Review

Effects of pH, High Pressure Processing, and Ultraviolet Light on Carotenoids, Chlorophylls, and Anthocyanins of Fresh Fruit and Vegetable Juices

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ABSTRACT

Health consciousness and the need to maintain immune system have increased the demand for fresh fruit and vegetable juices due to the presence of nutritional benefits including colorful pigments such as chlorophylls, carotenoids, and anthocyanins. Besides providing green, yellow, and red color in fruits and vegetables, these phytochemical compounds play an important role in health maintenance through their antioxidant activity. However, natural pigments have been known to be susceptible to adverse conditions including pH and temperature. Heat treatment is commonly applied in the processing of fresh juices in order to extend shelf-life. Nevertheless, thermal treatments have been reported to cause detrimental impacts on the quality of juices. Thus, non-thermal treatments such as high pressure processing (HPP) and ultraviolet (UV) light have become alternative methods to preserve the juices without high impact on nutritional and sensory characteristics. This review underlines the chlorophyll, carotenoid, and anthocyanin pigments in green, yellow, and red fresh juices obtained from several fruits and vegetables, with the influence of pH and non-thermal treatments (HPP and UV) on the color and stability of pigments in the fresh juices.

GRAPHICAL ABSTRACT
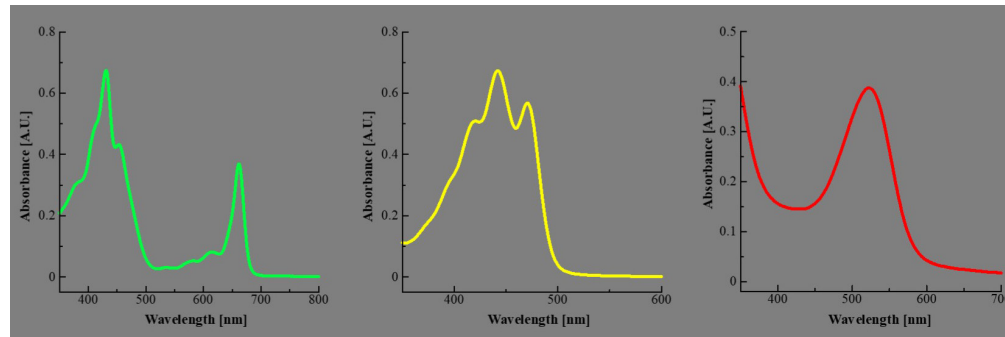
1. INTRODUCTION

There has been an increase in demand for fruit and vegetable juices since the beginning of 2020 due to the global corona virus disease pandemic [1]. Fruits and vegetables are considered as functional foods as they are capable of providing benefits for health due to sufficient supply of various nutrients and phytochemicals including pigments [2]. Chlorophylls, carotenoids, and anthocyanins are predominant groups of pigments that can mainly be found in fruits and vegetables, determining color of fruits and vegetables and providing beneficial impacts on human's health. It has been reported that low intake of fruits and vegetables is among ten risk factors impacting on the global mortality while sufficient consumption of those commodities could annually save around 2.7 million lives [2,3]. Therefore, daily intake of fruits or vegetables play a pivotal role in maintaining health and boosting immune system.

Chlorophylls, a photosensitive light harvesting pigment, are primarily present in green vegetables and have shown the ability to prevent carcinogenesis through multiple chemical mechanisms [4]. They consist of a porphyrin ring chelating an Mg atom with a 20-carbon phytol tail at C17. This results in hydrophobic properties of chlorophylls and enable it to integrate with biological lipid

membranes [4]. Meanwhile, carotenoids are naturally synthesized in plants and are placed in subcellular organelles (plastids) including chromoplasts and chloroplasts. In chromoplasts, carotenoids are accumulated in the form of oily droplets or crystalline while in chloroplasts they are mainly connected with proteins and play a role in photosynthesis, in association with chlorophylls [5,6]. In contrast to chlorophylls which chiefly deliver green color, carotenoids offer wider spectra of color, from yellow, orange to red. They consist of long, aliphatic and conjugated double bonded chains, playing a role as light-absorbing chromophores, and constituted with eight isoprene units of the molecular formula $C_{40}H_{56}$ [7], known as lipid antioxidants, singlet oxygen scavengers and potent free radical quenchers [8–10]. Besides carotenoids, red color is also provided by anthocyanins, one of the main phenolic groups in fruits and vegetables that are responsible for purple, blue, and red colors [11], which the colors are influenced by substitution in the B-ring of the aglycon and the acylation condition [12]. Anthocyanins are water-soluble glycosides and acylglycosides of anthocyanidins such as delphinidin, cyanidin, pelargonidin, petunidin, malvidin, and peonidin [13,14]. Thus, these pigments determine the color of fresh fruit and vegetable juices.

