Plant material is frozen and homogenized in liquid nitrogen.

Suspension in 1.5L of 4.5% PCA.

Homogenization in rotor-stator homogenizer for 30 min. at max. speed.

Removal of cellular debris by centrifugation (15 min., 15 000×g) and filtration of supernatant through Miracloth.

Precipitation of protein by addition of TCA to 25%, v/w and overnight incubation.

Centrifugation (25 000×g, 30 min., 30 ml glass tubes, 13 centrifugations were required to collect precipitate from the whole sample).

Protein precipitates rinsed twice by adding 30 ml of -20°C acetone and centrifugation (15 min., 25 000×g).

Six glass tubes containing precipitates vacuum dried and stored in -20°C.

Suspension transferred to microcentrifuge tubes, then glass tubes rinsed twice with 500µl of 4.5%G.

Suspension sonicated in BioRuptor (as before) and incubated for 30 min. in termomixer (1400 rpm.).

Centrifugation for 15 min. at 25 000×g supernatants pooled in new tube.

Addition of 2 ml of 50% Bio-Rex 70 in 4.5%G. Incubation for 4h Total volume at this stage ~12.5 mL.

Suspension transferred to column, resin allowed to settle (approximately 40 min.).

Proteins eluted with 10 ml portions of buffers.

HPLC

Unbound proteins

Fractions collected, frozen in liquid nitrogen, vacuum dried and stored in -20°C.