Carotenoids from Different Pumpkin Varieties Exert a Cytotoxic Effect on Human Neuroblastoma SH-SY5Y Cells

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Table S1. Correlation between color parameters (L, a, b, C, H) of two different species (*C. moschata* and *C. maxima*).

		L*	a*	b*	C*	H*
L*	*C. moschata*	-	0.9822	0.5958	0.3544	0.9919
	C. maxima	-	0.9171	0.9561	0.9505	0.9573
a*	*C. moschata*	0.9822	-	0.4948	0.2597	0.9884
	C. maxima	0.9171	-	0.8213	0.8056	0.9457
b*	*C. moschata*	0.5958	0.4948	-	0.9403	
	C. maxima	0.9561	0.8213	-	0.9993	
C*	*C. moschata*	0.3544	0.2597	0.9403	-	0.3572
	C. maxima	0.9505	0.8056	0.9993	-	0.9147
H*	*C. moschata*	0.9919	0.9884	0.6003	0.3572	0.6003
	C. maxima	0.9573	0.9457	0.9286	0.9147	0.9286

Table S2. Correlation between spectrophotometric parameters (TCC, ABTS, ORAC) of two different species (*C. moschata* and *C. maxima*).

		TCC	ABTS	ORAC
TCC	*C. moschata*	-	0.8618	0.5666
	C. maxima	-	0.7601	0.6122
ABTS	*C. moschata*	0.8618	-	0.5980
	C. maxima	0.7601	-	0.8513
ORAC	*C. moschata*	0.5666	0.5980	-
	C. maxima	0.6122	0.8513	-

TCC, total carotenoid content measured by spectrophotometric assay; ABTS, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt; ORAC, Oxygen Radical Absorbance Capacity

Table S3. Regression equation, R^2, linearity range, LOD, and LOQ of lutein and zeaxanthin dipalmitate, analyzed by HPLC-DAD.

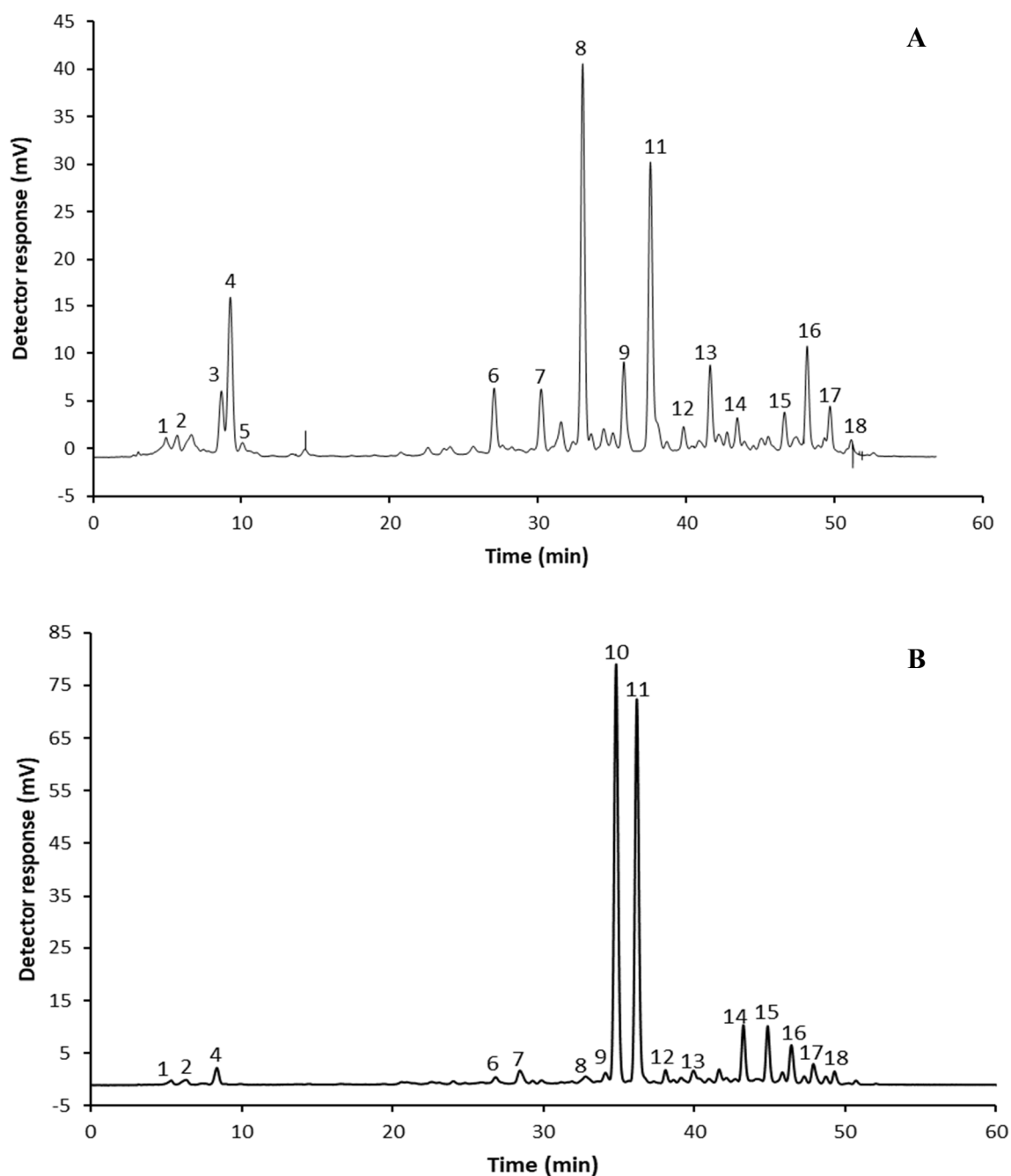| Standard	Regression Equation	R^2	Linearity range (µg/mL)	LOD (ng/mL)	LOQ (ng/mL)
Lutein	y = 182.59 (±5.69)x - 36.15 (±20.72)	0.996	0.24 - 5.90	0.37	1.13
Zeaxanthin dipalmitate	y = 11.03 (±0.44)x -27.66 (±2.34)	0.994	0.95 – 95.0	0.70	2.12

