

NMR-based metabolomic study of purple carrot optimal harvest time for a utilization as a source of bioactive compounds.

Compound <sup>a</sup>	Assignment <sup>b</sup>	<sup>1</sup> H δ (ppm)	Multiplicity <sup>c</sup>	<sup>13</sup> C δ (ppm)
<b>Organic acids</b>				
<b>2,3 Dihydroxyvaleric acid (2,3 DHIVA)</b>	$\gamma,\gamma'$ -CH <sub>3</sub>	<b>1.14</b>	<b>s</b>	20.65
	$\beta$ -C	\	\	\
	$\alpha$ -CH	\	\	\
<b>Acetic acid (AA)</b>	<b>CH<sub>3</sub></b>	<b>1.92</b>	<b>s</b>	25.98
<b>Caffeic acid (CafA)</b>	CH-1	7.19	d	117.15
	CH-2	7.07	d	124.122
	CH-3	6.96	dd	118.85
	CH-4	7.31	d	143.34
	<b>CH-5</b>	<b>6.27</b>	<b>d</b>	124.32
<b>Citric acid (CA)</b>	$\alpha,\gamma$ -CH	<b>2.67</b>	<b>d</b>	44.77
	$\alpha',\gamma'$ -CH	2.71	d	44.77
<b>Chlorogenic acid (CGA)</b>	CH <sub>2</sub> -2'	2.02,2.17	m	40.11
	CH-3'	5.33	m	40.14
	CH-4'	3.88	dd	75.43
	CH-5'	4.23	m	72.93
	<b>CH-8</b>	<b>6.39</b>	<b>d</b>	73.82
	CH-7	6.94	d	117.56
	CH-2	7.12	dd	118.90
	CH-5	7.19	d	117.81
	CH-6	7.65	d	149.59
<b>p-Coumaric acid (pCA)</b>	<b>CH-1, CH-2</b>	<b>8.05</b>	<b>d</b>	133.15
	CH-3, CH-4	6.95	d	116.21
	CH-5	7.52	d	143.23
	CH-6	6.29	d	116.42
<b>Dimethyl amine (DMA)</b>	<b>2 CH<sub>3</sub></b>	<b>2.72</b>	<b>s</b>	41.77
<b>Dimethyl Malonic acid (DMMA)</b>	<b>2 CH<sub>3</sub></b>	<b>1.46</b>	<b>s</b>	23.81
<b>Dimethyl Succinic acid (DMSA)</b>	<b>2 CH<sub>3</sub></b>	<b>1.26</b>	<b>s</b>	20.27
	CH <sub>2</sub>	\	s	\
<b>Formic acid (FA)</b>	<b>CH</b>	<b>8.46</b>	<b>s</b>	171.90
<b>Fumaric acid (FumA)</b>	<b>CH=CH</b>	<b>6.51</b>	<b>s</b>	137.94

<b>Lactic acid (LA)</b>	<b>CH<sub>3</sub></b>	<b>1.32</b>	<b>d</b>	22.95
	CH	4.11	q	77.04
<b>Malic acid (MA)</b>	<b>α- CH</b>	<b>4.31</b>	<b>dd</b>	69.33
	β,β'- CH	2.38,2.69	dd	40.86
<b>Neochlorogenic acid (nCGA)</b>	CH <sub>2</sub> -2'	1.92,2.09	m	40.11
	CH-3'	4.04	pd	75.43
	CH-4'	3.61	dd	72.93
	CH-5'	5.35	m	43.23
	CH <sub>2</sub> -6'	2.09,2.23	m	40.14
	<b>CH-8</b>	<b>6.43</b>	<b>d</b>	115.95
	CH-7	7.66	d	117.56
	CH-2	7.22	d	118.90
	CH-5	6.96	dd	117.81
CH-6	6.15	d	149.59	
<b>Quinic acid (QA)</b>	<b>CH<sub>2</sub>-1</b>	<b>1.89, 2.09</b>	<b>dd</b>	41.48
	CH-2	4.02	m	67.88
	CH-3	3.55	m	76.16
	CH-4	4.15	m	71.36
	CH <sub>2</sub> -5	2.00, 2.06	dd	38.21
<b>Succinic acid (SA)</b>	<b>2 CH<sub>2</sub></b>	<b>2.39</b>	<b>s</b>	36.31
<b><u>Amino acids</u></b>				
<b>Alanine (Ala)</b>	<b>β-CH<sub>3</sub></b>	<b>1.49</b>	<b>d</b>	19.05
	α- CH	3.80	q	53.56
<b>Aspartic acid (Asp)</b>	<b>β'-CH</b>	<b>2.68</b>	<b>dd</b>	39.31
	β-CH	2.72	dd	39.31
	α-CH	3.91	m	55.09
<b>Asparagine (Asn)</b>	β'-CH	2.86	dd	37.44
	<b>β-CH</b>	<b>2.89</b>	<b>dd</b>	37.44
	α-CH	4.01	m	54.09
<b>Glutamine (Gln)</b>	γ- CH <sub>2</sub>	2.11	m	29.31
	<b>β,β-CH<sub>2</sub></b>	<b>2.45</b>	<b>m</b>	34.02
	α-CH	3.81	m	57.19
<b>Histidine (His)</b>	CH-6	3.19	dd	30.25
	CH-7	3.99	dd	57.29
	CH-5	7.09	d	120.03
	<b>CH-2</b>	<b>8.01</b>	<b>d</b>	138.31

