Chemische analyse in onderwijs en toegepast onderzoek

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Ben van den Broek

Ben.vandenbroek@wur.nl

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Healthy & delicious foods

Sustainable food chains

Biorefinery

Biobased chemicals

Biobased materials

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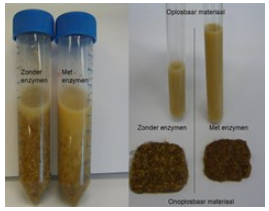
- Biobased Chemicals
  - Chemicals that can be used as building blocks for bulk and fine chemicals. These chemicals are derived from biomass (polysaccharides, lipids, proteins lignin etc.)

- Biobased Materials
  - Research and development of materials and products like paper, construction material and plastics based on renewable resources



- Biorefinery & Bioenergy
  - Chemical, thermal and enzymatic fractionation of biomass for the production of biobased intermediates
  - Production of biofuel and chemicals by fermentation

Introduction

- Dry matter and ash content
- Extractives
- Carbohydrates
- Proteins
- Fatty acids/lipids
- Lignin
- Phenolic compounds

Introduction

- Different methods are available for the characterization of food wastes and residues
- To be able to compare results knowledge about the principle of the methods is needed (pro and cons)
- Aim is to provide some background information about the methods that can be used

Pre-treatment

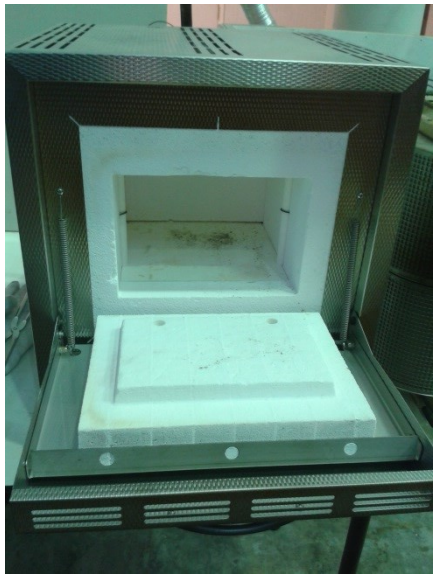
- Proper sample preparation
  - Drying
  - Particle size reduction
- Drying
  - Open air drying
  - Convection oven drying at 45 or 50-60^{o}C
  - Oven drying 105^{o}C or freeze drying
- Milling
  - < 2 mm particle size

Dry matter and ash content

- For comparison of analytical data often the content is expressed as % dry weight

- However, the content can also be expressed as % ash free weight

- Dry matter content by weighting
  - Drying at 105ºC for 16h
    - Cooling in desiccator
  - Drying by infrared moisture balance



Dry matter and ash content

- Ash content
  - 105ºC for 16h (dry weight determination)
  - 4h at 575ºC in muffle furnace
    - 4h warming up to 575ºC
    - 4h at 575ºC
    - Cooling down to 105ºC

- Ash weight at 900ºC can be performed for determination of e.g. carbonates
  - Ash value is weight obtained at 575ºC minus 900ºC

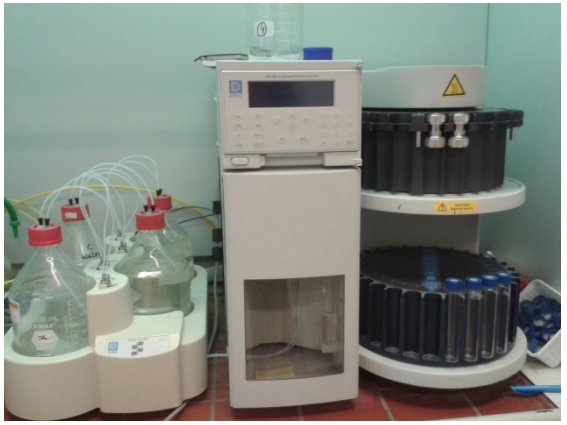
Extractives

- Extractives
  - A quantitative analysis on soluble non-structural components
  - The necessity to remove material from biomass prior to analysis on structural components like lignin
- Ethanol
  - Removal waxy material
- Water
  - Inorganic material (e.g. soil and fertilizers)
  - Soluble proteins and sugars (e.g. sucrose)

Extractives

- Ethanol only needed for woody feed stocks
- Ethanol and water needed for herbaceous feed stocks