However, although fruits and vegetables visually exhibit similar color of juices, they provide different composition of pigments as each commodity has its own characteristics. Furthermore, as pigments are generally susceptible to adverse conditions such as temperature and pH, the color of fresh juices can simply change during juice processing due to the degradation of pigments. Thermal pasteurisation is one of preservation methods that has been commonly used to extend the shelf-life of juices as it is able to inactivate microorganisms and undesired enzymes [15]. Nevertheless, heat exposure has resulted in undesired impacts on nutritional and sensory qualities of juices [16]. Color is one of sensory attributes that has been frequently used to determine the quality of agricultural and food products due to the direct correlation between color and flavour [17,18]. In fresh juices, color is directly correlated with pigments which are readily degraded by heat treatments. Therefore, non-thermal treatments such as ultraviolet (UV) light and high pressure processing (HPP) have been considered as alternative preservation methods for juices [19]. This review underlines the chlorophyll, carotenoid, and anthocyanin pigment in green, yellow, and red fresh juices obtained from several fruits and vegetables, with the influence of pH and non-thermal treatments (HPP and UV) on the color and stability of pigments in the fresh juices.

2. PIGMENT COMPOSITIONS IN GREEN, YELLOW, AND RED JUICES

The identified pigment compositions in several fruit and vegetable juices is presented in Table 1. This includes nine commodities to represent green (Jerusalem artichoke, broccoli, *Tiliacora triandra* leaves, young barley shoots and leaves), yellow (carrot and orange), and red (blueberry, strawberry, red raspberry, grape, and black currant) juices. In green juices obtained from green leafy vegetables, it can be seen that chlorophyll (Chl) *a* and *b* are found to be predominant pigments in the juices. However, some studies show that carotenoids can also be found in green juices as these pigments present along with chlorophylls to assist

chlorophylls in photosynthesis and photoaxis [20,21]. Kaszás et al. [22] reported that carotenoids including xanthophyll (lutein) were not only detected in fresh leaves of Jerusalem artichoke but also in its juice. In addition, another study revealed the presence of carotenoids such as lutein and neoxanthin along with Chl *a* and Chl *b* in the juice of suji (*Pleomele angustifolia*) leaves prepared for encapsulation [23].

Carotenoids are also found to be the main pigments in yellow fresh juices. In carrot juice, provitamin A carotenoids, α-carotene and β-carotene, appeared as the most dominant pigments, followed by ζ-carotene, phytofluene, phytoene, and lutein [24]. In fresh orange juice, 5,6-epoxide carotenoids, which were *cis*-violaxanthin and antheraxanthin, served as the major carotenoids and contributed on yellow-orange color [25]. Epoxycarotenoids are commonly present in fresh juices. During juice preparation, the sheered plant cells release organic acids and carotenoids. With the presence of epoxycarotenoids and acidity, the isomerization of 5,6-epoxy groups into 5,8-epoxy groups occurs, even with the presence of trace amount of acid [26] and over storage at $-18°C$ [25]. Therefore, 5,8-epoxycarotenoids are more commonly found in fresh juices. As reported by Mercadante and Rodriguez-Amaya [27], violaxanthin (5,6:5′,6′-diepoxy-5,6,5′,6′-tetrahydro-β,β-carotene-3,3′-diol) was not detected in fresh mango juice, although it was a major carotenoid in mango fruits. As a result, 5,8:5′,8′-diepoxide isomer auroxanthin (5,8:5′,8′-diepoxy-5,8,5′,8′-tetrahydro-β,β-carotene-3,3′-diol) which was not detected in the fresh fruit became a dominant pigment in the juice. Meanwhile, antheraxanthin (5,6-epoxy-5,6-dihydro-β,β-carotene-3,3′-diol) presented as a predominant carotenoid in a number of orange cultivars was not found in the juices kept for a long period of time. Consequently, 5,8-epoxide isomer of antheraxanthin which is mutatoxanthin (5,8-epoxy-5,8-dihydro-β,β-carotene-3,3′-diol) was discovered in the old juices [25]. Therefore, the presence of 5,8-epoxy groups can be used to determine the age of juices, in which the higher the concentration of 5,8-epoxycarotenoids and the lower the concentration of 5,6-epoxycarotenoids, the older the age of the juices [25].

In contrast to carotenoids which become major pigments in several yellow juices, anthocyanins are generally found in red fruit juices. As presented in Table 1, it can be seen that various amount and type of anthocyanins, particularly acylglycosides of anthocyanidins, are contained in red fresh juices derived from blueberry, strawberry, red raspberry, grape, and black currant. The most diverse profile of anthocyanin pigments is shown in blueberry and grapes as most of the type of anthocyanins, dephinidin, cyanidin, petunidin, peonidin, and malvidin, are provided in the juices, while in strawberry juice there are only two anthocyanidins found which are cyanidin and pelargonidin. This shows that each red fruit exhibits a unique anthocyanin profile which differs the characteristics from each other.

