded as some of the richest food sources of anthocyanins.

The red-fleshed fruits generally have much higher flesh/peel content ratios of anthocyanins, 0.1–0.2,[8,19] than yellow-fleshed varieties, 0.003–0.06.[15,19]. Whereas up to 97% of the total fruit anthocyanin content is located in the peel of a variety such as Angeleno,[15] this figure can be as low as 29–57% in the darker red-fleshed varieties and genotypes.[8,20] In darker red-fleshed plums the flesh anthocyanin content has been reported to be as high as 107 mg per 100 g.[8] This flesh anthocyanin content is somewhat novel given the fact that the majority of the anthocyanin content of other anthocyanin-rich fruits such as berries and grapes is often highly concentrated in the skin/peel, with little present in the flesh.

The carotenoid content (Table 2) ranges from 0.09 to 1.9 mg per 100 g fresh weight, with the major carotenoid (>90%) being β-carotene and with small amounts of β-cryptoxanthin also quantified.[21] The concentrations are relatively small and not significant from a dietary perspective. The colour of the carotenoids is generally masked by the presence of anthocyanins.

On comparison of Rubysweet (blood plum) and Byrongold (yellow-fleshed), the blood plum was seen to have higher proanthocyanidin content in both peel and flesh.[22] The proanthocyanidin content (Table 3) of Black Diamond and other 'black plums' (not specified as Japanese plum) was shown to compare favourably in terms of content to other fruits, being greater than 200 mg per 100 g fresh weight.[23] Among 21 analysed fruits, the proanthocyanidin content of the black plums was only lower than that of chokeberries, cranberries and lowbush blueberries.[23] Similarly, among 56 Spanish food products, 'plum' (not specified as Japanese plum) had the second highest flavanol content after broad bean.[24] Japanese plum was shown to have proanthocyanidins containing A-type linkages,[15,23] which are present in a limited numbers of foods, including cranberries, and are associated with bacterial anti-adhesion activity in the urinary tract.[25]

Hydroxycinnamic acid derivatives and flavonols have also been quantified in Japanese plum (Table 3). The quercetin glycosides that have been identified include quercetin pentosyl-hexoside, quercetin-3-glucoside, quercetin-3-rutinoside, quercetin pentosyl-pentoside, quercetin-3-xyloside, quercetin-3-arabionside, quercetin acetyl-hexoside and quercetin-3-rhamnoside.[12,15,17]

Table 2. Total phenolic content, anthocyanin content and total carotenoid content of commercial Japanese plum varieties (per 100 g fresh weight)

Variety	Study	Peel colour	Flesh colour	Total phenolic content (mg gallic acid equivalent or †chlorogenic acid equivalent)			Anthocyanin content (generally as mg cyanidin-3-glucoside equivalent)			Total carotenoid content (generally as mg β-carotene equivalent)		
				Peel	Flesh	Whole	Peel	Flesh	Whole	Peel	Flesh	Whole
	Diaz-Mula *et al.* (2009)											
Angeleno	Ref. 19	Black	Yellow	360	100		340	3.4		3	0.4	
Black Amber	Ref. 19	Black	Yellow	521	100		437	6.5		6.2	1	
Larry Ann	Ref. 19	Black	Yellow	420	124		210	12.5		9.9	1.1	
Black Diamond	Ref. 19	Black	Red	270	50		131	17.7		4.3	0.23	
	Tomas-Barberan and Espin (2001)											
Angeleno	Ref. 15	Black	Yellow				162	0.5				
Red Beaut	Ref. 15	Red	Yellow				12.9					
Black Beaut	Ref. 15	Black	Yellow				69.1	2.8				
Santa Rosa	Ref. 15	Red	Yellow				27.3					
	Gil *et al.* (2002)											
Angeleno	Ref. 21	Black	Yellow	332	41	82				0.41	0.06	0.11
Red Beaut	Ref. 21	Red	Yellow	166	41	57				0.22	0.08	0.09
Black Beaut	Ref. 21	Black	Yellow	318	77	109				0.44	0.2	0.23
Santa Rosa	Ref. 21	Red	Yellow	163	38	55				0.27	0.056	0.083
	Netzel *et al.* (2012)											
Angeleno	Ref. 9	Black	Yellow						9.3			
Black Amber	Ref. 9	Black	Yellow						13			
Santa Rosa	Ref. 9	Red	Yellow						6			
Queen Rosa	Ref. 9	Red	Yellow						7			
Frontier	Ref. 9	Purple	Red						14.1			
Autumn Giant	Ref. 9	Red	Yellow						14			
Tegan Blue	Ref. 9	Black	Yellow						12			
Satsuma	Ref. 9	Red	Red						22			
Queen Garnet*	Ref. 9	Black	Red						152			
	Vizzotto *et al.* (2007)											
Black Splendor*	Ref. 11	Black	Red			372†			227			0.1
Frontier*	Ref. 11	Purple	Red			423†			209			0.6
Byron Gold	Ref. 11	Green	Yellow			288†			2.4			0.4
Crimson*	Ref. 11	Red	Red			282†			60			0.5
Morris*	Ref. 11	Red	Red			456†			201			0.7
Burgundy*	Ref. 11	Red	Red			377†			264			0.3

