proceedings

MDPI

Proceedings

Carotenoid Profiling of Orange-Coloured Capsicums: in Search of High Zeaxanthin Varieties for Eye Health †

Rimjhim Agarwal [1,*], Hung T. Hong [1], Alice Hayward [2], Stephen Harper [3], Neena Mitter [2] and Tim J. O'Hare [1]

[1] Centre for Nutrition and Food Sciences, Queensland Alliance for Agriculture and Food Innovation, The University of Queensland, Brisbane, QLD, Australia; email1@gmail.com (H.T.H.); email2@gmail.com (T.J.O.)

[2] Centre for Horticultural Sciences, Queensland Alliance for Agriculture and Food Innovation, The University of Queensland, Brisbane, QLD, Australia; email3@gmail.com

[3] School of Agriculture and Food Science, The University of Queensland, Brisbane, QLD, Australia; email4@gmail.com

* Correspondence: Rimjhim.agarwal@uq.net.au

† Presented at the 1st International Electronic Conference on Food Science and Functional Foods, 10–25 November 2020; Available online: https://foods_2020.sciforum.net/.

Received: date; Accepted: date; Published: date

Abstract: Age related Macular Degeneration (AMD) is the leading cause of blindness in developed countries, such as Australia. Lutein and zeaxanthin are the only two carotenoids found in the macular region of the eye. Studies have shown that intake of 10 mg and 2 mg per day of lutein and zeaxanthin, respectively, can reduce the rate of progression of AMD. The supply of these carotenoids can only be met through dietary sources or supplements, as these compounds cannot be synthesized by humans. Although lutein is relatively abundant in dietary sources, zeaxanthin has limited sources. In this study, eight orange and three red capsicum varieties were analyzed for their carotenoid profiles by UHPLC-DAD-APCI-MS. It was observed that the principal carotenoid for seven of the orange varieties was zeaxanthin, and capsanthin for the three red varieties. One orange variety, which had a darker orange hue, had capsanthin and violaxanthin as its principal carotenoids instead of zeaxanthin. Zeaxanthin concentration (the principal carotenoid) in the 7 orange varieties varied from 2.6 ± 0.5 mg/100 g to 25.27 ± 9.4 mg/100 g FW, suggesting that as little as 7 g of high zeaxanthin line could meet the recommended daily dietary intake of 2 mg/person/day.

Keywords: carotenoids; zeaxanthin; bell peppers; eye health; age related Macular Degeneration; capsicums

1. Introduction

Among all the reasons behind blindness in old people in Australia, age related Macular Degeneration (AMD) is one of the leading cause [10]. The macula of the eye is the central region of the retina, thus the degeneration of macula leads to central blindness. At present, about one in seven Australian or 1.29 million people over the age 50 have some evidence of this disease. It is estimated that approximately 17% of these people (over 200,000) will experience vision impairment. Almost 15% of Australians over 80 years (around 160,000) have vision loss or blindness from AMD [2].

Lutein, zeaxanthin and meso-zeaxanthin (isomer of zeaxanthin) are carotenoid pigments derived from plants that form the macular pigments [3]. They play a protective role in shielding the retina from light damage and reducing progression of AMD [18]. Lutein constitutes 36%, zeaxanthin

18%, and meso-zeaxanthin 18% of macular pigment [12]. The remaining 20% is constituted by the oxidative metabolites such as oxo-lutein, (3-hydroxy-β,ε-carotene-3′-one), epilutein, and ε,ε-carotene-3,3′-dione, 9- and 13-Z isomers of both lutein and zeaxanthin [3]. The ratios and distributions of the macular pigments are not uniform and can vary widely between individuals. The ratio of lutein: zeaxanthin (including meso-zeaxanthin) is 1:2.4 in the central macula versus 2:1 in the periphery of the human retina [5]. Lutein and zeaxanthin are yellow and orange, respectively, and absorb blue light wavelengths of around 445 nm and 450 nm [12].

Dietary intake of 10 mg and 2 mg per day of lutein and zeaxanthin, respectively, has been associated with reduced progression of AMD [6] and as these carotenoids cannot be synthesized by humans, their supply through dietary sources or supplements is vital [15]. Green leafy vegetables such as spinach and kale are good sources of lutein, however dietary zeaxanthin is much less common, and is limited to a few orange coloured fruits and vegetables, such as orange capsicums and sweet corn [16].

