

Value-added products from biomass (BIO-VALUE)

BioValue joint meeting of Projekt 1 + Projekt 2
November 12, 2014

BIO-VALUE

A Strategic Platform for Research and Innovation on biorefining

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Protein platform, KU

Characterization of protein fractions:

- Extraction protocols (juice, fibre-associated)
 - Accelerated solvent extraction (ASE) picture!
 - Van Soest detergent fiber analysis (ADF, NDF)
- Total protein (total N)
- Amino acid composition
- Identification of protein type (expansin, extensin,...)
- Cell wall proteomics

Protein platform, KU

Target biomasses

- Triticale, other grass species, clover-grass
- Nitrogen levels, crop developmental stage, harvest method, conservation method
- Protein associated with different cell wall fractions (cellulose, hemicellulose, lignin)

In collaboration with Søren Ugilt Larsen, AgroTech, analyses of:

- Protein profile of tall fescue and Italian ryegrass grown at different N levels
- Protein profile of strip harvested barley – fresh and ensiled samples



Field experiment plan 2013 & 2014

- 100, 160 and 220 kg N applied ha⁻¹

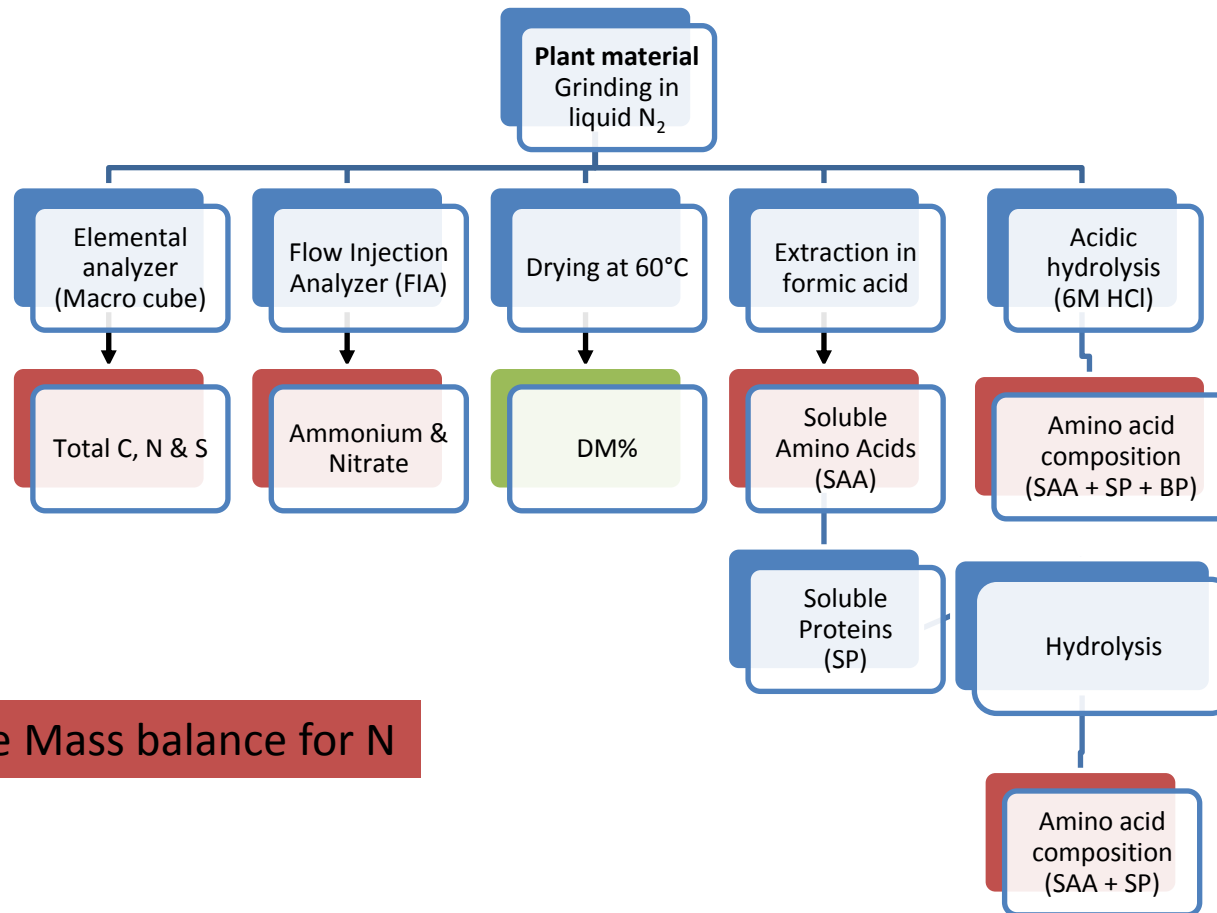
1-45 Mariboss N1	2-45 Tuareg N2	3-24 Nakskov N2	4-24 Nakskov N2
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1-43 Genius N3	2-43 Evolution N3	3-44 Gedser N3	4-44 Gedser N3
1-42 Hybery N3	2-42 Hybery N1	3-43 Tuareg N3	4-43 Tuareg N3
1-41 Audi N1	2-41 Evolution N2	3-42 Pierrot N1	4-42 Pierrot N1
1-40 Gedser N2	2-40 JB Asano N1	3-41 Ambition N3	4-41 Ambition N3
1-39 Pierrot N2	2-39 Audi N1	3-40 Tuareg N1	4-40 Tuareg N1
1-38 JB Asano N2	2-38 Hybery N2	3-39 JB Asano N1	4-39 JB Asano N1
1-37 Jensen N1	2-37 Jensen N1	3-38 Genius N3	4-38 Genius N3
1-36 Ambition N1	2-36 Tuareg N3	3-37 SJ8544003 N3	4-37 SJ8544003 N3
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1-24 SJ8544003 N2	2-24 SJ8544003 N2	3-25 Mariboss N1	4-25 Mariboss N1
1-23 Hereford N1	2-23 JB Asano N3	3-23 Ambition N2	4-23 Ambition N2
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1-07 Tuareg N3	2-07 Pierrot N2	3-07 Genius N2	4-07 Genius N2
1-06 JB Asano N1	2-06 Nakskov N3	3-06 Jensen N1	4-06 Jensen N1
1-05 Gedser N3	2-05 SJ070909-38 N1	3-05 Hereford N2	4-05 Hereford N2
1-04 Evolution N3	2-04 SJ8544003 N1	3-04 SJ8544003 N1	4-04 SJ8544003 N1
1-03 SJ070909-38 N3	2-03 Audi N2	3-03 Audi N1	4-03 Audi N1
1-02 Ambition N2	2-02 Pierrot N3	3-02 Mariboss N3	4-02 Mariboss N3
1-01 SJ8544003 N1	2-01 Gedser N1	3-01 Nakskov N3	4-01 Nakskov N3