Table 2. *Continued*

Variety	Study	Peel colour	Flesh colour	Total phenolic content (mg gallic acid equivalent or †chlorogenic acid equivalent)			Anthocyanin content (generally as mg cyanidin-3-glucoside equivalent)			Total carotenoid content (generally as mg β-carotene equivalent)		
				Peel	Flesh	Whole	Peel	Flesh	Whole	Peel	Flesh	Whole
	Venter *et al.* (2013)											
Sun Breeze	Ref. 12	Yellow	Yellow						ND			
Laetitia	Ref. 12	Red	Yellow						7			
African Delight	Ref. 12	Red	Yellow						10			
Sapphire	Ref. 12	Red	Yellow						28			
Ruby Red*	Ref. 12	Red	Red						54			
Ruby Crunch	Ref. 12	Red	Red						32			
	Lozano *et al.* (2009)											
Black Amber	Ref. 75	Black	Yellow			202			29			
Larry Ann	Ref. 75	Black	Yellow			145			24			
Suplumeleven	Ref. 75	Black	Red			95			24			
Suplumsix	Ref. 75	Black	Red			135			11			
Fortune	Ref. 75	Red	Yellow			145			24			
	Other studies											
Black Amber	Refs 31, 35	Black	Yellow			39[35], 60[31]						
Black Diamond	Ref. 13	Black	Red						38			
Red Beaut	Ref. 38	Red	Yellow	241	63	79						
Black Splendor*	Ref. 10	Black	Red			450†			63			
Early Magic	Refs 17, 76, 77	Red	Yellow			192[76], 143[77]			31[17]			
Composite of Black Star, Santa Rosa, Gaviota, June Black	Ref. 14	Red	Red			320			19			
Crimson Globe	Ref. 65	Red	Red			349†			34			
Methley	Ref. 48	Red	Red			246			31			
Queen Garnet*	Refs 8, 9	Black	Red				538[8]	107[8]	181[8], 175–272[9]			

*Blood plums.
ND, not detected.

Table 3. Content of other phenolic compounds of commercial Japanese plum varieties (mg per 100 g fresh weight)

Variety	Study	Kaempferol (whole)	Quercetin glycosides (whole)	Quercetin glycosides (peel)	Chlorogenic acid (whole)	Neochlorogenic acid (whole)	Epicatechin (whole)	Catechin (whole)	Proanthocyanidins (total, sum of procyanidin monomers to decamers) (whole)	Flavanols (whole)
	Tomas-Barberan and Espin (2001)									
Angeleno	Ref. 15			18.6	0.03	1.91				40.3†
Red Beaut	Ref. 15			16.6	0.51	19.65				17.7†
Black Beaut	Ref. 15			35.2	0.12	12.28				58.2†
Santa Rosa	Ref. 15			29.9	0.53	15.05				24.6†
Wickson	Ref. 15			20.4	0.045	8.54				21.1†
	Venter *et al.* (2013)									
Sun Breeze	Ref. 12		6.4		ND	21.8	0.8	6.6		22.0‡
Laetitia	Ref. 12		21.5		ND	36.7	1.0	6.2		10.9‡
African Delight	Ref. 12		27.0		2.5	39.1	0.6	6.6		13.2‡
Sapphire	Ref. 12		17.0		ND	ND	5.8	7.1		14.1‡
Ruby Red*	Ref. 12		20.7		ND	40.1	5.5	5.4		11.3‡
Ruby Crunch	Ref. 12		20.8		ND	6.0	4.1	17.2		30.4‡
	Other studies									
Black Amber	Ref. 35	1.17	2.24		7.1		0.46	0.09		
Black Amber	Ref. 31		8.0							
Black Diamond	Ref. 13						2.4	17.6		
Black Diamond	Ref. 23								256.6	
Black plums	Ref. 23								237.9	
Early Magic	Ref. 17		12.8							
Composite of Black Star, Santa Rosa, Gaviota, June Black	Ref. 14	1.3	2.7							
Plum	Ref. 24						4.35	5.2		32.3§

