Plant & Food Research
Rangahau Ahumāra Kai

PFR SPTS No. 21469

Carotenoid and polyphenol content of heritage tomatoes: 2021

McGhie TK, Cordiner SB

September 2021

Confidential report for:

Mark Christensen, Heritage Food Crops Research Trust, Whanganui

PUBLICATION DATA

McGhie TK, Cordiner SB. September 2021. Carotenoid and polyphenol content of heritage tomatoes: 2021. A Plant & Food Research report prepared for: Mark Christensen, Heritage Food Crops Research Trust, Whanganui. Milestone No. 92023. Contract No. 39655. Job code: P/243001/01. PFR SPTS No. 21469.

Report prepared by:

Tony McGhie
Scientist, Metabolite Chemistry
September 2021

Report approved by:

Daryl Rowan
Science Group Leader, Biological Chemistry & Bioactives
September 2021

Contents

Executive summary

Carotenoid and polyphenol content of heritage tomatoes: 2021

McGhie TK, Cordiner SB
Plant & Food Research Palmerston North

September 2021

Carotenoids and metabolites were measured in 29 samples of tomato (*Solanum lycopersicum*) provided by Mark Christensen, Heritage Food Crops Research Trust. The highest concentration of tetra-*cis*-lycopene was 4.2 mg/100 g FW in the golden cultivar 'Moonbeam'.

The carotenoids phytoene, phytofluene and zeta-carotene, which are biosynthetic precursors to tetra-*cis*-lycopene, were measured for the first time in this study. The concentration of these carotenoids tended to be higher when tetra-*cis*-lycopene was present and lower for tomatoes that contained beta-carotene and all-trans-lycopene.

Several additional polyphenol metabolites were chemically identified and measured in these tomato samples. Chlorogenic acids (both mono-caffeoyl and di-caffeoyl) are the major polyphenols in these tomatoes. Relatively low concentrations of flavonoids were detected and the concentrations of these compounds were variable between cultivars.

For further information please contact:

Tony McGhie
Plant & Food Research Palmerston North
Private Bag 11600
Palmerston North 4442
NEW ZEALAND

Tel: +64 6 953 7700
DDI: +64 6 953 7684
Email: Tony.Mcghie@plantandfood.co.nz

1 Introduction

The Heritage Food Crops Research Trust are wanting to understand the health benefits of tomato (*Solanum lycopersicum*) for consumers and are interested in identifying tomato cultivars that have greater concentrations of carotenoids and polyphenols compared with conventionally grown commercial tomatoes. The New Zealand Institute for Plant and Food Research Limited (PFR) has measured carotenoids and polyphenols for several years by means of a series of projects (Cordiner 2020). Here we report the results for tomato samples collected during the 2021 summer. Additionally, the results from previous analyses indicated the presence of several unidentified metabolites. This report also includes the results of investigations to chemically identify these previously unidentified metabolites and increase the confidence of the identification for those metabolites that were previously tentatively identified.

2 Materials and methods

Twenty-nine samples of tomato were provided by Mark Christensen, Heritage Food Crops; details are provided in Table 1. All samples were stored at -20°C from when they were received at PFR, Palmerston North until analysis.

Lab reference: TK399

Table 1. Tomato sample supplied for analysis.

Cultivar name	Description
‘African Oracle’	HFCRT selection from Oracle showing more orange fruit. Location S
‘Amish Yellowish Orange Oxheart’	Amish heirloom variety. Early producing, oxheart-shaped golden tomato
‘Blazing Beauty’	Determinate variety
‘B'mato’	Grown in NZ from hybrid seed saved
‘Bob Logan’	Medium-sized nice golden tomato
‘Eye Drop’	HFCRT selection from Golden Eye Improved. Pink tinge on the shoulders
‘Golden Bell’	NZ natural cross from Oxheart and small orange tomato. Bright orange bell shaped tomato
‘Golden Ellipse’	HFCRT selection from Golden Eye Improved, elongated shape
‘Golden Eye (original)’	HFCRT selection. Small orange cherry tomato very high in beta-carotene
‘Golden Eye 3’	HFCRT selection from Golden Eye. Salty taste. Location S
‘Golden Eye Red’	HFCRT selection from Golden Eye, with red colour. Location S
‘Golden Grape’	Grape-sized bright orange fleshy tomato. Prolific and holds well on vine
‘Golden Light’	HFCRT selection from Orange Teardrop. Very similar to Orange Roma
‘Golden Sheen’	HFCRT selection from Small Sweet Orange. Version 5
‘Loxton Lass’	Determinate variety
‘Mini Olga’	HFCRT selection from Olga's Round Golden Chicken Egg tomato
‘Moonbeam’	HFCRT selection from Moonglow
‘Moonglow’	Smooth bright orange medium-sized fruits. Great flavour
‘New Oracle’	HFCRT selection from Oracle. Strong plant. Location M, big tunnel
‘Olga's Round Golden Chicken Egg’	Siberian heirloom. Heavy producer of round to oval golden tomatoes
‘Optical’	HFCRT selection from Golden Eye. Massive fruit in comparison. Location small tunnel
‘Oracle 1 SBO’	HFCRT selection from Oracle. Small bright orange fruit. Location M small tunnel
‘Oracle SPS’	HFCRT selection from Oracle. Strong plant. Location S
‘Orange Fleshed Purple Smudge’	Orange fruit with purple blotches on the shoulders
‘Orange Roma’	Italian Roma style tomato
‘Red Oracle’	HFCRT selection from Oracle showing red fruit
‘Small Sweet Orange V3’	HFCRT selection from Small Sweet Orange
‘Tangella’	Disease-resistant highly productive, cluster of small round orange fruit
‘Wally's Spanish’	Meaty bright orange beefsteak variety, reputed to be of Spanish origin

2.1 Chemical identification

2.1.1 Carotenoids

In previous studies of carotenoids in tomato (Cordiner 2020) quantitative data have been provided for teta-*cis*-lycopene, all-*trans*-lycopene, beta-carotene and lutein. The presence of additional carotenoids was noted but these metabolites were not quantified as they were not chemically identified. Data for these carotenoids are required and therefore experiments were conducted to chemically identify the additional carotenoids and develop a means for calculating their concentrations.

A sample from a previous study ('Amish Yellowish Orange Oxheart') was used as a basis for carotenoid identification. This sample was extracted with methanol:tetrahydrofuran (THF) (1:2 v/v) and analysed by Ultra High Performance Liquid Chromatography (UHPLC) with Photo Diode Array (PDA) detection. The spectral properties of the components that were detected were examined and compared with reference data for carotenoids to establish the chemical identification.

