

Table S1 The concentration and change ratio of 17 amino acids which changed significantly ($p < 0.05$) after serum specimens incubated at 4 °C for 24h.

| Temperature and delay time | Analyte ($\mu\text{mol/L}$) | | | | | | | | | | | | | | | | |
|----------------------------|-------------------------------|------------|-------------|------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|------------|-------------|------------|-------------|-------------|-------------|
| | His | Leu | Lys | Phe | Trp | Val | Gly | Ser | Tau | Asp | Glu | Cys | Ala | bAla | 3MHis | PEtN | Pro |
| 0h-1 | 61.02 | 80.82 | 108.65 | 54.64 | 30.33 | 160.47 | 236.42 | 145.47 | 36.09 | 2.24 | 7.45 | 112.17 | 216.87 | 11.88 | 1.85 | 1.29 | 117.91 |
| 0h-2 | 57.85 | 88.25 | 101.16 | 51.86 | 34.72 | 163.05 | 237.33 | 140.03 | 38.34 | 1.34 | 9.71 | 78.64 | 299.41 | 10.57 | 2.30 | 1.53 | 108.97 |
| 0h-3 | 46.26 | 103.85 | 92.96 | 62.05 | 39.45 | 181.50 | 295.67 | 187.40 | 52.97 | 0.68 | 14.01 | 79.71 | 343.24 | 15.52 | 2.61 | 1.79 | 96.49 |
| 0h-4 | 31.91 | 96.34 | 106.79 | 55.82 | 36.46 | 166.28 | 200.44 | 157.07 | 41.78 | 2.16 | 9.55 | 115.24 | 276.87 | 10.01 | 2.06 | 1.77 | 124.27 |
| mean | 49.26 | 92.31 | 102.39 | 56.09 | 35.24 | 167.82 | 242.47 | 157.49 | 42.29 | 1.60 | 10.18 | 96.44 | 284.10 | 12.00 | 2.20 | 1.59 | 111.91 |
| SD | 13.19 | 9.96 | 7.05 | 4.31 | 3.81 | 9.42 | 39.41 | 21.17 | 7.49 | 0.74 | 2.76 | 19.98 | 52.61 | 2.47 | 0.32 | 0.24 | 12.04 |
| 4°C-1h-1 | 75.78 | 73.27 | 116.15 | 50.00 | 36.20 | 141.02 | 290.14 | 187.76 | 52.39 | 0.75 | 7.60 | 76.29 | 255.77 | 2.19 | 3.44 | 1.45 | 84.74 |
| 4°C-1h-2 | 74.43 | 91.29 | 130.33 | 54.01 | 44.53 | 142.86 | 302.63 | 194.04 | 64.58 | 2.72 | 7.33 | 70.35 | 260.08 | 3.41 | 0.00 | 1.09 | 64.92 |
| 4°C-1h-3 | 52.81 | 87.30 | 171.72 | 53.51 | 40.30 | 152.14 | 323.08 | 159.37 | 63.01 | 1.63 | 11.52 | 57.46 | 342.91 | 3.43 | 1.68 | 0.87 | 85.95 |
| 4°C-1h-4 | 65.88 | 77.92 | 145.25 | 54.37 | 37.12 | 159.51 | 282.61 | 132.67 | 55.61 | 1.27 | 6.92 | 59.51 | 293.22 | 4.40 | 1.11 | 1.39 | 73.61 |
| mean | 67.23 | 82.44 | 140.86 | 52.97 | 39.54 | 148.88 | 299.61 | 168.46 | 58.90 | 1.59 | 8.34 | 65.90 | 287.99 | 3.36 | 2.08 | 1.20 | 77.30 |
| SD | 10.56 | 8.30 | 23.76 | 2.01 | 3.76 | 8.60 | 17.69 | 28.23 | 5.84 | 0.83 | 2.14 | 8.94 | 40.25 | 0.90 | 1.44 | 0.27 | 9.95 |
| T-test | 0.05 | 0.14 | 0.08 | 0.26 | 0.15 | 0.03 | 0.02 | 0.65 | 0.02 | 0.99 | 0.07 | 0.06 | 0.83 | 0.01 | 0.48 | 0.17 | 0.03 |
| change ratio (%) | 136% | 89% | 138% | 94% | 112% | 89% | 124% | 107% | 139% | 99% | 82% | 68% | 101% | 28% | 94% | 75% | 69% |
| 4°C-2h-1 | 71.22 | 74.72 | 95.66 | 44.48 | 32.15 | 160.17 | 247.01 | 130.19 | 34.27 | 3.26 | 10.15 | 81.33 | 265.86 | 7.50 | 2.80 | 1.47 | 108.77 |
| 4°C-2h-2 | 76.53 | 85.19 | 110.45 | 48.90 | 32.06 | 161.86 | 336.76 | 130.21 | 41.16 | 3.74 | 10.68 | 75.31 | 292.72 | 11.03 | 3.24 | 1.47 | 147.30 |
| 4°C-2h-3 | 35.97 | 86.59 | 119.32 | 54.06 | 35.92 | 134.26 | 323.99 | 125.14 | 47.44 | 2.58 | 8.32 | 74.89 | 330.49 | 3.07 | 1.59 | 1.54 | 82.88 |
