

Supplementary Material

Table S1. Amino acids monoisotopic masses, MRM_s, retention times (t_R) and conditions in the mass spectrometer along with their respective molecular formulas.

| Analyte | Molecular Formula | Monoisotopic mass | Precursor Ion | Product Ion | Collision Energy (V) | Tube Lens | t_R standard solutions (min) (Mean \pm S _d *) | t_R hydrolysed sample (min) (Mean \pm S _d *) |
|------------------|---|-------------------|---------------|-------------|----------------------|-----------|--|---|
| Phe | C ₉ H ₁₁ NO ₂ | 165.19 | 166.1 | 120.2 | 14 | 47 | 2.50 \pm 0.06 | 3.12 \pm 0.13 |
| Trp | C ₁₁ H ₁₂ N ₂ O ₂ | 204.23 | 205.1 | 188.1 | 12 | 52 | 2.58 \pm 0.06 | - |
| Ile | C ₆ H ₁₃ NO ₂ | 131.17 | 132.1 | 86.3 | 6 | 40 | 2.67 \pm 0.06 | 3.18 \pm 0.15 |
| Leu | C ₆ H ₁₃ NO ₂ | 131.17 | 132.1 | 86.3 | 11 | 49 | 3.19 \pm 0.07 | 3.38 \pm 0.17 |
| Asn | C ₄ H ₈ N ₂ O ₃ | 132.12 | 133.1 | 87.2 | 8 | 49 | 2.74 \pm 0.05 | - |
| Met | C ₅ H ₁₁ NO ₂ S | 149.21 | 150.1 | 56.4 | 17 | 46 | 3.15 \pm 0.07 | 3.70 \pm 0.12 |
| Val | C ₅ H ₁₁ NO ₂ | 117.15 | 118.1 | 72.3 | 11 | 46 | 3.22 \pm 0.03 | 3.99 \pm 0.14 |
| Pro | C ₅ H ₉ NO ₂ | 115.13 | 116.1 | 70.3 | 17 | 51 | 3.56 \pm 0.07 | 4.02 \pm 0.16 |
| Tyr | C ₉ H ₁₁ NO ₃ | 181.19 | 182.1 | 136.2 | 14 | 46 | 3.53 \pm 0.05 | 4.24 \pm 0.12 |
| Ala | C ₃ H ₇ NO ₂ | 89.094 | 90.1 | 44.5 | 10 | 40 | 4.55 \pm 0.10 | 5.26 \pm 0.11 |
| Thr | C ₄ H ₉ NO ₃ | 119.12 | 120.1 | 74.3 | 11 | 46 | 4.91 \pm 0.03 | 5.73 \pm 0.11 |
| Gly | C ₂ H ₅ NO ₂ | 75.07 | 76.1 | 30.6 | 12 | 40 | 5.22 \pm 0.04 | 5.85 \pm 0.15 |
| Ser | C ₃ H ₇ NO ₃ | 105.09 | 106.1 | 60.4 | 12 | 60 | 5.74 \pm 0.07 | 6.47 \pm 0.13 |
| Glu | C ₅ H ₉ NO ₄ | 147.13 | 148.0 | 84.0 | 15 | 70 | 5.94 \pm 0.09 | 6.85 \pm 0.11 |
| Asp | C ₄ H ₇ NO ₄ | 133.11 | 134.1 | 88.3 | 11 | 70 | 7.86 \pm 0.12 | 8.81 \pm 0.16 |
| Arg | C ₆ H ₁₄ N ₄ O ₂ | 174.20 | 175.1 | 70.3 | 23 | 54 | 8.35 \pm 0.11 | 9.18 \pm 0.11 |
| Gln | C ₅ H ₁₀ N ₂ O ₃ | 146.15 | 147.1 | 130.2 | 9 | 45 | 8.78 \pm 0.12 | 9.54 \pm 0.12 |
| Lys | C ₆ H ₁₄ N ₂ O ₂ | 146.11 | 147.1 | 84.3 | 17 | 48 | 8.79 \pm 0.11 | 9.54 \pm 0.14 |
| His | C ₆ H ₉ N ₃ O ₂ | 155.16 | 156.1 | 110.2 | 15 | 49 | 9.20 \pm 0.10 | 9.92 \pm 0.17 |
| Cys ₂ | C ₆ H ₁₂ N ₂ O ₄ S ₂ | 240.29 | 241.0 | 152.0 | 13 | 76 | 10.24 \pm 0.08 | - |
| Cys | C ₃ H ₇ NO ₂ S | 121.16 | 122.1 | 59.3 | 20 | 44 | 1.84 \pm 0.06 | - |

* For S_d calculation n=6.

