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Tomatine, chlorophyll, β-carotene and lycopene content in tomatoes during growth and maturation

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Abstract: Tomato (*Lycopersicon esculentum*) plants synthesise nutrients, pigments and secondary metabolites. These include the green pigment chlorophyll, the yellow pigment β-carotene, the red pigment lycopene and the colourless glycoalkaloid α-tomatine. The levels of these compounds are strongly influenced by the maturity of the tomatoes. Widely consumed Japanese tomato varieties Momotaro, Momotaro-T93 and First Memory at five different stages of ripeness, each harvested at 10, 20, 30, 40 and 50 days after flowering of the plants, were analysed for the contents of these compounds. Additionally, tomato clusters from different locations along the vine on the same plant were also evaluated. The results show that chlorophyll and tomatine concentrations decrease rapidly during the growth of the tomatoes. By contrast, β-carotene and lycopene levels are low in immature and high in mature tomatoes. The location of the tomato clusters and tomato variety did not significantly affect these results. The possible usefulness of these results to optimise health-promoting effects of tomatoes is discussed.

Keywords: β-carotene; chlorophyll; lycopene; α-tomatine; green tomatoes; red tomatoes; human health

INTRODUCTION

Tomatoes synthesise nutrients, pigments and secondary metabolites, all of which play a role both in the plant and in human and animal diets. These include the green pigment chlorophyll, the yellow pigment β-carotene, the red pigment lycopene and the colourless glycoalkaloids α-tomatine and dehydrotomatine in a ratio of about 10:1.[1–3] These compounds are reported to have important functional roles in the plant[4–6] as well as the potential to prevent disease and to promote health in animals and humans.[7–11] Since the content of these compounds strongly depends on the maturity of the tomatoes, a need exists to define the dynamics of their biosynthesis as a function of tomato maturation in different cultivars. Changes in metabolism, ripening and composition of tomatoes are affected by tomato variety as well as by moisture and nitrogen content of the soil and by the production of ethylene.[3]

We found that orally fed tomatine, which has a strong affinity for cholesterol *in vitro*, induced a significant reduction in plasma cholesterol in hamsters and that feeding high-tomatine green tomatoes induced a greater reduction in plasma LDL (bad) cholesterol and triglyceride levels than feeding low-tomatine red tomatoes.[10,12] In contrast to potato glycoalkaloids,[6,13] orally consumed tomatine appears to be non-toxic, presumably because it is eliminated in the faeces as an insoluble tomatine–cholesterol complex formed in the digestive tract. The safety of tomatine is also supported by our observation that pickled green tomatoes, widely consumed in many countries, have a high tomatine content.[14]

Since tomatine is both synthesised and degraded as tomatoes mature,[15] these considerations suggest the need to delineate the tomatine content at different stages of maturity of widely consumed Japanese tomato varieties. As part of this effort, concurrent changes in content of three other tomato components were also measured. The main objective of this study was to define changes in the content of the four tomato compounds of interest as a function of tomato maturation. Although attempts have been made to define these trends for some tomato constituents,[16–18] this was not done simultaneously for all four compounds of the present study. This information should help guide the optimisation of beneficial effects of tomatoes in human health.

EXPERIMENTAL

Materials

HPLC-grade acetonitrile, acetone, methanol, dichloromethane and *n*-hexane were purchased from

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Kanto Chemical Co (Tokyo, Japan). The solvents were filtered through a 0.45 μm membrane filter (Millipore, Bedford, MA, USA) and then degassed in an ultrasonic bath before use. Analytical-grade butyl hydroxytoluene, anhydrous sodium sulphate and diethyl ether were purchased from Wako Pure Chemical Industry (Osaka, Japan). β-Carotene (C4582 from carrots) and lycopene (L9879 from tomatoes) were obtained from Sigma (St Louis, MO, USA). Chlorophyll *a* and *b* and tomatine were the same compounds as in our previous studies.

Seeds of three tomato cultivars widely consumed in Japan were obtained from the following suppliers: Momotaro (resistant to tobacco mosaic virus (TMV)-Tm-1 type)) and Momotaro-T93 (resistant to TMV-Tm-2^a type) seeds from Takii Plant Breeding Corp (Kyoto, Japan); First Memory (resistant to TMV-Tm-2^a) seeds from Sakata Plant Breeding Corp (Yokohama, Japan).