*Blood plum.
†Total, as catechin equivalents.
‡Sum of procyanidins B1 and B2.
§Total, sum of 15 flavanols.
ND, not detected.

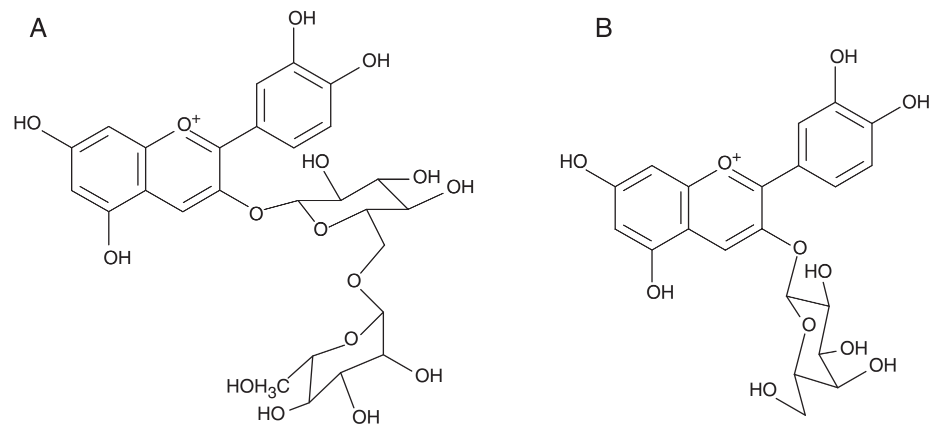

Figure 2. Chemical structures of (A) cyanidin-3-rutinoside and (B) cyanidin-3-glucoside, the predominant anthocyanins in Japanese blood plums.

Small amounts of resveratrol (0.1–6.2 μg g^{-1} peel dry weight) have been shown in the peel but not the pulp of Japanese plum,[26] with another study reporting levels of 13.1–20.0 ng g^{-1} dry weight in whole fresh 'plum' purchased from market (not specified as Japanese plum), together with *trans*-resveratrol-3-*O*-*β*-D-glucoside (117.2–340.8 ng g^{-1} dry weight) and *cis*-resveratrol-3-*O*-*β*-D-glucoside (59.3–137.2 ng g^{-1} dry weight).[27] The levels of resveratrol would not be significant in the diet compared with alternative sources such as red grapes.

Non-extractable polyphenols, including condensed tannins and hydrolysable polyphenols, are not generally determined in fruits when the content is analysed by conventional extraction techniques. However, they have recently been shown to contribute more than 82% of the total antioxidant activity in several *P. domestica* cultivars.[28] These 'missing dietary polyphenols' may have significant health benefits, particularly in regards to the colon, where these compounds and the attached fibre are subject to microbial degradation processes.[29] Non-extractable polyphenols have not yet been specifically quantified in Japanese plum and are an area that warrants investigation.

BREEDING

Several breeding programs have identified genotypes with high levels of phytochemicals for direct deployment as cultivars or for use as parents. The USDA plum-breeding program at Byron, Georgia has produced a range of blood plum selections. Fourteen of these were tested for total phenolic (298–563 mg chlorogenic acid equivalent per 100 g fresh weight) and anthocyanin (33–173 mg cyanidin-3-glucoside equivalent per 100 g) contents with a view to identifying plums that could be used to produce fruit with enhanced antioxidant, antimicrobial and colorant properties.[20]

Furthermore, very large ranges of anthocyanin content (2.4–611 mg per 100 g) and carotenoid content (0.1–1.5 mg per 100 g) have been seen within other genotypes from USDA collections.[11] Black Splendor released from the USDA, Fresno, California breeding program produces fruit with black peel and red flesh and is used primarily for fresh market production. Queen Garnet was released from a Queensland Government (Australia) breeding program[30] and is being grown as a processing plum for anthocyanin production.[8] It has also been used as a parent to produce further high-anthocyanin selections that are currently under evaluation. Quercetins (0.9–24 mg per 100 g) were the most abundant polyphenol in the majority of tested Western Australian Government plum-breeding lines.[31] Epicatechin (0–15.4 mg per 100 g) and chlorogenic acids (chlorogenic acid 0–3.8 mg per 100 g, neochlorogenic acid 0–22 mg per 100 g) were not found as commonly in this population, but Mubarak *et al.*[31] argued that all three polyphenols should be specifically targeted for selection because of their established human health benefits.