Limit of detection (LOD); limit of quantification (LOQ).

Table S4. Method validation for lutein and zeaxanthin dipalmitate: evaluation of precision (RSD %) and accuracy (recovery %) in the short- and log-term period (intra-day and inter-day precision and accuracy values).

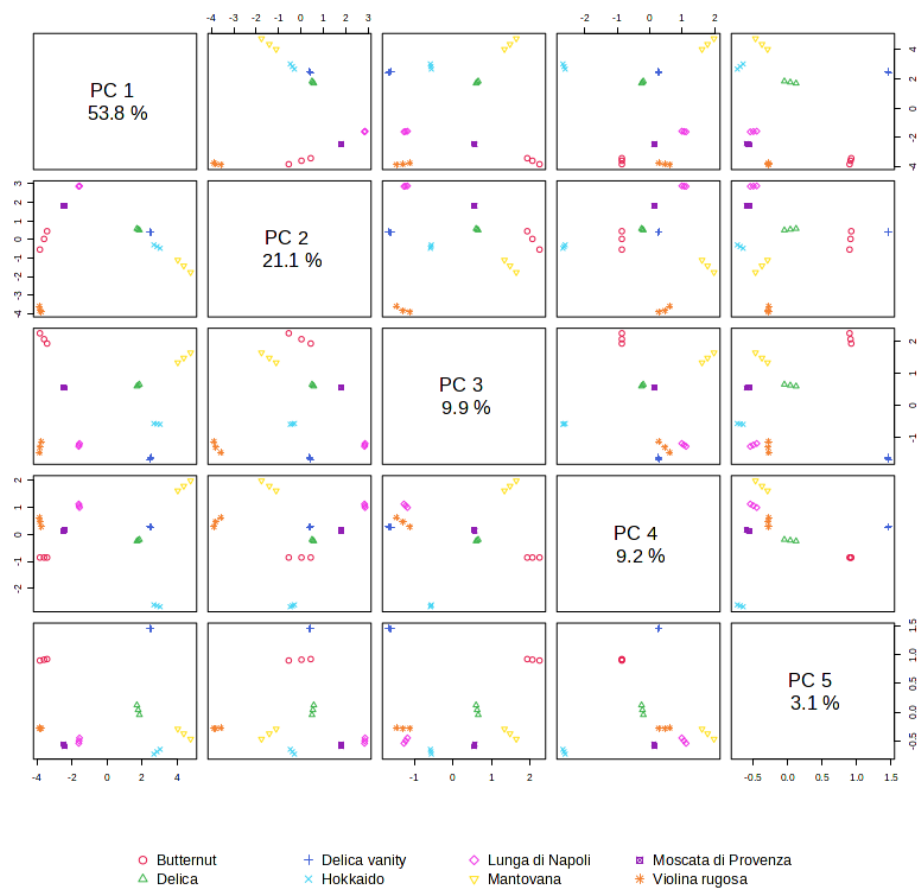| Theoretical conc. (µg/mL)	Intra-day mean conc. (µg/mL)	Intra-day Precision (RSD %)	Intra-day Accuracy (Recovery %)	Inter-day mean conc. (µg/mL)	Inter-day Precision (RSD %)	Inter-day Accuracy (Recovery %)
Lutein	4.56	1.55	96.57			
4.72	4.75	1.70	100.68	4.71	3.18	99.86
	4.83	2.93	102.32			
Zeaxanthin dipalmitate	72.17	2.18	100.84			
71.25	72.38	3.46	101.59	71.31	2.85	100.09
	69.73	1.65	97.86			

Intra-day and Inter-day evaluation: analysis of 3 replicates of the selected external standard within one day and for three consecutive days.

