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Note

Carotenoid Composition in Mature *Capsicum Annuum*

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We investigated the carotenoid composition of thirteen types of *Capsicum annuum* fruit at full maturity and discussed the ratio of β-carotene to capsanthin. Among the thirteen types examined, "Cherry sweet" and "Raruku" had the fruit with the highest values of total carotenoid content and capsanthin content. "Raruku" and "Sarara"had high capsanthin content ("Raruku" 63%, "Sarara" 59%). Although ten *C. annuum* varieties were included in the 13 fruit types sampled, a high correlation (r=0.960) was observed between β-carotene and capsanthin. In addition, the 1: 10 carotene capsanthin ratio was used as an index for the capsanthin content of ripe *C. annuum.*

Keywords: *Capsicum annuum*, β-carotene, capsanthin, carotenoid

Introduction

In phytotaxonomy, sweet peppers (*Capsicum annuum*) are classified as members of the Solanaceae family in the genus Capsicum, to which chili peppers also belong. The Japanese name for *C. annuum*, *pi-man*, came from the French word, *pimen*, which means chili peppers. While the classification of chili pepper cultivars differs among Japan, the United States of America, and European nations (Yazawa, 2000), the American classification has been more commonly adopted worldwide (Smith *et al.*, 1987) and is divided into the following groups: the Bell and Pimento group, for cultivars with large fruit, a smooth pericarp with no gaps and a thick sarcocarp; the Ancho group, for cultivars with rounded fruit with no gaps or wrinkles on the pericarp and a thin sarcocarp; the Anaheim group, for cultivars characterized as having long, thin fruit; the Jalapeño group, for green cultivars with a pungent taste when premature, and a length of up to 7.5 cm; and the cherry cultivar, for cultivars with relatively small round or oval fruit with lengths of up to 5 cm and a thick sarcocarp. In Japan, after various efforts at strain selection and selective breeding, a mid-sized Shishi group has been developed and is now extensively established. We have been investigating changes in carotenoid composition and capsanthin content associated with maturation in *C. annuum* cv. "Sarara", a midsized member of the Shishi group. Our findings revealed that lutein was the most abundant carotenoid in green fruit, while capsanthin was more abundant in red fruit. We further reported that the ratio of these carotenoids to β-carotene was consistent and the ratio of β-carotene: capsanthin was 1:10–11 (Suzuki and Mori., 2003). According to previous reports (Deli *et al.*, 2001; Hornero-Mendez *et al.*, 2000; Minguez-Mosquera and Hornero-Mondez., 1994), in four out of seven large-fruited Spanish cultivars in the mature stage, ratios of β-carotene: capsanthin were 1:10, which was the same as that observed for "Sarara" in this study. The present study examined carotenoid compositions and β-carotene: capsanthin ratios and investigated 13 cultivars of red *C. annuum* from the 9th to 10th week after blooming, the time at which the fruits mature.

Materials and Methods

Materials The mature specimens of *C. annuum L.* used in the present study were grown in the Japan Horticultural Production and Research Institute (Matsudo City, Chiba, Japan). The following 13 cultivars from the seven cultivar groups were used in the present study: "Sarara" and "Raruku" from the midsized Shishi group, "California Wonder", "Amezisuto", "Thigusa", "Chocolate Bell", and "Signal Type No. 607A" from the Bell group, "Miogi", which is a cross between the local Shishi group and the Bell group, "Edesalma" and "Cheese Pimento Type No. 684 A" from the Pimento group, "Cherry Sweet" from the Cherry group, "Jalapeño" from the Jalapeño group, and "Hungarian Yellow Wax Sweet" from the Long Wax group.

Samples were diced after removing seeds, freeze-dried, ground by a mill, and analyzed. The dry samples were stored at −20℃ until analyzed. Moisture was determined by gravimetric method after freeze-drying.

