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Bioactive compounds in food plants and their impact on human health

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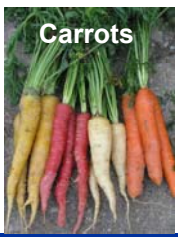


Bioactive natural products in plants

Plants produce secondary metabolites mainly for the protection against attack from microorganisms, herbivores etc.

> 200.000 secondary metabolites have so far been isolated from plants. **Potential bioactive compounds**.

Plants can contain > 1.000 secondary metabolites.

Bioactive secondary metabolites from plants:

Fatty acid derivatives (e.g., glycolipids such as galactolipids, fatty acids, polyacetylenes).

Origin: Acetate pathway.

Terpenoids and **steroids**. ***Origin***: Build up by isoprene units (C_5).

Polyphenols (e.g., flavonoids, isoflavones, anthocyanins, stilbenes, phenolic acids, lignans).

Origin: shikimic acid and/or acetate pathway.

Alkaloids. ***Origin***: Derived from amino acids + other pathways

Glucosinolates and **cyanogenic glycosides**. ***Origin***: Derived from amino acids.

Characteristics of bioactive natural products – Mode of action

✓ **Bioavailability**
Absorption from the gastrointestinal tract into plasma. **Bioactive compounds** has to be **relative hydrophobic** to be absorbed through the lipophilic cell membrane layers.

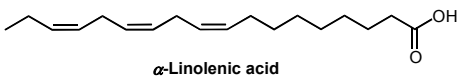
✓ **Binding to specific receptors**
(i) Size and shape resemblance those of endogen metabolites being able to bind to specific receptors.
(ii) Induction/inhibition of specific genes/enzymes/metabolites resulting in a physiological response.

✓ **Inhibition of ion-channels and disruption of cell membranes**

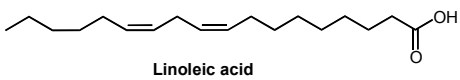
✓ **Antioxidant activity**
(i) **Direct antioxidant activity**. Redox active compounds that inactivate reactive oxygen species (ROS). Particular relevant in the prevention of cardiovascular diseases.
(ii) **Indirect antioxidant activity.** Compounds (not necessarily redox active), which induces the production of cytoprotective proteins (phase 2 enzymes).

✓ **Covalent binding to biomolecules**
Apoptosis (programme cell death), inhibition of cell proliferation, inhibition of metabolic processes, immune system stimulation.

Bioactive compounds in food plants – Fatty acid derivatives

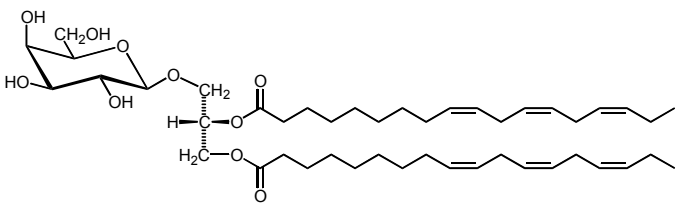


α-Linolenic acid



Linoleic acid

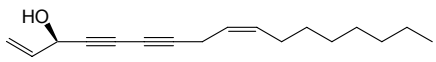
Bioactivity: Essential fatty acids. Precursor for **prostaglandins** that are important **regulators of metabolic processes**. ***Source***: Rapeseed, soya and flaxseed oil and many other plants.



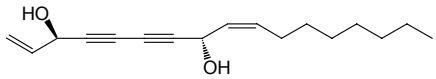
Monogalactosyldiacylglycerol (galactolipid)

Bioactivity: **Anti-inflammatory** and **anticancer** activity *in vitro*. *In vivo* activity unknown (bioavailable?). ***Source***: Mainly green vegetables.

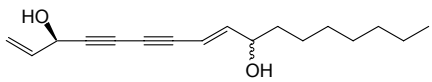
Bioactive compounds in food plants – Fatty acid derivatives



Falcarinol (= Panaxynol)



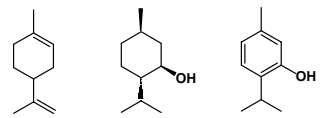
Falcarindiol



Panaxydiol

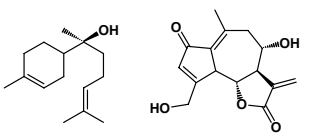
Bioactivity: **Anti-inflammatory**, **anticancer**, **anti-platelet-aggregatory**, **anti-bacterial, anti-fungal** and more. ***Mode of action***: Covalent-binding to biomolecules (strong alkylating agents). ***Source***: Food plants of the Apiaceae (Umbelliferae) family.