2.1.2 Polyphenols

Similarly to the carotenoids, in previous studies the presence of several unidentified polyphenols were noted but the chemical identity was not established conclusively. Three compounds in particular were noted and were tentatively identified as (*E*)-caffeoyl 3-glucoside, narigenin chalcone and quercetin 3-xylosylrutinoside.

The chemical identity of (E)-caffeoyl 3-glucoside was determined by isolating this metabolite using preparative HPLC from a kiwifruit (*Actinidia chinensis*) extract. The peak containing the putative (E)-caffeoyl 3-glucoside was collected from multiple cycles of the preparative HPLC, concentrated to dryness and then analysed by Nuclear Magnetic Resonance (NMR) spectroscopy and Liquid Chromatography – High Resolution Mass Spectrometry (LC-HRMS). The identity of quercetin 3-xylosylrutinoside and narigenin chalcone was established by analysis using LC-HRMS and inducing fragmentation of the mass spectral ions to investigate the molecular structure of the metabolites.

2.2 Metabolite analysis

The analytical methods used in this study were similar to those used in the previous study (Cordiner 2020). Details of the carotenoids and polyphenols measured are shown in Table 2 and Table 3 respectively.

Table 2. Carotenoids measured in tomatoes.

Compound	CAS	Formula	Exact mass	Detection wavelength (nm)
lutein	127-40-2	C40H56O2	568.4280	445
all-trans-lycopene	502-65-8	C40H56	536.4382	505
tetra-cis-lycopene	2361-24-2	C40H56	536.4382	436
phytoene	13920-14-4	C40H64	544.5008	286
phytofluene	540-15-6	C40H62	542.4852	348
zeta-carotene	13587-06-9	C40H60	540.4695	400
beta-carotene	7235-40-7	C40H56	536.4382	450

Table 3. Polyphenols measured in tomatoes.

Compound	Abbreviation	CAS	Formula	Exact mass	Equivalence
(E)-caffeoyl 3-glucoside	EC3-glu	143729-78-6	C15H18O9	342.0951	chlorogenic acid
(E)-caffeoyl 4-glucoside	EC4glu	147511-61-3	C15H18O9	342.0951	chlorogenic acid
3,4-dicaffeoylquinic acid	3,4-dCGA	14534-61-3	C25H24O12	516.1268	chlorogenic acid
3,5-dicaffeoylquinic acid	3,5-dCGA	2450-53-5	C25H24O12	516.1268	chlorogenic acid
4,5-dicaffeoylquinic acid	4,5-dCGA	57378-72-0	C25H24O12	516.1268	chlorogenic acid
catechin	Cat	154-24-4	C15H14O6	290.0790	epicatechin
chlorogenic acid	CGA	327-97-9	C16H18O9	354.0951	chlorogenic acid
cryptochlorogenic acid	cCGA	905-99-7	C16H18O9	354.0951	chlorogenic acid
epicatechin	epiCat	490-49-0	C15H14O6	290.0790	epicatechin
kaempferol 3-rutinoside	K-rut	17650-84-9	C27H30O15	594.1585	quercetin 3-rutinoside
naringenin	Nar	480-41-1	C15H12O5	272.0685	quercetin
neochlorogenic acid	nCGA	202650-88-2	C16H18O9	354.0951	chlorogenic acid
procyanidin B1	ProCy B1	20315-25-7	C30H26O12	578.1424	procyanidin B2
procyanidin B2	ProCy B2	29106-49-8	C30H26O12	578.1424	procyanidin B2
procyanidin B5	ProCy B5	12798-57-1	C30H26O12	578.1425	procyanidin B2
procyanidin B7	ProCy B7	12798-59-3	C30H26O12	578.1425	procyanidin B2
procyanidin C1	ProCy C1	37064-30-5	C45H38O18	866.2058	procyanidin B2
quercetin	Q	117-39-5	C15H10O7	302.0427	quercetin
quercetin 3-arabinopyranoside	Q-arapy	22255-13-6	C20H18O11	434.0849	quercetin 3-rutinoside
quercetin 3-galactoside	Q-gal	482-36-0	C21H20O12	464.0955	quercetin 3-rutinoside
quercetin 3-glucoside	Q-glu	482-35-9	C21H20O12	464.0955	quercetin 3-rutinoside
quercetin 3-rhamnoside	Q-rha	522-12-3	C21H20O11	448.1006	quercetin 3-rutinoside
quercetin 3-rutinoside	Q-rut	153-18-4	C27H30O16	610.1534	quercetin 3-rutinoside
quercetin 3-xyloside	Q-xyl	549-32-6	C20H18O11	434.0849	quercetin 3-rutinoside
quercetin 3-xylosylrutinoside	Q-xylrut	129235-39-8	C32H38O20	742.1956	quercetin 3-rutinoside
trans-4-p-coumaroyl quinic acid	t4CQA	1108200-72-1	C16H18O8	338.1002	trans-4-p-coumaroyl quinic acid
trans-5-p-coumaroyl quinic acid	t5CQA	5746-55-4	C16H18O8	338.1002	trans-4-p-coumaroyl quinic acid

3 Results

3.1 Metabolite identification

3.1.1 Carotenoids

The contour plot of the carotenoids present in 'Amish Yellowish Orange Oxheart' is shown in Figure 1. Tetra-*cis*-lycopene (TCLyc), the main target carotenoid, is readily apparent together with four additional carotenoids (U1 – U4). These five compounds can be clearly discerned in the 3-D display, however carotenoids U1, U2, and U3, show little chromatographic separation by retention time (x-axis). Attempts were made to improve this separation by adjusting the UHPLC solvent gradient conditions, but even though slightly better separation between U1 and U2 and U3 could be achieved, improved separation between U2 and U3 was not achieved. Fortunately each carotenoid has a distinctive UV/vis absorption profile and extraction of chromatograms at wavelengths specific for each carotenoid allows for quantation of the three carotenoids even when there is no chromatographic separation.