Quantitative determination of carotenoids Carotenoid concentrations were determined in the same manner as previously reported (Suzuki and Mori., 2003). Each sample was aliquoted, ground with quartz sand, magnesium carbonate and 0.5 M ascorbic acid, and extracted using

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mixed solvent containing hexane: acetone: ethanol: toluene (10 : 7 : 6 : 7). After being dissolved into ethanol, non-saponified product was obtained by alkaline saponification. The volume was adjusted with the mixed solvent containing hexane: acetone: ethanol: toluene (10 : 7 : 6 : 7): ethanol (4 : 6). The obtained solution was applied to HPLC analysis (Merck Hitachi, Japan) using a Carotenoid C30 column (4.6×250 mm, YMC Co., Ltd., U.S.A) with eluent A containing methanol: t-butyl methyl ether: water (75 : 15 : 10), and eluent B containing methanol: t-butyl methyl ether: water (8 : 90 : 2), under the following conditions: a gradient of 100%–0% of eluent A and 0%–100% of eluent B, a fractionation period of 30 minutes, a detecting wavelength of 460 nm, a column temperature of 30℃ and a flow rate of 1 ml/min. For identification, β-carotene (absorption coefficient $E^{1\%}_{1cm}$ of 2592 in hexane), zeaxanthin (absorption coefficient $E^{1\%}_{1cm}$ of 2350 in petroleum ether), and capsanthin (absorption coefficient $E^{1\%}_{1cm}$ 2072 in benzene), which are all authentic samples (Funakoshi Co., Ltd., Japan), were used, and the absorbance in the solution containing these samples was examined with a spectrophotometer. On the basis of Lambert-Beer's law, the total concentration of carotenoids was determined, and the quantity was analyzed using HPLC. After removing a part of the extracted solution and dissolving it in benzene, the total quantity of carotenoid was measured with absorption coefficient $E^{1\%}_{1cm}$ 2072 in benzene at a wavelength of 483 nm for capsanthin, which is the main carotenoid in mature fruit.

Results and Discussion

Carotenoid and water contents are shown in Table 1. The highest water content was observed in "Edesalma" and "Hungarian Yellow Wax Sweet" (92.0%), while the lowest content was observed in "Sarara" (89.0%). The mean value and standard deviation of water content was 90.5±0.93%. The highest total carotenoid content was observed in "Cherry Sweet" (45 mg/100 g) followed by "Raruku" (40 mg/100 g) and "Chocolate Bell" (39 mg/100 g). The lowest total carotenoid content of 13 mg/100 g was observed in "Hungarian Yellow Wax Sweet". The highest capsanthin content was observed in "Raruku" (25 mg/100 g), followed by "Cherry Sweet" (22 mg/100 g), "Chocolate Bell" (20 mg/100 g), and "Sarara" (19 mg/100 g). It was found that "Cherry Sweet" contained high levels of zeaxanthin (6.7 mg/100 g), which was approximately 10 times higher than the lowest level of found in "Sarara" (0.65 mg/100). Figure 1 shows the ratios of the various carotenoid compositions. The main carotenoid in mature *C. annuum* is capsanthin. It was found that capsanthin comprised approximately 40–60% of the total amount of carotenoids in the 13 cultivars. The percentages of capsanthin relative to other carotenoids in 11 cultivars, except for the "Raruku" and "Sarara" cultivars in the midsized Shishi group, were approximately 45–50%. Deli *et al.* (2001) reported that the relative percentage of capsanthin content in mature cultivars in the Bell group was approximately 40%. Thus, cultivars in the Bell group, the cross between the Local Shishi and the Bell groups, the Pimento group, the Cherry group, the Jalapeño group, and the Long Wax group, all used in the present study, exhibited consistent results relative to the findings by Deli *et al.* (2001). While it was previously reported that "Sarara" has a high capsanthin content (Suzuki and Mori, 2003), the results of the present study suggested that "Raruku" has an even higher capsanthin content. Consequently, it is assumed that cultivars in the midsized Shishi group are rich in capsanthin. The relative percentage of zeaxanthin in the "Thigusa", "Cheese Pimento Type No. 684A", "Cherry Sweet", and "Hungarian Yellow Wax Sweet" cultivars were approximately 12 to 15%, which was approximately 5% higher than that observed in other cultivars, "Sarara" and ""Raruku", with high capsanthin contents, were 2% and 7%. It has been reported that cultivars with high levels of the red capsanthin (Caps) and yellow zeaxanthin (Zeax) (Caps/Zeax) color pigments are highly selected when breeding red *C. annuum* (Hornero-Mendez *et al.,* 2000; Minguez-Mosquera *et al.,* 1984). The Caps/Zeax value in "Sarara" was 29, which was higher than that observed in other cultivars. However, since the color of "Sarara" is slightly dark red upon maturation due to the remaining chlorophyll (Ishikawa *et al.,* 1997), the relationship between carotenoid pigments and chlorophyll, in addition to the ratio of the red color pigment capsanthin (Caps) and yellow color pigment zeaxanthin (Zeax), need to be considered. Table 1 shows the ratios of capsanthin against β-carotene, which were 1 : 22 in mature fruit from "California Wonder," 1 : 31