Growth and harvesting of tomatoes

The seeds were planted in vermiculite in a phytotron on 27 March 1997. On 17 April the seedlings were transplanted to plastic pots 9 cm in diameter containing a soil mixture of organic matter, sand and perlite (1:2:1 v/v/v).

Plants were maintained in a greenhouse until 15 May, when plants from each cultivar were re-transplanted to grow in the field. Branches emerging from the main stem (ie trusses) were classified 1–6 from the base of the plant (1) to the apex (6). When flowers appeared, small paper tags with the date of flowering were tied to the plant.

For analysis the trusses were grouped by height from the ground. Three groups of tomato fruits were each analysed for size as follows. (a) trusses 1 and 2; (b) trusses 3 and 4; (c) trusses 5 and 6. Specifically, beginning 10 days after flowering and at four additional 10 day intervals thereafter, fruits were collected, weighed and measured for size (the major axis of the fruits was measured with a slice caliper). Thus, for each collection, the fruits were 10, 20, 30, 40 or 50 days old after flowering. Pericarp sections of four or five tomatoes of each cultivar of approximately equal size were used for the analyses. Although the sections analysed were of approximately equal size at each interval, weights and sizes of the pericarp sections analysed increased from one interval to the next as fruits grew.

Analysis of chlorophyll *a* and *b*

The tomato fruit pericarp section, crystalline sand and $MgCO_3$ (0.1 g) were macerated in a glass mortar with 80% acetone and then centrifuged at $18\,100 \times g$ for 10 min at 1 °C. The pellet was extracted three times with 10 ml of 80% acetone and centrifuged each time. The extracts were combined and adjusted with 80% acetone to 50 ml. This solution was then examined in a Beckman (Palo Alto, CA, USA) Model 24 spectrophotometer at 665 nm (chlorophyll *a*) and 642.5 nm (chlorophyll *b*). The concentration of each chlorophyll was calculated as described elsewhere.[19]

Analysis of α-tomatine

The method used for isolation and identification of tomatine was adapted from a previously described procedure.[20] Pericarp sections (1.0–20.0 g) from approximately equal-sized tomatoes harvested from three different trusses at 10 day intervals after flowering were used for the analysis of tomatine. Fruits of different weights (1.1–220.5 g) harvested at the green stage of trusses 1–6 from five different stages of ripening, each harvested at 10 day intervals after flowering, were also used for the analysis of tomatine. Samples were extracted with a mixture of chloroform/methanol (2:1 v/v), concentrated to 2–3 ml and dissolved in 40 ml of 0.2 M HCl, and the tomatine was precipitated with 2% (w/v) $(NH)_4OH$. The ammonia was dissipated and the pellet was dissolved in 2 ml of a mixture of tetrahydrofuran/acetonitrile/0.02 M KH_2PO_4 (50/30/20 v/v/v) and centrifuged at $18\,100 \times g$ for 10 min at 1 °C. The supernatant (50 μl) was used for HPLC analysis.

HPLC analysis was carried out using a Hitachi Model 655A-11 liquid chromatograph with a Model 655A-40 autosampler (Tokyo, Japan). The column (25 cm × 4.0 mm id) was made of stainless steel and packed with Nucleosil NH_2 (particle diameter 10 μm). Tomatine was eluted with tetrahydrofuran/acetonitrile/0.025 M KH_2PO4 (50:30:20 v/v/v) at a flow rate of 1 ml min^{-1}. The UV detector (Hitachi Model 655A UV monitor) was set at 208 nm. Three separate analyses were carried out for each sample.

To confirm the identity of tomatine, the peak collected from the HPLC column was hydrolysed with HCl into sugars and the aglycone tomatidine. The sugars were converted to alditol acetate derivatives. The nature and molar ratios of sugars were determined by gas–liquid chromatography (GLC) and the aglycone by GLC/mass spectrometry as described in detail elsewhere.[1,21]

Analysis of β-carotene and lycopene

Pericarp sections as described for tomatine were also used for extracting the carotenoids. The literature procedure[22–24] for the extraction and analysis of carotenes (β-carotene and lycopene) was modified as follows. Butyl hydroxytoluene (0.05 g), $MgCO_3$ (0.1 g), a small amount of crystal sand and 50 ml of acetone were added to the pericarp sections of each tomato fruit sample (total 5 g) and then homogenised in a mortar. The mixture was filtered through a glass filter and the solid residue was washed three or four times with 30 ml of acetone until no more coloured material was extracted. The extracts were combined and transferred to a decanting funnel containing diethyl ether. The ether solution was then partitioned with water. An equal volume of 20% KOH in methanol was added to the ether fraction, nitrogen gas was introduced into the headspace, and the

saponification was completed overnight at 5 °C. The unsaponifiable components were then washed out with water until the washings were at neutral pH, filtered through a bed of anhydrous $(Na)_2SO_4$ and evaporated to dryness in a rotary evaporator at 10 °C. The dried pigments were dissolved in 5 ml of dichloromethane and analysed by HPLC.