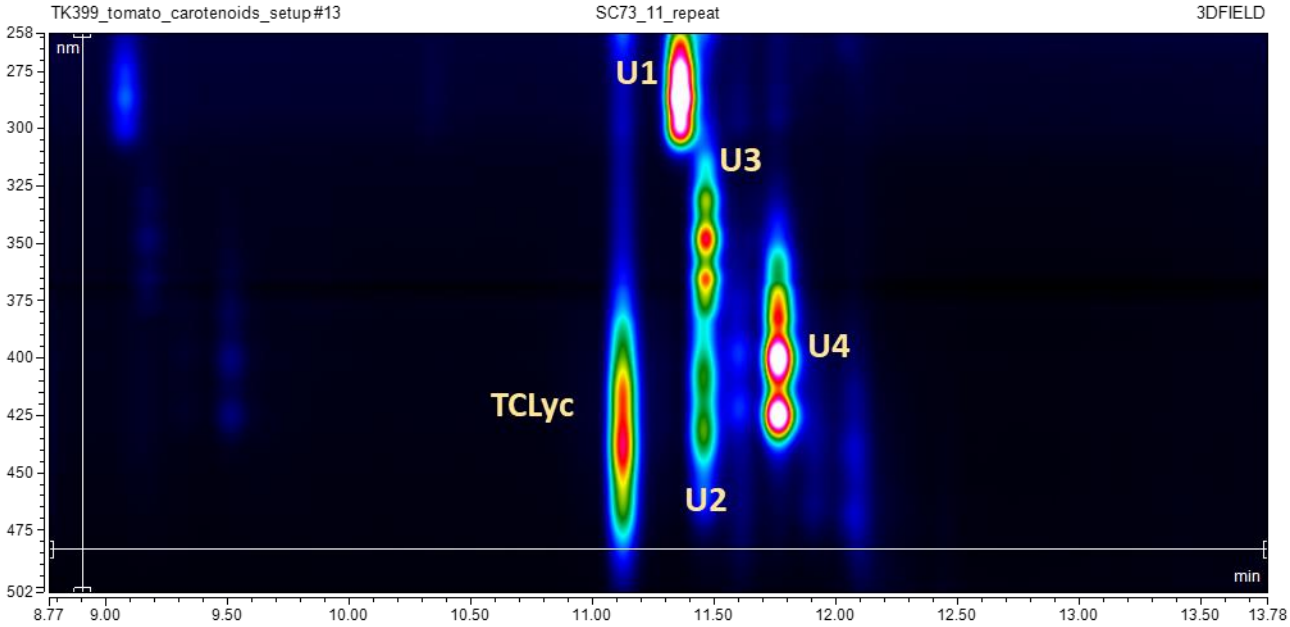

Figure 1. UHPLC chromatogram contour plot of the carotenoids in 'Amish Yellowish Orange Oxheart' tomato. The contour-plot show chromatographic separation as retention time on the x-axis, wavelength of absorption is on the y-axis, and the intensity of absorption is on the vertical z-axis; black is low absorption; white represents high absorption and various colours represent intermediate absorption. TCLyc = tetra-*cis*-lycpene; U1 = unknown#1; U2 = unknown#2; U3 = unknown#3; and U4 = unknown#4

During UHPLC analysis, spectral data are acquired as a function of time during the chromatographic analysis and the UV/vis spectrum at specific times can be used to obtain a spectral information characteristic for each of the carotenoids. Figure 2 shows wavelength-specific chromatograms and carotenoid-specific UV/vis spectra for each of the four unknown carotenoids. As U2 and U3 cannot be separated by UHPLC, the spectra at the retention time for U2/U3 contained elements of both carotenoids. These data were then compared with reference data (Rodrigues-Amaya 1999) to assign chemical identity (Table 4). In this way U1, U3, and U4 were identified, however no reference data matched U2 and this carotenoid remains unidentified.

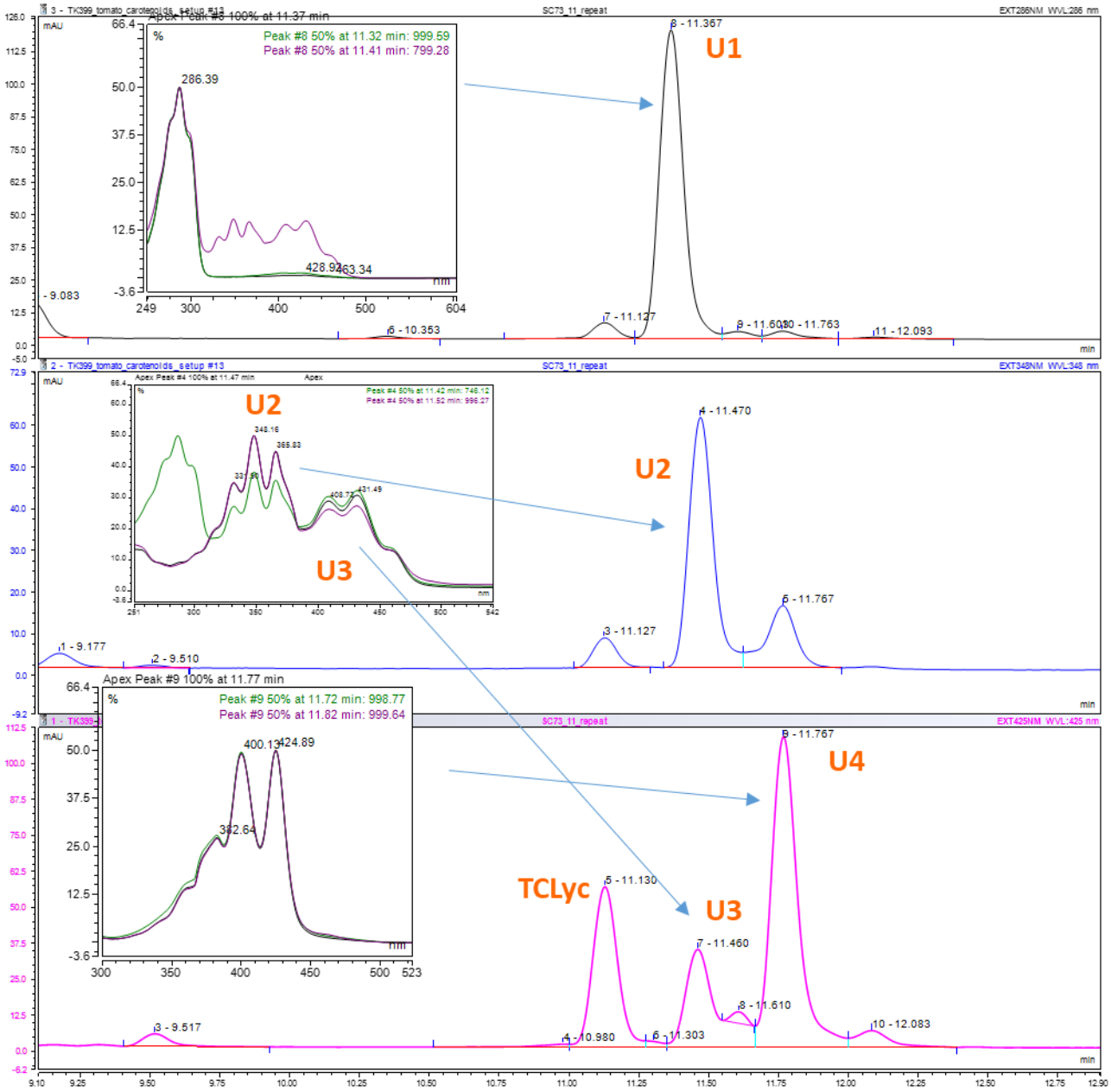

Figure 2. UHPLC chromatograms at wavelengths specific to the individual metabolites. Insert boxes show the observed spectra at retention times for each of the metabolites. Details of the wavelengths and the retention times that correspond to each of the metabolites are in Table 5.