According to Castañeda-Ovando et al. [38] and Khoo et al. [39], cyanidin, delphinidin, pelargonidin, peonidin, malvidin, and petunidin were the most common anthocyanidins presented in the fruit and vegetables, counting for 50%, 12%, 12%, 12%, 7%, and 7%, respectively. Highlighting on the red color, cyanidin, pelargonidin peonidin, and malvidin are the three anthocyanidins which contribute on that color. Cyanidin is a reddish-purple pigment which can be abundantly found in berries, red sweet potato, and purple

Table 1 │ Pigment compositions present in several green, yellow, and red fruit and vegetable juices

Fruit/vegetable	Color of juice	Pigments	Group of pigment	References
Jerusalem artichoke, broccoli, *Tiliacora triandra* leaves, young barley shoots and leaves	Green	Chlorophyll *a* Chlorophyll *b*	Chlorophyll	[22,28–30]
Carrot	Yellow	15-*cis*-phytoene Phytofluene isomer 1 Phytofluene isomer 2 Phytofluene isomer 3 ζ-carotene isomer 1 ζ-carotene isomer 2 ζ-carotene isomer 3 ζ-carotene isomer 4 ζ-carotene isomer 5 all-*trans*-α-carotene all-*trans*-β-carotene all-*trans*-lutein 9-*cis*-β-carotene 13-*cis*-β-carotene 15-*cis*-β-carotene 13,15-di-*cis*-β-carotene 9-*cis*-α-carotene 13-*cis*-α-carotene 15-*cis*-α-carotene 9-*cis*-lutein 13-*cis*-lutein	Carotenoid	[24,31]
Orange	Yellow	(all-*E*)-violaxanthin + (*Z*)-violaxanthin isomers Violaxanthin isomers + auroxanthin isomers Luteoxanthin + (*Z*)-antheraxanthin isomer (*Z*)-antheraxanthin isomer (*Z*)-antheraxanthin isomer + auroxanthin (*Z*)-luteoxanthin isomer A (*Z*)-luteoxanthin isomer B (*Z*)-ζ-carotene isomer (9*Z*)-violaxanthin (9*Z*) or (9′*Z*)-antheraxanthin Mutatoxanthin epimer A Mutatoxanthin epimer B Auroxanthin Lutein Zeaxanthin Zeinoxanthin α-carotene β-cryptoxanthin β-carotene β-carotene + ζ-carotene	Carotenoid	[25]
Blueberry	Red	Delphinidin 3-galactoside Delphinidin 3-glucoside Delphinidin 3-arabinoside Cyanidin 3-galactoside Cyanidin 3-glucoside Cyanidin 3-arabinoside Petunidin 3-galactoside Petunidin 3-glucoside Petunidin 3-arabinoside Peonidin 3-galactoside Peonidin 3-glucoside Malvidin 3-galactoside Malvidin 3-glucoside Malvidin 3-arabinoside	Anthocyanin	[32–34]
Strawberry	Red	Pelargonidin 3-glucoside Pelargonidin 3-rutinoside Cyanidin 3-glucoside	Anthocyanin	[32,35,36]

(Continued)

Table 1 | Pigment compositions present in several green, yellow, and red fruit and vegetable juices—*Continued*

Fruit/vegetable	Color of juice	Pigments	Group of pigment	References
Red raspberry	Red	Cyanidin 3-sophoroside Cyanidin 3-glucosyl-rutinoside Cyanidin 3-rutinoside Cyanidin 3-glucoside Pelargonidin 3-sophoroside	Anthocyanin	[32,33,36]
Grape	Red	Cyanidin 3-glucoside Delphinidin 3-glucoside Malvidin 3-glucoside Petunidin 3-glucoside Peonidin 3-glucoside	Anthocyanin	[32,33]
Black currant	Red	Cyanidin 3-glucoside Cyanidin 3-rutinoside Delphinidin 3-glucoside Delphinidin 3-rutinoside	Anthocyanin	[32,33,37]

corn [40,41]. Pelargonidin provides red color in several fruit and berries, while orange hue is shown in flowers [42–44]. Peonidin (3-*O*-methylated anthocyanidin) which is primarily contained in grapes, berries, and red wines has cherry red hue or magenta [39,42]. Malvidin, another *O*-methylated anthocyanidin, represents not only red pigment in red wine [45], but also purple color in some flowers [46].

3. COLOR AND PIGMENT CONTENT OF FRESH FRUITS AND VEGETABLES AND THE JUICES

Color has become a visual attribute used by consumers to value a product before deciding on purchase as it could help in describing the quality such as freshness of the product. It is also able to set the expectations of costumers towards the likely taste and flavour of the product [47]. It has been studied that color and flavour have strong correlation as the identification of flavour tends to decrease when colors of food products are different from the expectation [48]. For instance, there will be the tendency to describe white wine colored red using red wine odour instead of white wine odour [49]. Thus, besides influencing the flavour perception, color is one of the most important attribute that can lead consumers towards the products they expect and require, to be then purchased and consumed.