Table 1. Carotenoido contents of pericarpus of *Capsicum annuum*.

Cultivar	Water content,100g%	Carotenoid contents,mg/100g fresh wt				β—Carotene:Capsanthin
		Total carotenoid	Capsanthin	β-Carotene	Zeaxanthin	
Sarara	89.0	32±0.61	19±3.0	1.8±0.02	0. 65±0.10	1: 11
Raruku	90.1	40±0.94	25±1.2	2.0±0.25	2.7±0.13	1: 13
Amezisuto	90.9	23±0.18	11±0.69	0.99±0.056	0.80±0.14	1: 11
California Wonder	91.0	24±0.95	10±0.22	0.46±0.033	1.2±0.37	1: 22
Thigusa	89.8	29±0.44	13±0.36	1.4±0.031	4.1±0.49	1: 9
Chocolate Bell	90.7	39±1.9	20±1.2	1.7±0.12	0.80±0.051	1: 12
Signal type No.607A	90.2	19±0.86	8.7±0.71	1.2±0.13	0.77±0.042	1: 7
Miogi	90.6	21±0.32	10±2.4	1.0±0.075	0.84±0.12	1: 10
Edesalma	92.0	24±0.49	11±0.66	0.36±0.013	1.2±0.62	1: 31
Cheese pimento type No.684	89.3	32±0.70	16±1.1	1.0±0.084	3.7±0.61	1: 16
Cherry Sweet	89.6	45±1.2	22±0.05	1.8±0.076	6.7±0.83	1: 12
Jalapeno	91.0	25±0.40	12±0.84	1.2±0.033	1.9±0.61	1: 10
Hungarian Yellow Wax Sweet	92.0	13±0.86	5.5±0.46	0.63±0.042	1.5±0.11	1: 9

Data were expressed as means ±SD (*n*=5).