Table 4. Spectral data for the carotenoids detected in tomato samples and the assigned chemical identity.

Carotenoid	Retention time (min)	Lambda max (nm)	Carotenoid Identification	Reference Lambda-max (nm)	Detection Wavelength (nm)
tetra-*cis*lycopene	11.13	421(s), 428			436
U1	11.37	286	phytoene	(276), 286	286
U2	11.46	408, 432, 462(sh)	unknown		
U3	11.47	332, 348, 364	phytofluene	331, 348, 367	348
U4	11.77	382, 400, 424	zeta-carotene	377, 399, 425	400
beta-carotene	11.84	450, 475		450, 478	450
all-*trans*-lycopene	12.12	446, 470, 501		446, 472, 503	505
lutein	3.11	444, 473		445, 474	445

Three types of carotenoid profiles were identified in the samples. They are: 1) all-trans-lycopene containing, 2) tetra-cis-lycopene; phytoene; phytofluene; zeta-carotene containing, and 3) beta-carotene containing. Figure 3 contains contour-plot chromatograms for the main carotenoid profiles detected in the tomatoes analysed in this study. However, some samples appear to have elements of more than one profile. For example the contour plot chromatogram for 'Moonbeam' (Figure 4) shows that this sample appears to contain both tetra-*cis*-lycopene and beta-carotene. This is unexpected as accumulation of tetra-cis-lycopene is expected to prevent the branch of the carotenoid biosynthetic pathway leading to beta-carotene synthesis from operating. Consequently the spectral data for the carotenoid corresponding to beta-carotene in the samples were carefully examined.

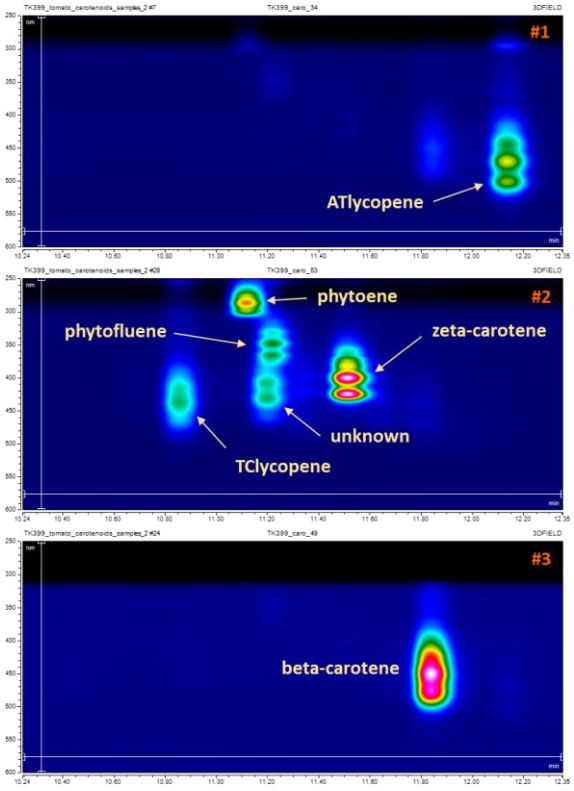

Figure 3. UHPLC contour chromatograms showing chemical identification of carotenoids in the three main types of profiles detected in the 29 tomatoes samples analysed in this study.

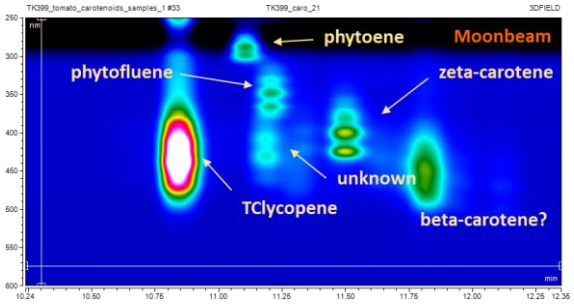

Figure 4. UHPLC contour chromatograms of 'Moonbeam'. The carotenoid with retention time of 11.84 min was initially quantified as beta-carotene, however, closer inspection suggests that this carotenoid is not beta-carotene.

A comparison of the UV/vis spectra for an authentic beta-carotene standard, an orange tomato sample and a tomato sample containing tetra-*cis*-lycopene (Figure 5) clearly shows that the putative beta-carotene in the tetra-*cis*-lycopene-containing tomatoes (Figure 5c) is not beta-carotene and has spectral maxima at 440 and 476 nm. The identity of this compound is uncertain as the measured spectra are similar to reference data for both neurosporene and several carotene epoxides. Neurosporene is located in the carotenoid biosynthetic pathway prior to tetra-*cis*-lycopene and since the other tetra-*cis*-lycopene precursors (phytoene, phytofluene and zeta-carotene) are also present in these samples, neurosporene is also likely to be present. Therefore this carotenoid is likely to be neurosporene, but the identification has limited confidence.

These data indicate that, in any sample, the identity of the carotenoid with a retention time of beta-carotene (11.84 min) can be either beta-carotene or an unknown carotenoid, possibly neurosporene. Therefore each result for beta-carotene was individually validated by careful review of the spectra and the carotenoid data present in Table 6 labelled accordingly.