The CIELAB color scale is frequently applied for food analysis to signify color of food products. It provides L^*, a^*, b^* coordinates, where L^* value describes lightness, a^* describes redness or greenness, and b^* describes yellowness or blueness. Hue angle (h^*), a qualitative attribute of color that involves the values of a^* and b^* into account, is associated with the traditional color expressions such as yellowish and reddish, through which the color of edible and descriptive part of fruits and vegetables can be represented [50]. Hence, a number of studies have applied the value of hue angle as a color attribute to associate with pigments in fruit and vegetables. Figure 1 represents hue values of several fruits and vegetables commonly processed into fresh juices in which red color is indicated with hue value <20° and >330°, yellow from 20° to 80°, while green from 80° to 160° [50]. In addition, to summarise the content of chlorophylls, carotenoids, and anthocyanins in those

commodities, Table 2 is presented and it can be seen that carotenoids were provided in all of the presented fruits and vegetables. However, the hue values and the pigment content are subject to change when the fruits and vegetables are processed into fresh juices due to the change in pH and preservation treatments. The effects of those factors on the color and pigment stability of fresh juices are explained below.

3.1. Color and Pigments of Fruit and Vegetable Juices Based on pH

Fresh juices obtained from fruits and vegetables typically represent color with certain hue which may be changed by pH of juices. In relation to chlorophylls and green color, the susceptibility of chlorophylls to pH is clearly shown at low pH. According to the study of Koca et al. [57], it was found that the rate constant of green color loss was increased with the decrease in pH, indicating that the green color was sensitive at lower pH condition. In addition, Gunawan and Barringer [58] revealed no significant difference in green color change at pH 7 and 8. Furthermore, Sweeney and Martin [59] found no significant decrease in chlorophyll at pH > 6.8 and argued that at higher pH chlorophyll degradation still occurs but at slower rate. The reason for the loss of green color during processing is chiefly contributed by the alteration of chlorophylls to pheophytins at lower pH due to the release of cellular acids and the synthesis of new acids [22,60]. In acid solution, the change of color from bright green to dull olive green or olive yellow occurs as result of the alteration of chlorophylls to pheophytins (the substitution of magnesium in the porphyrin ring of chlorophyll with two hydrogen ions) and further breakdown to pheophorbides (the cleavage of phytol chain of chlorophyll [60–62].

The effect of pH on color and pigments has also been studied in carotenoids-rich fresh juices. Yellow color of starfruit and mango juices, shown through b^* value, was relatively constant at low pH (around 2) and became more obvious at higher pH. Furthermore, starfruit juice provided hue value 120° while mango juice was 109° which both reduced to 89° and 80°, respectively, when the pH increased, indicating the change of color [63]. In addition, freshly prepared carrot juice with pH 6.07 and was adjusted to pH 3–8 resulted in the reduction of total carotenoid by 26% at neutral and
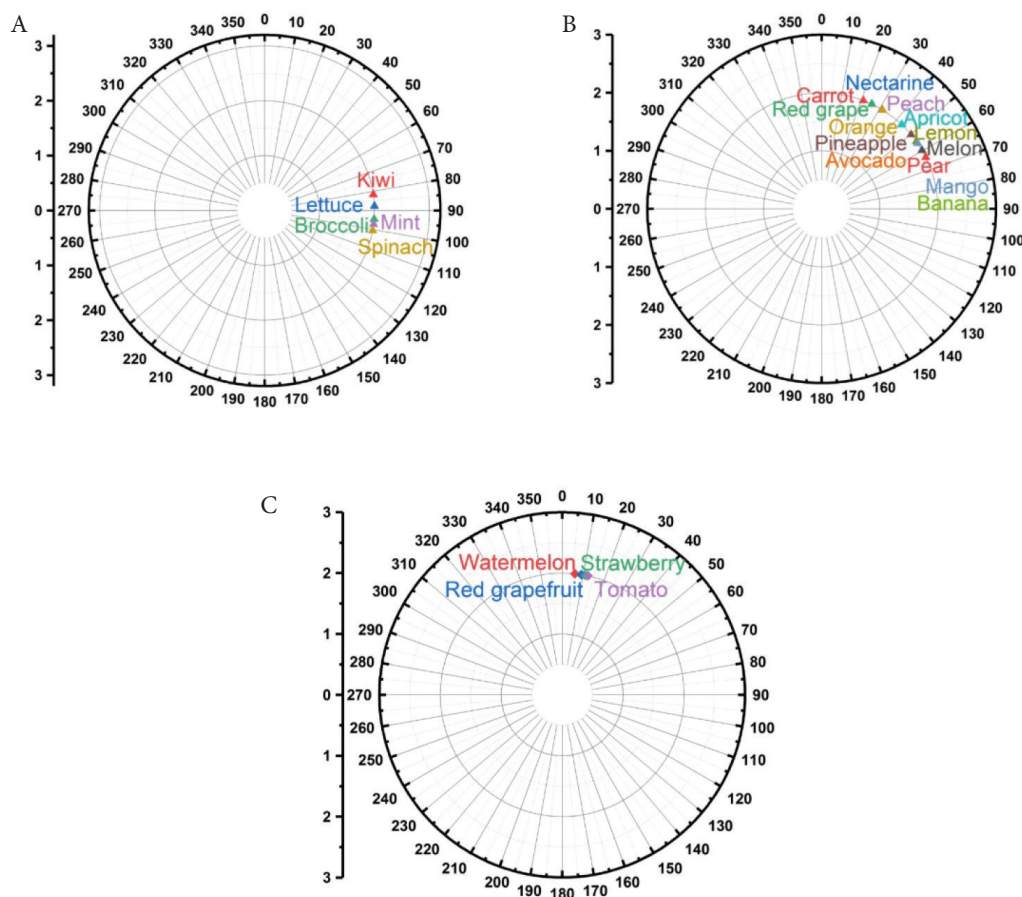
Figure 1 | Hue values of several fresh fruits and vegetables commonly processed into juices obtained from the reported study of Comert et al. [50]; (A) Green: kiwi, lettuce, broccoli, mint, spinach; (B) Yellow: carrot, nectarine, red grape, peach, orange, apricot, pineapple, lemon, avocado, mango, banana, melon, pear; (C) Red: watermelon, red grapefruit, strawberry, tomato.

