Plant Secondary Metabolites
-- Phytochemicals

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Which foods are healthier?

- Plant Foods
- Animal Foods
Albrecht Kossel (1891)
- Physiologist, Nobel Prize Laureate 1910 -

**Primary Compounds**

´… are present in all organisms; … life is probably not possible without them …´

**Secondary Compounds**

… may be missing; … are not required and of secondary importance for the maintenance of life …

Historically, in animal physiology no distinction was made between primary and secondary compounds
Plant physiology: superior biosynthetic capability of plants!
Response of Animals, Humans and Plants to Stress

**Impact / Stress**

- **Attack by animals, insects**
- **Infection with micro-organisms**
- **Excessive sunlight**

**Animals**

- Move / fight back / escape / use repellents

**Humans**

- Immune system ↑ / antibiotics
- Seek shade / use sunscreens (H) / wear appropriate clothes (H)

**Plants**

- Produce toxins, bitter compounds, enzyme inhibitors; make proteins unavailable;
- Produce antimicrobially active compounds;
- Produce UV absorbing compounds

(Sessile)
Role of Secondary Metabolites in Plants

- **Phyto-anticipins**
  - Preformed
  - Defence Systems
  - Protection against Pests

- **Phyto-alexins**
  - Induced

- Pigments
- Attractants

- Protection against UV irradiation
Role of Secondary Metabolites in Plants

✓ General protective roles (e.g. as antioxidants, free radical-scavenging, UV light absorbing)

✓ Defend the plant against pathogens (viruses, fungi and bacteria)

✓ **Allelopathic defenders** – defence against other plants for light, water, nutrient and space

✓ Defence against herbivores (insects, vertebrates) as **feeding deterrents** (many phytochemicals are bitter and/or toxic to potential herbivores)

✓ **Attractants** for pollinators and seed dispersing animals

✓ **Signal molecules** in the interactions between plants and their environment (such as legumes and symbiotic microorganisms)
Classification of Secondary Plant Metabolites

- Phenolic compounds
  - Flavonoids, phenolic acids, and others
- Terpenoids
  - Hemiterpenes to polyterpenes
  - Carotenoids
- Alkaloids
  - Nitrogen containing heterocyclic compounds
  - Caffeine
- Cyanogenic glycosides
  - Contain hydrogen cyanide (toxic!)
- Glucosinolates
  - Typical of Brassicaceae
- Non-proteinogenic amino acids
  - Often toxic; others typical of Alliaceae
- Toxic peptides and proteins
  - e.g. ricin in *Ricinus communis* L. (castor bean)

Characteristics of Secondary Plant Metabolites

Chemically very heterogenous (‘playground of evolution’)

Synthesis and storage in specialized compartments (‘excretion tissues’)

Not ubiquitously present (chemotaxonomy!)

Ontogenetic variability

Consequence of mutations
Occurrence of Secondary Metabolites

**ANIMAL KINGDOM**

- Batrachotoxin

**PLANT KINGDOM**

- Resveratrol
- Atropine
- Allicin
- Lycopene
Utilization of Plants

- Source of carbohydrates
- Source of proteins
- Source of lipids
- Fruits
- Vegetables and salads
- Alkaloid containing beverages
- Source of sweeteners
- Spices
  - Fodder
    - Pharmaceuticals
      - Fibre
      - Wood
      - Rubber
      - Resins, balms
      - Wax
      - Pigments
      - Insecticides
      - Energy, fuel

Food Use (Edible Plants)

Feed Use

Medicinal Use

Technical Use
Nutrient vs phytochemical

The role of phytochemicals as protective dietary constituents has become an increasingly important area of human nutrition research.

- Nutrients generally have very specific functions such as being an enzyme cofactor.

- Phytochemicals exhibit pleiotropic effects that in combination may reduce the risk of chronic disease.