| 4°C-2h-4 | 57.25 | 115.55 | 160.42 | 62.50 | 41.53 | 213.97 | 361.37 | 194.75 | 50.75 | 4.12 | 18.46 | 125.96 | 458.15 | 13.76 | 0.00 | 2.16 | 140.86 |
| mean | 60.24 | 90.51 | 121.46 | 52.48 | 35.41 | 167.56 | 317.28 | 145.07 | 43.40 | 3.43 | 11.90 | 89.37 | 336.80 | 8.84 | 2.55 | 1.66 | 119.95 |
| SD | 18.11 | 17.51 | 27.74 | 7.74 | 4.46 | 33.42 | 49.35 | 33.21 | 7.27 | 0.66 | 4.49 | 24.57 | 85.13 | 4.62 | 1.45 | 0.33 | 29.91 |
| T-test | 0.25 | 0.83 | 0.27 | 0.41 | 0.94 | 0.99 | 0.12 | 0.59 | 0.75 | 0.01 | 0.61 | 0.47 | 0.33 | 0.44 | 0.72 | 0.66 | 0.55 |
| change ratio (%) | 122% | 98% | 119% | 94% | 100% | 100% | 131% | 92% | 103% | 214% | 117% | 93% | 119% | 74% | 116% | 104% | 107% |

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|-------------------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|------------|-------------|
| 4°C-4h-1 | 65.80 | 78.19 | 120.38 | 45.90 | 28.57 | 129.41 | 246.65 | 124.79 | 36.72 | 3.07 | 12.60 | 62.30 | 299.31 | 13.99 | 1.53 | 1.62 | 81.63 |
| 4°C-4h-2 | 46.35 | 90.93 | 136.74 | 57.50 | 42.40 | 184.39 | 285.31 | 109.98 | 45.38 | 6.58 | 10.75 | 97.26 | 358.29 | 12.59 | 2.66 | 1.23 | 173.30 |
| 4°C-4h-3 | 67.81 | 97.15 | 105.77 | 53.44 | 33.31 | 160.26 | 294.43 | 172.18 | 46.82 | 4.04 | 10.19 | 64.86 | 417.76 | 12.24 | 2.85 | 1.62 | 108.62 |
| 4°C-4h-4 | 75.35 | 107.03 | 135.68 | 61.02 | 39.69 | 212.86 | 363.39 | 149.62 | 44.98 | 4.63 | 12.69 | 163.83 | 404.64 | 13.99 | 2.87 | 1.06 | 93.69 |
| mean | 63.83 | 93.32 | 124.64 | 54.46 | 35.99 | 171.73 | 297.45 | 139.14 | 43.47 | 4.58 | 11.56 | 97.06 | 370.00 | 13.20 | 2.48 | 1.38 | 114.31 |
| SD | 12.36 | 12.07 | 14.64 | 6.50 | 6.25 | 35.47 | 48.60 | 27.43 | 4.57 | 1.48 | 1.27 | 47.27 | 53.59 | 0.92 | 0.64 | 0.28 | 40.85 |
| T-test | 0.30 | 0.81 | 0.03 | 0.72 | 0.82 | 0.84 | 0.24 | 0.03 | 0.70 | 0.05 | 0.53 | 0.98 | 0.01 | 0.50 | 0.33 | 0.40 | 0.92 |
| change ratio (%) | 130% | 101% | 122% | 97% | 102% | 102% | 123% | 88% | 103% | 286% | 114% | 101% | 130% | 110% | 112% | 87% | 102% |
| 4°C-8h-1 | 47.71 | 86.44 | 104.07 | 55.62 | 32.18 | 156.82 | 179.13 | 133.26 | 43.59 | 6.74 | 13.26 | 89.86 | 222.40 | 13.05 | 3.16 | 0.96 | 135.75 |
| 4°C-8h-2 | 42.22 | 88.95 | 130.04 | 47.45 | 36.07 | 173.81 | 254.51 | 111.25 | 33.43 | 5.54 | 12.84 | 60.63 | 234.01 | 17.31 | 1.86 | 1.20 | 109.65 |
| 4°C-8h-3 | 59.04 | 101.98 | 123.23 | 55.05 | 33.23 | 142.54 | 249.41 | 123.63 | 44.40 | 5.89 | 11.14 | 61.15 | 402.51 | 11.87 | 3.09 | 1.09 | 144.71 |
| 4°C-8h-4 | 61.67 | 99.05 | 130.34 | 60.49 | 32.76 | 149.00 | 229.41 | 169.59 | 42.58 | 7.81 | 15.20 | 86.86 | 314.57 | 11.33 | 3.83 | 1.17 | 135.45 |
| mean | 52.66 | 94.11 | 121.92 | 54.65 | 33.56 | 155.54 | 228.11 | 134.43 | 41.00 | 6.50 | 13.11 | 74.62 | 293.37 | 13.39 | 2.98 | 1.10 | 131.39 |
| SD | 9.23 | 7.57 | 12.35 | 5.39 | 1.73 | 13.50 | 34.41 | 25.11 | 5.10 | 1.01 | 1.67 | 15.91 | 83.51 | 2.71 | 0.82 | 0.11 | 15.12 |
