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## Multi-omics analyses strategy reveals the molecular mechanism of the inhibition of *Escherichia coli* O157: H7 by anthocyanins from *Aronia melanocarpa* and its application

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## 2.4. Proteomics analysis

The proteomics method was carried out according to the previous description.<sup>21, 22</sup> In brief, 500 µL of lysis buffer were added to Escherichia coli O157: H7 cells. The samples were then subjected to ultrasound and centrifugation to obtain the supernatant. DTT was added to the sample to the final concentration of 10mM, iodoacetamide (IAM) was rapidly added to the sample after 1h (56 °C) water bath to the final concentration of 55mM, and the sample was kept in the dark room for 1h. Each group of samples took 30ug, added into 10K ultrafiltration tube, centrifuged at 14,000 g for 40 min at 4 °C, and discarded the waste liquid. TEAB (200 μL,100 mM) was added to the samples, and the waste liquid was discarded after centrifugation for 40 minutes (the process was repeated twice). Trypsin (1  $\mu g/\mu L$ ) Add to the sample and incubate it in 37 °C water bath for 24 hours for freeze-drying after digestion. TEAB (25 µL) was added to each sample for re-dissolution, and then acetonitrile (41 µL) was added. Vortex for 1 min and centrifugate. After mixing, centrifuge to the bottom of the tube and stand for 1 h at room temperature. Hydroxylamine (8 μL, 5 %) was added to the sample, and it was left standing at room temperature for 15 min, then the sample was mixed and vacuum dried to remove the solvents. The labeled peptides were separated into 16 fractions using high-performance liquid chromatography (HPLC). The 16 fractions were dried under vacuum and redissolved in 0.1% formic acid for MS analysis.