Various studies have determined the carotenoid content in red-colored capsicum fruit [8,11,13], while only few studies have focused on orange colored capsicums [7]. Moreover, the carotenoid profile of orange capsicums, which have been identified as a rich source of zeaxanthin, needs further investigation to better understand the possible variation in zeaxanthin concentrations that may occur across different varieties.

This current study is focused on characterizing the carotenoid profiles of eight orange and three red capsicum varieties, in order to investigate the differences in carotenoid profiles and zeaxanthin concentration between capsicums of similar appearance.

2. Materials and Methods

2.1. Plant Materials

Plants were grown from seeds collected from different seed companies (South Pacific Seeds, Seminis, Rijk Zwaan, De Ruiter and Climbing Fig) in Australia. Seeds were sown into seed trays in early August (ambient conditions 12 °C–22 °C) and after 12 weeks seedlings were transplanted into UQ23 potting mix comprising of 70% composted pine bark and 30% coco peat and other augments in a randomized block design. Basal fertilizer application was done with Osmocote® slow release fertilizer. 165 plants were grown in greenhouse at UQ, Gatton, Australia. Capsicum fruits were harvested in January-February (20 °C–30 °C) at the same maturity stage. Five plants per variety were harvested for the carotenoid analysis. For each plant, three fruits were selected and were measured for their hue angles using the Konica Minolta CR- 400 chromameter to ensure the same maturity stage.

2.2. Carotenoid Analysis

The pericarp of each fruit was dissected in cold room and were milled in presence of liquid nitrogen. A 'MM400 Retsch Mixer Mill' (Hann, Germany) was operated at 30 Hz for 60 s to achieve a fine dry powder. This fine powder (1 g each) was then dissolved in 6 mL ethanol in a 50 mL falcon tube and was vortexed for 20 s.

3 mL NaCl (5%) was added to the sample, followed by 10 mL extraction solvent (20:80 DCM:hexane). The solution was then centrifuged using an Eppendorf centrifuge (models 5804R and 5810R) at 4000 rpm for 5 min at 25 °C to separate layers. The supernatant was transferred to a new 50 mL falcon tube and pellet was re-extracted twice or until the pellet was colourless. Extracted solution was put into a centrifugal evaporator (mi Vac Duo concentrator Genevac, model DUP-23050-H00) fixed at 25–30 °C for 45 min or until dry. The carotenoids were then de-esterified using 15% methanolic KOH for 10 min at room temperature in dark.

The extracted carotenoid samples (saponified and non-saponified) were reconstituted in 50:50 MeOH:MTBE (0.1% BHT). All the samples were analyzed in technical replicates of three to ensure repeatability of the experiment. 5 µL of each sample was injected in the Waters AcquityTM UHPLC-PDA System (Waters, Milford, MA, USA). The mobile solvents were methanol 0.1% formic

acid (mobile phase A) and methyl tert-butyl ether (MTBE, mobile B). Column temperature hold was at 25 °C with the flow rate of 0.6 mL/min on a YMC C30 Carotenoid Column, 3 µm, 4.6 × 250 mm (Waters, Milford, MA, USA). The gradient elution of 40 min was started with isocratic at 20% B (mobile phase B: 0.1% formic acid in MTBE) for 1 min, from 20% B to 25% B in 20 min an 30% B in the next of 10 min before increasing sharply to 70% mobile phase B in 3 min, followed by conditioning for 1 min, and re-equilibrating for 6 min.

Identification of lutein, zeaxanthin, β-carotene, capsanthin and capsorubin were determined by using external standards and then the UV absorption spectrum [8]. A Shimadzu UHPLC-DAD-APCI-MS system (Shimadzu, Kyoto, Japan, System 3) carried out on A Nexera X2 UHPLC system consisting of a system controller (CBM-30A), three pumps (LC-30AD), an auto sampler (SIL-30AC), column heater (CTO-20AC), diode-array detectors (DAD) detector (SPD-M30A) and two degassers (DGU-20A3R and DGU-20A5R). The Nexera X2 UHPLC system coupled to a LCMS-8060 triple quadrupole mass spectrometer (Shimadzu, Kyoto, Japan) and the APCI source was used to identify the molecular mass and fragment ions of lutein, zeaxanthin, β-carotene, capsanthin, capsorubin and other carotenoids without external standards. The mobile solvents and gradient elution were used the same as the Waters Acquity™ UHPLC-PDA System. Standard curve dilutions were measured in the UHPLC to identify retention times and peak areas. Lutein, zeaxanthin, capsanthin, α and β-cryptoxanthin, α-carotene and β-carotene from the sample extracts were identified and quantified by comparison with the retention times and absorption spectra peaks from standards and their molecular masses and fragment ions.