Although blood plums present as good to excellent sources of dietary anthocyanins, and potentially other phytochemicals, breeding for high phytochemical content is not a major objective of large plum-breeding programs. Selection for high phenolics is possible, but extreme levels may be associated with increased astringency.[21] Breeding for plums with high levels of desired phytochemicals must be accompanied by selection for high yield and required agronomic traits. In red raspberry, high negative genetic correlations have been reported between yield and total phenolics, antioxidant activity and anthocyanin content.[32] There is scope to further improve the levels of desirable phytochemicals in Japanese plum, but studies on the inheritance of specific phytochemicals and their correlation with agronomic traits will aid in this process.

HORTICULTURAL PRACTICE AND GROWING

Maturity

The length of time Japanese plum remains growing and maturing on the tree has a large bearing on its phytochemical content.[33] Delaying fruit harvest results in significant increases in total phenolic, anthocyanin and carotenoid contents in Japanese plum varieties.[8,9,33,34] Total phenolic content of the peel and flesh increased 1.5–3.1- and 1.7–4.7-fold respectively over 38 days (from unripe to eating-ripe stage).[33] Total carotenoid content of the peel and flesh increased 2.2–17- and 1.5–6.6-fold respectively over 40 days.[33] Similar increases have been seen in the anthocyanin content of both peel and flesh.[8,33] In terms of total anthocyanin accumulation, the largest changes have been seen in Queen Garnet, with increases of 98 mg per 100 g over 28 days (from 175 to 272 mg per 100 g)[9] and 74 mg per 100 g over 20 days (from 107 to 181 mg per 100 g)[8] reported. Proanthocyanidin content in Rubysweet and Byrongold did not change during on-tree ripening.[22]

Harvest methods that are based on multiple picks using phytochemical maturity indices such as colour[8] may be practical ways of ensuring higher phytochemical levels in fresh fruit. Management tools such as aminoethoxyvinylglycine[35] may be useful for delaying preharvest fruit drop and thus allowing fruit to develop higher phytochemical levels by remaining on the tree longer.

The soluble solids content/acid ratio is the primary determinant of taste for consumers of fresh Japanese plums.[36] The pursuit of higher anthocyanin content with increased maturity may lead to fruit losing the appropriate soluble solids content/acid ratio, at least in certain cultivars, and thus being classified as over-mature.

Light/UV

Reduction of photosynthetic photon flux density by more than 30% resulted in significantly higher hue angle (lower colour developed) of Laetitia at harvest, during storage and after ripening.[37] As hue angle correlates inversely with anthocyanin content,[8] it is expected that the higher hue angle of shaded fruit would represent lower anthocyanin levels. Tree training, together with thinning and the avoidance of using netting, allows for maximum light and UV exposure.

Irrigation

The use of black lineal low-density polyethylene plastic mulching with reduced volume irrigation led to decreased peel (65% lower than control) and flesh (42% lower than control) total phenolic content in Red Beaut.[38]

Chemicals

The ethylene stimulator ethephon (Ethrel®) stimulates anthocyanin accumulation, with advanced development of 10 days seen in Santa Rosa when applied 3 weeks prior to commercial maturity.[39] However, abscisic acid did not stimulate anthocyanin development.[40] Aminoethoxyvinylglycine (Retain®) when applied 14 days prior to harvest of Black Amber had no effect on hue angle of peel, total phenolic content or chlorogenic acid content at harvest, and caffeic acid content was higher in treated fruit.[35] However, epicatechin, catechin, rutin, quercetin, naringenin and kaempferol were all lower in treated fruit. In Laetitia, when applied 7 days prior to harvest, aminoethoxyvinylglycine delayed peel colour development, which would suggest inhibited anthocyanin accumulation.[41]