Block 1 Block 2 Block 3 Block 4 (growth reg.)





Sample flow



Calculate Mass balance for N

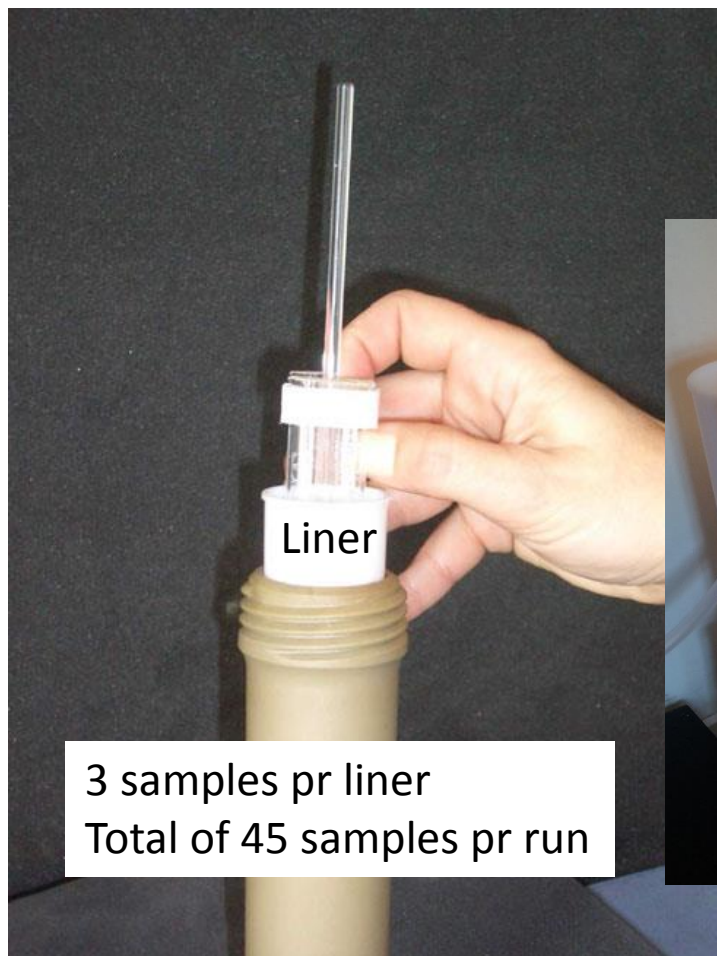
Establish a fast protein
hydrolyses platform for amino
acid composition analysis