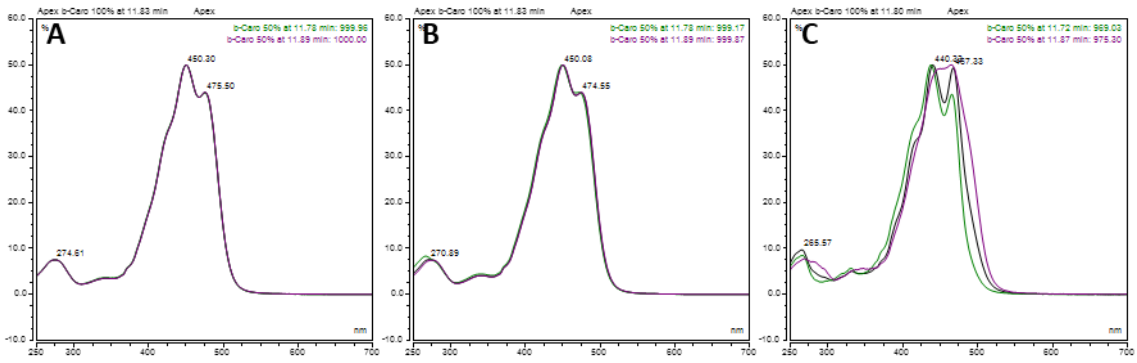

Figure 5. UV/vis spectra of carotenoids with a retention time of approximately 11.82 minutes. A) beta-carotene standard; B) orange tomato sample containing beta-carotene; C) tomato samples containing tetra-cis-lycopene. The carotenoid detected in C) has aretention time consistent with for beta-carotene, but the spectral properties indicate that this carotenoid is not beta-carotene.

3.1.2 Polyphenols

In a previous report (Cordiner 2020) data for three polyphenolic compounds were reported but the chemical identification was not certain due to the lack of authentic standards.

(E)-caffeoyl 3-glucoside

Previous analysis of tomato indicated that (*E*)-caffeoyl-3-glucoside was a major polyphenolic metabolite of tomato. Previous identification of the compound was based on accurate mass and isotope ratio measurements that were consistent with a molecular formula of $C_{15}H_{18}O_9$.

(E)-Caffeoyl-3-glucoside is known to be a major metabolite of kiwifruit. To increase the confidence of the identification of the tomato metabolite, an authentic sample of this metabolite was isolated and purified from kiwifruit and directly compared with the metabolite present in tomatoes.

A kiwifruit sample with high concentrations of the putative (E)-caffeoyl-3-glucoside was prepared and analysed by UHPLC with UV/vis detection. The target peak was identified and then the UHPLC was set up in preparative mode and multiple cycles of analysis, and target peak collection were carried out. All target peak collections were combined and concentrated to dryness. Between 100 and 300 µg of pure compound were prepared using this process and the isolated compound was characterised by NMR and MS spectroscopy.

NMR spectroscopy confirmed that the compound is a caffeoyl glucoside with the double bond in the caffeic acid moiety in the E (or trans) configuration and that the glucose moiety is attached at the 3 position of the caffeic acid. MS spectroscopy confirmed that the molecular formula is $C_{15}H_{18}O_9$, and the molecular formulae of fragments generated by ms/ms are consistent with caffeic and a hexose. Thus the metabolite isolated from kiwifruit is confirmed as being (E)-caffeoyl-3-glucoside (Figure 6).

When the (E)-caffeoyl-3-glucoside isolated from kiwifruit was compared with the corresponding metabolite in tomato, it was discovered that there are two caffeoyl glucoside isomers present in tomato. These two isomers were therefore identified as (E)-caffeoyl-3-glucoside and (E)-caffeoyl-4-glucoside. The metabolite concentrations previously reported correspond to (E)-caffeoyl-4-glucoside and not (E)-caffeoyl-3-glucoside as reported.

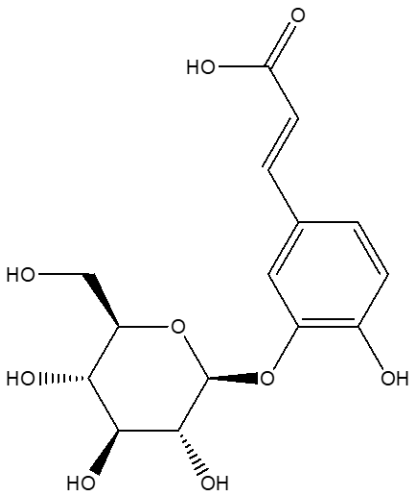

Figure 6. (E)-caffeoyl-3-glucoside; C15H18O9; CAS 143729-78-6.

Naringenin chalcone or naringenin

The flavonoids naringenin and naringenin chalcone are structural isomers (Figure 7) and have both been reported to be present in tomato. Since they have the same molecular formula, the compounds have identical exact masses. Furthermore the retention times on Reversed Phase – Liquid Chromatography (RP-LC) systems are very similar (Gómez-Romero et al. 2010). Consequently these two compounds can be easily confused with each other when authentic standards are not available.

Naringenin/naringenin chalcone was detected in these tomato samples by LC-MS using Exact Ion Chromatograms (EICs) at *m/z* 271.0612 at high mass resolution. A single peak was detected and the retention time was very close, but not an exact match, to the retention time of an authentic naringenin standard. Previous studies have reported that tomato contains both naringenin and naringenin chalcone, suggesting that two peaks should be observered. These results, and the previous reports that the retention times of naringenin and naringenin chalcone are very similar, suggest that these two compounds are not separated by our LC system and are therefore measured together. Since we have an authentic standard of naringenin available, this chromatographic peak that may contain both naringenin and naringenin chalcone and has been quantified as naringenin equivalents.

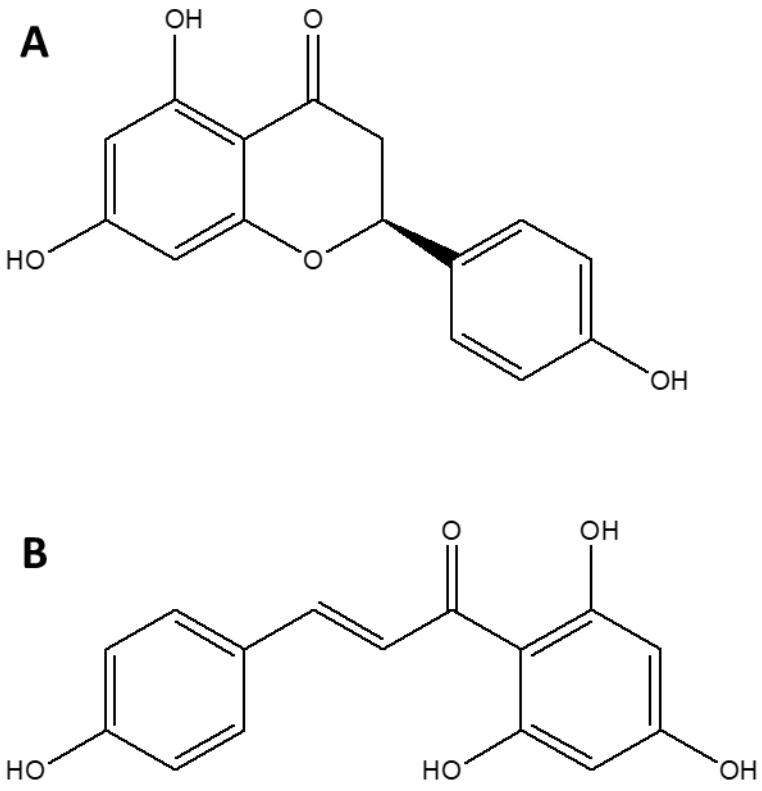

Figure 7. A) naringenin, CAS 480-41-1; B) naringenin chalcone, CAS 73692-50-9.