| T-test | 0.78 | 0.34 | 0.10 | 0.62 | 0.46 | 0.33 | 0.56 | 0.25 | 0.74 | 0.00 | 0.24 | 0.00 | 0.76 | 0.56 | 0.21 | 0.01 | 0.15 |
| change ratio (%) | 107% | 102% | 119% | 97% | 95% | 93% | 94% | 85% | 97% | 405% | 129% | 77% | 103% | 112% | 135% | 69% | 117% |
| 4°C-12h-1 | 85.78 | 98.95 | 80.54 | 66.52 | 38.09 | 180.57 | 282.61 | 193.84 | 41.18 | 10.94 | 20.91 | 99.06 | 495.80 | 11.18 | 2.01 | 1.24 | 162.52 |
| 4°C-12h-2 | 60.78 | 101.02 | 169.23 | 70.98 | 44.27 | 176.26 | 349.61 | 257.40 | 47.79 | 11.45 | 23.38 | 69.08 | 316.25 | 19.59 | 2.90 | 1.00 | 125.74 |
| 4°C-12h-3 | 73.89 | 97.04 | 203.76 | 58.64 | 31.78 | 206.02 | 306.36 | 176.70 | 54.19 | 9.58 | 19.01 | 107.41 | 281.11 | 13.35 | 2.26 | 1.12 | 172.17 |
| 4°C-12h-4 | 63.40 | 108.90 | 110.00 | 68.42 | 39.14 | 214.29 | 464.29 | 166.93 | 44.61 | 9.16 | 18.28 | 71.92 | 443.96 | 16.31 | 4.19 | 0.89 | 138.89 |
| mean | 70.96 | 101.48 | 140.88 | 66.14 | 38.32 | 194.28 | 350.72 | 198.72 | 46.94 | 10.28 | 20.39 | 86.87 | 384.28 | 15.11 | 2.84 | 1.06 | 149.83 |
| SD | 11.39 | 5.21 | 55.83 | 5.32 | 5.13 | 18.71 | 80.63 | 40.67 | 5.54 | 1.09 | 2.28 | 19.24 | 102.09 | 3.65 | 0.98 | 0.15 | 21.29 |
| T-test | 0.04 | 0.19 | 0.31 | 0.13 | 0.48 | 0.04 | 0.15 | 0.24 | 0.08 | 0.00 | 0.02 | 0.56 | 0.28 | 0.33 | 0.32 | 0.06 | 0.08 |
| change ratio (%) | 144% | 110% | 138% | 118% | 109% | 116% | 145% | 126% | 111% | 641% | 200% | 90% | 135% | 126% | 129% | 67% | 134% |
| 4°C-24h-1 | 92.06 | 108.00 | 149.52 | 62.22 | 40.20 | 203.95 | 339.24 | 200.80 | 48.57 | 7.40 | 26.35 | 96.04 | 401.99 | 8.15 | 2.64 | 0.70 | 197.16 |
| 4°C-24h-2 | 94.55 | 118.46 | 124.90 | 73.80 | 42.86 | 188.68 | 339.29 | 128.90 | 49.77 | 17.23 | 26.33 | 135.08 | 401.34 | 8.55 | 4.07 | 0.65 | 162.66 |
| 4°C-24h-3 | 84.04 | 106.33 | 101.27 | 71.66 | 45.08 | 189.33 | 402.82 | 137.41 | 51.81 | 8.40 | 28.31 | 114.23 | 455.82 | 11.35 | 3.14 | 0.78 | 151.06 |

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|-------------------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|------------|-------------|-------------|-------------|-------------|-------------|------------|-------------|------------|-------------|
| 4°C-24h-4 | 66.82 | 125.81 | 186.39 | 65.16 | 45.82 | 202.08 | 364.53 | 152.49 | 45.35 | 11.61 | 27.38 | 68.43 | 319.80 | 13.19 | 2.97 | 0.79 | 193.55 |
| mean | 84.37 | 114.65 | 140.52 | 68.21 | 43.49 | 196.01 | 361.47 | 154.90 | 48.88 | 11.16 | 27.09 | 103.45 | 394.74 | 10.31 | 3.21 | 0.73 | 176.11 |
| SD | 12.53 | 9.17 | 36.38 | 5.42 | 2.53 | 8.13 | 30.03 | 32.12 | 2.70 | 4.43 | 0.95 | 28.27 | 56.11 | 2.39 | 0.62 | 0.07 | 22.77 |
| T-test | 0.00 | 0.04 | 0.09 | 0.04 | 0.00 | 0.04 | 0.00 | 0.91 | 0.14 | 0.02 | 0.00 | 0.79 | 0.03 | 0.39 | 0.03 | 0.00 | 0.00 |
| change ratio (%) | 171% | 124% | 137% | 122% | 123% | 117% | 149% | 98% | 116% | 696% | 266% | 107% | 139% | 86% | 146% | 46% | 157% |

Table S2 The concentration and change ratio of 18 amino acids which changed significantly ($p < 0.05$) after serum specimens incubated at 22 °C for 24h.