Microwave assisted acid
hydrolysis



Sample handling

Samples weighted out
in 2 ml HPLC vials



3 samples pr liner
Total of 45 samples pr run

6 M HCl in bottom of each
liner (gas fase hydrolysis)



Sample handling / preparation / protection

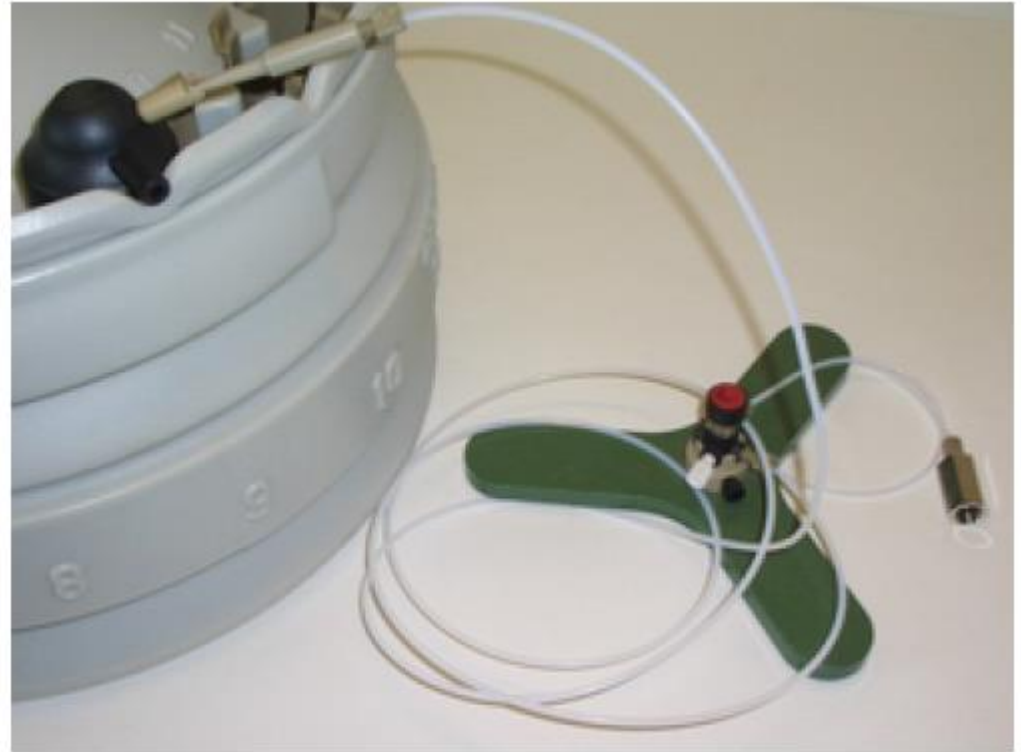


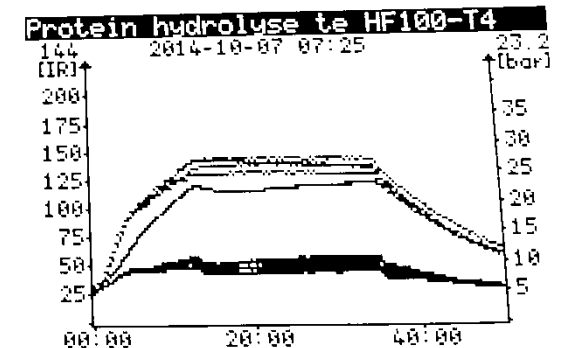
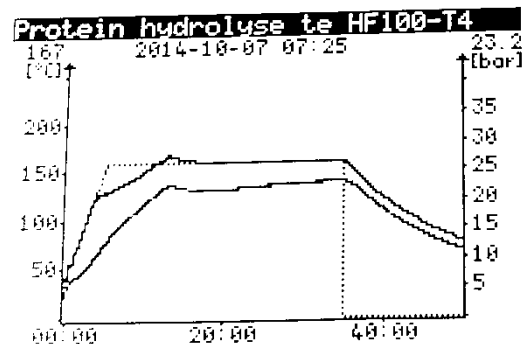