HPLC was carried out with a Hitachi Model 635-11 instrument equipped with a 20 µl sampling loop (Rheodyne Model 7120, Palo Alto, CA, USA). A reverse phase C_{18} column packed with Intersil ODS 2 (250 mm × 4.6 mm id, particle size 5 µm; GL Science Co, Osaka, Japan) was used. β-Carotene and lycopene were eluted with acetonitrile/methanol/dichloromethane/*n*-hexane (50:40:5:5 v/v/v/v) at a flow rate of 1 ml min^{-1}. A visible light detector (Hitachi wavelength-tunable effluent monitor), set at 453 nm at a chart speed of 5 mm min^{-1} and connected to a Hitachi D-2500 chromato-integrator, was used to determine the spectrum of each HPLC peak. Quantification of the carotenes was achieved by comparing sample peak areas with those of known amounts of the standards β-carotene and lycopene.

The specific carotenes were identified by comparing HPLC retention times with those of known standards.

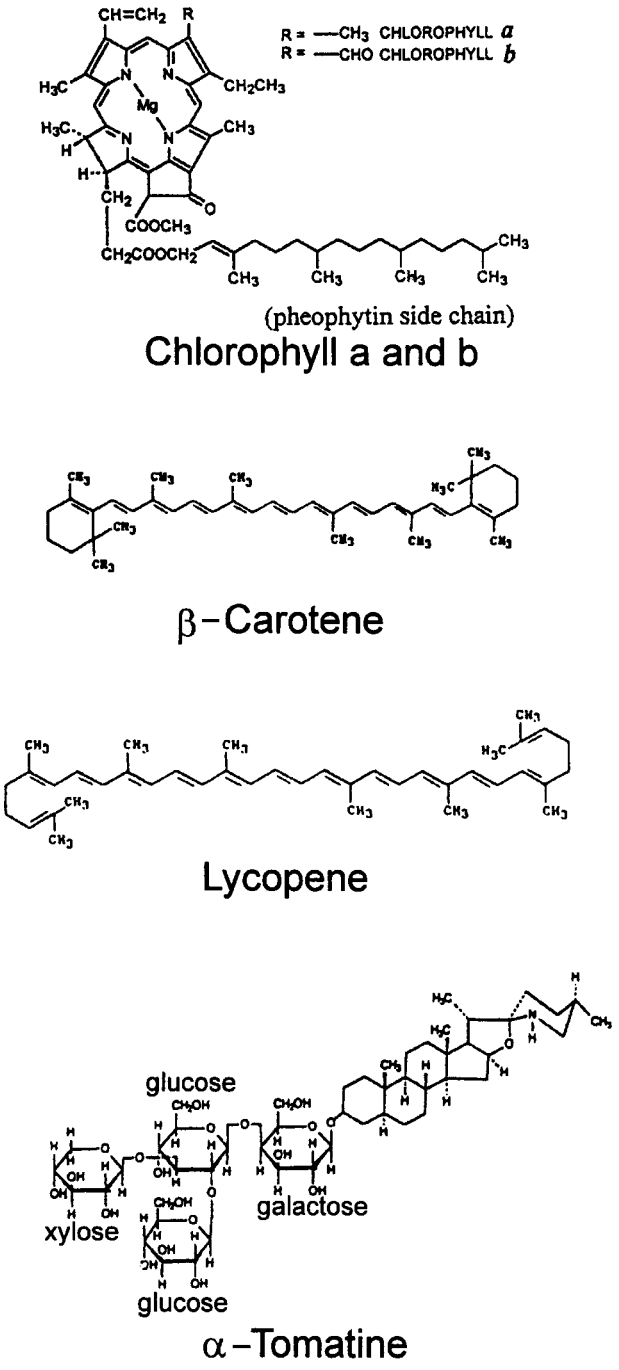

Figure 1. Structures of chlorophyll *a* and *b*, β-carotene, lycopene and α-tomatine.

