Carotenoids from different pumpkin varieties exert a cytotoxic effect on human neuroblastoma SHSY-5Y 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 |

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| **Table S3** Regression equation, R2, linearity range, LOD, and LOQ of lutein and zeaxanthin dipalmitate, analyzed by HPLC-DAD |
| **Standard** | **Regression Equation** | **R2** | **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)

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| --- |
| **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 %)** |
| Lutein4.72 | 4.56 | 1.55 | 96.57 |  |  |  |
| 4.75 | 1.70 | 100.68 | 4.71 | 3.18 | 99.86 |
| 4.83 | 2.93 | 102.32 |  |  |  |
| Zeaxanthin dipalmitate71.25 | 72.17 | 2.18 | 100.84 | 71.31 | 2.85 | 100.09 |
| 72.38 | 3.46 | 101.59 |
| 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

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Carotenoids** | **Molecular formula** | **Rt (min)** | **λmax****(nm)** | **[M+H]+****(*m/z*)** | **Fragments****(*m/z*)** |
| neoxanthin | C40H56O4 | 5.05 | 416, 442, 470 | 601 | 583, 565, 221 |
| violaxanthin | C40H56O4 | 5.83 | 420, 444, 472 | 601 | 583, 565, 491 |
| antheraxanthin | C40H56O3 | 8.96 | 445, 473 | 585 | 567, 549, 493 |
| lutein | C40H56O2 | 9.61 | 422sh, 445, 473 | 569 | 551, 553, 463 |
| zeaxanthin | C40H56O2 | 10.53 | 426sh, 450, 477 | 569 | 551, 553 |
| α-carotene | C40H56 | 35.07 | 422sh, 445, 473 | 537 | 537, 444, 177 |
| β-carotene | C40H56 | 37.80 | 426sh, 450, 477 | 537 | 444, 177 |
| Violaxanthin myristate | C55H84O3 | 27.46 | 416,442,470 | 812 | 794, 776, 565 |
| Lutein palmitate | C56H86O3 | 30.63 | 420, 444, 472 | 809 | 551, 533, 495 |
| Antheraxanthin myristate | C55H84O3 | 33.42 | 420, 446, 472 | 797 | 779, 705  |
| Antheraxanthin palmitate | C56H86O5 | 36.17 | 420, 446, 471 | 825 | 807, 733  |
| Violaxanthin di-myristate | C70H112O6 | 40.13 | 413, 435, 464 | 1022 | 1004, 793, 775 |
| Antheraxanthin di-laurate | C70H112O6 | 42.01 | 418, 442, 470 | 949 |  931, 857, 533 |
| Lutein di-laurate | C70H112O4 | 45.61 | 420, 445, 474 | 942 | 734, 533 |
| Lutein myristate-laurate  | C68H108O4 | 47.17 | 421, 443, 474 | 960 | 761, 733, 533 |
| Lutein di-myristate | C71H114O4 | 48.74 | 422, 446, 474 | 990 | 761, 669, 533 |
| Lutein myristate-palmitate | C70H112O4 | 50.32 | 421, 443, 467 | 1018 | 790, 762, 533 |
| Lutein di-palmitate | C72H116O4 | 51.79 | 428, 448, 476 | 1046 | 790, 533 |

**Table S5** HPLC retention times, ultraviolet (UV)/visible light (Vis) spectra, and MS spectral data of carotenoids from pumpkin pulp

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**A**



**B**

**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.