Quercetin 3-xylosylrutinoside

In previous studies (Cordiner 2020) a prominent metabolite was chemically tentatively identified as quercetin 3-xylosylrutinoside. This metabolite was investigated in more detail to increase the confidence in this tentative identification.

The LCMS data from the polyphenol analyses processed using metabolomics software (MetaboScape, Bruker Daltonics) to characterise the tentative quercetin 3-xylosylrutinoside metabolite and the results are shown in Table 5. Details of the proposed structure and the possible fragmentation are shown in Figure 8.

Table 5. LCMS data for the metabolite tentatively identified as quercetin 3-xylosylrutinoside.

Parameter	Data
Retention time	3.53 minutes
Exact mass (neutral monoisotopic)	742.19569
Calculated molecular formula	$C_{32}H_{38}O_{20}$
Mass difference (measured versus calculated)	0.05 mDa
Isotope ratio (mSigma)	7.80
Fragment ions (one major fragment only)	300.02745
Fragment ion formula	$C_{15}H_8O_7$
Fragment ion mass difference (measured versus calculated)	-0.50 mDa

These data confirm that the metabolite is a quercetin glycoside and that the glycoside moiety is attached at a single location on the quercetin as the only major fragment is the quercetin aglycone. Although there are other possible combinations for the glycoside portion, it is probable that rutinoside is a component which means the other component is a pentose sugar, such as xylose. Thus this metabolite is identified as quercetin 3-xylosylrutinoside (CAS: 129235-39-8).

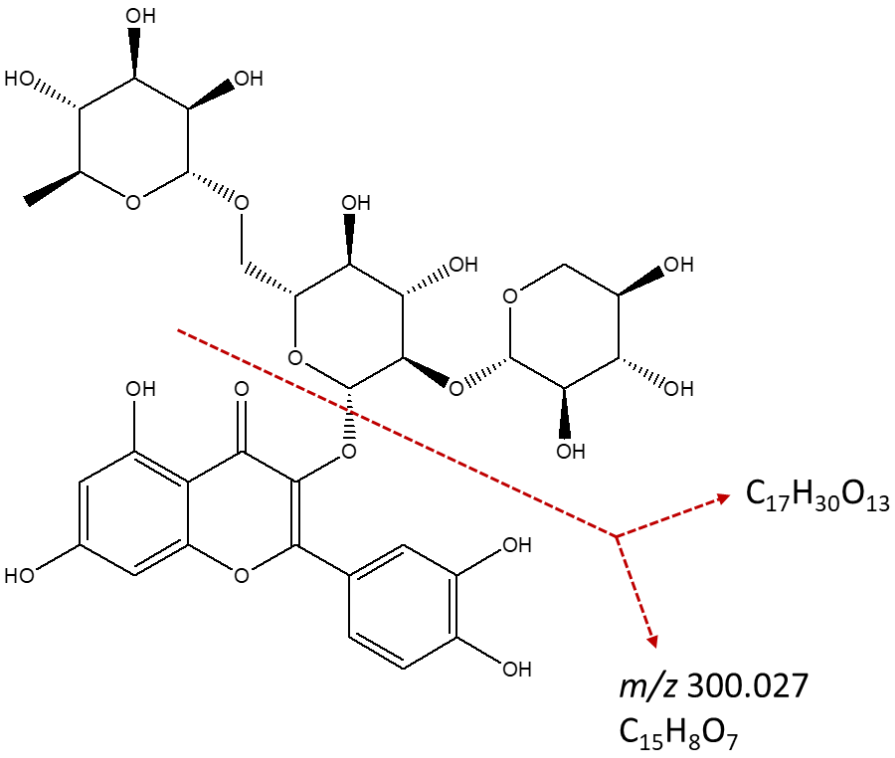

Figure 8. Chemical structure of quercetin 3-xylosylrutinoside CAS 129235-39-8, and probable molecular fragmentation.

Chlorogenic acids

Analysis of the polyphenolic data using the MetaboScape software indicated that a further three peaks in the LCMS chromatograms were related to chlorogenic acid. Examination of these three peaks revealed that they are dicaffeoylquinic acid with a chemical structure shown in Figure 9. These metabolites are chlorogenic acid with an additional caffeic acid attached to the quinic acid core. Three isomers were detected with the caffeoyl moieties attached to different positions on the quinic acid. The individual isomers were identified by considering the ms/ms fragments and the published identification scheme for chlorogenic acid (Clifford et al. 2003)

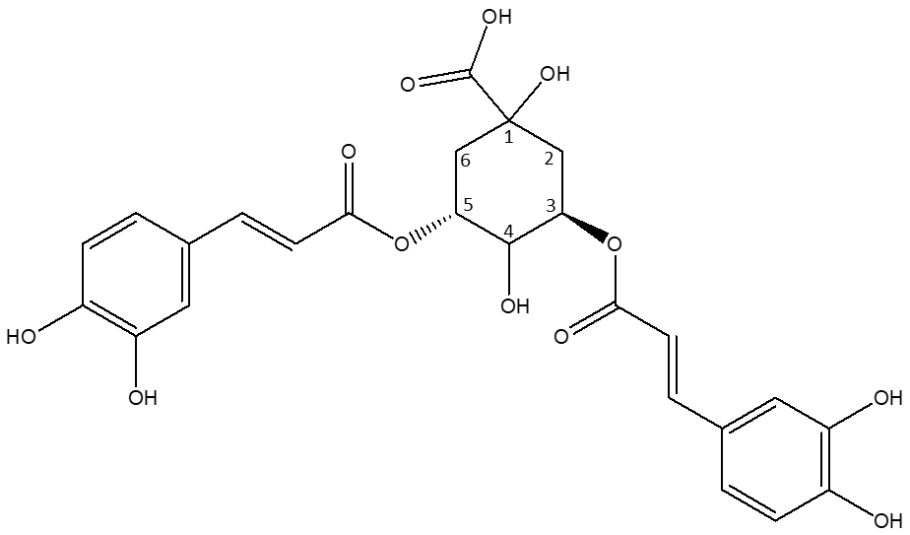

Figure 8. Chemical structure of 3,5 dicaffeoylquinic acid (3,5-dCGA) CAS 2450-53-8-5. The other two isomers have the caffeic acid moieties attached at the 3,4 and 4,5 positions of the quinic acid core.