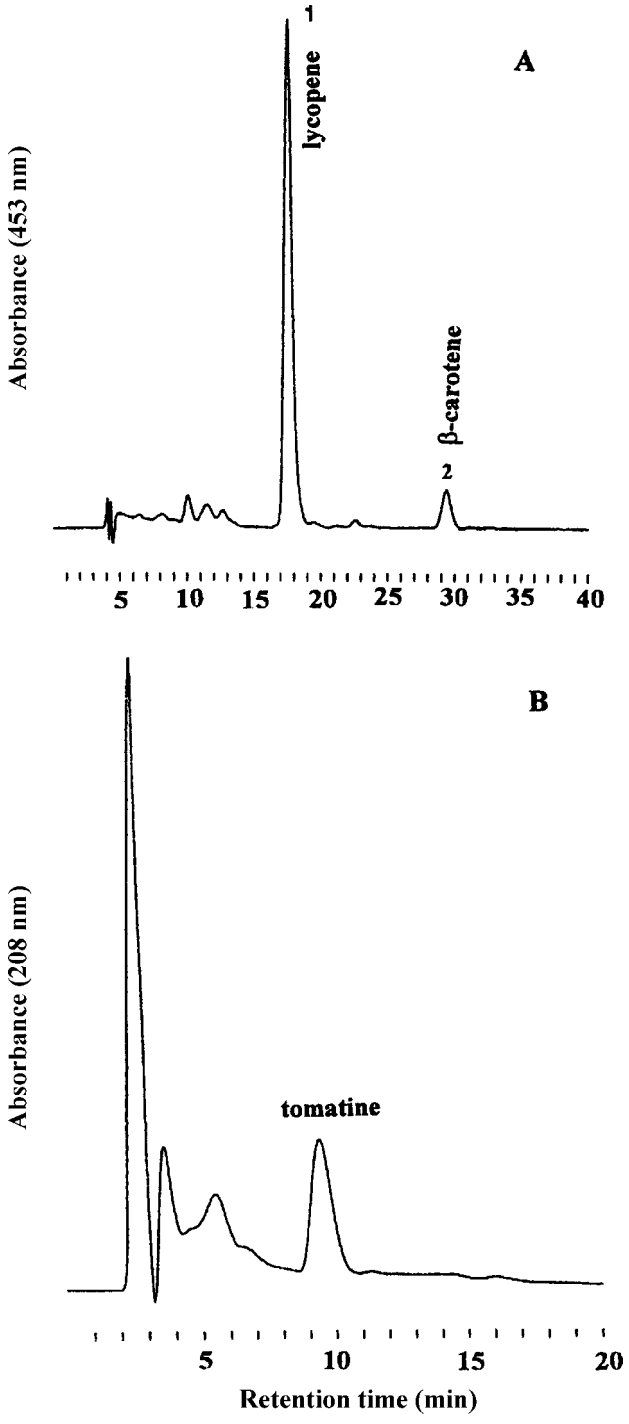

Figure 2. HPLC chromatograms of (A) lycopene and β-carotene and (B) α-tomatine isolated from tomatoes.

Table 1. HPLC retention times and UV maxima (λ_{max}) of lycopene and β-carotene standards, and corresponding values for peaks of tomato extracts

Compound	Retention time (min)	λ_{max} (nm) n-Hexane	Dichloromethane	Chloroform	Identification
Lycopene	17.42	445, 473	445, 471, 503	449, 486, 520	
β-Carotene	29.92	425, 452, 479	450, 478	465, 492	
Peak 1	17.63	445, 473	445, 471, 503	449, 486, 520	Lycopene
Peak 2	29.39	425, 452, 479	450, 478	465, 492	β-Carotene

Table 2. Effect of maturation on fruit weight, fruit size and β-carotene, lycopene and chlorophyll contents of Momotaro tomatoes (mg per 100 g fresh pericarp weight)[a]

Days after flowering	Fruit weight (g)	Fruit size (mm)	β-Carotene	Lycopene	Chlorophyll a+b	Chlorophyll a/b
10	1.7±1.6	15.0±5.7	0	0	8.3±1.0	2.32
20	46.4±3.5	45.1±1.6	0	0	6.3±1.1	2.63
30	77.1±21.6	58.9±5.9	0	0	2.1±0.9	2.50
40	96.9±26.5	59.4±4.8	0	0	2.3±1.0	1.88
50	136.1±24.5	66.3±5.5	1.2±0.1	5.8±0.3	0.1±0.0	0.66

[a] Values are average±SE for tomatoes from 18 plants for each ripening stage for triplicate determinations.

