Analysis of plant phenols and flavonoids

Contents

Chemical structure and properties of phenolic compounds

- one or more aromatic or heterocyclic rings
- one or more hydroxy or methoxy groups (*polyphenols*)
- occurrence in the form of glycosides and aglycones as well
  most often bound sugars: Glc. Gal, Rha, Ara, Xyl, Rut
- reducing agents
- antioxidants
- chelating agents
- substrates of enzymatic browning reactions
- effects on sensory quality of food

Groups of phenolic compounds

- phenolic acids
  - benzoic acid derivatives
  - cinnamic acid derivatives
- tannins
  - hydrolyzable tannins (gallotannins, ellegotannins)
  - condensed tannins (proanthocyanidins)
- coumarins
- flavonoids and derived compounds
  - anthocyanins and anthocyanidins

  - catechins
  - flavanons, chalcons and dihydrochalcons
  - flavons
  - flavonols
- isoflavonoids
- prenylated flavonoids (e.g. isoxanthohumol)
- stilbene derivatives (e.g. resveratrol)
- other compounds

HPLC determination of phenolic compounds

Sample preparation

Isolation

- extraction by water (or aqueous solution of acids or salts), MeOH, EtOH, acetone, ethylacetate, dimethylsulphoxide...
  addition of antioxidant (TBHQ, BHA, ascorbic acid)
- enzyme hydrolysis (glycosidases, proteases, amylases)
- acid hydrolysis (glycosides → aglycones)
- alkaline hydrolysis: deacylation of glycosides and catechins (e.g. epigallocatechin gallate)
  transformation of flavanons to chalcons occur

Purification of the extract

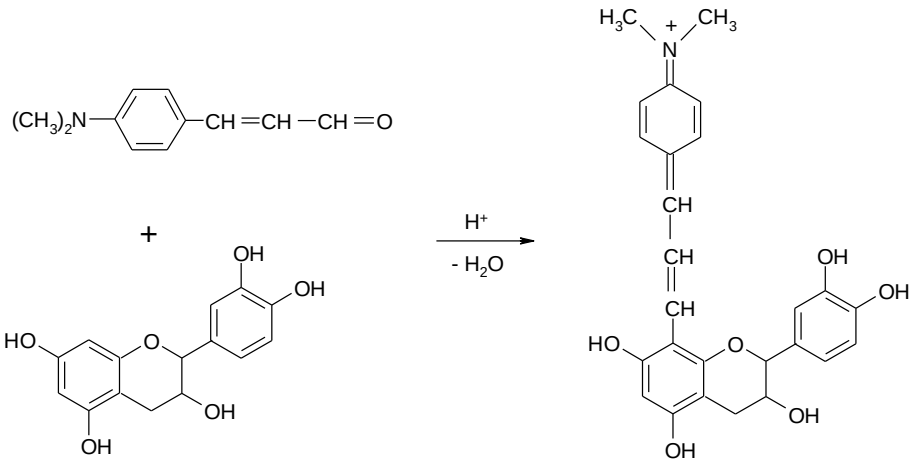
- precipitation of tannins by proteins followed by protein hydrolysis
- liquid-liquid extraction (water-BuOH) – separation of aglycones
- gel chromatography
- solid phase extraction (SPE)
  - sorption: polyamide, polyvinylpyrrolidone, SiC18,
    anion exchanger (phenolic acids)
  - elution: ethylacetate and other semi-polar solvents
    acids solutions (phenolic acids)

HPLC separation of phenolic compounds

- **reversed-phase chromatography**
  - stationary phase: most often SiC_{18}
  - mobile phase:
    water or aqueous solution of HCOOH, CH_3COOH, $HCOONH_4$...
    and organic solvent: MeOH, iPrOH, acetonitrile (ACN) or tetrahydrofurane (THF)
  - order of elution:
    substituted benzoic acids, substituted cinnamic acids, flavonoids
    order of elution of classes of flavonoids with analogous skeleton substitution:
    glycosides of antocyanidins, flavanons, flavonols and flavons folowed by the respective aglycons
    order of elution in the group of anthocyanidins and anthocyanins:
    glycosides of delphinidin, cyanidin, petunidin, pelargonidin, peonidin and malvidin followed by the respective aglycones
- **ion-exchange chromatography**: phenolic acids