POSTHARVEST

Temperature

During storage at 0 °C, hue angle of Black Amber decreased over 28 days, which presumably represented increased anthocyanin content of the peel.[35] The total phenolic content increased (3.8-fold) together with chlorogenic acid (5.5×), *p*-coumaric acid (1.4×) and rutin (1.7×). However, epicatechin, caffeic acid, catechin, ferulic acid and kaempferol all decreased. There was no change in the anthocyanin content of Sordum (Sultan) after 20 days at 3 °C.[42] However, significant increases in peel anthocyanin content (but not flesh anthocyanin content) were seen after 35 days at 2 °C in Black Amber, Larry Ann, Angeleno and Black Diamond cultivars.[19] Significant increases in total phenolic content were also seen in both peel and flesh, but there was no change in total carotenoid levels.[19] Following the complete storage regimen of 35 days at 2 °C and then 4 days at 20 °C, there was 54–82% increase in peel anthocyanin content, 22–96% increase in peel total phenolic content and 40–180% increase in flesh total phenolic content.[19]

For Royal Diamond, 2 days at 20 °C prior to storage for 28 days at 5 °C and then 1 day at 20 °C led to a significant increase in anthocyanin content.[43] Storage for an extra 4 days at 20 °C (following 28 days at 5 °C and then 1 day at 20 °C) had a larger impact, increasing levels 10-fold. During storage at 20 °C for 5 days, the following range of changes in phytochemical levels occurred in Angeleno, Black Beaut, Santa Rosa and Red Beaut: hydroxycinnamic acid derivatives, peel 0 to +21%, flesh −6 to +8%; flavan-3-ols, peel +12 to +21%, flesh +2 to +32%; flavonol glycosides, peel −21 to +30%; anthocyanins, peel −9 to +29%, flesh −16 to 0%.[15]

In Black Splendor, storage at 18 °C for 15 days resulted in a 2.6-fold increase in anthocyanin content from an initial level of 62.5 mg per 100 g.[10] Anthocyanins accumulated at a rate of 12 mg per 100 g over the first 4 days of storage and then at 5 mg per 100 g during days 4–15. Total phenolic content did not change significantly during storage. Similar significant increases in anthocyanin content have been shown during storage of Queen Garnet[8] and Sordum.[42] In Queen Garnet, anthocyanin levels increased by 6–69% over 8 days at 21 °C depending on the starting maturity of the fruit, with less mature fruit increasing the most.[8] In Sordum, although significant increases were seen at both 20 and 30 °C, the increase was larger at 20 °C, which correlated with phenylalanine ammonia-lyase (PAL) activity.[42] Ascorbic acid and total carotenoid content have increased during 20 °C storage in Amber Jewel, Angeleno and Black Amber.[44] The highest levels of ascorbic acid and carotenoid content were reached by 7–9 and 5–9 days respectively. Proanthocyanidin content did not change in Rubysweet or Byrongold during 18 °C storage for 3–6 days.[22]

Chemicals

Ethylene applied during 28 days of storage at 5 °C resulted in increased PAL activity and corresponding anthocyanin accumulation in Royal Diamond fruits that were then additionally ripened for 1 or 5 days at 20 °C.[43] Methyl jasmonate treatment prior to storage at 20 °C has generally resulted in heightened ripening responses with increased phytochemical levels.[44] Ascorbic acid and total carotenoid content were significantly increased, above levels in untreated fruit, in Black Amber and Amber Jewel, with maximum levels achieved after 7–9 days of storage. It also significantly increased the drop in hue angle (representative of increase in anthocyanin content of peel) in Amber Jewel and Angeleno but not in Black Amber.

The use of 1-methylcyclopropene (1-MCP) has inhibited the development of phytochemical levels in several varieties.[43,45] Treatment of fruit has inhibited anthocyanin formation in storage at 0, 5 and 20 °C in Harrow Sun and inhibited the drop in hue angle of peel of Joanna Red[43] and Tegan Blue[45] and pulp of Tegan Blue[45] during ambient ripening and cold storage. In Tegan Blue, 1-MCP also inhibited development of ascorbic acid and total carotenoids.