- Accelerated Solvent Extraction (ASE) can be used
  - Extracts can by dried
    - Amount can be gravimetric determined
  - Extracted sample can be used for further analysis



Carbohydrate analysis

- Cellulose determination - Updegraff method
- Selective removal of pectin, starch and hemicelluloses
  - Acid nitric acid treatment at 100°C
- Remaining insoluble cellulose is solubilized in 72 % (w/w) sulphuric acid and the amount of glucose is determined
- Lignin can be determined by filtration after solubilisation of cellulose

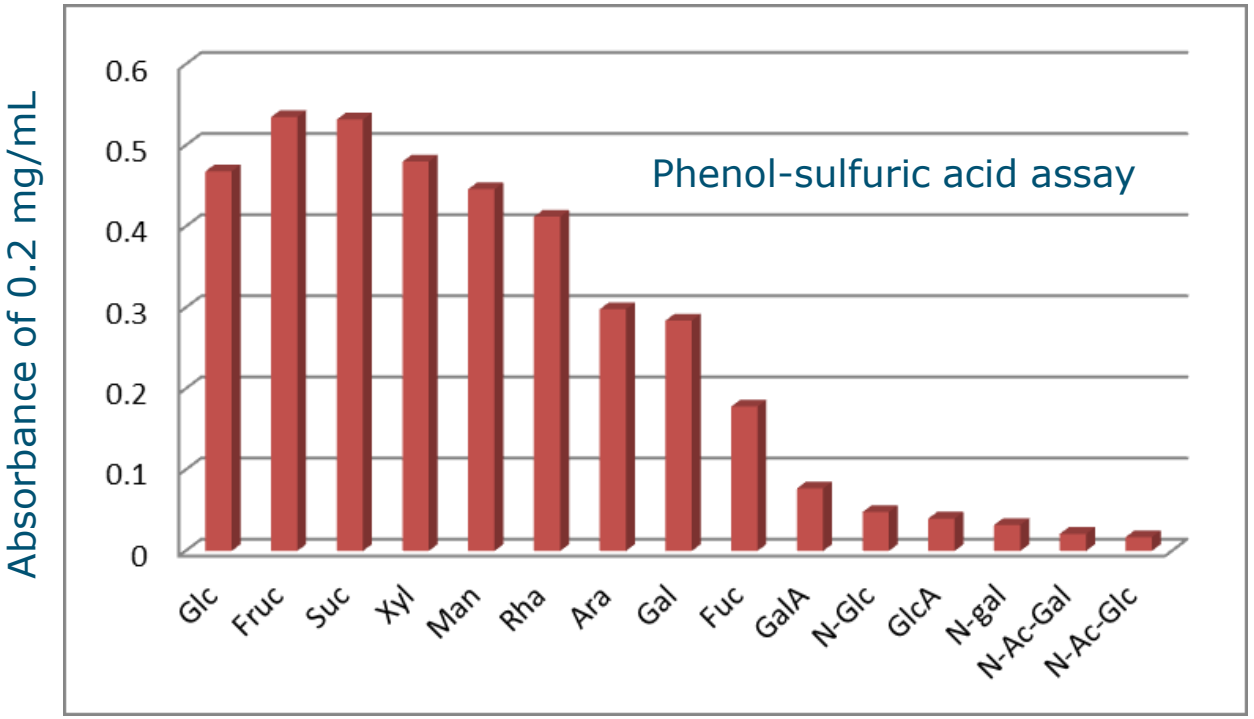
Semimicro determination of cellulose in biological materials. (1969) Anal. Biochem. 32:420-424.

Carbohydrate analysis

- Sugar analysis is depending on the hydrolysis condition
- Carbohydrate analysis (composition) is mainly based on determination of their constituent sugar residues obtained after chemical hydrolysis of the native polymer/oligomer
- Some carbohydrates have very acid-resistant glycosidic linkages (e.g. uronic acids)
- In some cases acid labile sugars are destroyed using too strong acid conditions

Carbohydrate analysis

- Colorimetric assay
  - Neutral sugars: Dubois et al. (phenol-sulfuric acid)



Colorimetric Method for Determination of Sugars and Related Substances. (1956) Anal. Chem., 28(3) 350–356

Carbohydrate analysis

- Uronic acids: Blumenkranz and Asboe-Hansen (mhdp-assay)