Bioactive compounds in food plants – Terpenoids



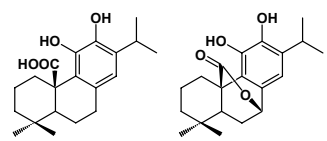
Limonene **(–)-Menthol** **Thymol**

Monoterpenes (C_{10})



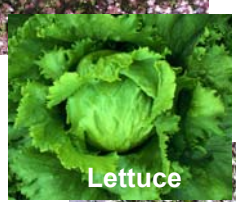
(–)-α-bisabolol **Lactucin**

Sesquiterpenes (C_{15})



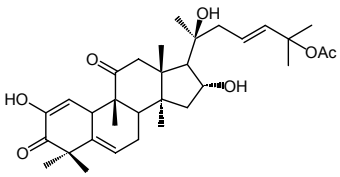
Carnosoic acid **Carnosol** **Steviol**

Diterpenes (C_{20})



Bioactivity: **Anti-inflammatory**, **anti-bacterial, anti-fungal**, and **anti-diabetic** activity. ***Mode of action***: Disruption of membranes, covalent binding to biomolecules, and binding to specific receptors. ***Source***: Mainly herbs and spices.

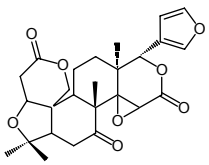
Bioactive compounds in food plants – Triterpenoids



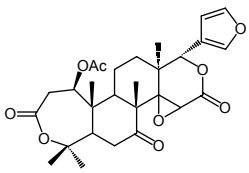
Cucurbitacin E

Cucurbitacins present in cucumber, melon, and marrow. **Bitter tasting**, **purgative** and extremely **cytotoxic**.

Limonoids present in citrus fruits, seeds, and juice. **Bitter tasting** and **antifeedant activity**. **Anticancer effect** and prevent **cardiovascular diseases** (reduces LDL cholesterol levels).



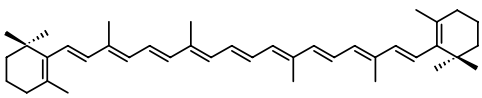
Limonin



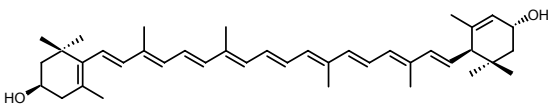
Nomilin

Bioactive compounds in food plants – Tetraterpenes

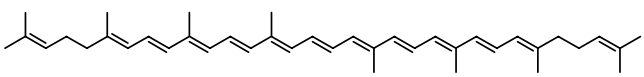
Carotenoids are widespread in plants and have **provitamin A** activity, **antioxidant** capacity, and possible **anticancer effect** (e.g., lycopene)



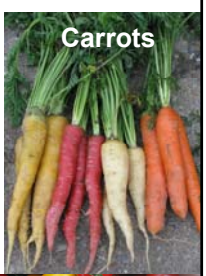
***β*-Carotene** (orange)



Lutein (yellow)

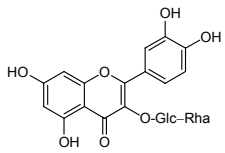


Lycopene (red)

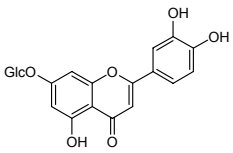


Tetraterpenes (Carotenoids, C_{40})

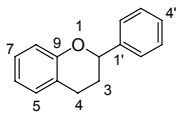
Bioactive compounds in food plants – Flavonoids



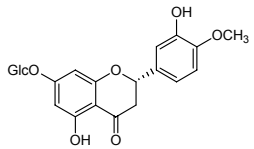
Quercetin 3-*O*-rutinoside (rutin, flavonol)



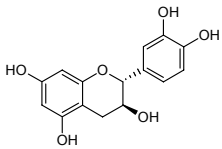
Luteolin 3-*O*-glucoside (flavone)



C_6-C_3-C_6 flavonoid skeleton



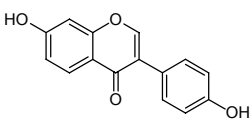
Hesperetin 7-*O*-rutinoside (flavanone)



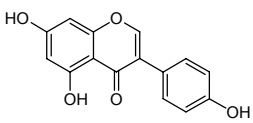
(+)-Catechin (flavan-3-ol)

Bioactivity: **Antioxidants**. Anti-inflammatory, anticancer and immunostimulatory effect and prevention of cardiovascular diseases? **Glycosides low bioavailability**.
Source: Widely distributed in plants.