During 0 °C storage of aminoethoxyvinylglycine-treated (applied 14 days before harvest) Black Amber fruit, there was generally little effect on the accumulation/loss of the various polyphenols measured compared with untreated fruit.[35]

Modified atmosphere packaging

The use of modified atmosphere packaging (MAP) has shown significant reduction in anthocyanin and phenolic content accumulation in Friar stored at 0°C.[46] Furthermore, accumulation of peel and flesh phenolic content (Blackamber, Larry Ann, Songold, Golden Globe) and peel anthocyanin content (Blackamber, Larry Ann) during 2 °C storage has been inhibited.[47] In untreated fruit, carotenoid content increased at 2 °C in both peel and flesh of Songold and Golden Globe but decreased in both peel and flesh of Blackamber and Larry Ann.[47] However, MAP significantly reduced these changes in carotenoid content in all varieties.

Summary

Other than the much larger anthocyanin accumulation in the flesh of blood plums, there are no clear differences, from the current data, between blood plums and other Japanese plums in the effect of postharvest treatment on phytochemical levels. However, further studies are warranted to more fully investigate this.

PROCESSING

Canned, juice and jam

While European plum cultivars are the predominant processed plum, being commonly dried to prunes, the iconic processed Japanese blood plum products have been canned whole black or purple plums or dark red jams made from Black Doris or Angeleno and similar cultivars. These plums stay firm in retort processing, preserving the shape of the fruit, and heating accentuates the attractive red colour through extraction of anthocyanins into the juice or jam. Processing of two Japanese plum cultivars into jam resulted in only 28 and 46% respectively of the total anthocyanins remaining after jam production, yet 73 and 95% of the total phenolics were preserved.[48] Heating during jam making therefore substantially decreases the anthocyanin content but the majority of the polyphenolic content is preserved, albeit with an altered chemical composition.[48]

Juicing of Japanese plums can be carried out cold, but hot break processing of plums has been shown to increase juice yield.[49] Alternatively, treatment with commercial pectinase enzyme solutions can also improve yields in separating the liquid juice from the residual pomace.[50,51] A juice prepared from Queen Garnet using heating and enzyme treatment had an outstanding anthocyanin content of 279 mg per 100 mL.[9] Heating during production of juice and other products causes loss of anthocyanins, with a time dependent decrease shown on storage. In comparative tests, anthocyanins from Santa Rosa plum peel showed the highest stability on extended storage, with 47% loss of monomeric anthocyanins over 17 weeks at 20 °C compared with 64% loss of bilberry and 99% loss of strawberry anthocyanins.[52] In contrast, the colour intensity of the plum peel anthocyanins did not change, indicating the conversion of monomeric forms to equally coloured material.[52]

Non-thermal processing technologies

More recently, advanced processing technologies such as high-pressure processing have become available to preserve the fresh flavours and colours of fruit during processing. However, in a trial with Songold (a Japanese plum but not a blood plum), no difference in total phenolic content or carotenoid content was seen between high-pressure processed and thermally treated juice.[53] Another technology, microwave hydrodiffusion, has been trialled with a European plum variety, but juice yield was significantly lower than that obtained by thermal treatment/enzyme treatment/press process.[54,55] Furthermore, the content of phytochemicals was generally lower in juice made using microwave hydrodiffusion, with estimated yields of the different phytochemicals as follows: 26–57% of anthocyanins recovered, 48–180% of flavonols recovered, 44–275% of phenolic acids recovered and 5–23% of procyanidins recovered.[55]

Use as colorants

The intense red colour of Japanese blood plum juices and extracts provides a potential use as a natural red colorant in other foods and food blends. These can be used as sources of natural colorant with a similar hue to the synthetic colorant erythrosine (FD&C Red 3, E127).[20] The stability of plum anthocyanins is also greater than that of commercial red grape colorant with respect to time, temperature and pH.[20] The loss of colour from plum peel anthocyanins extracted at pH 4.5 under conditions simulating use in low-acid foods such as yoghurts was slower than that of most other tested sources, showing the potential for use as a colour extract.[52] The storage stability of dried plum anthocyanin powder in a squash-based low-acid model solution showed that the use of plum pomace and crude anthocyanin pigments for coloration in processed food was promising.[56] These properties of plum anthocyanins make them an attractive proposition for use as natural red colorants in higher-pH dairy products such as milks and low-acidity yoghurts.