| Temperature and delay time | Analyte ($\mu\text{mol/L}$) | | | | | | | | | | | | | | | | | |
|----------------------------|-------------------------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|------------|
| | His | Ile | Leu | Lys | Met | Phe | Thr | Trp | Val | Asn | Asp | Glu | Gln | Orn | Cys | Ala | 3MHis | PEtN |
| 0h-1 | 61.02 | 35.81 | 80.82 | 108.65 | 29.22 | 54.64 | 182.80 | 30.33 | 160.47 | 62.26 | 2.24 | 7.45 | 574.30 | 33.42 | 112.17 | 216.87 | 1.85 | 1.29 |
| 0h-2 | 57.85 | 41.93 | 88.25 | 101.16 | 28.00 | 51.86 | 164.22 | 34.72 | 163.05 | 38.83 | 1.34 | 9.71 | 669.98 | 31.54 | 78.64 | 299.41 | 2.30 | 1.53 |
| 0h-3 | 46.26 | 49.81 | 103.85 | 92.96 | 31.79 | 62.05 | 187.42 | 39.45 | 181.50 | 66.36 | 0.68 | 14.01 | 710.53 | 36.86 | 79.71 | 343.24 | 2.61 | 1.79 |
| 0h-4 | 31.91 | 42.17 | 96.34 | 106.79 | 29.60 | 55.82 | 105.05 | 36.46 | 166.28 | 57.14 | 2.16 | 9.55 | 539.71 | 32.82 | 115.24 | 276.87 | 2.06 | 1.77 |
| mean | 49.26 | 42.43 | 92.31 | 102.39 | 29.65 | 56.09 | 159.87 | 35.24 | 167.82 | 56.15 | 1.60 | 10.18 | 623.63 | 33.66 | 96.44 | 284.10 | 2.20 | 1.59 |
| SD | 13.19 | 5.73 | 9.96 | 7.05 | 1.58 | 4.31 | 37.90 | 3.81 | 9.42 | 12.14 | 0.74 | 2.76 | 79.95 | 2.27 | 19.98 | 52.61 | 0.32 | 0.24 |
| 22°C-1h-1 | 84.51 | 49.53 | 113.20 | 120.97 | 30.20 | 59.07 | 156.89 | 33.04 | 169.03 | 68.15 | 4.99 | 12.30 | 467.53 | 19.93 | 79.48 | 261.56 | 3.27 | 1.78 |
| 22°C-1h-2 | 84.87 | 41.46 | 83.64 | 139.82 | 23.87 | 51.49 | 92.40 | 28.58 | 182.96 | 48.02 | 3.30 | 11.48 | 583.33 | 29.69 | 74.56 | 324.72 | 2.20 | 0.99 |
| 22°C-1h-3 | 43.38 | 43.46 | 102.04 | 124.53 | 31.76 | 59.93 | 148.30 | 42.63 | 185.62 | 75.46 | 4.58 | 13.09 | 403.51 | 37.43 | 78.53 | 239.60 | 2.42 | 1.29 |
| 22°C-1h-4 | 42.81 | 52.95 | 108.95 | 123.66 | 36.58 | 69.70 | 130.93 | 45.91 | 212.67 | 59.59 | 5.33 | 15.99 | 542.97 | 53.07 | 148.27 | 389.16 | 3.26 | 1.45 |
| mean | 63.89 | 46.85 | 101.96 | 127.24 | 30.60 | 60.05 | 132.13 | 37.54 | 187.57 | 62.80 | 4.55 | 13.22 | 499.34 | 35.03 | 95.21 | 303.76 | 2.79 | 1.38 |
| SD | 24.02 | 5.32 | 13.05 | 8.52 | 5.25 | 7.47 | 28.60 | 8.09 | 18.25 | 11.80 | 0.89 | 1.96 | 79.90 | 14.00 | 35.44 | 67.41 | 0.56 | 0.33 |
| T-test | 0.12 | 0.42 | 0.34 | 0.03 | 0.71 | 0.35 | 0.27 | 0.52 | 0.13 | 0.03 | 0.01 | 0.16 | 0.15 | 0.86 | 0.93 | 0.69 | 0.26 | 0.43 |
| change ratio (%) | 130% | 110% | 110% | 124% | 103% | 107% | 83% | 107% | 112% | 112% | 284% | 130% | 80% | 104% | 99% | 107% | 127% | 86% |
| 22°C-2h-1 | 58.87 | 47.16 | 117.81 | 179.17 | 39.87 | 67.36 | 147.89 | 46.70 | 169.18 | 40.73 | 6.15 | 17.19 | 811.48 | 27.39 | 124.68 | 449.90 | 1.98 | 1.41 |
| 22°C-2h-2 | 67.68 | 45.19 | 86.73 | 112.12 | 33.61 | 50.97 | 219.86 | 38.16 | 182.42 | 50.42 | 5.46 | 12.54 | 1020.62 | 45.18 | 73.27 | 348.84 | 3.95 | 1.57 |
| 22°C-2h-3 | 57.54 | 52.94 | 99.59 | 101.09 | 37.42 | 57.85 | 97.38 | 36.75 | 183.55 | 48.58 | 3.64 | 19.92 | 563.47 | 38.80 | 97.30 | 277.61 | 2.96 | 1.61 |
| 22°C-2h-4 | 93.40 | 51.67 | 106.77 | 148.21 | 31.68 | 59.39 | 175.32 | 40.10 | 200.79 | 70.95 | 4.15 | 14.48 | 681.72 | 54.55 | 122.26 | 244.70 | 3.49 | 1.36 |