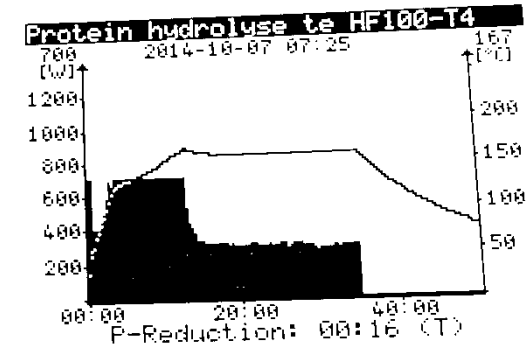
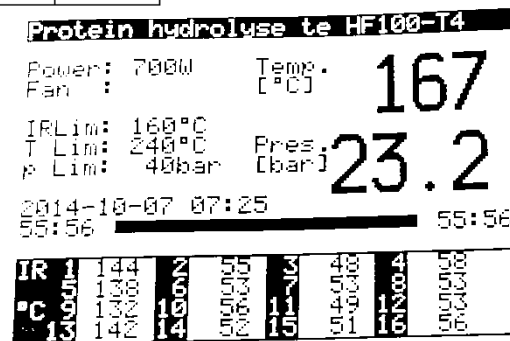
Fig. 4: Purging the vessel with inert gas

The Microwave oven used for hydrolysis



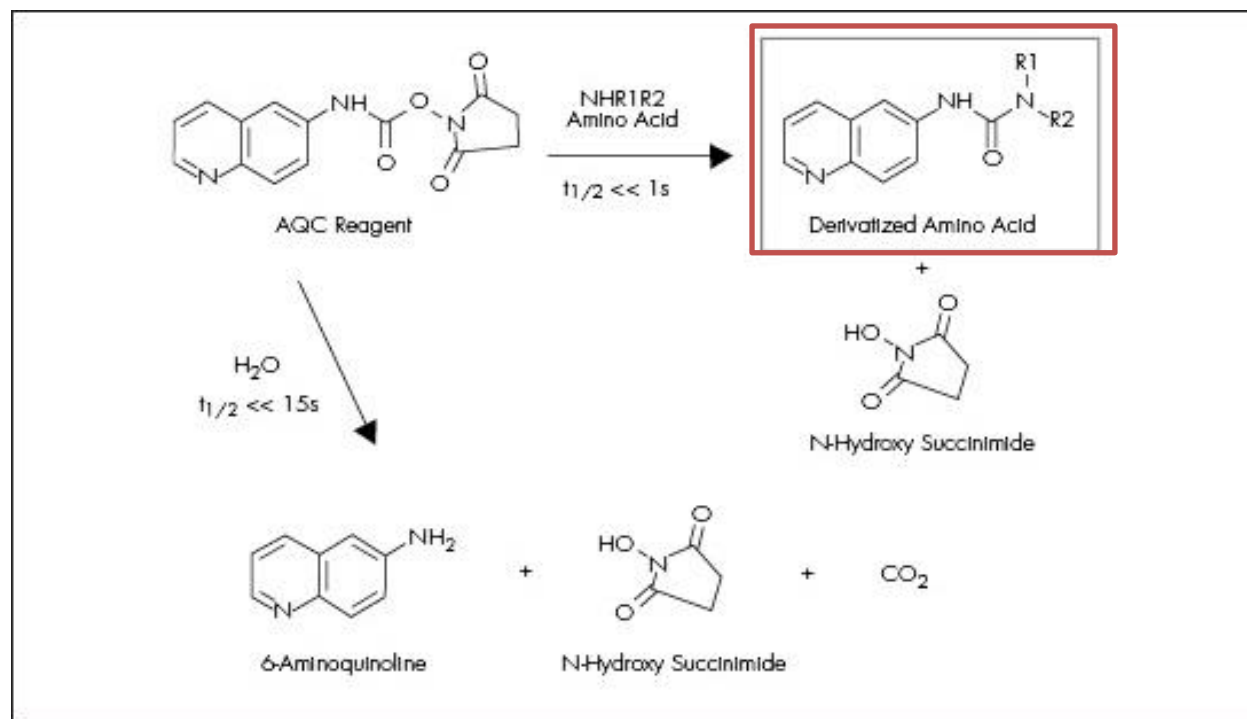
Method description

Step	Temp [°C]	Ramp [min]	Hold [min]	Fan
1	160	5	30	1
2	0	0	15	3



Amino acid determination

Precolumn Sample preparation (derivatization)



