Experimental

System CE

- Capillary Electrophoresis Beckman PA 800 *enhanced* (AB Sciex, Germany, Darmstadt) equipped with PDA-Detector (200-400 nm)
- Uncoated fused-silica capillary; 60/70 cm, Ø 50 µm
- Prior to injection:
  1. washing step, NaOH 0.25 mol/L (2 min) and
  2. equilibration step, separation buffer (2 min)
- separation buffer: 100 mM $Na_2B_4O_7$ (pH ca. 8.8) + 1.0 % β-cyclodextrin
- temperature: 20 °C, Voltage: 30 kV (approx. 100 µA)
- Injection at anodic side for 20 s (hydrodynamic mode, 0.5 psi)
- Detection at λ = 225 nm on the cathode side (10 cm before the capillary end)

System UPLC

- Acquity UPLC® HClass (Waters), PDA eλ detector [λ 200 - 400 nm], QDa detector [ESI+, ESI-, single quadrupole, 100 - 800 Da], quaternary solvent manager, sample manager FTN (Inj.-Vol.: 1 µL), column manager (40°C)
- Mobile Phase: A: H_2O + 0.1% HCOOH, B: CH_3CN + 0.1% HCOOH
- Gradient elution:
  time[min]/%A:%B: 0/99:1, 2/99:1, 10/50:50, 12/0:100, 13/0:100, 13.1/99:1, 15/99:1
- Flow rate: 0.5 mL/min
- Stationary phase: Waters Acquity UPLC® HSS-T3, 2.1×100mm, 1.8 µm
- Detection PDA, λ 200 – 400 nm, evaluation at λ = 225 nm
- Detection QDa: neg. mode, selected *m/z* values

Sample preparation A (hot water extraction)
The contents of 1 or 2 capsules are accurately weighed into a 100 mL iodine-count flask, 30 mL of boiling water is added and heated for 30 min in a boiling water bath (shaking occasionally). After the addition of 20 mL of water at room temperature, 2.00 mL of I.S. solution[1] is added, mixed well and extracted for another 5 min in an ultrasonic bath. The solution is filtered through a membrane filter (0.45 µm) into a sample vial[2]. For qualitative UPLC analysis 1 mL of the filtrate is used, for quantitative CE analysis 100 µL of the filtrate is mixed with 100 µL CE separation buffer[3]

Sample preparation B (cold water extraction – "fermentation" conditions)
The contents of 1 or 2 capsules are accurately weighed into a 100 mL iodine-count flask, 30 mL of water is added and incubated for 30 minutes at 38°C in a drying oven (shaking occasionally). After the addition of 20 mL of water, 2.0 mL of I.S. solution[1] is added, mixed well and extracted for another 5 min in an ultrasonic bath. The solution is filtered through a membrane filter (0.45 µm) into a sample vial[2] and diluted (1:1, v/v) with separation buffer before analysis[3]. This work-up serves for the indirect detection of an active myrosinase through the degradation of glucosinolates. By comparing both electropherograms (A / B), the absence of GSL peaks provides indirect evidence of enzymatic degradation. A quantitative determination of the enzyme activity was not carried out.

[1] Propyl 4-hydroxybenzoate (propylparaben) in water-methanol 1:1 (v/v); c= 1.5 – 2 mg/mL.
[2] Discard the first drops of the filtrate, alternatively centrifuge.
[3] Avoidance of substance losses due to precipitation in the cooled autosampler vial.