3. Results and Discussion

3.1. Carotenoid Profile of Varieties

Figure 1 depicts, the difference between the carotenoid profiles of orange and red capsicums varieties. Seven of eight orange coloured capsicums had similar carotenoid profiles, with zeaxanthin (50–75%) as their principal carotenoid (BP, 199-9, PS, Orandino, DSP, Daina, and Boogie). Zeaxanthin, an orange colour carotenoid, supports the observed orange colour of these varieties. Red capsicums (Hugo, Plato and Warlock) on contrast had capsanthin (50–70%) as their principal carotenoid. The majority of red carotenoid pigments (capsanthin and capsorubin) in these varieties, supports the observed red colour. Interestingly, the variety '179-8', which was a darker orange hue than the other orange capsicums, had violaxanthin (33%) and capsanthin (39%) as its major carotenoids. The mixture of these yellow and red carotenoids namely violaxanthin and capsanthin respectively likely explains the resultant darker hue observed in this variety.

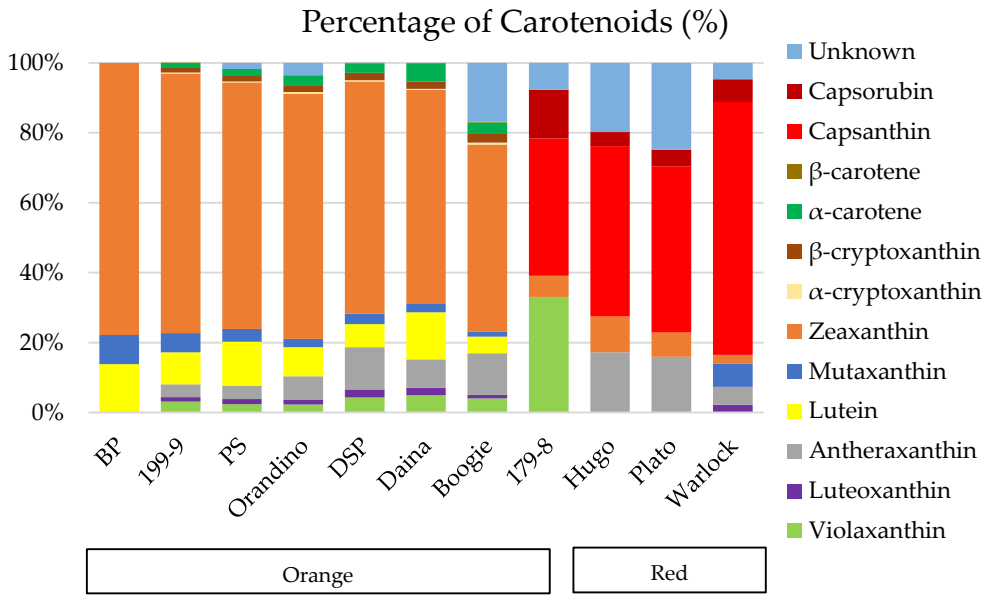

Figure 1. Percentage of carotenoids (after de-esterification) in different orange and red-coloured capsicums.

Lutein was observed only in the seven orange capsicum varieties, and was absent in the red and dark-orange varieties. Similar results for orange and dark-orange capsicums have been previously reported by Rodriguez-Uribe et al. [17], with zeaxanthin (45–60%) being the major carotenoid in orange capsicums, and violaxanthin (22%) and capsanthin (27%) being the major carotenoids in the dark orange variety. Similar observations of the principal carotenoids were also reported in other studies with regard to orange and red colored capsicums [4,11,14]. However, in a separate study conducted by Guzman et al. [7], β-carotene, an orange coloured carotenoid, was the principal carotenoid in most of the orange colored capsicums. This is in contrast to the current study, where beta-carotene was a minor carotenoid in all varieties assessed. Thus, it can be inferred from the past and present studies that not all orange coloured capsicums are due to accumulation of zeaxanthin, other carotenoids such as β-carotene or a mix of yellow and red carotenoids can also lead to orange colour in capsicums.