- Colour assay more specifice for uronic acids such as galacturonic acid (pectin) and glucuronic acid (arabinoxylan)

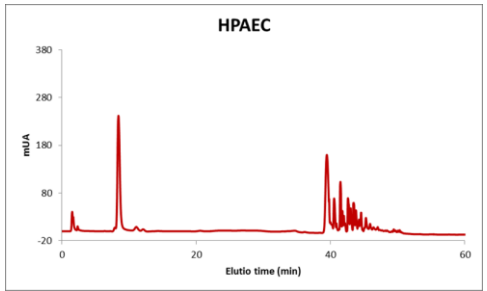
    - High amount of neutral sugars can influence the measurement

New method for quantitative determination of uronic acids. (1973) Anal. Biochem. 54:484–489

Carbohydrate analysis

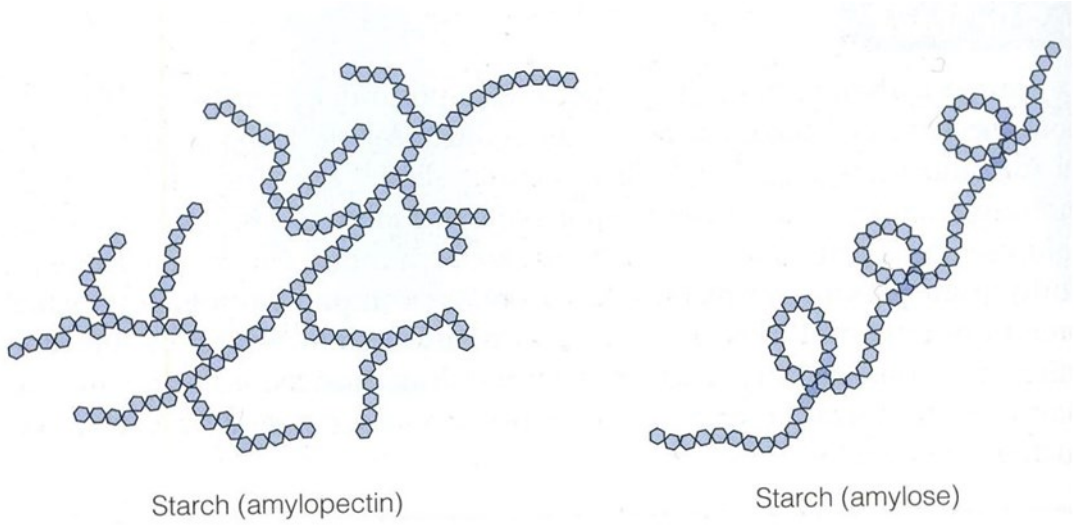
- High performance anion exchange chromatography (HPAEC) and pulsed amperiometric detection
  - Neutralization by barium carbonate

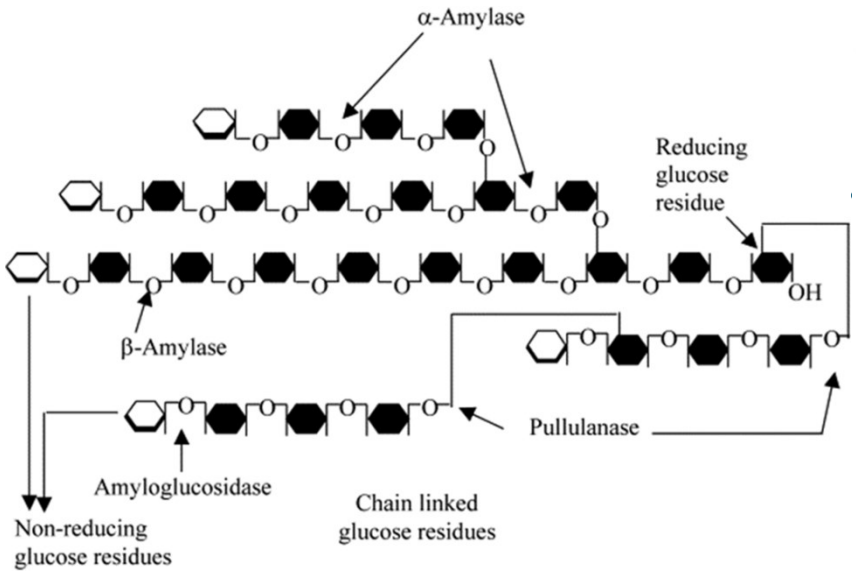
- The CarboPac PA1 column separates monosaccharides and oligosaccharides by high-performance anion-exchange (HPAE) using a high-pH eluent
- At high pH, carbohydrates become charged and are separated as the oxyanion
- The system is ideally suited for use with pulsed amperometric detection (PAD)