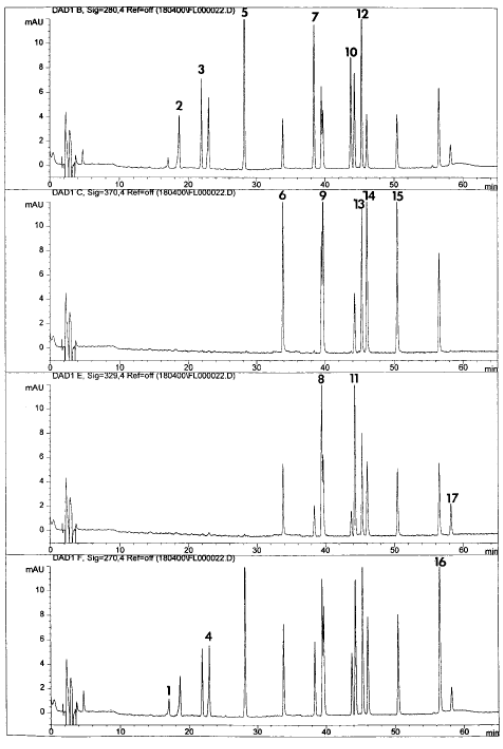
Detection of phenolic compounds in HPLC

- **spectrophotometric detection** (conventional UV/VIS or diode array detectors)
  - absorption of analytes:
    hydroxy-benzoic acids: 270-280 nm
    hydroxy-cinnamic acids: 270-280 nm, 305-330 nm
    coumarines: 220-230 nm, 310-350 nm
    anthocyanins: 500-530 nm
    flavons, flavonols, chalcons: 270-280 nm, 310-390 nm
    isoflavons: 245-270 nm, 300-350 nm
    other flavonoids: 270-280 nm, 310-350 nm
  - post-column derivatization:
    reaction of catechins with *p*-DMCA: detection at 640 nm



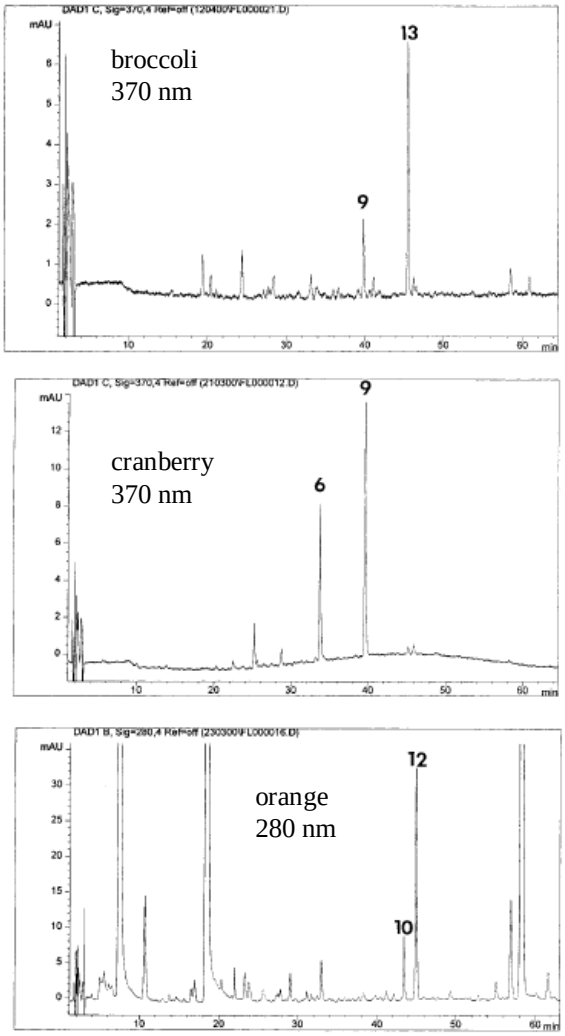
- **fluorimetric detection** – flavonoids λ_{ex} 270-310 nm, λ_{em} 310-360 nm
- **electrochemical detection**
  - amperometric detection – Au or glassy carbon electrode
  - electro-array detector – a series of working electrodes at stepwise values of potential
- **MS**