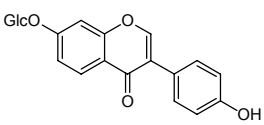
Bioactive compounds in food plants – Isoflavones



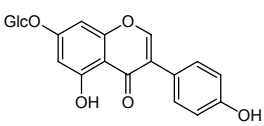
Daidzein



Genistein



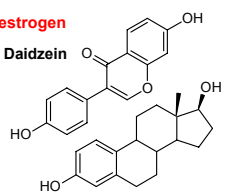
Daidzein 7-*O*-glucoside



Genistein 7-*O*-glucoside

Phyto-oestrogen

Daidzein

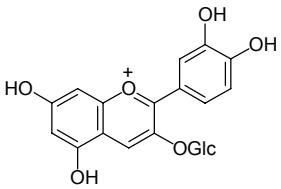


Oestradiol

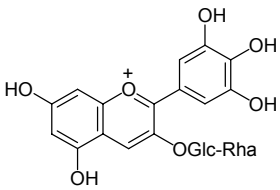
Steroidal oestrogen

Bioactivity: **Regulates oestrogenic response** being able to bind to oestrogen receptors. Prevent the incidence of **breast cancer** and **prostate cancer**. Effect against **type 2 diabetes**?
Source: Legumes (in particular soybeans).

Bioactive compounds in food plants – Anthocyanins



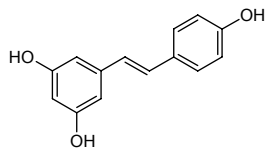
Cyanidin 3-*O*-glucoside



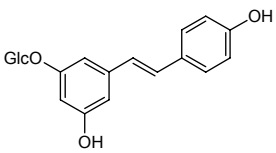
Delphinidin 3-*O*-rutinoside

Bioactivity: **Antioxidants**. Anti-inflammatory and anticancer activity and prevention of cardiovascular diseases? **Low bioavailability**. ***Source***: Widely distributed in plants and present in high concentrations in berries.

Bioactive compounds in food plants – Stilbenes



***trans*-Resveratrol**

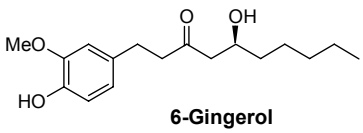


***trans*-Resveratrol 3-*O*-glucoside**

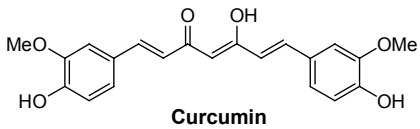
Bioactivity: **Antioxidant**, **anti-inflammatory** and **anticancer** activity. Prevent the development of **cardiovascular diseases** (inhibiting LDL-oxidation and platelet aggregation). ***Source***: Grapes, wine, soya and peanut products and in the Itadori plant (roots used to produce tea in Asia).

Bioactive compounds in food plants – Gingerols and Curcuminoids

Bioactivity: **Anti-inflammatory, antiulcer** and **anticancer** activity. ***Source***: Ginger family (Zingiberaceae)

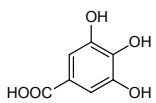


6-Gingerol

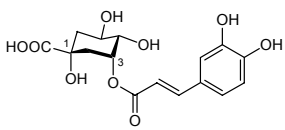


Curcumin

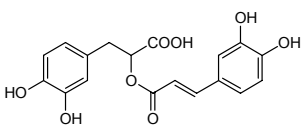
Bioactive compounds in food plants – Phenolic acids



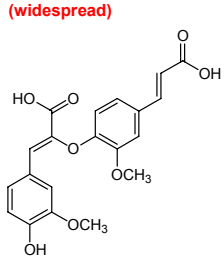
Gallic acid
(e.g., red grapes)



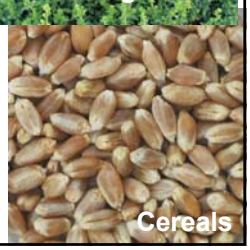
3-*O*-Caffeoylquinic acid
(widespread)



Rosmarinic acid
(herbs and spices)

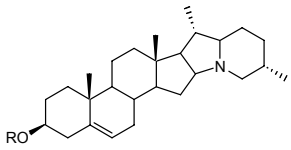


8-O-4' Diferulic acid
(cereals)



Bioactivity: **Antioxidants**. Prevents the development of cardio-vascular diseases. Anticancer and immunostimulatory effect? Bioavailable?