<b>Isoleucine (Ile)</b>	$\delta$ -CH <sub>3</sub>	0.95	t	13.85
	<b><math>\gamma</math>-CH<sub>3</sub></b>	<b>1.02</b>	<b>d</b>	17.38
	$\gamma'$ -CH	1.25	m	27.01
	$\gamma''$ -CH	1.49	m	27.01
	$\beta$ -CH	1.99	m	38.71
	$\alpha$ -CH	3.69	m	63.04
<b>Leucine (Leu)</b>	<b><math>\delta, \delta'</math>-CH<sub>3</sub></b>	<b>0.97</b>	<b>m</b>	23.85, 24.59
	$\gamma$ -CH	1.72	m	26.81
	$\beta$ -CH <sub>2</sub>	1.73	m	42.60
	$\alpha$ -CH	3.74	m	56.21
<b><math>\gamma</math>-aminobutyric acid (GABA)</b>	<b><math>\beta</math>-CH<sub>2</sub></b>	<b>1.95</b>	<b>t</b>	26.38
	$\gamma$ -CH <sub>2</sub>	2.30	m	37.06
	$\alpha$ -CH <sub>2</sub>	3.01	t	42.21
<b>Phenylalanine (Phe)</b>	CH-2,6	7.32	d	130.3
	CH-4	7.38	d	128.6
	<b>CH-3,5</b>	<b>7.42</b>	<b>d</b>	130.3
	$\beta$ -CH <sub>2</sub>	3.27	m	37.1
	$\alpha$ -CH	3.98	dd	56.8
<b>Threonine (Thr)</b>	<b><math>\gamma</math>-CH<sub>3</sub></b>	<b>1.33</b>	<b>d</b>	22.15
	$\alpha$ -CH	3.60	m	63.46
	$\beta$ -CH	4.27	m	68.94
<b>Tyrosine (Tyr)</b>	CH-2,6	7.22	d	130.0
	<b>CH-3,5</b>	<b>7.08</b>	<b>d</b>	117.0
	$\beta$ -CH <sub>2</sub>	3.15	dd	37.1
	$\alpha$ -CH	3.93	dd	56.8
<b>Tryptophan (Trp)</b>	CH-5	7.20	t	124.9
	CH-6	7.27	t	127.9
	<b>CH-7</b>	<b>7.53</b>	<b>d</b>	114.7
	CH-4	7.73	d	121.2
<b>Valine (Val)</b>	$\gamma$ -CH <sub>3</sub>	0.99	d	19.41
	<b><math>\gamma'</math>-CH<sub>3</sub></b>	<b>1.05</b>	<b>d</b>	20.75
	$\beta$ -CH	2.29	m	31.89
	$\alpha$ -CH	3.62	m	63.36
<b><u>Carbohydrates</u></b>				
<b>Fructose (F)</b>	CH-1	3.69	m	62.94