3.2. Zeaxanthin Concentration of Varieties

From Table 1, it can be calculated that the varieties requiring the lowest amount of tissue to be ingested to meet the daily suggested intake of zeaxanthin are 'Orandino' and 'Boogie' (nearly 7 g), whereas 25 g and 30 g tissue would be required to meet the same suggested intake for the '199-9' and 'DSP' varieties. Similarly, between 260–440 g of tissue would be required with the red capsicums to meet the same daily zeaxanthin requirement. Interestingly, despite its intense orange colour, the dark orange variety '179-8' had the lowest zeaxanthin concentration, so that as much as 900 g would be required to meet the suggested zeaxanthin intake. Considering an average size of 400 g of capsicums in Australian market, it would mean consumption of few slices of high zeaxanthin orange varieties will be sufficient as compared to a whole capsicum in case of red lines to meet the recommended zeaxanthin daily dietary intake.

Table 1. Zeaxanthin concentration (mg/100 g FW) and the tissue needed to meet a daily dietary intake of zeaxanthin of 2 mg/per person/per day.

Variety	Colour	Total Carotenoid Conc.	Zeaxanthin Conc.	Tissue Needed (g)
Orandino	Orange	40.0 ± 12.3	28.0 ± 8.5	7
Boogie	Orange	50.3 ± 22.5	27.0 ± 9.4	7
PS	Orange	29.1 ± 13.7	20.5 ± 10.3	10
Daina	Orange	22.0 ± 14.1	13.5 ± 8.4	15
199-9	Orange	10.4 ± 3.2	8.0 ± 2.7	25
DSP	Orange	9.3 ± 1.5	6.2 ± 1.0	30
BP	Orange	2.4 ± 0.1	1.9 ± 0.1	100
179-8	Dark-Orange	3.7 ± 1.2	0.2 ± 0.1	910
Hugo	Red	7.5 ± 1.3	0.8 ± 0.2	260
Plato	Red	9.5 ± 2.0	0.7 ± 0.3	300
Warlock	Red	17.8 ± 7.6	0.5 ± 0.4	440

It is also worth noting that varieties with high percentage of zeaxanthin (Figure 1) do not necessarily have high zeaxanthin concentration for instance variety "BP" has the highest percentage of zeaxanthin (80%) among all the varieties but its zeaxanthin concentration is only 1.9 ± 0.1 mg/100 g FW, which is significantly less than the highest zeaxanthin concentration of "Orandino and Boogie" (28.0 ± 8.5 and 27.0 ± 9.4 mg/100 g FW respectively). This observation suggests, that zeaxanthin production relative to FW, not relative to total carotenoids produced, is an important consideration in the dietary value of various cultivars.

4. Conclusions

From the present study, it can be concluded that although orange capsicums are a considerably better source of zeaxanthin compared to red capsicums, and had similar carotenoid profiles to each other, there was still considerable variation in zeaxanthin concentration between the orange varieties. It is also worth noting that orange colour does not necessarily indicate high zeaxanthin concentration for instance in the dark orange variety investigated in this study, rather it was due to a mixture of red (capsanthin and capsorbin) and yellow (violaxanthin) carotenoids. Thus, depending on the zeaxanthin concentration, different capsicum varieties will have different dietary value in meeting the suggested daily dietary requirement of zeaxanthin.

Funding: This study was funded in part by 'Naturally Nutritious' (Hort Innovation, project HN15001).