Table S2. Precision and trueness data for the analysis of AA in spiked B-90 bread wheat sample.

| Target Amino acid | Added (mg/100 g) | Repeatability (n=3) | | | Intermediate precision (n=3) | | |
|-------------------|------------------|---------------------|--------------|---------|------------------------------|--------------|---------|
| | | Found (mg/100 g) | Recovery (%) | RSD (%) | Found (mg/100 g) | Recovery (%) | RSD (%) |
| Gly | 20.0 | 19.0 | 94.8 | 8.7 | 19.2 | 96.0 | 7.9 |
| | 40.0 | 43.9 | 109.8 | 2.9 | 44.5 | 111.2 | 6.6 |
| Ala | 20.0 | 19.9 | 99.6 | 6.2 | 20.2 | 100.8 | 4.8 |
| | 40.0 | 46.5 | 116.2 | 4.4 | 47.0 | 117.6 | 3.4 |
| Ser | 20.0 | 21.3 | 106.3 | 2.3 | 21.52 | 107.6 | 1.4 |
| | 40.0 | 40.5 | 101.3 | 6.5 | 41.04 | 102.6 | 3.5 |
| Pro | 20.0 | 20.1 | 100.7 | 7.2 | 20.4 | 101.9 | 3.2 |
| | 40.0 | 39.2 | 98.1 | 13.9 | 39.7 | 99.3 | 9.8 |
| Val | 20.0 | 20.1 | 100.3 | 2.9 | 20.3 | 101.5 | 3.6 |
| | 40.0 | 39.7 | 99.3 | 6.8 | 40.2 | 100.5 | 8.7 |
| Thr | 20.0 | 19.3 | 96.5 | 4.6 | 19.5 | 97.7 | 2.8 |
| | 40.0 | 41.9 | 104.7 | 6.6 | 42.4 | 106.0 | 6.7 |
| Asp | 20.0 | 20.6 | 103.2 | 0.9 | 20.9 | 104.5 | 2.7 |
| | 40.0 | 46.2 | 115.4 | 1.7 | 46.7 | 116.8 | 4.3 |
| Glu | 20.0 | 18.8 | 93.8 | 0.6 | 19.0 | 95.0 | 1.8 |

| | | | | | | | |
|------------|------|------|-------|------|------|-------|------|
| | 40.0 | 35.6 | 88.9 | 4.8 | 36.0 | 90.0 | 7.2 |
| Ile | 20.0 | 19.4 | 97.0 | 2.3 | 19.6 | 98.2 | 5.2 |
| | 40.0 | 39.3 | 98.2 | 5.3 | 39.8 | 99.4 | 8.9 |
| Leu | 20.0 | 19.4 | 97.0 | 2.3 | 19.6 | 98.2 | 6.1 |
| | 40.0 | 48.7 | 121.8 | 2.8 | 49.3 | 123.3 | 5.4 |
| Gln | 20.0 | 18.9 | 94.4 | 6.5 | 19.1 | 95.6 | 5.6 |
| | 40.0 | 38.9 | 97.3 | 9.2 | 39.4 | 98.5 | 8.7 |
| Lys | 20.0 | 20.8 | 104.2 | 3.7 | 21.1 | 105.5 | 3.1 |
| | 40.0 | 34.3 | 85.7 | 5.6 | 34.7 | 86.8 | 13.7 |
| Met | 20.0 | 20.6 | 103.0 | 9.8 | 20.9 | 104.3 | 5.5 |
| | 40.0 | 48.1 | 120.2 | 5.7 | 48.7 | 121.7 | 7.6 |
| His | 20.0 | 18.8 | 93.9 | 1.8 | 19.0 | 95.1 | 2.3 |
| | 40.0 | 44.5 | 111.3 | 5.5 | 45.1 | 112.7 | 2.7 |
| Phe | 20.0 | 20.6 | 102.9 | 2.3 | 20.8 | 104.2 | 8.4 |
| | 40.0 | 40.1 | 100.2 | 11.1 | 40.6 | 101.4 | 11.3 |
| Arg | 20.0 | 19.1 | 95.3 | 7.3 | 19.3 | 96.5 | 5.6 |
| | 40.0 | 39.0 | 97.6 | 10.2 | 39.5 | 98.8 | 4.3 |
| Tyr | 20.0 | 20.4 | 102.0 | 4.0 | 20.7 | 103.3 | 3.8 |
| | 40.0 | 39.9 | 99.9 | 8.3 | 40.4 | 101.1 | 9.7 |