Unlike the traditional vitamins, they are not essential for short term well-being, but there is increasing evidence that modest long-term intakes may exhibit a potential for modulating human metabolism in a manner favorable for the prevention or reduction in the risk in the risk of degenerative diseases such as cardiovascular diseases, diabetes, obesity, and cancer (Anderson et al. 1999).
Polyphenols

- Ubiquitous distribution in the plant kingdom
- Secondary plant metabolites
- Skeletal structure: One or more hydroxyl groups are attached to one or more aromatic rings
- High reactivity under certain conditions

Phenol

“Polyphenols”

“Polyphenolics”

“Phenolic compounds”

“Plant phenolics”
Classification of Phenolic Compounds

- **C$_6$-C$_1$ Compounds (Hydroxybenzoic Acids)**
- **C$_6$-C$_3$ Compounds (Hydroxycinnamic Acids)**

**Phenolic Acids**

**C$_6$-C$_1$-C$_6$ Compounds**
- **Xanthones**

**C$_6$-C$_2$-C$_6$ Compounds**
- **Stilbenes**

**C$_6$-C$_3$-C$_6$ Compounds**
- **Flavonoids**
- **Numerous Sub-classes!**
C₆-C₁ Compounds: Hydroxybenzoic Acids

- Salicylic acid: Willow barks
- 4-Hydroxybenzoic acid: Berries
- Protocatechuic acid: Onion/potato skins/dates
- Gallic acid: Mango peels, grapes
Phenolic acids are usually present in the bound form and are typically components of complex structures such as lignins and hydrolyzable tannins. They can also be found as derivatives of sugars and organic acids in plant foods.

Gallagic acid
(4 gallic acids)

Pomegranate

Punicalin
(gallagic acid bound to a glucose)
$C_6$-$C_3$ Compounds: Hydroxycinnamic Acids

- $p$-Coumaric acid
- Caffeic acid
- Ferulic Acid
Frequently found in plant materials (small quantities)

Caffeoylquinic acid

- Artichoke leaf extract: 2.4 - 6 % (dry matter)

Exceptions:
- Coffee (raw, unroasted): 5 - 8 % (dry matter)

Phenolic acid are usually present in the bound forms and are typically components of complex structures (lignins and hydrolyzable tannins).
Principal components in turmeric

- Diferuloylmethane

- Curcuminoids
  - 3 major curcuminoids:
    - Curcumin (R1,R2=OCH₃)
    - Demethoxycurcumin (R1 =H, R2 = OCH₃)
    - Bis-demethoxycurcumin (R1,R2 = H)
You have to drink 100 L of red wine per day to afford protective effects (Corder et al., 2003).
C₆-C₃-C₆ Flavonoid Compounds: comprising of 15 carbons, with 2 aromatic rings connected by a 3-carbon bridge.
Flavanones

- Are mainly represented by naringenin, hesperetin and eriodictyol
- Flavanones are exclusively found in citrus fruits in their glycosidic forms
- The absence of C2,3 double bonds
- The peel is by far the richest part of citrus fruit in terms of the flavanone content
Flavones

- Similar to flavonols and differ only in the absence of –OH at the 3-position on the C-ring
- Not widely distributed and their dietary intake is very low
- Mainly represented by apigenin and luteolin
- Typical sources:

Celery    Parsley    Artichoke
Flavonols

- Most widespread flavonoids in foods
- Accumulation in the outer and aerial tissues / layers (skins, leaves) because their biosynthesis is stimulated by light
- Generally present at relatively low concentrations
- Always in the form of glycosylated conjugates
- Typical sources:
Flavan-3-ols

- **The most common flavonoid** consumed in the American and the Western diet
- Affect food quality such as astringency, bitterness, sourness, sweetness, salivary viscosity, aroma, and color formation
- Found abundantly in fruits such as apricots, sour cherries, grapes, blackberries
- Barley is the only common cereal
- Dark chocolate is a rich source of procyanidins
- Beer and red wine are also rich in flavan-3-ols
- Green tea infusions and black tea

![Chemical structures of flavonoids](image)

- Procyanidin
- (-)-Epicatechin
- Flavonols
- Flavan-3-ols
Flavan-3-ols

- Structurally, the most complex ranging from the simple monomers (cathchin) to oligomeric and polymeric proanthocyanidin (condensed tannins)
- Can undergo esterification with gallic acid to form catechin gallates, and hydroxylation reaction to form gallocatechin.