3.2 Metabolite concentrations

Carotenoid concentrations for the 29 tomato cultivars are shown in Table 6.

Authentic standards for tetra-*cis*-lycopene, phytoene, phytofluene and zeta-carotene were not available and the concentrations for these carotenoids were calculated using the all-*trans*-lycopene standard with corrections based on relative extinction coefficients at the wavelengths used to detect each carotenoid (Table 1). The accuracy of the absolute concentration values should be treated with caution and comparison with quantitative data from other studies should only be done with care. Fortunately, the relative concentrations for these carotenoids reported in a US study using 'Tangerine' tomatoes (Cooperstone et al. 2016) are similar to the results reported here.

The concentrations of tetra-*cis*-lycopene were generally similar to previous years, however the relative concentration between cultivars seems to vary year-to-year. The differences between tetra-*cis*-lycopene, all-*trans*-lycopene and beta carotene tended to align with the colour of the tomato. For example, red tomatoes contain all-*trans*-lycopene, orange tomatoes often contain beta-carotene, and golden tomatoes contained tetra-*cis*-lycopene.

The addition of data for phytoene, phytofluene and zeta-carotene provides several interesting perspectives on tomato carotenoid biosynthesis. The concentrations of phytoene, phytofluene and zeta-carotene are somewhat correlated with each other, and tend to be elevated when tetra-*cis*-lycopene is present and lower for tomatoes that contain beta-carotene and all-trans-lycopene. However, these three metabolites (phytoene, phytofluene and zeta-carotene), which are intermediates in the carotenoid pathway, were detected in all samples, regardless of the carotenoid that accumulates as a result of the carotenoid biosynthetic pathway and generates the visual colour of the particular tomato.

Phytoene, phytofluene and zeta-carotene have a lower intensity of colour than other carotenoids due to a lower number of double bonds in their molecular structure. Therefore, they have a lower impact on the colour of the fruit but appear to be present at higher concentrations. It is not known if these higher concentrations have an impact on other factors such as health. These compounds are generally not available in a pure form and consequently appear to have been little studied with respect to health benefits.

Polyphenol concentrations for the 29 tomato cultivars are shown in Table 7. The phenolic compounds detected generally agree with those reported by Gómez-Romero et al. 2010.

The major polyphenolic metabolites in these tomatoes are caffeic acid conjugates with quinic acid. A number of monocaffeoyl and dicaffeoyl quinic acid conjugates were detected. Only (E)-caffeoyl-3-glucoside was reported previously (Cordiner 2020), whereas both (E)-caffeoyl-3-glucoside and (E)-caffeoyl-4-glucoside are included in this study. The concentrations for these metabolites presented here are lower than those previously reported. A standard is not available for quantitation. For this study (E)-caffeoyl-3-glucoside and (E)-caffeoyl-4-glucoside were quantified as chlorgenic acid equivalents, whereas in the previous study (E)-caffeoyl-3-glucoside was quantified as caffeic acid equivalents. This undoubtedly accounts for the differences in the reported concentrations between the studies.

The concentrations reported for naringenin probably include both naringenin and naringenin chalcone, as described above. Naringenin/naringenin chalcone is a key intermediate in the flavonoid biosynthetic pathway and interestingly the concentrations found vary substantially; however the concentrations of naringenin/naringenin chalcone do not appear related to the concentrations quercetin 3-rutinoside, the main flavonoid in these tomatoes.

Table 6. Carotenoid concentrations (mg/100 g fresh weight) for the 29 cultivars analysed in this study. n.d. = not detected.

Cultivar	tetra-cis-lycopene	phytoene	phytofluene	beta-carotene*	all-trans-lycopene	lutein	zeta-carotene
'African Oracle'	2.6	5.2	5.3	2.0*	0.0	0.1	7.0
'Amish Yellowish Orange Oxheart'	2.2	14.9	15.1	1.6	0.2	0.1	15.7
'Blazing Beauty'	2.7	12.5	11.9	0.3	0.0	0.0	11.9
'B'mato'	3.0	11.5	12.6	0.7	0.1	0.1	12.7
'Bob Logan'	3.0	11.0	10.7	0.4	0.0	n.d.	10.6
'Eye Drop'	0.0	0.8	0.4	4.8*	0.2	0.1	0.1
'Golden Bell'	3.6	13.6	14.7	0.8	0.1	0.1	14.3
'Golden Ellipse'	1.3	7.1	7.4	0.9	0.0	0.1	5.9
'Golden Eye 3'	1.3	7.7	7.7	1.6	0.1	0.1	3.5
'Golden Eye Red'	0.0	1.6	1.6	1.0*	3.3	0.2	0.2
'Golden Eye'	0.2	1.9	1.6	4.3*	0.0	0.1	2.5
'Golden Grape'	3.1	6.6	7.9	1.0	0.1	0.1	4.0
'Golden Light'	3.2	10.0	10.0	0.8	0.1	0.0	11.1
'Golden Sheen' (small sweet orange v3)	2.4	7.1	8.0	0.5	0.0	0.1	5.8
'Loxton Lass'	2.7	18.9	17.4	1.0	0.2	0.1	19.0
'Mini Olga'	2.9	4.1	5.0	1.2	0.1	0.2	5.5
'Moonbeam'	4.2	3.3	4.0	1.0	0.1	0.1	2.9
'Moonglow'	3.1	10.1	11.3	0.5	0.1	0.0	9.7
'New Oracle'	0.0	1.0	0.8	5.8*	0.2	0.1	0.1
'Olga's Round Golden Chicken Egg'	2.6	8.2	9.9	0.8	0.1	0.1	10.0
'Optical'	0.0	1.2	0.8	5.2*	0.2	0.1	0.1
'Oracle 1 SBO'	0.0	6.8	5.9	6.2*	0.1	0.1	1.4
'Oracle SPS'	0.0	0.5	0.3	4.6*	0.1	0.1	0.0
'Orange Fleshed Purple Smudge'	3.4	16.3	15.7	0.8	0.1	0.1	14.9
'Orange Roma'	2.2	6.4	5.9	0.8	0.1	0.1	6.0
'Red Oracle'	0.1	3.5	3.7	1.3*	5.8	0.1	1.2
'Small Sweet Orange V3'	2.2	9.2	9.8	0.9	0.1	0.1	8.3
'Tangella'	3.6	5.8	6.6	1.3	0.2	0.1	7.2
'Wally's Spanish'	3.1	19.8	19.8	0.3	0.0	0.0	20.3

* beta-carotene identity confirmed; otherwise identity uncertain, use with caution.