Bioactive compounds in food plants – Alkaloids



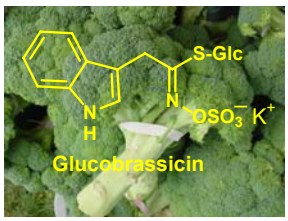
α-Solanine, R = Glucose, Rhamnose, Galactose–

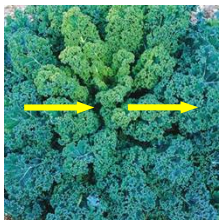
α-Chaconine, R = Glucose, Rhamnose, Galactose–

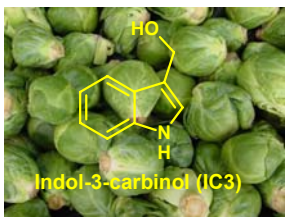
Glycoalkaloids (steroidal alkaloids)

Bioactivity: **Toxic to humans** in high concentrations. Potential **anticancer effect** in low concentrations. ***Source***: Many plants of the genus *Solanum* (Solanaceae), including potatoes and tomatoes.

Bioactive compounds in food plants – Glucosinolates

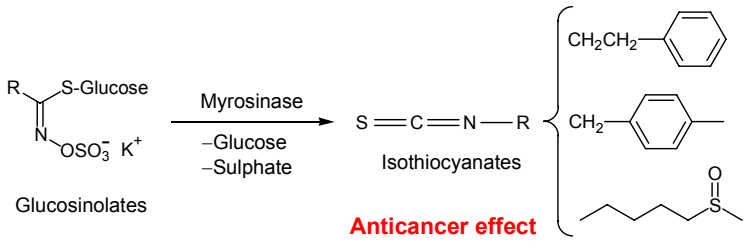






Anticancer effect

- Potent inducers of detoxification enzymes resulting in excretion of potential carcinogens prior to harmful effects. Removal of xenobiotics.
- Antiproliferative effects on cancer cells (apoptosis).
- Phyto-oestrogenic effect. Prevention of breast/prostate cancer (IC3).



✓ **State of the art within the field of bioactive compounds in food plants**

– Direct antioxidants (especially glycosides) are not major contributors to the health effects of plant-based foods due to: (**i**) low bioavailability, (**ii**) metabolization and absorption *in vivo* unclear, (**iii**) general low bioactivity compared to other bioactive compounds etc.

– Health effects of plant-based foods are most likely due to the presence of bioactive compounds with bioactivity unrelated to antioxidant activity.

✓ **Hypotheses**

Bioactive secondary metabolites with different bioactivities and mode of actions are major contributors to the health promoting effects of plant-based foods.

✓ **Future work**

(**i**) Focus on identification of highly bioactive compounds with effects towards specific diseases and their possible mode of action.

(**ii**) Bioavailability and metabolization of bioactive compounds.

Bioactive compounds in plants: Anticancer effect and other health promoting effects of carrots

✓ Carrots are used for foods all over the world and have been used in centuries in traditional medicine in the West.

✓ Carrot is closely related with food plants and medicinal plants of the Apiaceae and Araliaceae plant families that are known to possess important pharmacological effects such as:

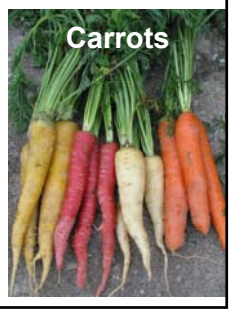
Anti-inflammatory effect

Anticancer effect

Anti-platelet-aggregatory effects

Anti-diabetic effects

..... and many other pharmacological effects



Anticancer effect of carrots

- ✓ Epidemiological studies have shown that the intake of vegetables rich in α- and β-carotene, and/or a high level of β-carotene in blood samples, is correlated with a reduced risk of cancer.
- ✓ Intake of α- and β-carotene is closely correlated with a high intake of carrots.
- ✓ **Human intervention studies**: supplements of β-carotene increased the incidence of cancer!

↓

'β-Carotene paradox'

↓

Do carrots contain other potential substances with the ability to prevent cancer?

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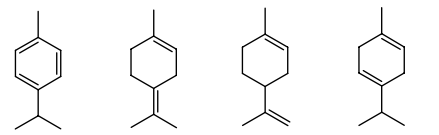
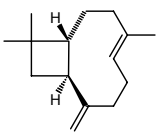
Potential bioactive compounds of carrots

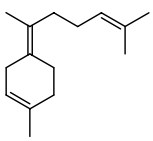
Monoterpenes



p-Cymene | Terpinolene | Limonene | γ-Terpinene



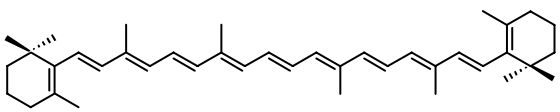
β-Caryophyllene



(*E*)-γ-bisabolene

Sesquiterpenes

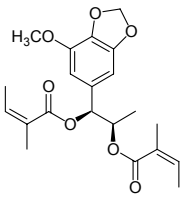
Tetraterpenes



β-Carotene

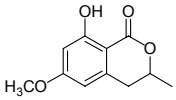
Potential bioactive compounds of carrots

Cytotoxic



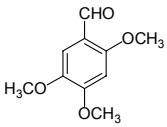
2-Epilaserine

Anti-fungal



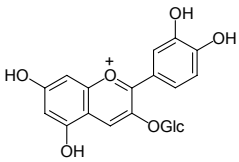
6-Methoxymellein

Anti-inflammatory



Gazarin

Anti-inflammatory?
Antioxidant?