BIOACTIVITY OF JAPANESE BLOOD PLUMS

Anthocyanins

As introduced earlier, anthocyanins are not only the characteristic phytochemicals in Japanese blood plums but are one of the most abundant phenolic compounds in nature, being responsible for the red to dark blue colours of plums and many other fruits and vegetables. Approximately 640 individual anthocyanins have been identified to date.[57] Numerous studies, mostly *in vitro*, and some animal experiments have demonstrated a broad range of biological functions of anthocyanins, including antioxidant, anti-inflammatory, antimicrobial and anticarcinogenic activities.[58,59] In addition, there is emerging evidence from recently published epidemiological and intervention studies that anthocyanin-rich foods and derived products may contribute to protection against hypertension, cardiovascular disease, inflammation and oesophageal cancer in humans.[60,61]

Most of the biological activities listed in Table 4 are based on *in vitro* cell culture experiments. However, Japanese blood plum anthocyanins must be bioavailable in some form to exert their biological effects *in vivo*. Detailed information on the absorption and metabolism of anthocyanins from raw and processed blood plums is still very limited. Preliminary results with Queen Garnet plum juice in healthy male subjects indicated an extensive metabolism of the native plum anthocyanins, mainly to glucuronidated and methylated compounds.[9,62]

The pharmacological activities of dried plums (mainly European plums), such as the prevention of bone loss, decreasing plasma cholesterol and reducing the severity of constipation, are well documented,[63,64] whereas *in vivo* studies investigating the biological activity of Japanese plums are rare.

In vivo bioactivity

In a human study comprising three different age groups, young (20 ± 10 years, $n = 6$), middle-aged (45 ± 10 years, $n = 6$) and elderly (75 ± 10, $n = 6$), the intake of 195 g of Crimson Globe twice a day for 5 days increased significantly the urinary 6-sulfatoxymelatonin concentration and total antioxidant capacity (ABTS assay) compared with the corresponding basal (before consumption) and post-assay (second day after the last ingestion of plums) values.[65] The daily plum intake of each participant contained 681 mg of total phenolics (chlorogenic acid equivalent)

Table 4. Selected biological activities of cyanidin-based anthocyanins[a] and its aglycon, cyanidin, as reported in the literature[78,79b]

Bioactivity
Free radical-scavenging activity
Reduction of reactive oxygen species (ROS) production/activity
Prevention of lipid peroxidation
Protection against oxidative DNA damage
Protective effect against oxidative endothelial dysfunction and vascular failure
Modulation of human DNA topoisomerase I and II activity in the prevention of DNA damage
Antineurodegenerative activity
Prevention of inflammation
Anti-obesity and anti-diabetes effects
α-Glucosidase-inhibitory activities
Regulation of glucose/glycogen homeostasis
Protection against human ocular diseases
Stimulatory effect on the regeneration of rhodopsin
Inhibitory effects against human cancer cell lines

[a] Cyanidins are the main anthocyanins in Japanese blood plums and derived products.
[b] A detailed list of references can be found in the comprehensive review by Galvano *et al.*[79]

and 66 mg of anthocyanins respectively. The increase in urinary 6-sulfatoxymelatonin, the main urinary metabolite of melatonin, is regarded as a biomarker for increased blood melatonin levels. It is speculated that melatonin, a biogenic amine, has beneficial effects in the treatment of cardiovascular and neurodegenerative conditions as well as being involved in the aging process.[65]

The consumption of 400 mL of Queen Garnet plum juice (containing 2660 mg of total phenolics and 1117 mg of anthocyanins) by two healthy male subjects resulted in increased urinary antioxidant capacity (total phenolics, FRAP and ORAC) and decreased malondialdehyde excretion (biomarker for oxidative stress) within 24 h as compared with water as the polyphenol/antioxidant-free control.[9]

In another human study with ten healthy male subjects, Ko *et al.*[66] compared the effect of nine fruit juices on the antioxidant activity in blood plasma. After consumption of 150 mL of pear, apple, grape, peach, plum, kiwi fruit, melon and watermelon juice, blood samples were collected at 0, 30, 60, 90 and 120 min. The antioxidant activity of the samples was determined using the 2′, 7′-dichlorodihydrofluorescein (DCHF) assay. Eight fruit juices, including plum juice, suppressed the generation of reactive oxygen species in the plasma samples. The plum variety was not specified in this study. However, the authors stated that the plum juice was from the 'high antioxidant capacity group', indicating a polyphenol-rich blood plum variety.