Waters UPLC

Hydrolysed sample are added MilliQ water and further diluted in borat buffer before derivation

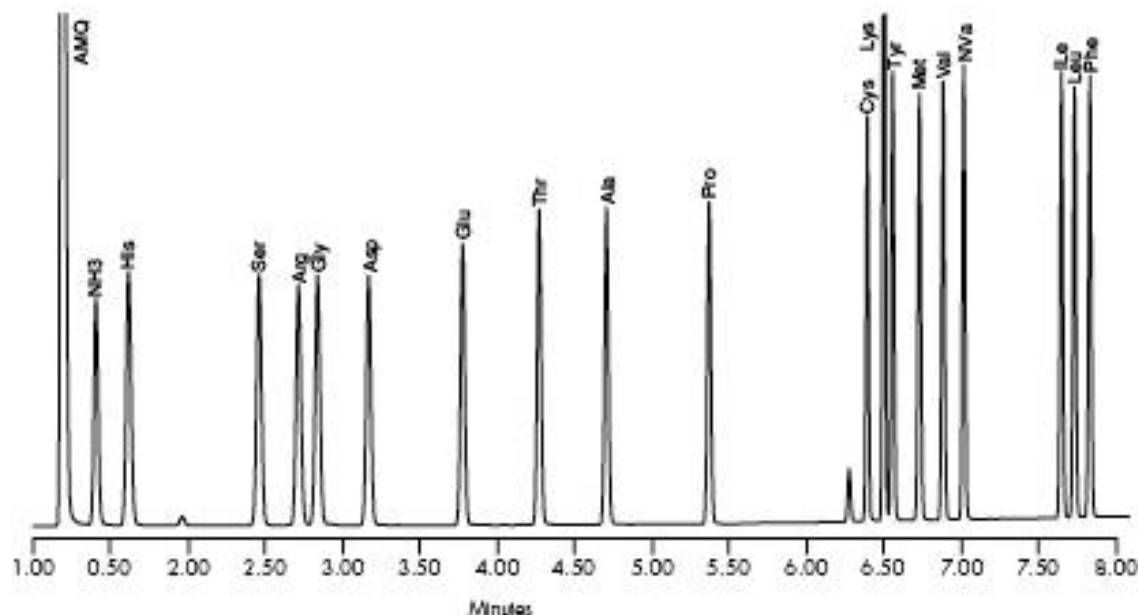


Processing: Standard

Chromatographic Conditions

Column: AccQ-Tag Ultra Column, 2.1 x 100 mm
 Mobile Phase A: AccQ-Tag Ultra Eluent A
 Mobile Phase B: AccQ-Tag Ultra Eluent B
 Flow Rate: 700 μ L/min
 Injection Vol.: 0.5 μ L
 Sample Conc.: 50 pmol on column
 Temperature: 60° C
 Detection: UV @ 260 nm
 Instrument: ACQUITY UPLC System for Amino Acid Analysis