Cyanidin 3-*O*-glucoside

+ other cyanidin derivatives

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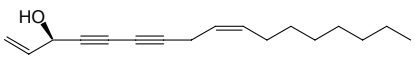
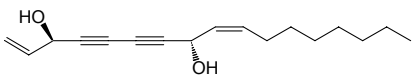
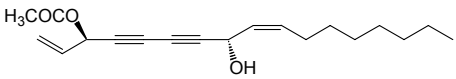
Potential bioactive compounds of carrots



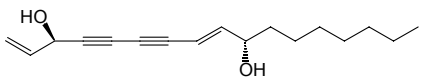
Falcarinol (= Panaxynol)



Falcarindiol



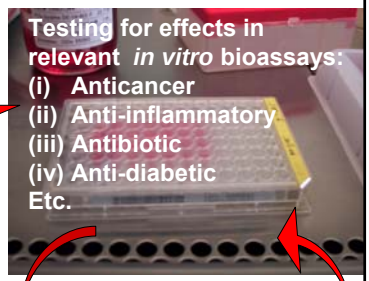
Falcarindiol 3-acetate



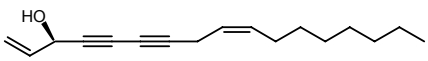
Panaxydiol

Bioassay-guided fractionation for identification of potential anticancer principles of carrots

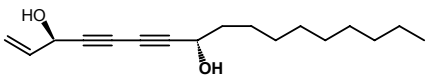
Processing/extraction



Fractionation and testing



Falcarinol (= Panaxynol)

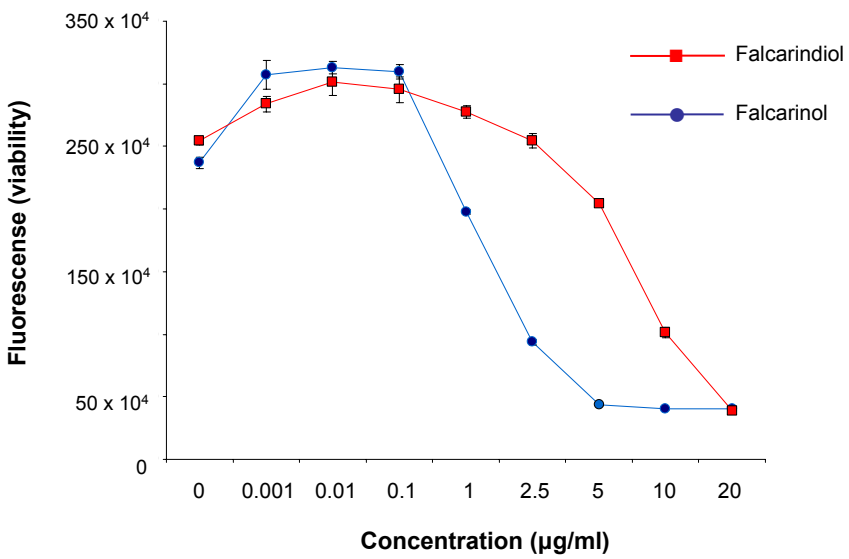


Falcarindiol

Potential anticancer and anti-inflammatory principles in carrots

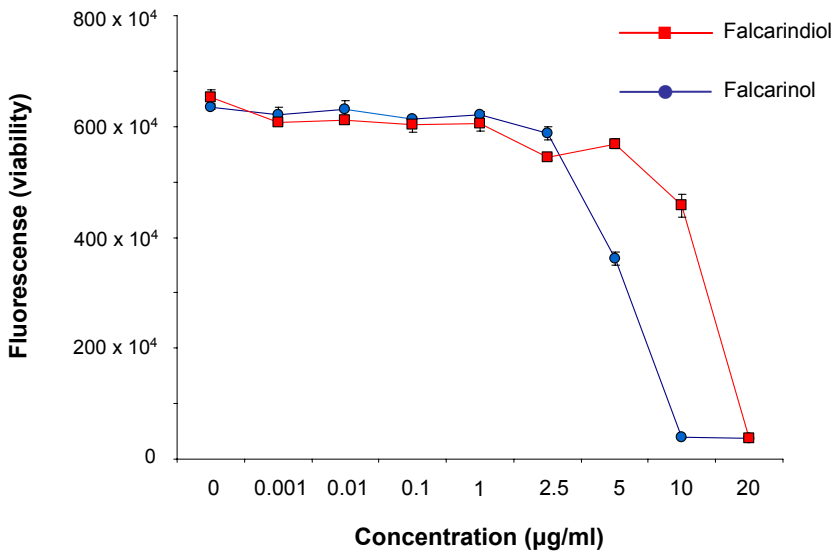
+

Effect of falcarinol and falcarindiol on the proliferation of human colon cancer cells (Caco-2)