Example of HPLC separation of flavonoid standards



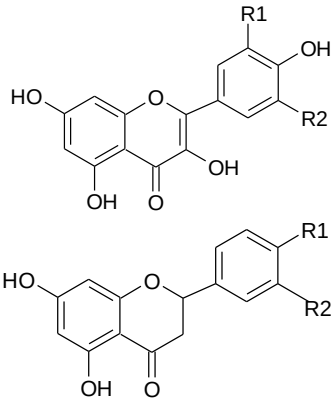
Column: Inertsil ODS-3 (4×150 mm, 3 μm), *t* = 35°C
mobile phase flow rate: 0.7 ml/min
A:50 mM H_3PO_4, pH = 2,5; B: CH_3CN
Programme: start-5 min: 5 % B, 5-55 min: linear gradient from 5 to 50 % B, 55 min –end: 50 % B
injection volume 10 μl

1 epigallocatechin
2 catechin
3 epicatechin
4 epigallocatechingallate
5 epicatechingallate
6 myricetin
7 eridictyol
8 luteolin
9 quercetin
10 naringenin
11 apigenin
12 hesperetin
13 kaempherol
14 isorhamnetin
15 rhamnetin
16 galangin
17 tangeretin



HPLC of flavonoids in food samples
(conditions as in the previous figure)

6 myricetin
9 quercetin
10 naringenin
12 hesperetin
13 kaempherol



Flavonols

myricetin R1=R2=OH
quercetin R1=OH, R2=H
kaempherol R1=R2=H

Flavanons

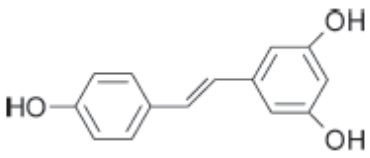
naringenin R1=OH, R2=H
hesperetin R1=OCH_3
R2= OH

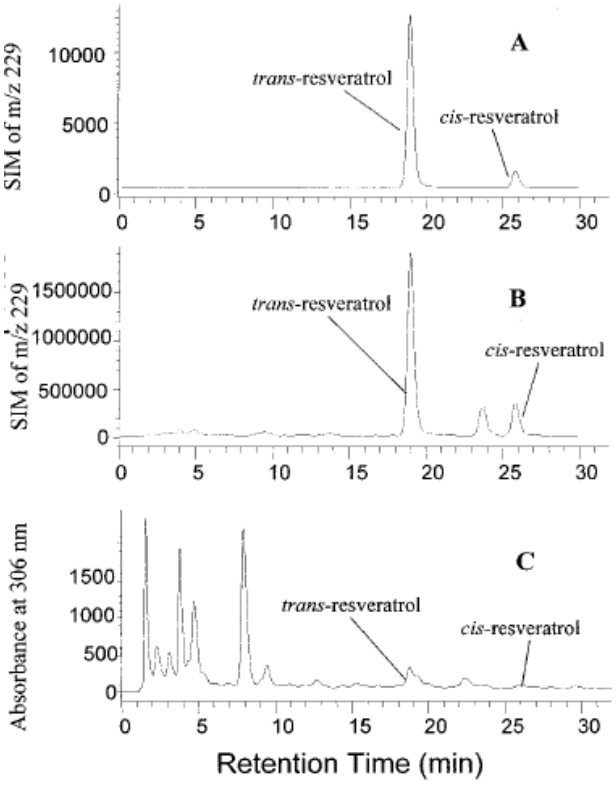
Sample preparation:
extraction 60 % EtOH (+ 2g/l BHA)
in nitrogen atmosphere
hydrolysis 6 M HCl 2 h at 90 °C
dilution in MeOH
filtration 0.2 μm

Importance of phenolic compounds analysis

- plant phenols as possible protective anti-cancer compounds
- phenolic compounds as markers of the crude food material – check of authenticity of food
  - naringin (and naringenin) *vs*. hesperidin (hesperetin) – presence of grapefruit juice in orange juice
  - floridzin or floretin – marker of apple juice
  - arbutin (=hydroquinon-glucoside) – marker of pear
  - chromatographic profiles of anthocyanins – detection of another kind of fruit
- determination of phenols as natural antioxidants or sensory-active compounds (carnosol, carnosic acid, sinapins)

Determination of resveratrol





Comparison of LC/MS (A, B) and LC/UV determination of resveratrol
A – standards of cis and trans resveratrol
B, C – sample of grape juice

Hypersil ODS (2.1×100 mm, 5 μm)
mobile phase: A – 0,5 % HCOOH, B – MeOH
gradient from 25 % B to 39 % B
flow rate 0.25 ml/min

Sample preparation:
homogenization in water
hydrolysis by β-glukosidase
ethylacetate extraction
evaporation to dryness
dissolution of the residue in the mixture of MeOH-0.5 mM HCOOH (7+3)