Carbohydrate analysis

- Starch





Enzymes to degrade starch into glucose units

Carbohydrate analysis

1. Starch + $H_2O \rightarrow$ maltodextrins
   - α-Amylase

2. Maltodextins $\rightarrow$ D-glucose
   - Amylogycosidase

3. D-Glucose + O_2 + H_2O D-gluconate + H_2O_2
   - Glucose oxidase

4. 2 H_2O_2 + *p*-hydroxybenzoic acid + 4-aminoantipyrine $\rightarrow$ quinoneimine dye + 4 H_2O
   - Peroxidase

E.g. www.megazyme.com

Protein analysis

- Protein content can be analysed by determination of the amino acid content
- Proteins are converted into their amino acid homologs by the use of 6 M HCl at 110^{o}C under vacuum
- The amino acids are derivatised (e.g. *ortho*-phtalaldehyde or fluorenlymethyoloxycarbonyl chloride (FMOC)
- The derivatised amino acids are separated using a reversed phase HPLC and quantified using an UV detector

Protein analysis

- %N can be determined by Kjeldahl en DUMAS en the %N can be converted into protein content by using a N-to-protein conversion factor

- Kjeldahl is based on digestion in concentrated sulfuric acid, distillation of NH_3 and hereafter titration

Protein analysis

- Degradation

$$\text{Sample} + H_2SO_4 \rightarrow (NH_4)_2SO_4(aq) + CO_2(g) + SO_2(g) + H_2O(g)$$

- Destillation

$$(NH_4)_2SO_4(aq) + 2NaOH \rightarrow Na_2SO_4(aq) + 2H_2O(l) + 2NH_3(g)$$

- Capture of ammonia

$$B(OH)_3 + H_2O + NH_3 \rightarrow NH_4^+ + B(OH)_4^-$$

- Titration

$$B(OH)_4^- + NH_4^+ + HCl \rightarrow NH_4Cl\ (aq) + H[B(OH)_4]$$

Protein analysis

- DUMAS is based on combustion, reduction and separation and detection of N_2

- Combustion (oxidation)
  - 1800^{o}C
  - fixed amount of pure oxygen
  - Nitrogen atoms are converted
    - N_2
    - N_xO_y
  - H_2O and CO_2

Protein analysis

- Reduction
  - Pure copper
    - N_xO_y converted into N_2
    - excess of oxygen to water
- Separation
  - Filters to remove CO_2 and water
- Detection
  - GC by thermal conductivity detector

Protein analysis

- Results Dumas nitrogen determination are usually a little bit higher than with Kjeldahl, since even the heterocyclic compounds and nitrogen compounds (e.g. nitrites and nitrates and amino sugars) are detected

- In the Kjeldahl method, such compounds are converted into the ammonium ion incompletely or not at all

Protein analysis

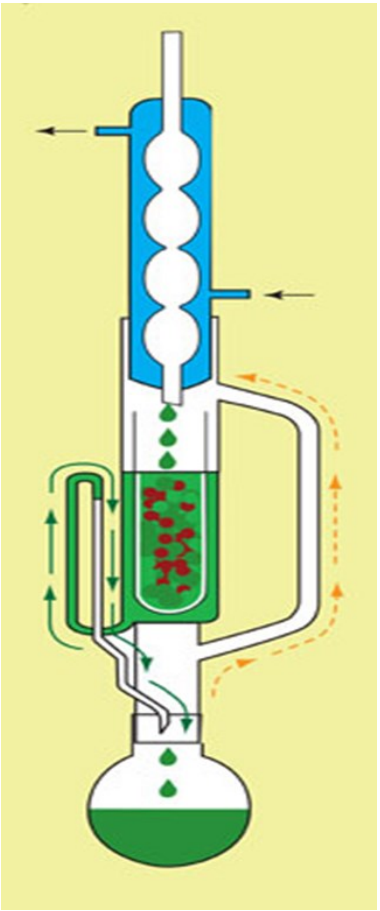
- Soluble protein can be determined by:
  - Lowry which is based on copper complex formation with the peptide bonds by oxidation of aromatic protein residues
  - Bradford is based on dye binding onto charged amino acid residues