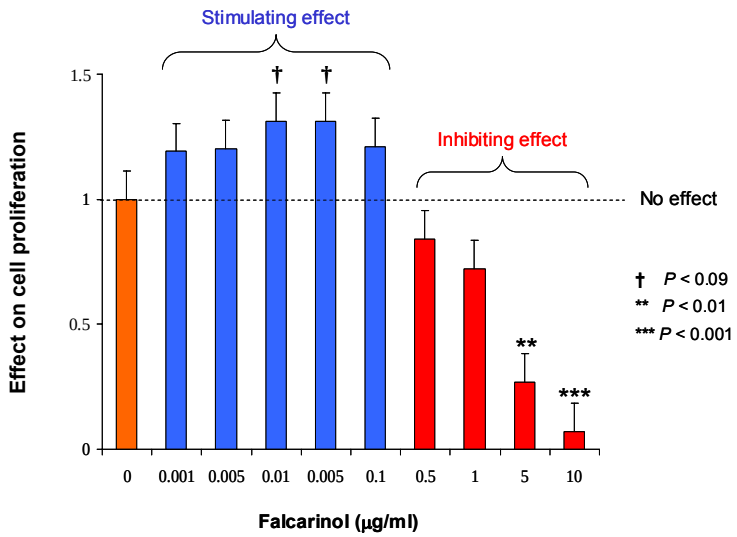
Larsen, Purup & Christensen (2009). *J. Agric. Food Chem.* **57**, 8290-8296

Effect of falcarinol and falcarindiol on the proliferation of normal small intestinal cells



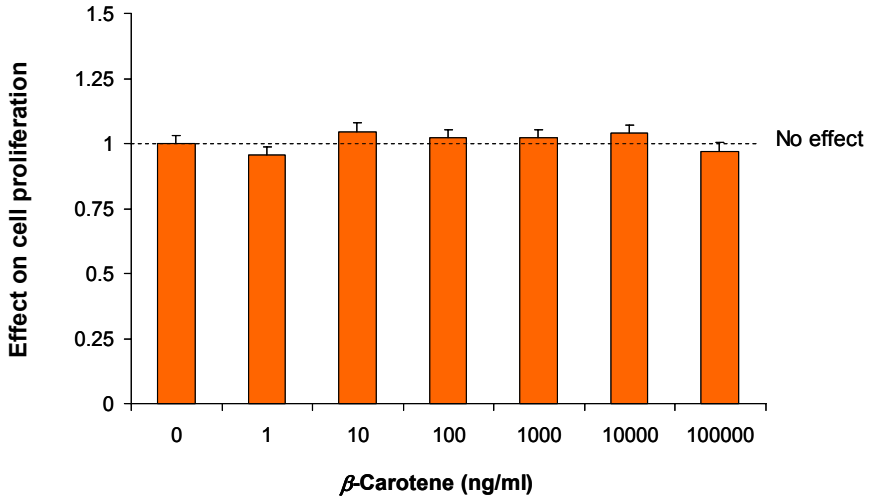
Larsen, Purup & Christensen (2009). *J. Agric. Food Chem.* **57**, 8290-8296

Effects of falcarinol on cell proliferation of normal cells and Caco-2 cells



Hansen *et al.* (2003). *J. Sci. Food Agric.* **83**, 1010-1017; Young *et al.* (2007). *J. Agric. Food Chem.* **55**, 618-623

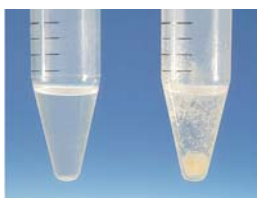
Effects of β-carotene on cell proliferation of normal and Caco-2 cells