	C-2	\	\	104.23
	<b>CH-3</b>	<b>4.22</b>	<b>d</b>	77.24
	CH-4	4.06	m	75.04
	CH-5	3.90	m	83.37
	CH-6	3.82	m	63.88
<b><math>\alpha</math>-Glucose (<math>\alpha</math>-G)</b>	<b>CH-1</b>	<b>5.25</b>	<b>d</b>	93.10
	CH-2	3.55	m	72.49
	CH-3	3.72	m	73.84
	CH-4	3.42	m	70.67
	CH-5	3.84	m	72.52
	CH <sub>2</sub> -6	3.73, 3.90	m	96.97
<b><math>\beta</math>-Glucose (<math>\beta</math>-G)</b>	<b>CH-1</b>	<b>4.69</b>	<b>d</b>	96.97
	CH-2	3.26	m	75.17
	CH-3	3.50	m	76.84
	CH-4	3.42	m	70.70
	CH-5	3.48	m	74.57
	CH <sub>2</sub> -6	3.74, 3.91	m	61.80
<b>Sucrose (S)</b>	<b>GLC CH-1</b>	<b>5.42</b>	<b>d</b>	93.22
	CH-2	3.59	m	72.11
	CH-3	3.79	m	73.54
	CH-4	3.48	m	70.26
	CH-5	3.85	m	73.38
	CH <sub>2</sub> -6	3.82	m	61.18
	FRU CH <sub>2</sub> -1'	3.69	m	62.44
	C-2	\	\	104.85
	CH-3'	4.22	m	77.45
	CH-4'	4.06	m	75.04
	CH-5'	3.90	m	82.44
	CH <sub>2</sub> -6	3.82	m	63.38
	<b><u>Lipids &amp; Sterols</u></b>			
<b>Saturated fatty acid (SFA)</b>	CH <sub>3</sub>	0.87	t	14.05
	n-CH <sub>2</sub>	1.26	m	29.32
	CH <sub>2</sub> -CH <sub>2</sub> -CO <sub>2</sub> <sup>-</sup>	1.62	m	24.61
	<b>CH<sub>2</sub>-CO<sub>2</sub><sup>-</sup></b>	<b>2.30</b>	<b>t</b>	33.52
<b>Monounsaturated <math>\omega</math>-9 fatty acid (<math>\omega</math>-9 FA)</b>	CH <sub>3</sub>	0.88	t	14.07
	n-CH <sub>2</sub>	1.27	m	29.35
	<b>CH<sub>2</sub>-CH=CH</b>	<b>2.03</b>	<b>m</b>	27.14