Isolating each HPLC peak and comparing absorbance maxima in the visible spectrum in three different solvents (chloroform, dichloromethane and *n*-hexane) provided further confirmation of identity.

RESULTS AND DISCUSSION

Fig 1 shows the structures of the tomato constituents evaluated in this study. Figs 2(A) and 2(B) show the HPLC chromatograms of the carotenes and α-tomatine respectively. Table 1 summarises the HPLC retention times and the λ_{max} values in three solvents of the lycopene and β-carotene standards, as well as the corresponding values for the tomato extracts. The data provide a basis for identifying the peaks of tomato extracts in Fig 2(A) associated with lycopene and β-carotene. The ratio of pericarp to whole tomato weights was 91% for tomatoes harvested 10 days after anthesis and 99% for all others.

Biosynthesis and degradation of tomato compounds during ripening

Table 2 shows that during the five maturity stages, artificially defined as starting 10 days after flowering (stage 1) and followed by four more stages each 10 days apart, the average weight of the tomatoes increased 80-fold, from 1.7 to 136.1 g. The corresponding increase in the size of the tomatoes was from 15.0 to 66.3 mm of the major axis. Fig 3 shows the relationship between fruit weight and length for tomatoes harvested from the first and second trusses.

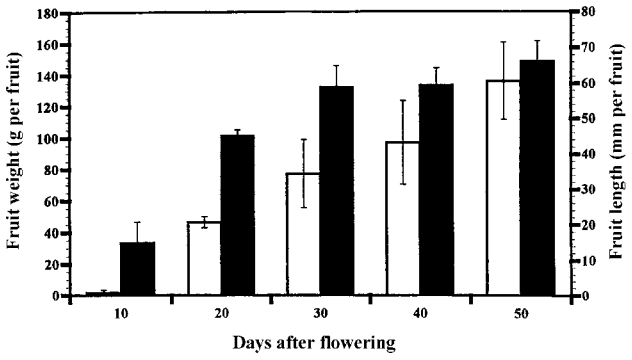

Figure 3. Weights (white) and lengths (black) of Momotaro tomato fruits harvested at different stages of maturity after flowering of tomato plants.

Chlorophyll *a* and *b*

Table 2 shows that the tomatoes harvested during the first stage contained 8.3 mg chlorophyll per 100 g fresh pericarp weight. The chlorophyll content then decreased by about 25% during stage 2 and by about 75% during stages 3 and 4. It then dropped precipitously to a value near zero during the final stage (50 days after flowering).

The last column in Table 2 also shows that the ratio of chlorophyll *a* to *b* remains at about 2.5 during the first 30 days after harvest, then decreases to 1.88 on day 40 and to 0.66 on day 50.

Chlorophyll is evidently degraded enzymatically during tomato ripening. The loss of chlorophyll is accompanied by a corresponding loss of the green colour.

α-Tomatine

Table 3 shows an approximate inverse relationship between fruit weight and tomatine content of Momotaro green tomatoes. This decrease in tomatine does not appear to be greatly influenced by tomato variety or location of the tomato clusters (trusses) on the vine, as revealed by an examination of Fig 4, which shows results for the three varieties and six trusses evaluated in this study. However, it is possible to conclude from this figure that trusses 3 and 4 of cultivars Momatoro and First Memory contain more tomatine than trusses 1, 2, 5 and 6. It is relevant to note that tomatine

Table 3. Relationship between Momotaro tomato fruit weight and α-tomatine content[a]

Fruit weight (g)	α-Tomatine	
	mg per 100 g fresh pericarp weight	mg per fruit
1.5–1.9	89.9±3.6	1.6
3.3–3.8	71.9±1.0	2.5
5.5–6.4	61.6±2.8	3.7
9.6–9.9	44.0±1.5	4.3
16.2–16.4	25.2±2.5	4.1
33.9–43.9	12.3±0.4	4.7
59.4–66.0	9.8±0.1	6.1
100.3–109.0	12.1±0.4	12.6
160.8–162.1	11.5±0.1	18.5
218.0–220.5	14.9±0.3	32.6

[a] Values are average±SE for tomatoes from 18 plants (n=3).

degradation in tomato fruits is catalysed by both plant and fungal enzymes[4,15,25] and that the tomatine contents of calyxes, flowers, leaves, roots and stems of the tomato plant are very high.[2,14] These considerations imply that chlorophyll- and tomatine-containing green tomatoes and possibly also chlorophyll- and tomatine-containing potatoes[26] may impart a dual benefit to the consumer, by providing protection against both cancer and arteriosclerosis.