Hansen *et al.* (2003). *J. Sci. Food Agric.* **83**, 1010-1017

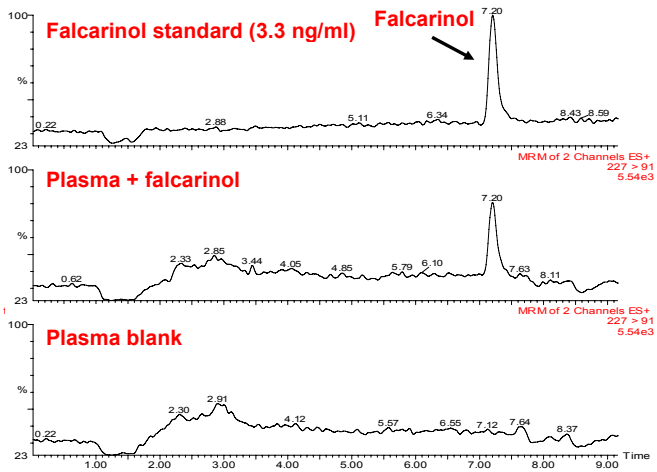
Bioavailability of polyacetylenes determined by multiple reaction monotioring (MRM) LC-MS/MS

Plasma samples



Proteins removed by precipitation with acetonitrile

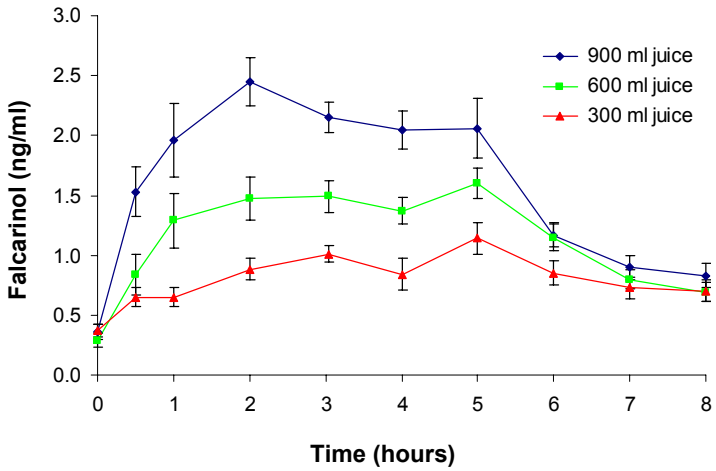
m/z 227 $[M + H - H_2O]^+$ — Mass transition → *m/z* 91



Christensen & Brandt (2006). *J. Pharm. Biomed. Anal.* **41**, 683-693

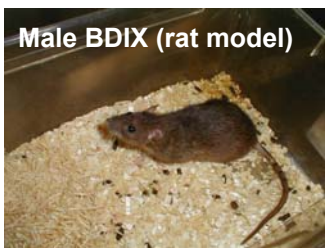
Bioavailability studies of falcarinol in humans

Concentration of falcarinol in plasma as function of time after ingestion of a breakfast meal consisting of carrot juice (13 mg/L). n = 14.

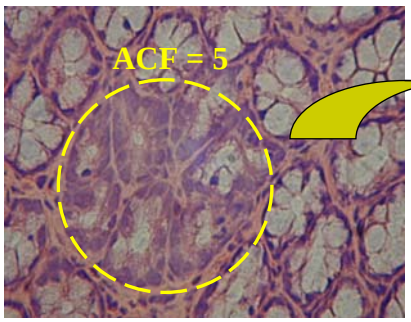


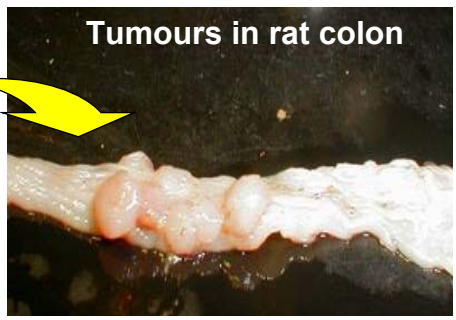
Brandt *et al.* (2004). *Trends Food Sci. Technol.* **15**, 384-393

Potential anticancer effect of carrots and polyacetylenes demonstrated in rats induced with colon cancer

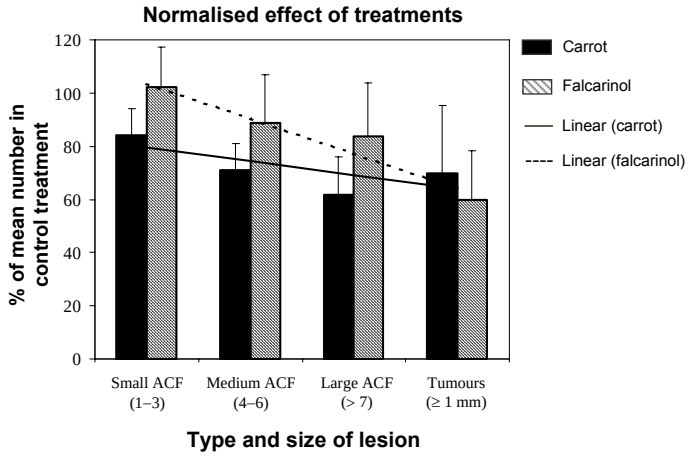


ACF (»Aberrant Crypt Foci«)
= biomarkers for advanced steps on the progression towards cancer