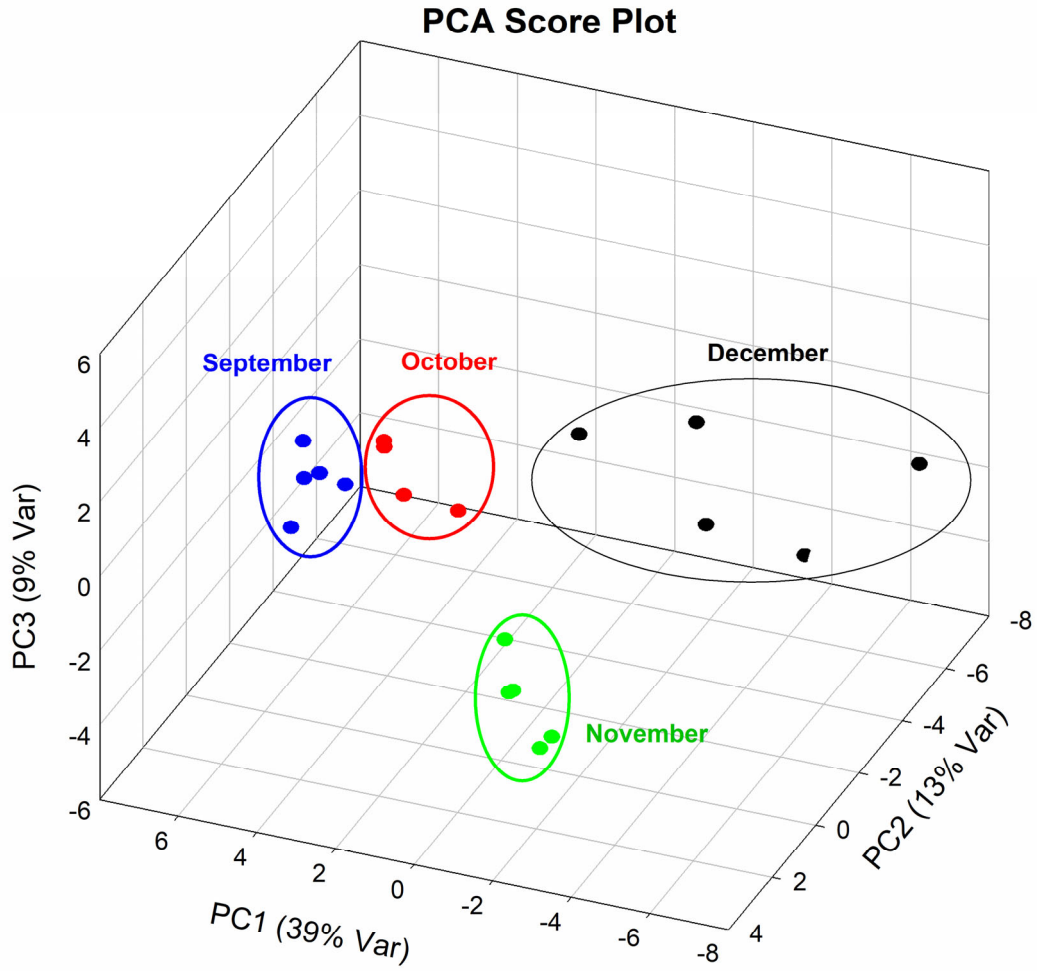
	CH=CH	5.35	m	129.84; 127.43
	CH <sub>2</sub> -CH <sub>2</sub> -CO <sub>2</sub> <sup>-</sup>	1.62	m	24.61
	CH <sub>2</sub> -CO <sub>2</sub> <sup>-</sup>	2.31	t	33.96
<b>Polyunsaturated ω-6 fatty acid (ω-6 FA)</b>	CH <sub>3</sub>	0.86	t	14.06
	n-CH <sub>2</sub>	1.36	m	29.37
	CH <sub>2</sub> -CH=CH	2.04	m	29.45
	CH=CH	5.37	m	130.29; 128.45
	<b>=CH-CH<sub>2</sub>-CH=</b>	<b>2.76</b>	<b>t</b>	25.68
	CH <sub>2</sub> -CH <sub>2</sub> -CO <sub>2</sub> <sup>-</sup>	2.06	m	24.75
	CH <sub>2</sub> -CO <sub>2</sub> <sup>-</sup>	2.31	t	34.05
<b>Polyunsaturated ω-3 fatty acid (ω-3 FA)</b>	CH <sub>3</sub>	0.95	t	14.27
	n-CH <sub>2</sub>	1.37	m	29.22
	CH <sub>2</sub> -CH=CH	2.04	m	27.24
	CH=CH	5.36	m	130.45; 128.33
	<b>=CH-CH<sub>2</sub>-CH=</b>	<b>2.82</b>	<b>t</b>	26.89
	CH <sub>2</sub> -CH <sub>2</sub> -CO <sub>2</sub> <sup>-</sup>	2.03	m	24.56
	CH <sub>2</sub> -CO <sub>2</sub> <sup>-</sup>	2.30	t	33.94
<b>β-Sitosterol (β-ST)</b>	CH <sub>2</sub> -1	1.08, 1.85	m	37.19
	CH <sub>2</sub> -2	1.51, 1.84	m	31.50
	CHOH-3	3.52	m	71.81
	CH <sub>2</sub> -4	2.28	m	42.37
	CH-6	5.34	m	121.79
	CH <sub>2</sub> -7	1.52, 1.98	m	31.98
	CH-8	1.46	m	31.78
	CH-14	0.99	m	56.74
	CH <sub>2</sub> -15	1.57	m	24.25
	CH <sub>2</sub> -16	1.26, 1.85	m	28.37
	<b>CH<sub>3</sub>-18</b>	<b>0.68</b>	<b>s</b>	12.20
CH <sub>3</sub> -25	1.01	s	19.12	
<b>Campsterol (Camp)</b>	CH <sub>2</sub> -1	1.08, 1.85	m	37.19
	CH <sub>2</sub> -2	1.51, 1.84	m	31.50
	CHOH-3	3.52	m	71.81
	CH <sub>2</sub> -4	2.28	m	42.37
	CH-6	5.34	m	121.79
	CH <sub>2</sub> -7	1.52, 1.98	m	31.98
	CH-8	1.46	m	31.78
	CH-14	0.99	m	56.74