| mean | 69.37 | 49.24 | 102.73 | 135.15 | 35.64 | 58.89 | 160.11 | 40.43 | 183.99 | 52.67 | 4.85 | 16.03 | 769.32 | 41.48 | 104.38 | 330.26 | 3.09 | 1.49 |
| SD | 16.64 | 3.67 | 13.03 | 35.58 | 3.69 | 6.73 | 51.27 | 4.40 | 12.97 | 12.89 | 1.16 | 3.22 | 195.77 | 11.40 | 24.15 | 90.83 | 0.85 | 0.12 |
| T-test | 0.25 | 0.05 | 0.35 | 0.11 | 0.04 | 0.50 | 1.00 | 0.29 | 0.11 | 0.74 | 0.01 | 0.03 | 0.26 | 0.29 | 0.21 | 0.54 | 0.10 | 0.44 |
| change ratio (%) | 141% | 116% | 111% | 132% | 120% | 105% | 100% | 115% | 110% | 94% | 302% | 158% | 123% | 123% | 108% | 116% | 140% | 93% |
| 22°C-4h-1 | 108.38 | 49.08 | 111.76 | 134.34 | 29.45 | 72.13 | 209.70 | 37.64 | 197.53 | 34.87 | 11.44 | 20.82 | 625.32 | 33.50 | 59.07 | 411.42 | 2.36 | 1.04 |

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|-------------------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|------------|-------------|-------------|-------------|-------------|------------|-------------|-------------|------------|
| 22°C-4h-2 | 95.65 | 55.10 | 131.13 | 120.22 | 28.38 | 65.53 | 206.28 | 37.24 | 177.00 | 61.28 | 10.43 | 13.20 | 742.39 | 71.84 | 103.08 | 389.51 | 1.94 | 1.10 |
| 22°C-4h-3 | 79.08 | 56.42 | 109.30 | 149.53 | 29.62 | 69.91 | 191.63 | 42.84 | 187.17 | 52.65 | 12.27 | 18.29 | 553.81 | 30.70 | 51.39 | 389.58 | 3.07 | 1.02 |
| 22°C-4h-4 | 73.52 | 48.59 | 118.24 | 135.79 | 31.75 | 59.45 | 154.98 | 35.79 | 202.65 | 58.84 | 11.68 | 30.60 | 811.56 | 45.83 | 110.18 | 404.34 | 2.56 | 0.70 |
| mean | 89.16 | 52.30 | 117.61 | 134.97 | 29.80 | 66.75 | 190.65 | 38.38 | 191.09 | 51.91 | 11.46 | 20.73 | 683.27 | 45.47 | 80.93 | 398.71 | 2.48 | 0.97 |
| SD | 15.89 | 4.04 | 9.77 | 11.98 | 1.41 | 5.59 | 25.04 | 3.08 | 11.39 | 11.93 | 0.77 | 7.30 | 115.57 | 18.77 | 29.98 | 10.97 | 0.47 | 0.18 |
| T-test | 0.00 | 0.01 | 0.05 | 0.03 | 0.88 | 0.04 | 0.06 | 0.15 | 0.06 | 0.72 | 0.00 | 0.08 | 0.54 | 0.33 | 0.42 | 0.04 | 0.28 | 0.04 |
| change ratio (%) | 181% | 123% | 127% | 132% | 100% | 119% | 119% | 109% | 114% | 92% | 715% | 204% | 110% | 135% | 84% | 140% | 113% | 61% |
| 22°C-8h-1 | 35.02 | 36.30 | 78.80 | 98.18 | 21.62 | 55.47 | 100.30 | 29.90 | 163.51 | 34.56 | 7.81 | 19.17 | 613.79 | 37.47 | 83.15 | 271.32 | 1.67 | 0.61 |
| 22°C-8h-2 | 49.34 | 35.86 | 82.92 | 84.52 | 23.58 | 59.60 | 151.74 | 32.50 | 156.99 | 39.49 | 8.18 | 19.90 | 430.26 | 36.76 | 51.00 | 288.40 | 2.68 | 0.61 |
| 22°C-8h-3 | 72.82 | 50.61 | 111.35 | 129.02 | 33.41 | 70.28 | 200.87 | 36.90 | 193.72 | 71.06 | 12.23 | 32.02 | 984.57 | 46.96 | 40.31 | 318.60 | 2.69 | 0.98 |
| 22°C-8h-4 | 78.46 | 42.77 | 96.69 | 126.55 | 23.49 | 53.97 | 171.34 | 33.98 | 195.80 | 52.26 | 11.26 | 20.63 | 603.38 | 39.84 | 70.90 | 176.82 | 4.08 | 0.60 |
| mean | 58.91 | 41.38 | 92.44 | 109.57 | 25.52 | 59.83 | 156.06 | 33.32 | 177.51 | 49.34 | 9.87 | 22.93 | 658.00 | 40.26 | 61.34 | 263.78 | 2.78 | 0.70 |
| SD | 20.31 | 6.92 | 14.75 | 21.79 | 5.33 | 7.36 | 42.31 | 2.92 | 20.12 | 16.29 | 2.21 | 6.09 | 233.42 | 4.66 | 19.29 | 61.18 | 0.99 | 0.19 |
| T-test | 0.60 | 0.58 | 0.97 | 0.61 | 0.13 | 0.23 | 0.91 | 0.03 | 0.29 | 0.42 | 0.01 | 0.01 | 0.77 | 0.02 | 0.00 | 0.57 | 0.33 | 0.00 |