The data in Table 3 will facilitate the selection of tomatoes at different stages of maturity for their tomatine content. Obviously, this work points to the need for additional studies to better define the possible beneficial roles of these tomato constituents, especially tomatine, in animals and humans.

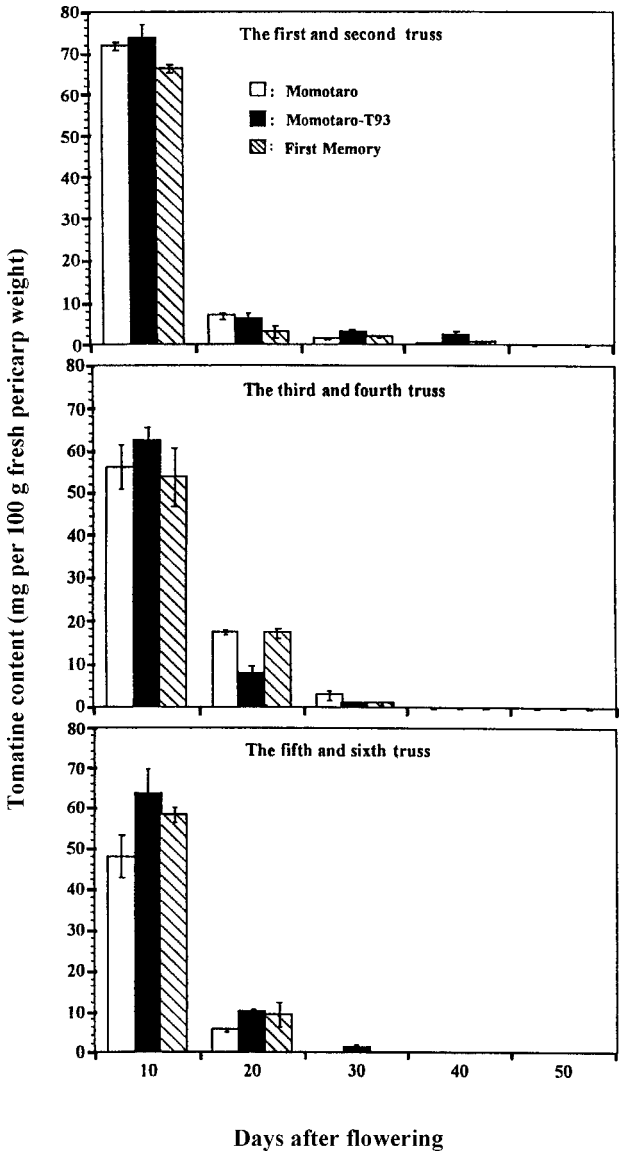

Figure 4. Degradation rates of tomatine harvested from six trusses of three tomato varieties during a 50 day period after flowering of tomato plants.

β-Carotene and lycopene

Tomatoes harvested during the first four stages of ripening did not contain detectable quantities of β-carotene or lycopene. However, those harvested 50 days after flowering contained 1.2 mg β-carotene and 5.8 mg lycopene per 100 g fresh pericarp weight (Table 2). These findings confirm the well-known fact that ripe red tomatoes contain high levels of lycopene.[27] However, these amounts for Momotaro tomatoes appear to be three to four times higher than for tomatoes commonly consumed in the USA.[7,16,28,29]

CONCLUSIONS

With respect to health benefits of tomatoes at different stages of maturity, the consumer is caught in a dilemma, since unripe tomatoes contain chlorophyll and α-tomatine but no lycopene, whereas ripe tomatoes contain no chlorophyll, low levels of tomatine and high levels of lycopene. This and related studies therefore imply that it would benefit human health to (a) consume both green and red tomatoes and (b) create through plant breeding and/or plant molecular biology techniques[30–32] improved tomatoes which contain the health-promoting ingredients β-carotene, chlorophyll, lycopene and tomatine.

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