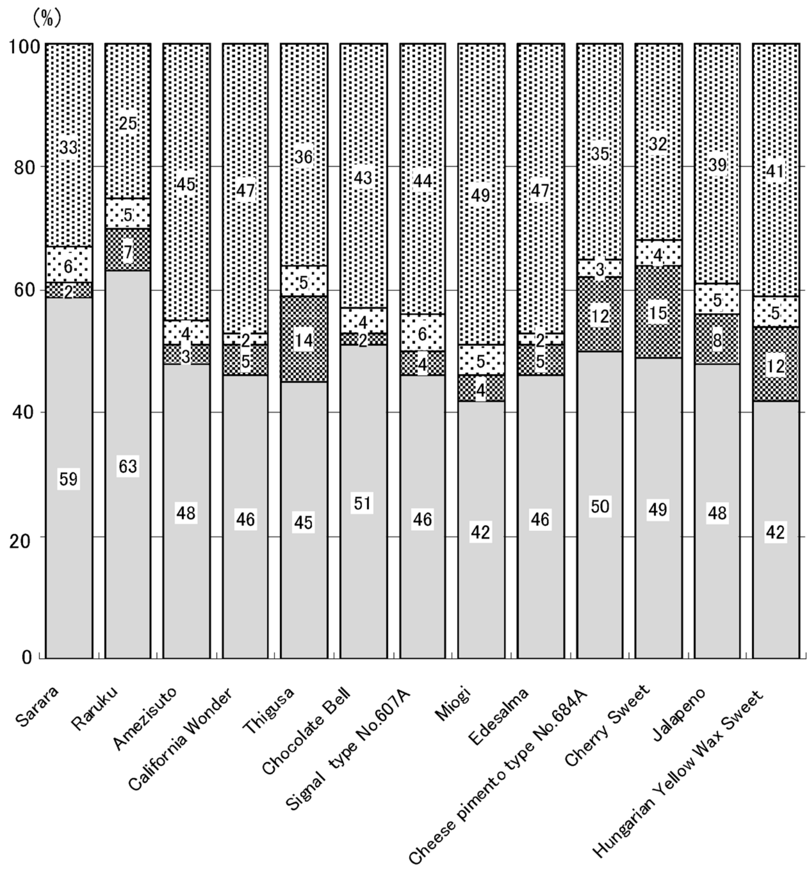

Fig. 1. The rate of Carotenoid content in *Capsicum annuum*.
Carotenoid: ⁝ Capsanthin: ▩ Zeaxanthin: ▦ β-Carotene: □ Others

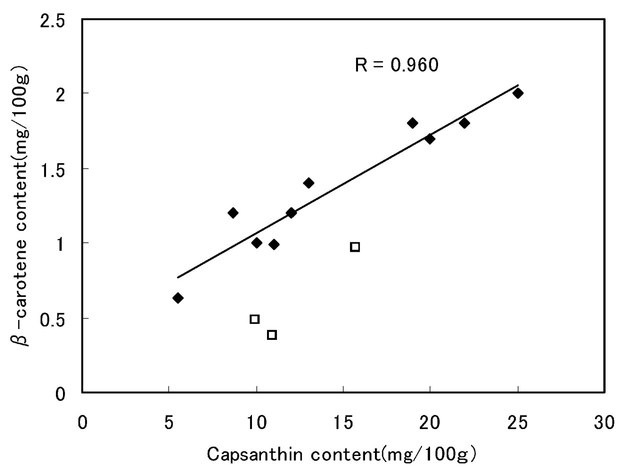

Fig. 2. The correlation of β-carotene and capsanthin. Significant positive correlations (r=0.960) were observed between β-carotene and capsanthin contents in the 10 cultivars excluding "California Wonder", "Edesalma", and "Cheese Pimento Type No. 684A". ■ 10 cultivars; □ "California Wonder", "Edesalma", and "Cheese Pimento Type No. 684A".

for "Edesalma," 1:16 for "Cheese Pimento Type No. 684A," and between 1:7 to 1:13 (mean 1:10) for the 10 other cultivars. Figure 2 shows the relationship between the β-carotene and capsanthin contents. Significant positive correlations (r=0.960) were observed between β-carotene and capsanthin contents in the 10 cultivars excluding "California Wonder", "Edesalma", and "Cheese Pimento Type No. 684A". It was therefore suggested that the ratio of β-carotene and capsanthin in mature *C. annuum* was approximately 1:10. "Edesalma" and "Cheese Pimento Type No. 684A", which did not exhibit this ratio, belong to the Pimento group. Among the five cultivars in the Bell group, only the "California Wonder" cultivar did not exhibit this consistency with respect to β-carotene and capsanthin ratios. Based on these observations, irrespective of the total quantity of carotenoids and their relative proportion of capsanthin in mature fruit from *C. annuum,* it is assumed that the ratio of β-carotene to capsanthin is constant and the ratio of 1:10 could thus potentially be used as an index to analyze the content of these carotenoids. It may be possible to assume the quantity of capsanthin by analyzing β-carotene. In a similar manner, Hornero-Mendez *et al.* (2000) demonstrated an increase in the ratio of xanthophyll esterification associated with maturation in five *C. annuum* cultivars (Hornero-Mendez and Minguez-Mosquera, 2000). Based on these results we propose that biosynthesis of carotenoid pigments in *C. annuum* is maintained within relatively specific limits.

Summary

Carotenoid composition in mature *C. annuum* exhibited the following tendencies:

1) The total carotenoid and capsanthin content in "Cherry Sweet" and "Raruku" cultivars was high.

2) The levels of capsanthin to total carotenoids in 13 mature *C. annuum* cultivars were approximately 40–60%. Markedly high levels of capsanthin (60%) were observed in "Raruku" and "Sarara" cultivars, which belong to the midsized Shishi group.

3) Regarding the relationship between β-carotene and capsanthin, 10 of the 13 cultivars exhibited a strong positive correlation ($r=0.9603$) at a ratio of 1:10.

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