Table 2 | Total chlorophylls, carotenoids, and anthocyanins of several fresh fruits and vegetables commonly processed into juices

Fruit/vegetable	Total chlorophylls (mg/100 g)	Total carotenoids (mg/100 g)	Total anthocyanins (mg/100 g)	References
Watermelon		4.92		[51]
Red grapefruit		2.12		[51]
Strawberry		0.03	20–60	[51]
Tomato		4.26		[51]
Carrot		13.86		[51,52]
Nectarine		0.38	6.8	[51,53]
Red grape		0.11	26.7	[51,54]
Peach		0.32	4.8	[51,53]
Orange		0.33		[51]
Apricot		1.30		[51]
Pineapple		0.035		[51]
Lemon		0.03		[51]
Avocado		0.38		[51]
Mango		0.68		[51]
Banana		0.07		[51]
Melon		0.06		[51]
Pear		0.06		[51]
Kiwi	0.99	0.17		[51,55]
Lettuce	37.7	6.15		[51,56]
Broccoli	12.8	1.3		[51,52]
Mint	267–283	50–60		[51,56]
Spinach	108	11.5		[51,52]

slightly basic condition (pH 7 and 8) and an increase in the total carotenoid by 22%, 27%, 22%, and 18% at pH 3–6, respectively [64]. Meanwhile, fresh blended juices of carrot, lemon, orange, and apple with low pH (3.15, 3.98 and 4.95) showed no reduction of carotenoid after 8 days of storage at 4°C. However, at the lowest pH (3.15), 16% decrease of total carotenoids occurred [64]. These findings show that, despite the degradation of carotenoids in fruit and vegetable juices was considerably low (<25%), in acidic condition the total carotenoid content tended to increase which may be caused by the enhancement in the solubility of crystallized carotenoids available in the vacuoles of plant material [65].

Further study in the relationship between pH and carotenoids can be seen through the addition of acid in fresh juices. In fresh orange juice, the addition of citric acid initially accelerated the formation of 5,8-epoxy isomers, reducing the level of 5,6-epoxycarotenoids, during 48 h of storage. Afterward, there was a considerably decrease in the level of both epoxycarotenoids and showed no significant changes in their levels relative to the control [25]. This indicates that due to the lipophilic properties of carotenoids, these pigments were not dissolved in the juice and located in the particles of pulp, contributing on the turbidity of the juice [25]. Besides the changes in epoxycarotenoids, there were also changes in the levels of major carotenoids due to the acidified juice during storage. The levels of the dihydroxycarotenoids zeaxanthin (β,β-carotene-3,3′-diol) and lutein (β,ε-carotene-3,3′-diol) reduced in the juice, while the amounts of the monohydroxycarotenoids β-cryptoxanthin (β,β-caroten-3-ol) and zeinoxanthin (β,ε-caroten-3-ol) remained more constant [25]. Hence, the acid treatment leads to the color changes of fresh orange juice during 48 h of storage as it was able to be differentiated visually. The contact between the acids and the carotenoids triggers the isomerization of 5,6- to 5,8-epoxycarotenoids, changing the yellow-orange color of violaxanthin (major carotenoid in orange juice) into light yellow of 5,8:5′,8′-diepoxy isomer auroxanthin [25]. However, after 48 h of storage, the color between the control and the acidified juice was found to be not significantly different as the juices appeared visually identical at 96 h [25]. In contrast to this result, previous study of Meléndez-Martínez et al. [66] about the enrichment of orange juice with ascorbic acid yielded in the significant color changes after 48 h of storage and this might be caused by the involvement of ascorbic acid in the changes of structure that preferred the association between the acids and the carotenoids in the pulp particles, accommodating the isomerization of 5,6- to 5,8-epoxycarotenoids. This shows that the color change of the yellow juices containing carotenoids is not only determined by the pH but also by the storage time and the type of acids.