Table 7. Polyphenol concentrations (mg/100 g fresh weight) for the 29 cultivars analysed in this study. n.d. = not detected. Table 2 lists the analytes that were analysed for and a number of these were not detected. These are: catechin, epicatechin, procyanidin B1, procyanidin B2, procyanidin B5, procyanidin B7, procyanidin C1, quercetin 3-arabinopyranoside, quercetin 3-galactoside, quercetin 3-glucoside, quercetin 3-rhamnoside, quercetin xyloside and trans-4-p-coumarylquinic acid.

Cultivar	CGA	nCGA	cCGA	3,4-dCGA	3,5-dCGA	4,5-dCGA	EC3-glu	EC4-glu	t5CQA	K-rut	Nar	Q-rut	Q-xylrut
‘African Oracle’	7.45	0.19	4.00	0.73	1.00	6.15	3.59	4.02	1.57	0.14	0.85	4.66	0.76
‘Amish Yellowish Orange Oxheart’	0.78	0.16	1.58	0.53	0.38	4.18	4.06	6.07	0.02	0.07	0.00	1.32	0.53
‘Blazing Beauty’	1.41	0.06	1.56	0.28	0.20	1.29	2.65	5.94	0.44	0.04	0.00	0.35	0.22
‘B'mato’	4.31	0.10	2.93	0.57	0.57	8.04	3.13	4.36	0.23	0.07	0.79	1.31	0.31
‘Bob Logan’	0.47	0.05	0.96	0.19	0.10	0.42	2.72	2.17	0.03	0.00	0.00	0.19	0.22
‘Eye Drop’	12.18	0.14	1.67	0.15	1.07	1.79	3.85	2.42	5.99	0.05	0.00	1.71	0.79
‘Golden Bell’	0.33	0.08	0.98	0.26	0.10	0.38	2.54	0.68	0.01	0.00	0.14	0.25	0.17
‘Golden Ellipse’	2.24	0.07	1.67	0.39	0.31	1.23	2.25	2.66	0.03	0.04	0.00	1.81	1.12
‘Golden Eye 3’	8.30	0.09	1.13	0.08	0.57	0.76	2.30	2.84	2.39	0.06	0.00	2.25	1.07
‘Golden Eye Red’	4.20	0.15	3.19	0.49	0.52	3.85	3.28	4.98	1.15	0.11	0.00	2.95	1.21
‘Golden Eye’	25.30	0.17	0.00	0.19	2.59	4.64	5.25	7.59	2.68	0.29	0.00	6.54	2.07
‘Golden Grape’	2.52	0.13	3.16	0.93	0.62	5.86	2.73	1.65	1.12	0.14	4.69	3.81	0.43
‘Golden Light’	0.92	0.14	1.71	0.41	0.21	0.94	2.41	1.54	0.08	0.00	0.00	0.28	0.37
‘Golden Sheen’	0.65	0.06	0.85	0.17	0.10	0.83	1.80	2.35	0.01	0.18	0.72	2.65	0.57
‘Loxton Lass’	0.43	0.08	1.24	0.26	0.11	0.87	3.45	5.67	0.04	0.01	0.00	0.25	0.15
‘Mini Olga’	1.89	0.16	3.70	0.92	0.52	3.30	3.84	5.27	0.19	0.05	0.10	2.09	0.57
‘Moonbeam’	1.91	0.16	3.17	0.82	0.44	2.04	3.36	4.60	0.05	0.04	0.08	1.33	0.37
‘Moonglow’	0.61	0.06	1.05	0.23	0.13	0.76	1.77	1.87	0.02	0.02	0.13	0.27	0.14
‘New Oracle’	18.26	0.14	0.00	0.22	2.23	3.80	4.38	5.52	4.24	0.08	0.00	2.74	1.12
‘Olga's Round Golden Chicken Egg’	0.92	0.10	1.57	0.31	0.18	0.72	2.75	2.25	0.07	0.01	0.00	0.43	0.20
‘Optical’	10.55	0.17	2.26	0.22	1.39	2.33	4.21	2.55	2.34	0.14	0.00	7.06	2.14
‘Oracle 1 SBO’	5.24	0.40	6.04	2.03	1.70	10.38	7.47	6.09	0.61	0.40	0.31	10.65	1.63
‘Oracle SPS’	14.00	0.15	0.00	0.14	1.29	2.04	3.81	5.64	3.45	0.14	0.00	5.39	1.37
‘Orange Fleshed Purple Smudge’	0.56	0.07	1.22	0.32	0.15	1.06	2.35	3.04	0.11	0.02	0.00	0.46	0.26
‘Orange Roma’	1.20	0.08	1.85	0.39	0.22	1.03	2.60	1.18	0.03	0.01	0.00	0.35	0.29
‘Red Oracle’	8.25	0.07	1.21	0.12	0.81	1.11	1.81	1.98	1.13	0.06	0.46	2.46	0.58
‘Small Sweet Orange V3’	2.62	0.20	3.17	0.70	0.60	7.22	2.93	2.20	0.35	0.15	1.26	2.48	0.42
‘Tangella’	0.99	0.14	2.62	0.66	0.31	1.82	3.84	5.29	0.00	0.04	0.34	1.58	0.47
‘Wally's Spanish’	0.82	0.09	1.50	0.29	0.14	0.67	3.02	2.11	0.24	0.01	0.00	0.18	0.05

4 References

Clifford MH, Johnston KL, Knight S, Kuhnert. 2003. Hierarchical secheme ofr LC-MSn identification of chlorogenic acids. Journal of Agricultural and Food Chemistry 51: 2900-2911.

Cooperstone JL, Francis DM, Schwartz SJ. 2016. Thermal processing differentially affects lycopene and other carotenoids in *cis*-lycopene containing *tangerine* tomatoes. Food Chemistry 210: 466-472.

Cordiner SB 2020. Carotenoid and polyphenol content of heritage tomatoes. Plant and Food Research Client Report No. 19332. Plant and Food Research Contract No 38061.

Gómez M, Segura-Carretero A, Fernández-Gutiérrez A. 2010. Metabolite profiling and quantification of phenolic compounds in methanol extracts of tomato fruit. Phytochemistry 71: 1848-1864.

Rodrigues-Amaya DB 1999. A guide to carotenoid analysis in foods. Washington, OMNI Research.

A smart green future. Together.