Electronic supplementary information

Quantitative determination of the sulfur-containing antioxidant ergothioneine by HPLC/ICP-QQQ-MS

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Examples of external calibration plots for the quantitative determination of ergothioneine, HPLC/ICP-QQQ-MS chromatograms of native and spiked 1+199 dilutions of an ergothioneine-treated cell culture medium and high resolution mass spectra of an aqueous ergothioneine standard solution, an ergothioneine-treated cell pellet and a water extract of oyster mushrooms



Fig. S-1 Examples of external calibration plots for (a) low and (b) high ergothioneine concentrations



Fig. S-2 HPLC/ICP-QQQ-MS chromatograms of native and spiked 1+199 dilutions of an ergothioneine-treated cell culture medium (recovery for dilutions of ergothioneine-treated cell culture medium spiked with 145 μ g S L⁻¹ ergothioneine after syringe filtration and dilution: $103 \pm 2\%$ (n = 3)); native 1+199 dilution of ergothioneine-treated cell culture medium (solid black line) and 145 μ g S L⁻¹ ergothioneine spiked 1+199 dilution of ergothioneine-treated cell culture medium (solid light gray line); chromatographic conditions as follows: Gemini C6-Phenyl 4.6 × 150 mm; column temperature, 30°C; injection volume, 10 μ L; mobile phase, eluent A: 20 mM ammonium formate pH 9.0 and eluent B: methanol (gradient elution using the 6-port-valve); flow rate, 0.5 – 0.7 mL min⁻¹



Fig. S-3 HPLC/ESI-Orbitrap MS signals of a 100 μ g S L⁻¹ aqueous ergothioneine standard solution. (a) simulated spectrum for ergothioneine, (b) full scan spectrum of a 100 μ g S L⁻¹ aqueous ergothioneine standard solution and (c) MSMS fragmentation pattern of a 100 μ g S L⁻¹ aqueous ergothioneine standard solution. Chromatographic conditions as follows: Gemini C6-Phenyl 4.6 × 150 mm; column temperature, 30°C; injection volume, 10 μ L; mobile phase, 20 mM ammonium formate 1% methanol pH 9.0; flow rate, 0.7 mL min⁻¹



Fig. S-4 HPLC/ESI-Orbitrap MS signals of an ergothioneine-treated cell pellet. (a) full scan spectrum and (b) MSMS fragmentation pattern. Chromatographic conditions as follows: Gemini C6-Phenyl 4.6×150 mm; column temperature, 30°C; injection volume, 10 µL; mobile phase, 20 mM ammonium formate pH 9.0; flow rate, 0.7 mL min⁻¹



Fig. S-5 HPLC/ESI-Orbitrap MS signals of a water extract of oyster mushroom sample 1. (a) full scan spectrum and (b) MSMS fragmentation pattern. Chromatographic conditions as follows: Gemini C6-Phenyl 4.6×150 mm; column temperature, 30°C; injection volume, $10 \,\mu$ L; mobile phase, 20 mM ammonium formate 1% methanol pH 9.0; flow rate, 0.7 mL min⁻¹