Regarding to anthocyanins, the change of color based on pH conditions is also influenced by the type of anthocyanins due to an ionic nature in the molecular structure of anthocyanins [67]. In acidic condition certain type of anthocyanins exhibit red color while in neutral pH some of them tend to appear in purple hue. In basic pH, the color can change into blue although this color can also be found in neutral condition. Therefore, anthocyanins, particularly cyanidin and delphinidin [44], become more stable in acidic condition. Cyanidin appears red at pH < 3, violet at pH 7 and 8, and blue at pH > 11 [68]. However, it also depends on the water concentration as the decrease in the concentration of water enhances the deprotonation rate of the flavylium cation which is the predominant form of anthocyanins in lower pH solution, reducing

color stability [42,69]. Peonidin also exhibits cherry red hue at acidic condition and deep blue at pH 8; however, it was reported that it has higher stability at high pH than other anthocyanidins [39]. This may be caused by the increase in the proportion of flavylium cations due to the acylation of anthocyanin, as peonidin is one of the acylated anthocyanins, resulting in the retention of redness at basic condition [39].

3.2. The Effect of Non-Thermal Treatments on the Color and Pigments of Fruit and Vegetable Juices

3.2.1. Ultraviolet light

Thermal pasteurization is the most widely used method of extending the shelf life of juices due to the ability to inactivate food spoilage enzymes and microorganisms [15]. However, thermal treatment causes detrimental effects on the nutritive and sensory characteristics of juices, including the loss of color [16,70]. Therefore, non-thermal processing for preservation of juices have become interest, following the growing demands for high quality, nutritious and fresh-like food products [71]. UV-C irradiation is one of non-thermal treatments that has been used for preserving juices [72]. Presented in the range of 200–280 nm wavelength of light spectrum, UV-C lights provide germicidal effect against microorganisms including bacteria, moulds, viruses and yeasts as the lights absorbed by the DNA of microorganisms impede transcription and translation of DNA [72–75]. In addition, contrary to thermal treatment, it has been reported that the color of juices is able to be maintained under UV-C treatment [73].

As chlorophyll is susceptible to heat treatments, these treatments generate the formation of pheophytin in processed vegetables as natural organic acids and fatty acids might be released during heat treatments. In addition, the presence of chlorophyllase enzyme in green vegetables contributes on the formation of pheophorbide by cutting the phytyl group of pheophytin, resulting in olive-brown color. Besides chlorophyllase, peroxidase (POD) and lipoxygenase (LOX) enzymes also generate chlorophyll degradation in green plants [76]. Therefore, these enzymes are required to be inactivated without high process temperatures. In wheatgrass juice, it is found that the total content of chlorophyll in the juice treated using UV-C was not significantly different from the untreated juice, whereas by using thermal treatment the loss of chlorophyll occurred by 12.4% [77]. Concerning on the color of juice, by thermal treatment (75°C, 15 s), the lighter-reddish change in color appeared in wheatgrass juice as a result of the conversion of chlorophyll into pheophytins, with a^* (−2.90) and L^* (17.65), while in the control, a^* and L^* value were −6.63 and 19.81, respectively. Meanwhile, under UV-C treatment (69.2 mJ/cm²) there was no significant different in color, with a^* (−6.15) and L^* (22.39) [77]. This indicates that UV-C treatment could maintain the color and chlorophyll content of green juice.

The ability of UV treatment in retaining color of juices was also shown in several fresh yellow juices made from orange, pineapple, and orange/carrot blend [73,74,78]. According to the study of Bhat et al. [79], starfruit juice which was exposed under UV light at 2.158 J/m² for 30 and 60 min resulted in the increase of L^* value with time, indicating the more transparent juice due

to the degradation of coloured polymers responsible for enzymatic browning. Another study of Ibarz et al. [80] provided the decrease of a^* and b^* values in the same treatment of apple, peach, and lemon juices with different browning degree. The decrease of these values implies the destruction of melanoidin polymers as browning is shown through the increase of a^* and b^* values, shifting the color from green to reddish and blue to yellowish, respectively [80,81]. Besides color shifting, the increase in brightness of juices following the exposure time of UV was also observed, indicating that the browning of the fruit juices was counteracted by UV light. It is argued that the most influential factors to inactivate enzymatic browning caused by the activity of polyphenol oxidase (PPO) and POD enzymes using UV light are exposure time, temperature, dose, wavelength, juice composition, and soluble solids [19,80,82]. Meanwhile, under heat treatment, the lightness of carrot juice was reported to decrease. In addition, the value of a^* and b^* were also found to decrease with the increase in temperature and heating time, indicating the color shifting to be more greenish and bluish. Following this, the hue value showed the significant decrease, turning the color to be lighter, from orange to yellow [31]. Munsch et al. [83] reported that the alteration of color in carrot juice during processing can be associated not only with the content of carotenoid but also the formation of *cis* carotenoid isomers. Hence, the preservation using UV-C light could retain the color of juices containing carotenoids by inactivating the activity of enzymes responsible for enzymatic browning (PPO and POD) and limiting the formation of *cis* carotenoid isomers.