| change ratio (%) | 120% | 98% | 100% | 107% | 86% | 107% | 98% | 95% | 106% | 88% | 616% | 225% | 106% | 120% | 64% | 93% | 126% | 44% |
| 22°C-12h-1 | 50.83 | 41.24 | 93.88 | 175.88 | 30.36 | 62.19 | 127.27 | 36.05 | 181.61 | 66.68 | 6.45 | 19.23 | 484.18 | 43.94 | 102.12 | 307.41 | 3.40 | 0.79 |
| 22°C-12h-2 | 51.79 | 47.80 | 111.33 | 151.81 | 30.74 | 67.16 | 131.38 | 33.85 | 195.28 | 39.13 | 13.16 | 29.19 | 556.23 | 42.48 | 82.28 | 327.01 | 3.81 | 0.58 |
| 22°C-12h-3 | 61.65 | 43.87 | 91.15 | 150.54 | 30.79 | 65.12 | 112.45 | 33.92 | 193.60 | 64.68 | 12.33 | 29.10 | 482.97 | 59.04 | 80.07 | 298.11 | 0.00 | 0.66 |
| 22°C-12h-4 | 23.60 | 38.33 | 83.10 | 121.12 | 31.88 | 54.24 | 90.43 | 27.50 | 149.32 | 37.34 | 11.15 | 15.33 | 591.13 | 40.37 | 111.04 | 277.36 | 1.28 | 0.62 |
| mean | 46.97 | 42.81 | 94.86 | 149.84 | 30.94 | 62.18 | 115.38 | 32.83 | 179.95 | 51.96 | 10.77 | 23.21 | 528.63 | 46.46 | 93.88 | 302.47 | 2.83 | 0.66 |
| SD | 16.33 | 4.02 | 11.89 | 22.41 | 0.65 | 5.67 | 18.51 | 3.70 | 21.31 | 15.89 | 3.00 | 7.03 | 53.94 | 8.52 | 15.14 | 20.62 | 1.80 | 0.09 |
| T-test | 0.73 | 0.91 | 0.80 | 0.03 | 0.22 | 0.19 | 0.04 | 0.50 | 0.33 | 0.49 | 0.01 | 0.02 | 0.20 | 0.03 | 0.45 | 0.56 | 0.94 | 0.01 |
| change ratio (%) | 95% | 101% | 103% | 146% | 104% | 111% | 72% | 93% | 107% | 93% | 672% | 228% | 85% | 138% | 97% | 106% | 128% | 42% |
| 22°C-24h-1 | 106.76 | 54.77 | 118.03 | 153.43 | 41.16 | 78.77 | 202.06 | 43.18 | 195.82 | 43.94 | 9.33 | 96.75 | 692.92 | 47.42 | 57.06 | 323.70 | 2.56 | 0.00 |
| 22°C-24h-2 | 86.60 | 51.23 | 99.43 | 140.42 | 32.50 | 63.42 | 117.22 | 38.60 | 195.85 | 36.90 | 8.93 | 28.24 | 693.25 | 45.91 | 43.64 | 311.45 | 3.46 | 0.16 |
| 22°C-24h-3 | 105.63 | 60.59 | 137.78 | 182.07 | 43.36 | 80.63 | 192.15 | 42.49 | 187.31 | 57.10 | 8.01 | 36.51 | 915.43 | 72.67 | 51.97 | 420.14 | 3.33 | 0.34 |
| 22°C-24h-4 | 95.35 | 51.79 | 118.78 | 188.19 | 42.03 | 72.01 | 133.05 | 34.54 | 210.30 | 58.83 | 14.41 | 26.60 | 676.13 | 72.42 | 30.56 | 530.76 | 3.03 | 0.00 |

| | | | | | | | | | | | | | | | | | | |
|-------------------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|------------|-------------|-------------|-------------|-------------|------------|-------------|-------------|------------|
| mean | 98.58 | 54.59 | 118.51 | 166.03 | 39.76 | 73.71 | 161.12 | 39.70 | 197.32 | 49.19 | 10.17 | 47.03 | 744.43 | 59.61 | 45.81 | 396.51 | 3.09 | 0.25 |
| SD | 9.50 | 4.29 | 15.66 | 22.83 | 4.92 | 7.79 | 42.25 | 3.99 | 9.54 | 10.55 | 2.88 | 33.43 | 114.28 | 14.95 | 11.57 | 101.85 | 0.40 | 0.16 |
| T-test | 0.01 | 0.01 | 0.02 | 0.02 | 0.01 | 0.01 | 0.95 | 0.24 | 0.04 | 0.21 | 0.01 | 0.13 | 0.05 | 0.03 | 0.03 | 0.12 | 0.00 | 0.00 |
| change ratio (%) | 200% | 129% | 128% | 162% | 134% | 131% | 101% | 113% | 118% | 88% | 634% | 462% | 119% | 177% | 47% | 140% | 140% | 16% |

Table S3-1 The concentration and change ratio of 14 amino acids in the serum altered after three freeze-thaw cycles compared with the samples without freeze-thaw process ($p < 0.05$). The freeze-thaw cycle including samples were brought from $-80\text{ }^{\circ}\text{C}$, placed at $4\text{ }^{\circ}\text{C}$ for one hour and delivered to $-80\text{ }^{\circ}\text{C}$ for 12 hours.