	CH <sub>2</sub> -15	1.57	m	24.25
	CH <sub>2</sub> -16	1.26, 1.85	m	28.37
	<b>CH<sub>3</sub>-18</b>	<b>0.70</b>	<b>s</b>	12.21
	CH <sub>3</sub> -25	1.01	s	19.12
<b>Miscellaneous Metabolites</b>				
<b>Luteolin 7-O-glucoside (L7G)</b>	CH-6	6.43	d	\
	CH-3	6.64	s	\
	CH-8	6.80	d	\
	CH-5	6.93	d	\
	CH-6	7.39	d	\
	CH-2	7.43	bs	\
	<b>Glucosyl CH-1</b>	<b>5.65</b>	<b>bs</b>	111.31
	Glucosyl CH-6	4.40	d	\
	Glucosyl CH-6'	4.32	d	\
	Glucosyl CH-2	4.19	t	\
Glucosyl CH-4	4.04	m	\	
Glucosyl CH-5	4.04	m	\	
<b>1,2-Propanediol (PD)</b>	CH'-1	3.38	dd	\
	CH-1	3.68	dd	\
	CH-2	3.91	m	\
	<b>CH<sub>3</sub></b>	<b>1.21</b>	<b>d</b>	21.46
<b>Choline (Chn)</b>	<b>N(CH<sub>3</sub>)<sub>3</sub></b>	<b>3.20</b>	<b>s</b>	56.70
<b>Uridine (Uri)</b>	CH <sub>2</sub> -14	3.80-3.90	dd	63.75
	CH-5	4.12	m	87.16
	CH-4	4.22	dd	72.10
	CH-3	4.45	dd	76.52
	CH-10	5.85	d	105.12
	<b>CH-2</b>	<b>5.91</b>	<b>d</b>	93.25
	CH-11	7.84	d	144.36
<b>Inosine Triphosphate (ITP)</b>	CH-7	8.48	s	142.71
	CH-12	8.22	s	148.86
	<b>CH-2</b>	<b>6.13</b>	<b>d</b>	90.93
	CH-4	4.58	m	76.75
	CH-5	4.35	m	73.20
	CH-17	4.25	m	88.23
<b>Nicotinamide adenine dinucleotide</b>	<b>CH-2</b>	<b>9.45</b>	<b>s</b>	151.42
	CH-4	9.21	dd	153.25

<b>(NAD)</b>	CH-6	8.95	dd	138.89
	CH-5	8.48	m	126.84
<b>Monoacylglycerol (MAG)</b>	<b>CH<sub>2</sub></b>	<b>3.65-3,55</b>	<b>dd</b>	65.45
	CH <sub>2</sub>	4.05-4.15	dd	70.32
	CH	3.82	m	75.12
<b>Triglyceride (TG)</b>	CH	5.13-5.21	m	77.45
	<b>2CH<sub>2</sub></b>	<b>4.15-4.29</b>	<b>dd</b>	68.23
<b>Guanosine n-phosphate (GXP)</b>	CH-17'	4.18	m	67.40
	CH-17	4.24	m	67.40
	CH-5	4.36	m	73.10
	CH-4	4.58	dd	76.35
	<b>CH-2</b>	<b>5.92</b>	<b>d</b>	89.33
	CH-7	8.12	s	140.39
<b>Carotenoids (Crt)</b>	CH <sub>2</sub> -2,2'	1.47	m	39.62
	CH <sub>2</sub> -3,3'	1.62	m	19.27
	CH <sub>2</sub> -4,4'	2.02	m	33.18
	CH-7,7'	6.15	d	126.68
	CH-8,8'	6.14	d	137.78
	CH-10,10'	6.14	d	130.88
	<b>CH-11,11'</b>	<b>6.68</b>	<b>m</b>	125.04
	CH-12,12'	6.35	d	137.26
	CH-14,14'	6.25	d	132.45
	CH-15,15'	6.63	m	130.02
	CH <sub>3</sub> -16,16',17,17'	1.03	s	29.01
	CH <sub>3</sub> -18,18'	1.72	s	21.77
CH <sub>3</sub> -19,19'	1.97	s	12.81	
<b>Falcarinol (Flc)</b>	CH-1 a,b	5.47, 5.26	m	117.01
	<b>CH-2</b>	<b>5.95</b>	<b>dd</b>	136.25
	CH-3	4.92	m	63.61
	CH <sub>2</sub> -8	3.05	d	17.71
	CH-9	5.38	m	121.9
	CH-10	5.52	m	133.05
	CH <sub>2</sub> -11	2.03	m	27.22
	CH <sub>2</sub> -12	1.35	m	29.30
	CH <sub>2</sub> -(13-16)	1.28	m	29.21
	CH <sub>3</sub> -17	0.89	t	14.10

**Table S1:** Metabolites identified in the  $^1\text{H}$  NMR spectrum of the aqueous and chloroform extracts of purple carrots. In bold are evidenced the resonances chosen for metabolite quantification; s: singlet, bs: broad singlet, d: doublet, t: triplet, q: quadruplet, dd: doublet of doublets, m: multiplet.





**Figure S1:** Score plot of PCA analysis carried out on purple carrot samples. Carrots harvested in September are evidenced in blue, October in red, November in green and December in black.