In addition, related to carotenoid content, the change of this coloured compound content has been revealed in several studies. The minor reduction of total carotenoid content was found in pineapple juice during UV treatment at 53.42 mJ/cm^2, and the amount was still significantly higher compared to the thermal treatment. Interestingly, it was increased within 14 days of storage [84]. The other increase result is presented in the study conducted by Nornisa et al. [85] which shows an increase in total carotenoid content in orange, carrot, and celery juice blend when the samples were treated under UV-C for certain duration of time, by 84% for 30 min and 3% for 60 min of exposure. The enhancement of total carotenoids by 35% was still found after 7 days of storage particularly at UV-C treatment for 15 min. An increase in the carotenoid extraction during storage may be caused by the inactivation of enzymes responsible for the deterioration of carotenoids [85,86]. However, to be noted that effects of UV-C treatment may vary among the juices and it highly depends on the amount of UV-C light absorbed by the products as the major components of fruit juices may limit the absorption of UV-C [87,88].

In several red juices containing anthocyanins, UV light treatment resulted in the slight decrease in total anthocyanin content in pomegranate juice by 8.4% at 62.4 kJ/L, while 23.6% occurred under heat treatment [89]. The similar trend of anthocyanin loss was also exhibited in red grape juice, in which the higher loss occurred when the juice was subjected to thermal treatment (11.9%), compared to 8.7% by UV light at 25.2 kJ/L [90]. In watermelon juice, UV treatment at 37.5 kJ/L yielded in the maintenance of the redness (a^* value) during 37 days of storage [91]. The retention of red color may be caused by the presence of phenolic compounds which are able to stabilise anthocyanins, preventing the

degradation of anthocyanins during UV-C exposure [92]. Phenolic compounds are complexing agents or copigments which are able to stabilize anthocyanins in plants through molecular copigmentation mechanism [93]. Besides phenols, a copigment could be one of alkaloids, flavonoids, organic acids, amino acids, polysaccharides, metals, nucleotides, and anthocyanins themselves [94]. This stabilization mechanism occurs through the association between electron pi systems in copigments with electron-poor flavylium ion, providing protection from the nucleophilic addition of water on the flavylium ion so that the formation of colourless pseudobase resulting in color loss is prevented [95]. In addition, the complexation of an anthocyanin with a copigment causes the wavelength shift of maximum absorbance (a bathochromic shift) and the increase in color intensity (a hyperchromic effect) [96]. However, discoloration of pigments due to photo degradation of anthocyanins may occur during long-term exposure of UV-C light, shown in cranberry and grape juices [97]. As reported by Koutchma [72], at 253.7 nm, photons of UV light are absorbed by organic molecules, affecting conjugated (double-ring and aromatic-ring molecules) and disulphide bonds and reducing the content of anthocyanins. During thermal treatment, anthocyanins form colorless or undesirable brown-colored polymeric pigments, although these color may also be found during UV treatment. The polymeric colour value in pomegranate juice was increased from 11.3% to 14.2% after UV-C treatment at 62.35 J/mL, whereas it reached to 24.5% after thermal treatment [89], showing that red color was more retained by UV treatment. Meanwhile, the change in anthocyanin profile during UV treatment can be seen in pomegranate juice. There were six pigments provided in this juice, i.e. delphinidin-3-glucoside, delphinidin-3,5-diglucoside, cyanidin-3-glucoside and cyanidin-3,5-diglucoside as major pigments, as well as pelargonidin-3-glucoside and pelargonidin-3,5-diglucoside as minor pigments. At 62.35 J/mL, the highest loss provided by delphinidin-3-glucoside and the lowest by pelargonidin-3-glucoside [89].

3.2.2. High pressure processing

High pressure processing is a non-thermal alternative that has been applied for fruit and vegetable juices preservation through enzyme inactivation and microbial destruction with relatively slight changes in nutritional and sensory quality due to its minimal impact on covalent bonds [70,98]. Noncovalent bonds such as ionic, hydrogen, and hydrophobic bonds are reported to be very sensitive to pressure [99,100]. In addition, molecular weight of food components also determines the effect of pressure exposure on the quality of food products. Components with high molecular weight particularly components important for functionality determination such as proteins are considered to be pressure sensitive, while low molecular weight food compounds especially those responsible for nutritional and sensory quality are pressure insensitive [99,100]. Therefore, it has become an alternative to conventional thermal processing technology as it is able to preserve the natural freshness of juices.

Concerning on green juices, the content of chlorophylls was able to be retained in spinach juice during HPP which might be caused by the lack of disruption of the chlorophyll-protein complexes photosystem II [101]. Furthermore, the total chlorophyll was reported to be slightly increased during high pressure treatment in wheatgrass

and cucumber juice [77,102], which was probably caused by the release of chlorophyll from plant cells with time of treatment [103]. However, although there was an increase in the amount of chlorophyll in wheatgrass juice, under high pressure 500 MPa for 1 min, the color of juice was reported to be not significant different from the control and also those treated using UV-C [77]. Nevertheless, PPO and POD enzymes are known to be moderately stable to high pressure but it is dependent on the source of the enzymes [104,105]. Therefore, to prevent browning that may appear during storage, combination of techniques with thermal treatment or the other non-thermal treatments such as UV-C have been developed and applied in preservation of juices [19,105].