| sample style | Analyte ($\mu\text{mol/L}$) | | | | | | | | | | | | | | |
|--------------------------------|-------------------------------|---------------|----------------|---------------|---------------|---------------|---------------|----------------|----------------|---------------|---------------|---------------|----------------|----------------|---------------|
| | Arg | His | Ile | Leu | Lys | Met | Phe | Glu | Gln | Thr | Trp | Val | Gly | Ser | Ala |
| 0h-sample-1 | 82.78 | 61.02 | 35.81 | 80.82 | 108.65 | 29.22 | 54.64 | 7.45 | 574.30 | 182.80 | 30.33 | 160.47 | 236.42 | 145.47 | 216.87 |
| 0h-sample-2 | 69.45 | 57.85 | 41.93 | 88.25 | 101.16 | 28.00 | 51.86 | 9.71 | 669.98 | 164.22 | 34.72 | 163.05 | 237.33 | 140.03 | 299.41 |
| 0h-sample-3 | 102.10 | 46.26 | 49.81 | 103.85 | 92.96 | 31.79 | 62.05 | 14.01 | 710.53 | 187.42 | 39.45 | 181.50 | 295.67 | 187.40 | 343.24 |
| 0h-sample-4 | 127.54 | 31.91 | 42.17 | 96.34 | 106.79 | 29.60 | 55.82 | 9.55 | 539.71 | 105.05 | 36.46 | 166.28 | 200.44 | 157.07 | 276.87 |
| 0h-sample-mean | 95.47 | 49.26 | 42.43 | 92.31 | 102.39 | 29.65 | 56.09 | 10.18 | 623.63 | 159.87 | 35.24 | 167.82 | 242.47 | 157.49 | 284.10 |
| SD | 25.24 | 13.19 | 5.73 | 9.96 | 7.05 | 1.58 | 4.31 | 2.76 | 79.95 | 37.90 | 3.81 | 9.42 | 39.41 | 21.17 | 52.61 |
| freeze-thaw cycles-sample-1 | 110.15 | 114.18 | 92.76 | 190.90 | 205.93 | 44.40 | 65.32 | 43.90 | 673.37 | 257.71 | 49.16 | 270.74 | 183.53 | 180.78 | 414.83 |
| freeze-thaw cycles-sample-2 | 100.30 | 100.00 | 84.42 | 169.97 | 90.14 | 40.63 | 60.22 | 31.92 | 485.66 | 216.15 | 46.15 | 271.53 | 204.60 | 169.92 | 353.04 |
| freeze-thaw cycles-sample-3 | 97.89 | 60.59 | 98.13 | 178.57 | 160.28 | 44.23 | 69.68 | 35.07 | 340.71 | 115.91 | 51.54 | 274.76 | 270.59 | 101.97 | 391.84 |
| freeze-thaw cycles-sample-4 | 123.62 | 81.22 | 75.33 | 176.85 | 217.96 | 53.07 | 60.70 | 32.48 | 459.33 | 115.92 | 41.57 | 281.87 | 209.77 | 80.73 | 420.20 |
| freeze-thaw cycles-sample-mean | 107.99 | 89.00 | 87.66 | 179.07 | 168.58 | 45.58 | 63.98 | 35.84 | 489.77 | 176.42 | 47.11 | 274.72 | 217.12 | 133.35 | 394.98 |
| SD | 11.69 | 23.26 | 9.97 | 8.72 | 57.90 | 5.29 | 4.44 | 5.54 | 137.68 | 71.90 | 4.30 | 5.07 | 37.41 | 49.47 | 30.54 |
| T-test | 0.28 | 0.02 | 0.00 | 0.00 | 0.09 | 0.01 | 0.01 | 0.01 | 0.27 | 0.64 | 0.02 | 0.00 | 0.15 | 0.52 | 0.06 |
| change ratio (%) | 13.12% | 80.66% | 106.60% | 93.98% | 64.64% | 53.72% | 14.06% | 252.12% | -21.47% | 10.35% | 33.68% | 63.70% | -10.45% | -15.33% | 39.03% |

Table S3-2 The concentration and change ratio of 14 amino acids in the serum altered after three freeze-thaw cycles compared with the samples without freeze-thaw process ($p < 0.05$). The freeze-thaw cycle including samples were brought from $-80\text{ }^{\circ}\text{C}$, placed at $4\text{ }^{\circ}\text{C}$ for one hour and delivered to $-80\text{ }^{\circ}\text{C}$ for 12 hours.