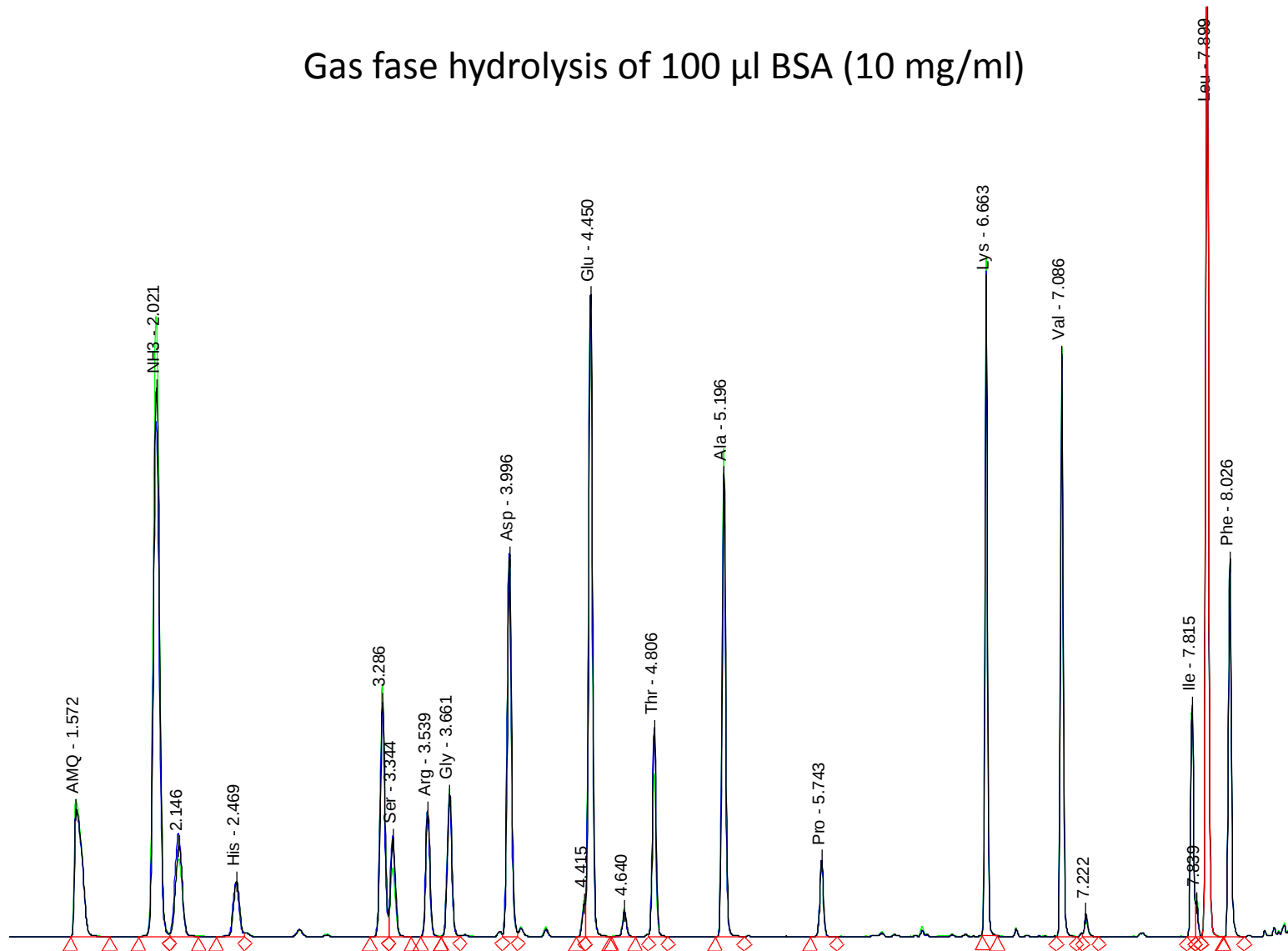
Fast method: 9.5 min/sample (150 samples/day)
 Sample concentration: 0 – 50 pmol / amino acid
 Samples stable for a week at room temperature
 Price: ~200 DKK sample