In yellow juices, HPP treatment has been found to cause a significant impact on carotenoids due to the pressure levels applied. In accordance to Stinco et al. [24], the highest carotenoid degradation (41%) in carrot juice was provided by the HPP at the low pressure (300 MPa), while the higher pressure (600 MPa) resulted in the lower degradation of carotenoids (26%). As the carotenoid content impacted by the treatment, the change of color will follow. Observing the lightness (L^*) of the juice, the low pressure (300 MPa) yielded lower L^* compared to that treated with higher pressure (600 MPa), which means that darker color of juice was provided by juice treated with lower pressure and this was caused by the enzymatic browning due to the activity of PPO and POD enzymes [24]. Besides, the color alteration in carrot juice was also caused by the degradation of pigments (particularly ζ-carotene) as the low pressure still allowed the exposure of the pigments to oxygen, enzymes and other agents [24]. This indicates that low pressure cannot prevent the alteration of the color especially during storage as the amount of pressure may be insufficient to inactivate the enzymes. Therefore, as high pressure alone, in certain samples, is considered insufficient to inactivate the enzymes, emerging techniques of combination with another treatment is required in the application of HPP in the preservation of juices.

Meanwhile, in the studies related to anthocyanins, HPP has resulted in limited effect on the degradation of anthocyanins and visual color differences compared to thermal processing. However, significant differences of total anthocyanin content and visual color are detected during storage. Thermal processing of strawberry and raspberry juices at 80–140°C for 20 min was found to have impact on the significant decrease of cyanidin-3-glucoside, pelargonidin-3-glucoside, and pelargonidin-3-arabinoside, while at 140°C almost all anthocyanins content was degraded [106]. Subjected to HPP treatment, pelargonidin-3-glucoside and pelargonidin-3-rutinoside in raspberries and strawberries were found to be stable at 800 MPa [107]. Nevertheless, temperatures of treatment have been noticed to play a crucial role in the stability of anthocyanins. Anthocyanins treated with HPP up to 600 MPa at room temperature experienced no significant change of content, while when the temperature was increased up to 70°C, 25% of anthocyanins were lost [108,109]. This loss was reported to be 20% higher compared to the conventional thermal processing at the same temperature which indicates that HPP combined with higher temperatures escalates the degradation of anthocyanins [110].

The similar effect of combination of HPP with thermal treatment on the color dan anthocyanin content of juices has also been proven in other studies. Torres et al. [111] revealed that orange juice treated with 600 MPa at 20°C for 15 min exhibited 99% retention of cyanidin-3-glucoside. In pomegranate juice, treatment of HPP at 400 MPa (25°C, 5 min) resulted in 37% losses of total anthocyanins content [112]. This implies that slightly higher temperature had impact on the higher loss of anthocyanins, although, the level of pressure applied was lower. HPP (400–700 MPa) coupled temperature treatment (20–100°C) which was applied in strawberry and raspberry juices for 20 min resulted in the loss of cyanidin-3-glucoside, pelargonidin-3-glucoside, pelargonidin-3-arabinoside, cyanidin-3-sophorosdie, and cyanidin-3-rutinoside by 80% at >80°C and 23% at <80°C [106]. High pressure and/or temperatures induce the conversion of monomeric anthocyanins to more condensed components, resulting in the formation of a new pyran ring by cycloaddition, changing the color of juice [109,113]. Meanwhile, related to the pressure-time exposure, a higher and longer pressure (400–600 MPa, 2–15 min) applied in Chinese bayberry juice resulted in the significant reduction of a^*, b^* and L^* values which means that the color of juice became less red and tended to reddish brown after HPP treatment [114]. Therefore, the application of high pressure in fresh juices should be taken into account when it is combined with temperature and time exposure. The advantages of application using HPP can be achieved if the juices treated with pressure not more than 600 MPa at cold and room temperature although the enzyme inactivation cannot be achieved at the mild temperature. Furthermore, the combination with temperature above 50°C should be avoided as thermal degradation and condensation reaction of anthocyanins would be accelerated [113].

4. CONCLUSION

The change of pH that occurred during juice processing as well as the application of HPP and UV light as non-thermal treatment to preserve fresh fruit and vegetable juices were found to provide effect on the content of chlorophylls, carotenoids, and anthocyanins, along with the color of the juices. Low pH solution seemed to obviously affect the color of fresh juices containing chlorophylls, on the contrary to those containing carotenoids and anthocyanins where the color was able to be more retained. In the application of UV light, most of the studies reported that the reduction of pigment content and the alteration of color in the juices were able to be impeded, even during storage, due to the inactivated PPO and POD enzymes. However, it should be noted that the effect of UV treatment may vary among the juices and it highly depends on the amount of UV light absorbed by the products which is determined by exposure time, temperature, dose, wavelength, juice composition, and soluble solids. On the other hand, under the sole HPP treatment, the color of juices was still found to change during storage as high pressure was insufficient to inactivate the enzymes. Therefore, it is suggested to combine HPP with other non-thermal methods as they have been proven to preserve color and minimize the degradation of pigments.

CONFLICTS OF INTEREST

The authors declare they have no conflicts of interest.

AUTHORS' CONTRIBUTION

RDC designed the concept, wrote, and revised the manuscript. MNUP and DML contributed to correct the manuscript.

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