Effect of treatments with carrot or falcarinol in physiological relevant concentrations of four types of (pre)cancerous lesions in rat colons



Increasing steps on the progression towards cancer. The trend for reducing the size of lesion was significant (P = 0.028)

Kobæk-Larsen *et al.* (2005). *J. Agric. Food Chem.* **53**, 1823-1827

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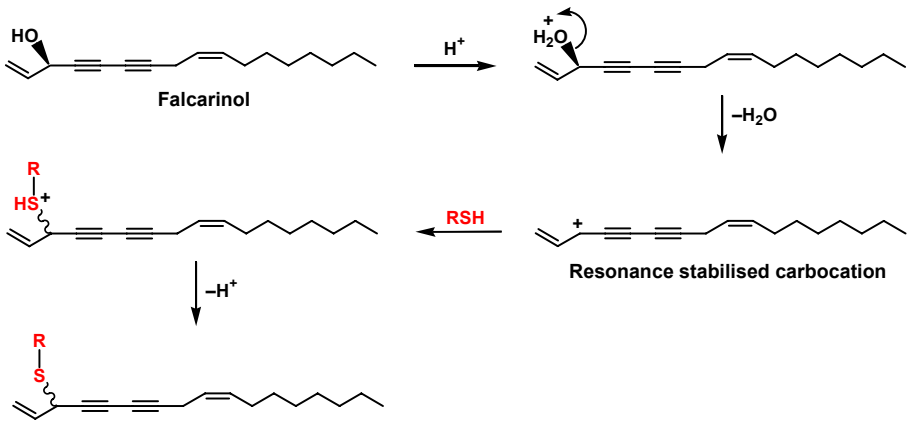
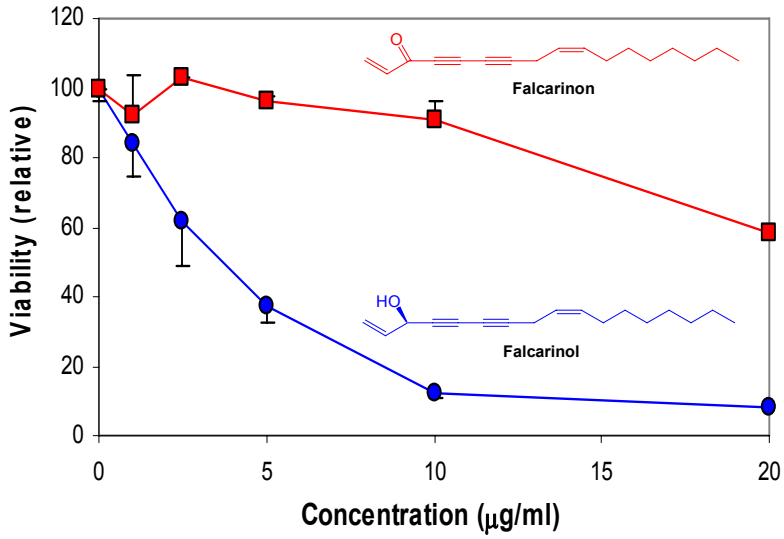
Falcarinol-type polyacetylenes strong alkylating agents



Falcarinol coupled to biomolecules (e.g., a protein)

Effect of falcarinol and falcarinon on the proliferation of human colon cancer cells (Caco-2)



Larsen, Purup & Christensen (2009). *J. Agric. Food Chem.* **57**, 8290-8296

Health promoting effects of falcarinol-type polyacetylenes probably related to their lipophilic and alkylating properties

- **Immunomodulatory effect:** Enhanced production of T-lymphocytes and macrophages through interaction with proteins, e.g., formation of hapten-protein complexes (antigens).

- **Inactivation of proteins/enzymes:**
  - (**i**) Inhibition of enzymes responsible for the proliferation of cancer cells such as COX-2 and nuclear factor κB. **Anti-inflammatory activity!**
  - (**ii**) Inhibition of COX-1 and COX-2 related to the anti-inflammatory effect of falcarinol-type polyacetylenes, and hence also their possible anti-platelet aggregatory effects. Regulation of prostaglandin production.

- **Apoptosis (programme cell death).**
  - (**i**) DNA damage.
  - (**ii**) Induction of cell cycle arrest.
  - (**iii**) Cell damage caused by increased production of macrophages.

..... and many other possible explanations.

Thank you for your attention!