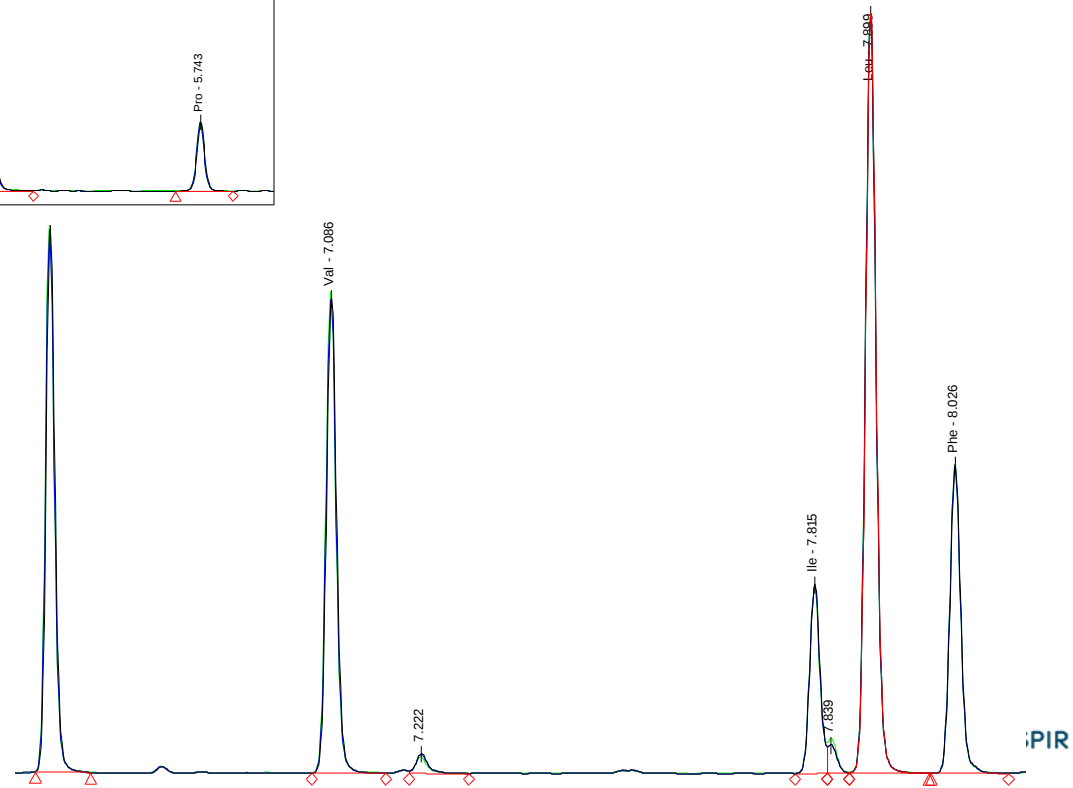
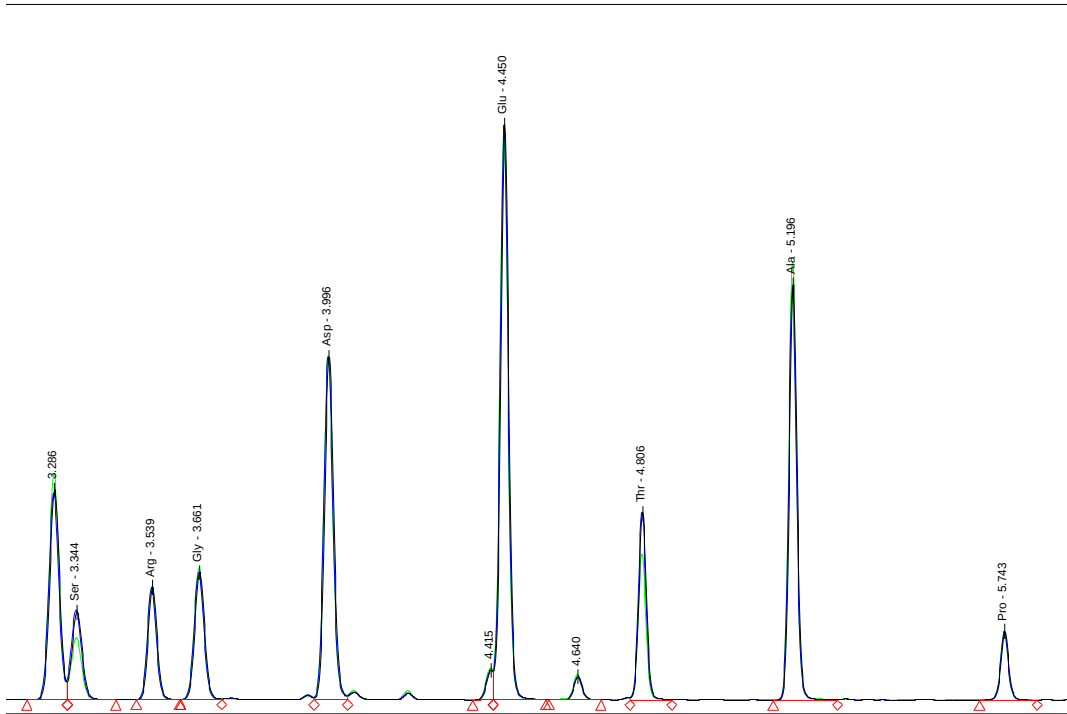




Test: Bovine Serum Albumin

Gas phase hydrolysis of 100 µl BSA (10 mg/ml)







Next

- Further testing of BSA hydrolysis (sequence known)
- Green samples from Foulum (AA composition known)
- Validation of method against 'Foulum samples'
- Outcome: Method paper with different green biomasses and grain materials
- Then processing a lots of samples!



Thank you for your attention