(-)-epicatechin

(-)-epigallocatechin

(-)-epigallocatechin gallate
Anthocyanins

- Fruit and flower, red, blue, purple
- Located mainly in the skins; less frequently in the flesh
- Glycosidically bound to sugars (→ water solubility, stability)
- Substitution preferably at positions 3 and 5, rarely at position 7
- 6 major aglycones found in foods
- Have a positive charge in their structure at acidic pH

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Anthocyanins in Food

- Rhubarb
- Blueberries
- Grapes
- Red Wine
- Plums
- Blackberries
- Potatoes
- Cherries
- Red Cabbage
- Eggplants
- Elderberries
- Strawberries
$C_6-C_3-C_6$ Compounds: Flavonoids

(Jaganath and Crozier 2010)
Isoflavones

- Having the B-ring attached at C3 rather than the C2 position
- Found only in leguminous species
- Well known for their estrogenic activity due to their ability to bind to estrogen receptor
- Have putative role in the prevention of breast cancer and osteoporosis

Typical sources:

Soybean  Red clover  Kudzu
Previous Opinions about the Role of Phenolic Compounds in Foods and Feeds

**Antinutritive Effects**

- Reduction of protein bioavailability
- Inhibition of digestive enzymes (proteases, lipases, amylases)
- Toxic

**Undesired from a Technological Point of View**

- Turbidity (haze formation)
- Discoloration / browning
- Astringency
- Bitterness

1986: “Flavonols – Mutagens in our daily diet”

Mutagenic Flavonol Aglycones in Infusions and in Fresh and Pickled Vegetables

M. FIESCHI, A. CODIGNOLA, and A.M. LUPPI MOSCA
Trend: Polyphenol-rich Foods – Good for Our Health?

-The “evergreens”: Green tea and red wine
  - Green tea rich in flavanols
  - Role in weight loss, cardiac health, arthritis, skin protection; beneficial effects under conditions of stress discussed
  - Protection against some forms of cancer?

- Red wine rich in anthocyanins, flavonol glycosides, resveratrol, phenolic acids, procyanidins
  - Reduced risk of cardiovascular disease through moderate wine consumption
  - Role of alcohol vs. role of polyphenols?

“... it would seem reasonable to recommend that patients who currently drink try to move towards moderate consumption of wine over that of beer and spirits.”

Lindberg & Amsterdam, 2008
Trend: Polyphenol-rich Foods – Good for Our Health?

- The “shooting stars”: Cocoa and coffee
  - Cocoa rich in flavanols (catechin, epicatechin), dimeric and polymeric pro-cyanidins
  - High antioxidant activity in vitro
  - Improved antioxidant status, reduced inflammation, correlation with reduced heart disease risk
  - Cocoa polyphenols vs. other components of cocoa; synergistic effects?
  - Cocoa vs. chocolate (high calorie food!)

- Coffee rich in caffeic and chlorogenic acids
- Coffee consumption associated with reduced risk for type 2 diabetes, gallstones, CVD, colon cancer, Parkinson’s disease; improvement of cognitive function and endurance performance
- Adverse effects of caffeine for people with hypertension!
The Touch of Exoticism: Tropical Fruits and “Superfruits”

- Certain nutrient-rich fruits which have a high antioxidant activity and an appealing taste and/or flavour
- Blueberry, pomegranate, cranberry, noni fruit, mangosteen, seabuckthorn, goji, açaí, and others
- Usually rich in vitamin C, carotenoids, anthocyanins and/or other phenolic compounds
Trend: Increasing Polyphenol Levels in Plants

- **Breeding**
  - Long-term approach

- **Genetic engineering**
  - More selective but still not a rapid solution
  - Sustainability of genetic manipulations?
  - Consumer acceptance?