| sample style | Analyte ($\mu\text{mol/L}$) | | | | | | | | | | | | | | | |
|--------------------------------|-------------------------------|--------|--------|---------|--------|---------|--------|--------|-------------------|-------------------|--------|--------|-------|---------|-------|---------|
| | Tau | Tyr | Asn | Cit | Orn | Cys | Abu | Pro | βAla | βAib | EtN | Hyp | 1MHis | 3MHis | PEtN | Asp |
| 0h-sample-1 | 36.09 | 43.12 | 62.26 | 18.14 | 33.42 | 112.17 | 9.01 | 117.91 | 11.88 | 1.66 | 11.24 | 7.34 | 1.83 | 1.85 | 1.29 | 2.24 |
| 0h-sample-2 | 38.34 | 42.94 | 38.83 | 45.92 | 31.54 | 78.64 | 15.90 | 108.97 | 10.57 | 0.60 | 8.77 | 6.86 | 1.81 | 2.30 | 1.53 | 1.34 |
| 0h-sample-3 | 52.97 | 52.68 | 66.36 | 71.23 | 36.86 | 79.71 | 20.45 | 96.49 | 15.52 | 0.92 | 12.97 | 6.67 | 1.25 | 2.61 | 1.79 | 0.68 |
| 0h-sample-4 | 41.78 | 58.51 | 57.14 | 17.86 | 32.82 | 115.24 | 11.68 | 124.27 | 10.01 | 1.65 | 16.15 | 6.23 | 2.36 | 2.06 | 1.77 | 2.16 |
| 0h-sample-mean | 42.29 | 49.31 | 56.15 | 38.29 | 33.66 | 96.44 | 14.26 | 111.91 | 12.00 | 1.21 | 12.28 | 6.77 | 1.81 | 2.20 | 1.59 | 1.60 |
| SD | 7.49 | 7.64 | 12.14 | 25.61 | 2.27 | 19.98 | 5.01 | 12.04 | 2.47 | 0.53 | 3.10 | 0.46 | 0.46 | 0.32 | 0.24 | 0.74 |
| freeze-thaw cycles-sample-1 | 63.37 | 61.69 | 53.38 | 25.19 | 50.00 | 65.42 | 27.87 | 116.52 | 5.71 | 1.49 | 14.02 | 7.07 | 0.00 | 2.03 | 2.04 | 0.00 |
| freeze-thaw cycles-sample-2 | 71.52 | 69.18 | 41.80 | 16.37 | 42.76 | 50.98 | 18.30 | 144.63 | 3.26 | 2.26 | 11.40 | 8.99 | 0.00 | 1.50 | 1.69 | 0.00 |
| freeze-thaw cycles-sample-3 | 64.92 | 61.43 | 69.23 | 26.20 | 55.91 | 49.08 | 22.62 | 98.73 | 3.83 | 3.28 | 11.26 | 8.64 | 0.00 | 1.88 | 1.83 | 0.00 |
| freeze-thaw cycles-sample-4 | 55.05 | 66.97 | 48.85 | 9.02 | 46.82 | 56.06 | 37.36 | 110.15 | 2.49 | 2.82 | 18.17 | 6.45 | 0.00 | 0.00 | 1.44 | 0.00 |
| freeze-thaw cycles-sample-mean | 63.71 | 64.82 | 53.32 | 19.19 | 48.87 | 55.39 | 26.54 | 117.51 | 3.82 | 2.46 | 13.71 | 7.79 | 0.00 | 1.81 | 1.75 | 0.00 |
| SD | 6.77 | 3.87 | 11.63 | 8.10 | 5.55 | 7.31 | 8.21 | 19.52 | 1.38 | 0.77 | 3.23 | 1.22 | 0.00 | 0.93 | 0.25 | 0.00 |
| T-test | 0.03 | 0.04 | 0.46 | 0.19 | 0.00 | 0.01 | 0.13 | 0.63 | 0.01 | 0.10 | 0.27 | 0.20 | 0.00 | 0.16 | 0.53 | 0.02 |
| change ratio (%) | 50.65% | 31.45% | -5.04% | -49.87% | 45.20% | -42.57% | 86.06% | 5.00% | -68.14% | 103.84% | 11.65% | 14.95% | -100% | -17.97% | 9.84% | -100.0% |