References

1. Abdel-Aal, E.-S.M.; Akhtar, H.; Zaheer, K.; Ali, R. Dietary Sources of Lutein and Zeaxanthin Carotenoids and Their Role in Eye Health. *Nutrients* **2013**, *5*, 1169–1185, doi:10.3390/nu5041169.
2. About Macular Degeneration. Available online: https://www.mdfoundation.com.au/content/what-is-macular-degeneration (accessed on 14 October 20202).
3. Arunkumar, R.; Gorusupudi, A.; Bernstein, P.S. The macular carotenoids: A biochemical overview. *Biochim. et Biophys. Acta (BBA) Mol. Cell Biol. Lipids* **2020**, *1865*, 158617, doi:10.1016/j.bbalip.2020.158617.
4. Bonaccorsi, I.; Cacciola, F.; Utczas, M.; Inferrera, V.; Giuffrida, D.; Donato, P.; Dugo, P.; Mondello, L. Characterization of the pigment fraction in sweet bell peppers (*Capsicum annuum* L.) harvested at green and overripe yellow and red stages by offline multidimensional convergence chromatography/liquid chromatography-mass spectrometry. *J. Sep. Sci.* **2016**, *39*, 3281–3291, doi:10.1002/jssc.201600220.
5. A Bone, R.; Landrum, J.T.; Fernandez, L.; Tarsis, S.L. Analysis of the macular pigment by HPLC: Retinal distribution and age study. *Investig. Ophthalmol. Vis. Sci.* **1988**, *29*, 843–9.
6. Chew, E.Y.; Clemons, T.E.; SanGiovanni, J.P.; Danis, R.P.; Ferris, F.L.; Elman, M.J.; Antoszyk, A.N.; Ruby, A.J.; Orth, D.H.; Bressler, S.B.; et al. Secondary Analyses of the Effects of Lutein/Zeaxanthin on Age-Related Macular Degeneration Progression. *JAMA Ophthalmol.* **2014**, *132*, 142–149, doi:10.1001/jamaophthalmol.2013.7376.
7. Guzman, I.; Hamby, S.; Romero, J.; Bosland, P.W.; O'Connell, M.A. Variability of carotenoid biosynthesis in orange colored Capsicum spp. *Plant Sci.* **2010**, *179*, 49–59, doi:10.1016/j.plantsci.2010.04.014.
8. Ha, S.-H.; Kim, J.-B.; Park, J.-S.; Lee, S.-W.; Cho, K.-J. A comparison of the carotenoid accumulation in Capsicum varieties that show different ripening colours: Deletion of the capsanthin-capsorubin synthase gene is not a prerequisite for the formation of a yellow pepper. *J. Exp. Bot.* **2007**, *58*, 3135–3144, doi:10.1093/jxb/erm132.
9. Howe, J.A.; Tanumihardjo, S.A. Carotenoid-Biofortified Maize Maintains Adequate Vitamin A Status in Mongolian Gerbils. *J. Nutr.* **2006**, *136*, 2562–2567, doi:10.1093/jn/136.10.2562.
10. Keel, S.; Xie, J.; Foreman, J.; Van Wijngaarden, P.; Taylor, H.R.; Dirani, M. Prevalence of Age-Related Macular Degeneration in Australia: The Australian National Eye Health Survey. *JAMA Ophthalmol.* **2017**, *135*, 1242–1249, doi:10.1001/jamaophthalmol.2017.4182.
11. Kim, J.-S.; An, C.G.; Park, J.-S.; Lim, Y.P.; Kim, S. Carotenoid profiling from 27 types of paprika (Capsicum annuum L.) with different colors, shapes, and cultivation methods. *Food Chem.* **2016**, *201*, 64–71, doi:10.1016/j.foodchem.2016.01.041.
12. Landrum, J.T.; Bone, R.A. Lutein, zeaxanthin, and the macular pigment. *Arch. Biochem. Biophys.* **2001**, *385*, 28–40.
13. Mínguez-Mosquera, M.I.; Hornero-Méndez, D. Changes in Carotenoid Esterification during the Fruit Ripening of Capsicum annuum Cv. Bola. *J. Agric. Food Chem.* **1994**, *42*, 640–644, doi:10.1021/jf00039a007.
14. Minguez-Mosquera, M.I.; Hornero-Mendez, D. Formation and transformation of pigments during the fruit ripening of *Capsicum annuum* cv. Bola and Agridulce. *J. Agric. Food Chem.* **1994**, *42*, 38–44.
15. Perera, C.O.; Yen, G.M. Functional Properties of Carotenoids in Human Health. *Int. J. Food Prop.* **2007**, *10*, 201–230, doi:10.1080/10942910601045271.
16. Perry, A.; Rasmussen, H.; Johnson, E.J. Xanthophyll (lutein, zeaxanthin) content in fruits, vegetables and corn and egg products. *J. Food Compos. Anal.* **2009**, *22*, 9–15, doi:10.1016/j.jfca.2008.07.006.

17. Rodriguez-Uribe, L.; Guzman, I.; Rajapakse, W.; Richins, R.D.; O'Connell, M.A. Carotenoid accumulation in orange-pigmented Capsicum annuum fruit, regulated at multiple levels. *J. Exp. Bot.* **2011**, *63*, 517–526, doi:10.1093/jxb/err302.
18. Tosini, G.; Ferguson, I.; Tsubota, K. Effects of blue light on the circadian system and eye physiology. *Mol. Vis.* **2016**, *22*, 61–72.