- **Stressing plants by biotic and abiotic elicitors**
  - “Phytoalexin-enriched functional foods”
  - Enhancing bioactives in medicinal plants

  - Boue et al. (2009)
  - Kuzel et al. (2009)
Trend: Extraction of Polyphenols from By-Products of Food Processing

- Polyphenols located primarily in outer layers of fruits and vegetables
- Often removed during processing
  - Peeling, e.g. potatoes
  - Pressing, e.g. apples, grapes
- Alternate route that can be realized in a more time-efficient manner
- Prerequisites
  - Immediate processing (microbial spoilage!) or drying
  - Development of economically feasible processes
    - Co-extraction of valuable components other than polyphenols?
Carotenoids

- Most widespread colored compounds in nature
- Great variety of compounds and colors: yellow-orange-red (green-blue: associated with proteins)
- De novo biosynthesis
  - Plants
  - Algae (Dunaliella, Hematococcus)
  - Fungi (Phycomyces, Neurospora)
  - Bacteria (Flavobacterium, Halobacterium)
  - > 100 million tons per year
- Animals cannot biosynthesize carotenoids!
  - Taken up with food (food chain)
  - Selective absorption/metabolism
- Chemically, carotenoids are terpenoid compounds → Lipophilic
Carotenoids in the Food Chain

**Biosynthesis**
(de novo)

- Microalgae
- Macroalgae
- Higher Plants
- Bacteria
- Fungi

**Metabolism**
(Herbivores)

- Animal A

**Metabolism**
(Carnivores)

- Animal B

**Higher Plants**

- Birds
- Insects

**Fungi**

**Micro-/Macro-algae**

- Marine Invertebrates
- Zooplankton
- Fish
Classification of Carotenoids and Selected Members

**Carotenes**

- **α-Carotene**
- **β-Carotene**
- **γ-Carotene**
- **ε-Carotene**
- **ζ-Carotene**
- **Lycopene**

**Xanthophylls**

- **Lutein**
- **Zeaxanthin**
- **Astaxanthin**
- **Canthaxanthin**
- **Violaxanthin**
Important Sources of Carotenoids in Human Nutrition

- **Capsanthin / Capsorubin**
- **β-Carotene**
- **Lutein / Zeaxanthin**
- **Lycopene**
- **Astaxanthin**
Effects of Food Matrix and Processing on the Bioavailability of Carotenoids

**Very high bioavailability**

- Formulated natural or synthetic carotenoids
- Natural or synthetic
- Papaya, peach, melon
- Sweet potato
- Tomato
- Carrots, peppers
- Tomato
- Carrots, peppers
- Spinach

- Formulated carotenoids in water-dispersible beadlets
- Carotenoids - oil form
- Fruits
- Tubers
- Processed juice with fat containing meal
- Mildly cooked yellow/orange vegetables
- Raw juice without fat
- Raw yellow/orange vegetables
- Raw green leafy vegetables

**Very low bioavailability**
Difference in bioavailability of carotenoids between fruits and vegetables

- In dark green leafy vegetables, carotenoids are contained in chloroplasts in crystalline form. In orange and yellow fruits, carotenoids are dissolved in oily droplets within the chromoplast structure.

- Carotenoids dissolved in oily droplets are more likely to be solubilized by the digestive milieu during intestinal transit.

- Carotenoids contained within chloroplasts, in crystalline sheets, or complexed to proteins may not be as accessible to the digestive processes that render these compounds bioavailable.

- Disruption of the food matrix will improve carotenoid bioavailability.