- Protein amount is based on the protein standard used (often BSA)

- Kjeldahl and DUMAS total protein
- Lowry and Bradford soluble protein

Lipid/oil analysis

- Most extraction procedures of the oil/lipid fraction are based on Bligh and Dyer
- Soxhlet is often used to extract the oil/lipid fraction
- Nuts can also be pressed to obtain the oil/lipid fraction



Bligh and Dyer. (1959) A rapid method for total lipid extraction and purification.
Can. J. Biochem. Physiol. 37:911-917

Lipid analysis

- Extraction of lipids/oil fraction
  - Disruption of sample
    - (Bead) milling
  - Solvent extraction
    - Chloroform:methanol (2:1)
    - Hexane
  - Solvent removal with vacuum using a rotary film evaporator

- Depending on sample, cell disruption and solvent different amounts of lipids, pigments, chlorophyll can be extracted

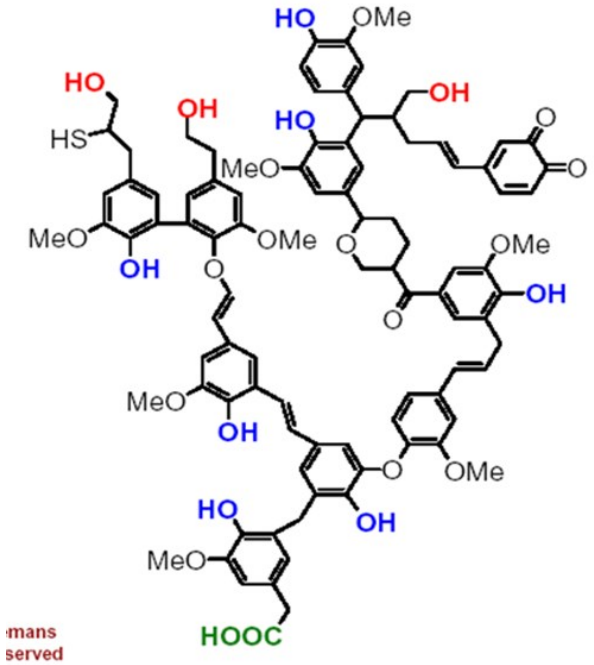
Lipid analysis

- Lipid classes can be separated by: Solid phase extraction (SPE (Silica))
  - Neutral lipids: chloroform
  - Glycolipids: acetone
  - Phospholipids: methanol

- Fatty acids methyl esters (FAMEs) can be determined by mechanical cell disruption of the sample, solvent based lipid extraction, transesterification of fatty acids into FAMEs and quantified and identified by GC-FID

Lignin

- Lignin is a network polymer that results from the dehydrogenative radical polymerisation of monolignols (e.g. *p*-coumaryl-, coniferyl- and sinapyl-alcohols), which are connected via carbon–carbon and ether linkages



Functionality

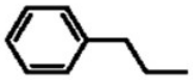
* Alcohol groups
1.08 per PPU

* Carboxyl
0.16 per PPU

* Carbonyl
0.14 per PPU

(McCarthy and Islam, 2000)

PPU = 185 g/mol



C_6-C_3 unit

Lignin

- Lignin in biomass is acid stable and its acid solubility is very small
- For the determination of the chemical composition of biomass, extractive free biomass samples are subjected to a two-step acidic hydrolysis. A small amount of the lignin (ASL) will be solved in the supernatant and the main insoluble part (AIL) will be found in the residue/pellet
- Acid Insoluble Lignin in the acid solution is determined by a gravimetric method
- The content of Acid Soluble Lignin is determined by spectrophotometer (UV absorption at 205 nm)

Conclusions

- Most methods have a kind of optimum for the determination of a component e.g.
  - hydrolysis condition
  - extraction solvent
- For comparison preferably the same methods should be used
- Be aware of the advantages and disadvantages of you method

Interesting Links

- http://www.slideshare.net/VinarsDawane/how-to-find-24913263
- http://www.slideshare.net/rahulbs89/extraction-of-plant-contituents?related=1
- http://www.slideshare.net/OmerSyria/efg-702?related=1

Thank you for your attention