Table S5. HPLC retention times, ultraviolet (UV)/visible light (Vis) spectra, and MS spectral data of carotenoids from pumpkin pulp.
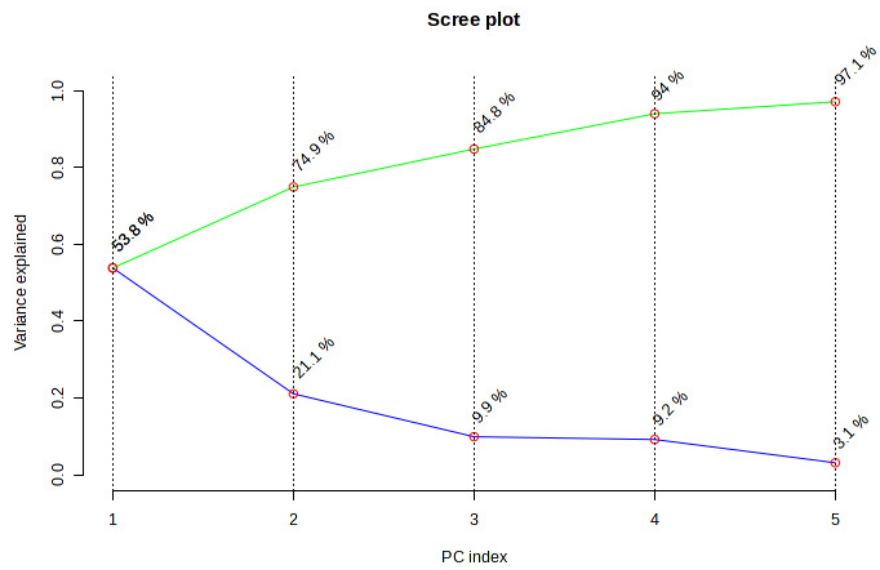
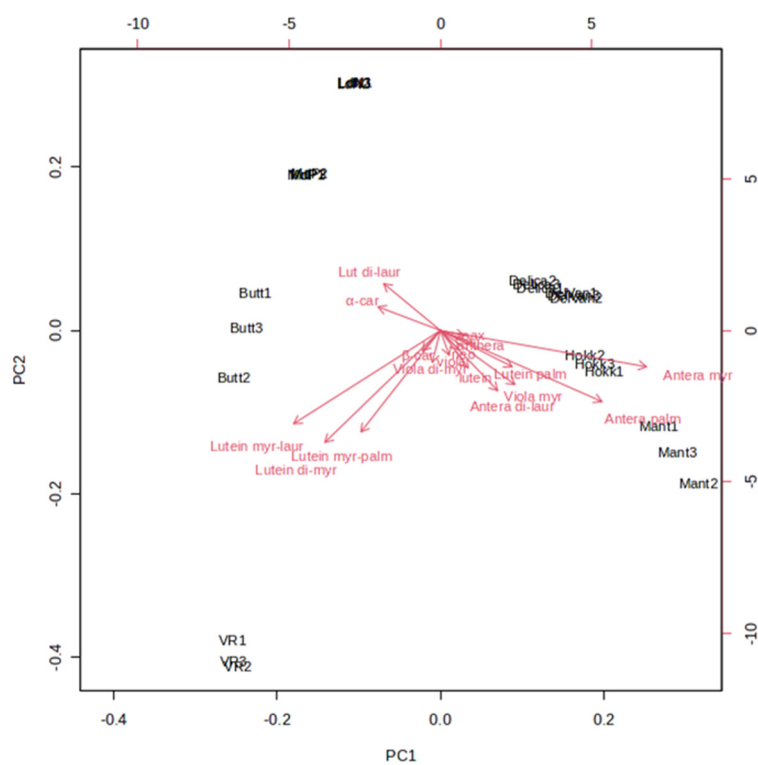| Carotenoids	Molecular formula	Rt (min)	λmax (nm)	$[M+H]^+$ (*m/z*)	Fragments (*m/z*)
neoxanthin	$C_{40}H_{56}O_4$	5.05	416, 442, 470	601	583, 565, 221
violaxanthin	$C_{40}H_{56}O_4$	5.83	420, 444, 472	601	583, 565, 491
antheraxanthin	$C_{40}H_{56}O_3$	8.96	445, 473	585	567, 549, 493
lutein	$C_{40}H_{56}O_2$	9.61	422sh, 445, 473	569	551, 553, 463
zeaxanthin	$C_{40}H_{56}O_2$	10.53	426sh, 450, 477	569	551, 553
α-carotene	$C_{40}H_{56}$	35.07	422sh, 445, 473	537	537, 444, 177
β-carotene	$C_{40}H_{56}$	37.80	426sh, 450, 477	537	444, 177
Violaxanthin myristate	$C_{55}H_{84}O_3$	27.46	416,442,470	812	794, 776, 565
Lutein palmitate	$C_{56}H_{86}O_3$	30.63	420, 444, 472	809	551, 533, 495
Antheraxanthin myristate	$C_{55}H_{84}O_3$	33.42	420, 446, 472	797	779, 705
Antheraxanthin palmitate	$C_{56}H_{86}O_5$	36.17	420, 446, 471	825	807, 733
Violaxanthin di-myristate	$C_{70}H_{112}O_6$	40.13	413, 435, 464	1022	1004, 793, 775
Antheraxanthin di-laurate	$C_{70}H_{112}O_6$	42.01	418, 442, 470	949	931, 857, 533
Lutein di-laurate	$C_{70}H_{112}O_4$	45.61	420, 445, 474	942	734, 533
Lutein myristate-laurate	$C_{68}H_{108}O_4$	47.17	421, 443, 474	960	761, 733, 533
Lutein di-myristate	$C_{71}H_{114}O_4$	48.74	422, 446, 474	990	761, 669, 533
Lutein myristate-palmitate	$C_{70}H_{112}O_4$	50.32	421, 443, 467	1018	790, 762, 533
Lutein di-palmitate	$C_{72}H_{116}O_4$	51.79	428, 448, 476	1046	790, 533