- Disruption must be balanced so that processing severity does not result in excessive degradative loss of carotenoids through heat or oxidation.
Physiological Functions and Health Benefits of Carotenoids

- **Provitamin A activity** (only ca. 50 out of >600 carotenoids)
  - β-Carotene, α-carotene, β-cryptoxanthin

- **Antioxidant activity**
  - e.g. Lycopene: effective quencher of singlett oxygen

- **Light filtering activity**
  - Lutein, zeaxanthin: protective role in age-related macular degeneration

- **Intercellular communication**
  - Increase in expression of the gene encoding a connexin protein

- **Cancer protective activities**

- **Protection against cardiovascular disease**
Age Related Macular Degeneration (AMD)

Disease that affects the central vision and a common cause of vision loss among people over age of 60

Slowly progressing loss of viable photoreceptors in the macula
New blood vessels begin to grow behind the retina and move into the macula where they are usually not seen; they tend to leak blood and fluid under the macular region, a process which quickly damages the normal macular function
Progress of AMD can even physically lift the macula forward in uneven ridges, causing the patient to have a distorted and stretched visual field

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Amsler Grid
β-Carotene and Health

- Quenching of oxygen-containing free radicals / singlet oxygen
- Regeneration of the antioxidant form of vitamin E (not vitamin C!)
- Scavenging of free radicals in human LDL and HDL as well as in cell membranes
- Protection of vitamin E from oxidation in lipoproteins
- Synergistic actions of β-carotene, vitamin E and vitamin C in the protection of lipids in membranes
- Enhancement of lymphocyte proliferation independent of its provitamin A action
- β-Carotene supplementation
  - inhibited UV-induced tumor formation in the mouse skin cancer model
  - resulted in significant increase in HDL
- Epidemiological evidence: inverse association between carotenoid intake and cancer risk
- ATBC and CARET trials: β-Carotene – „pro-“ or anti-carcinogen? Oxidation products of carotene as carcinogens??

### ATBC Study: α-Tocopherol β-Carotene Cancer Prevention Study

- **Size**: 29,133
- **Dosage**: 20 mg β-carotene ± 50 mg vitamin E
- **Duration**: 6 years
- **Cancer Site**: Lung, colorectum, prostate, bladder, others
- **Outcome**: Final lung cancer incidence was 16% higher in the β-carotene group. Prostate and stomach cancer incidence were also 23 and 25%, respectively, higher in the β-carotene group.

### CARET Study: β-Carotene and Retinol Efficacy Study

- **Size**: 18,314 (former) smokers, workers exposed to asbestos
- **Dosage**: 30 mg β-carotene + 25,000 IU vitamin A
- **Duration**: 4 years
- **Cancer Site**: Lung, breast colorectum, prostate
- **Outcome**: Increase in lung cancer incidence (28%) and total mortality (17%).
Lycopene

- Linear, highly unsaturated hydrocarbon containing 13 double bonds, 11 of which are conjugated

- **Physical properties:**
  - Molecular formula: $\text{C}_{40}\text{H}_{56}$
  - Molecular weight: 536.85 Da
  - Melting point: 172–175°C
  - Crystal form: Long red needles separate from a mixture of carbon disulfide and ethanol
  - Powder form: Dark reddish-brown
  - Solubility:
    - Soluble in chloroform, hexane, benzene, carbon disulfide, acetone, petroleum ether and oil
    - Insoluble in water, ethanol and methanol
  - Stability: Sensitive to light, oxygen, high temperatures, acids, catalysts and metal ions

- Crystalline state bright red colour typical of fresh tomatoes
- Usually present in plants in all-\textit{trans} configuration
- Light, high temperatures, catalysts, oxygen and redox-active metal ions ($\text{Cu}^{++}$, $\text{Fe}^{+++}$): Isomerization and oxidation
- Highest antioxidative capacity among all naturally occurring carotenoids (quenching of singlet oxygen)
Lutein

- Xanthophyll (β-carotene-3,3’-diol)
- Isomer: zeaxanthin (α-carotene-3,3’-diol)
- Abundantly present in dark, leafy green vegetables such as spinach and kale
- Marigold flowers: lutein esterified with fatty acids
  - Extracts used for fraudulent purposes in orange juices
- Second most prevalent carotenoid in human serum
- Antioxidant activity in both plants and humans
- Health benefits associated with lutein:
  - Heart health: reduces risk of atherosclerosis
  - Skin: reduces UV-induced damages
  - Eye health ("the eye-protective nutrient")

Purified lutein crystals from *Tagetes erecta*