Development of a high throughput method to study the inhibitory effect of phytochemicals on TMA formation.

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**Figure S1:** Effect of gallic acid and chlorogenic acid (**A – B**) in the peak area of betaine relativized to its internal standard betaine-d9. Relative changes in betaine/betaine-d9 versus choline 100 μM conditions (**C-D**). All fermentations were carried out at optimal conditions (fecal slurry 20 % and choline 100 μM). Results are expressed as % ± SEM (*n*=6). Different letters indicate statistical differences (p<0.05) by One-way (**C – D**) or Two-way (**A – B**) ANOVA (Tukey’s post hoc test). Factors for Two-way ANOVA were gallic acid or chlorogenic acid concentration and time. Abbreviations: DMB, 3,3-dimethyl-1-butanol; GA, gallic acid; and Chl, chlorogenic acid.

**Table S1**: Molecular weight (MW), retention time (RT) and optimized MRM condition for TMA and related compounds.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Compound** | **MW** | **RT (min)** | **MS/MS transition** | **CV (V)** | **CE (eV)** |
| Choline | 103.16 | 1.01 | 104.2>60.0 | 38 | 16 |
| Choline-d9 | 112.16 | 1.02 | 113.3>69.1 | 40 | 16 |
| L-carnitine | 161.20 | 2.17 | 162.3>85.0 | 34 | 20 |
| L-carnitine-d9 | 170.25 | 2.17 | 171.3>85.0 | 34 | 20 |
| Betaine | 117.15 | 1.07 | 118.2>59.4 | 44 | 18 |
| Betaine-d9 | 126.15 | 1.07 | 127.3>68.1 | 46 | 18 |
| γ-Butyrobetaine | 145.20 | 2.26 | 146.3>87.0 | 26 | 16 |
| TMAO | 75.11 | 1.35 | 76.2>58.9 | 40 | 10 |
| TMAO-d9 | 84.12 | 1.39 | 85.2>68.1 | 40 | 12 |
| TMA a | 59.11 | 0.76 | 146.3>118.2 | 34 | 16 |
| TMA-d9 a | 68.17 | 0.75 | 155.3>127.2 | 34 | 20 |

Abbreviations: TMAO, trimethylamine *N*-oxide; TMA, trimethylamine; MW, molecular weight; RT, retention time CV, cone voltage; and CE, collision energy. a Molecular weight listed is for the original molecules, and MS/MS transitions listed for derivatives (ethyl betaine and ethyl betaine-d9).

**Table S2**: Parameters for the quantification of TMA and TMA-related compounds in spiked fecal fermentation samples by UPLC-MS/MS.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Compound** | **Calibration curve** | **R2** | **Working linearity**  **range (μM)** | **LOD (μM)** | **LOQ (μM)** | **MDL (μM) a** | **MQL (μM) a** |
| Choline | y = 0.765x + 3.003 | 0.997 | 1.93 – 247.10 | 0.488 | 1.628 | 0.977 | 3.256 |
| L-carnitine | y = 5.932x – 4.723 | 0.997 | 0.24 – 497.72 | 0.024 | 0.081 | 0.049 | 0.162 |
| γ-Butyrobetaine | y = 0.197x + 0.181 | 0.998 | 0.27 – 37.92 | 0.032 | 0.105 | 0.063 | 0.210 |
| TMAO | y = 0.355x – 0.978 | 0.997 | 0.43 – 228.71 | 0.011 | 0.037 | 0.075 | 0.075 |
| TMA | y = 58.301x + 13.776 | 0.999 | 0.84 – 462.73 | 0.194 | 0.648 | 0.387 | 1.291 |

Abbreviations: TMAO, trimethylamine N-oxide; TMA, trimethylamine; R2, determination coefficient; LOD, limit of detection; LOQ, limit of quantification; MDL, method detection limit; and MQL, method quantification limit. a MDL and MQL for 25 μL of fecal fermentation media.