Figure S1: A typical HPLC-DAD chromatographic profile of pumpkin pulp. A, Hokkaido variety (*C. maxima* species); B, Moscata di Provenza variety (*C. moschata* species). 1, neoxanthin; 2, violaxanthin; 3, antheraxanthin; 4, lutein; 5, zeaxanthin; 6, violaxanthin myristate; 7, lutein palmitate; 8, antheraxanthin myristate; 9, antheraxanthin palmitate; 10, α-carotene; 11, β-carotene; 12 violaxanthin di-myristate; 13, antheraxanthin di-laurate; 14, lutein di-laurate; 15, lutein laurate myristate; 16, lutein di-myristate; 17, lutein palmitate myristate; 18, lutein di-palmitate.
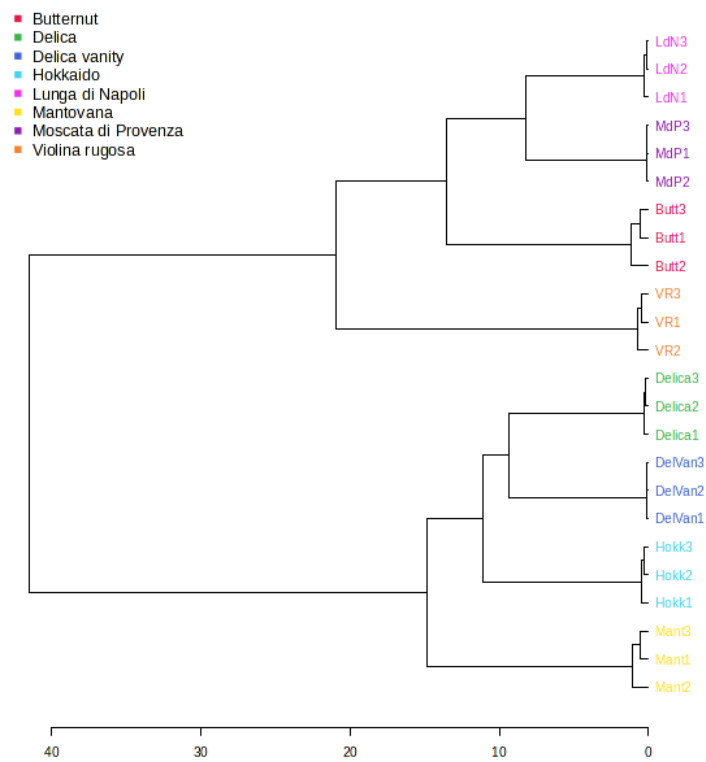
Figure S2: PCA overview. Display pairwise score plot for top 5 PCs.

Figure S3: PCA scree plot. The green line on top shows the accumulated variance explained; the blue line underneath shows the variance explained by individual PC.

Figure S4: Biplot for principal components (PC1 and PC2).

Figure S5: Hierarchical Clustering Dendrogram. Distance Measure: Euclidean; Clustering Algorithm: Ward.