It is unlikely that intact (plum) anthocyanins are responsible for the improved antioxidant status owing to their poor bioavailability.[67] Since anthocyanins are significantly metabolised once ingested, a synergistic effect of anthocyanin metabolites, non-anthocyanin phenolics/metabolites and other functional plum compounds such as vitamin C and carotenoids is more likely the reason for the observed antioxidant effects in humans.

The potential role of plant polyphenols in the treatment of age-related neurodegenerative and cognitive decline is an emerging research area.[67–69] The results of previous studies have suggested that especially the consumption of dark-coloured fruits and vegetables rich in phenolic compounds may mitigate age-related decline in brain function.[69] In most of these studies, fruits such as blueberries, blackberries, strawberries and Concord grapes (or derived products) were used as dietary sources of polyphenols.[67,68] However, a key study using European plum (Petite d'Agen variety) investigated the efficacy of dried plum powder and plum juice in mitigating cognitive deficits in aged male Fischer 344 rats.[69] The authors observed an improved working memory of the plum juice-fed rats in the Morris water maze, whereas the plum powder-fed animals were not different from the control group. Based on their results, the authors concluded that the difference in the observed *in vivo* efficacy of plum juice and dried plums is probably related to the quantity of phenolics consumed (~10 times more via the plum juice), the type and amount of different phenolics present in the juice and powder (e.g. no detectable anthocyanins in the dried plum powder) as well as the effects of processing on the chemical structure of the native plum phenolics (e.g. degradation) and subsequently their bioactivity. The reason for including this European plum study in the present review was to clearly demonstrate the efficacy of plum phenolics on brain function and thus highlight the potential bioactivity of Japanese blood plums and the pressing need to further investigate blood plums in similar studies.

A potential mode of action was suggested by Tsuda[67] in his comprehensive review of the recent progress in health benefit studies of dietary anthocyanins: anthocyanins (and probably other phenolics) might prevent neurodegeneration and brain aging via the inhibition of neuroinflammation and modulation of neural signalling (up- and down-regulation of specific molecules such as NF-κB, IL-1β and TNF-α). Furthermore, improved cerebral blood flow is also discussed as another beneficial effect of anthocyanin consumption.[67]

In vitro bioactivity

Similar to other anthocyanin/polyphenol-rich fruits, blood plum extracts exhibit strong *in vitro* bioactivity, such as immunostimulatory effects assessed by lymphocyte proliferation, tumour cell cytotoxicity and nitric oxide production,[70] chemopreventive activities against human breast cancer cell lines (inhibition of proliferation and induction of apoptosis)[71,72] as well as providing protection against oxidatively stressed human lung cells.[73] However, these *in vitro* effects mainly observed in selected human cell lines have to be interpreted with caution. Significant physiological 'features' such as dietary dosage, bioavailability, metabolism and actual tissue retention are usually not realised in these *in vitro* models, therefore the obtained results have very limited *in vivo* relevance.[74]

CONCLUSION

Japanese blood plums and derived products are rich dietary sources of phytochemicals, especially anthocyanins. Some varieties such as Queen Garnet can have levels of up to 300 mg per 100 g, exceeding or comparable to that of many berry fruits, which are regarded as some of the richest food sources of anthocyanins. The anthocyanin content can be maximised by harvesting mature fruit. The risk of crop loss in targeting this practice can be moderated by postharvest storage to raise the anthocyanin content of less mature fruit in the harvest mix.

Processing of Japanese blood plums can yield high-anthocyanin juices and related products that allow these fruits to be a significant dietary source outside of the fresh fruit season. Furthermore, in the continual search by the food industry for products and ingredients that provide functional attributes, including colour and potential health benefits, Japanese blood plums appear to be an underutilised fruit that has significant potential.

Anthocyanin-rich blood plums demonstrated significant bioactivity *in vitro* and *in vivo*. However, the exact mechanisms behind the observed biological effects are not fully understood yet. In particular, the potential biological activity of anthocyanin metabolites generated in the gut/colon is of specific interest, since these microbial catabolites are often better absorbed than the original compounds and therefore may exert both local and systemic effects. Although promising, the number of studies that have investigated the bioactivity of Japanese blood plums is small and future research is warranted. Further clinical trials focusing on bioavailability and metabolism of plum anthocyanins and other polyphenols, as well as well-designed human cell studies using physiological concentrations of the relevant *in vivo* metabolites, are warranted to get a better understanding of how these compounds exert their biological activity on a cellular and molecular level.

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