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Phenolic compounds in strawberry **fruits**

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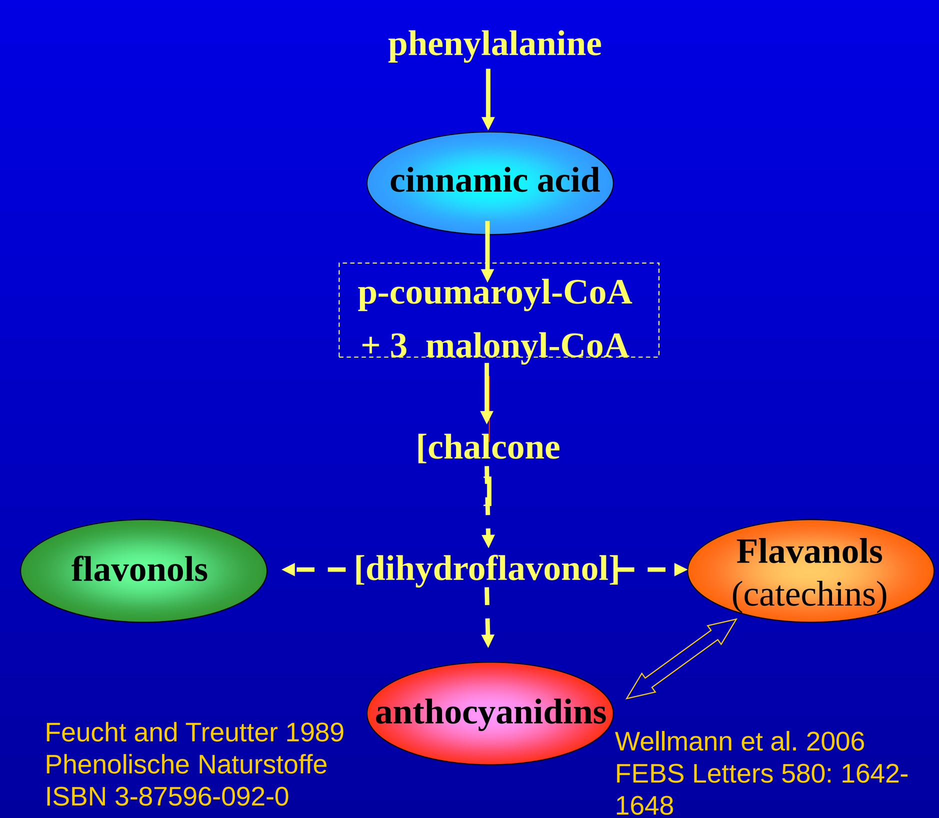
INTRODUCTION:

Phenylpropanoids are known to be involved in defense mechanisms of plants against various pathogens. Furthermore, phenolics become more and more popular as internal quality parameters because of their health promoting effects. A prerequisite to understand both activities is the identification of their structures, to estimate their contents in plants, their dynamic during organ development and localization.

This attempt was made on strawberries (*Fragaria x ananassa*) which are the most important commercial berry fruits in Germany.

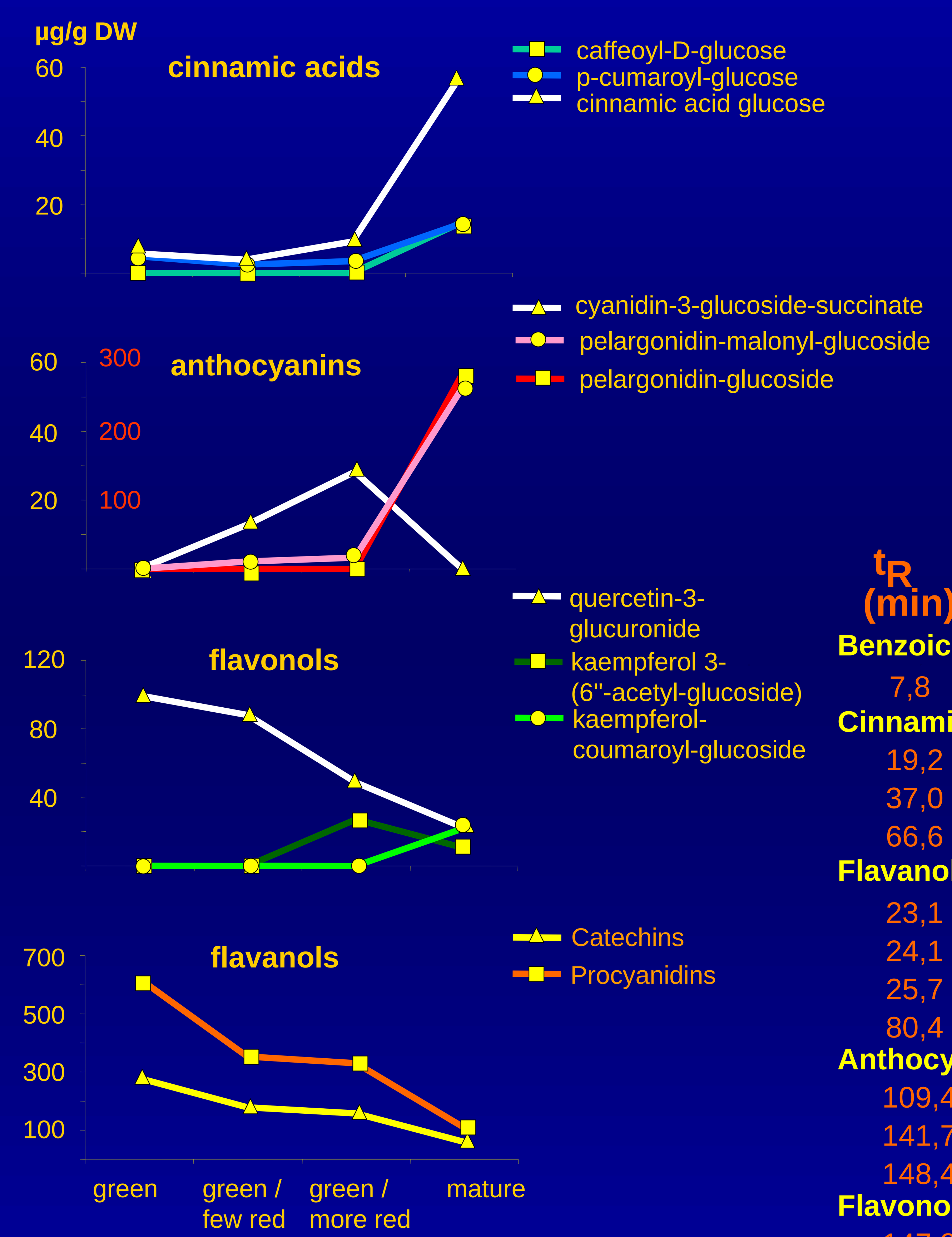


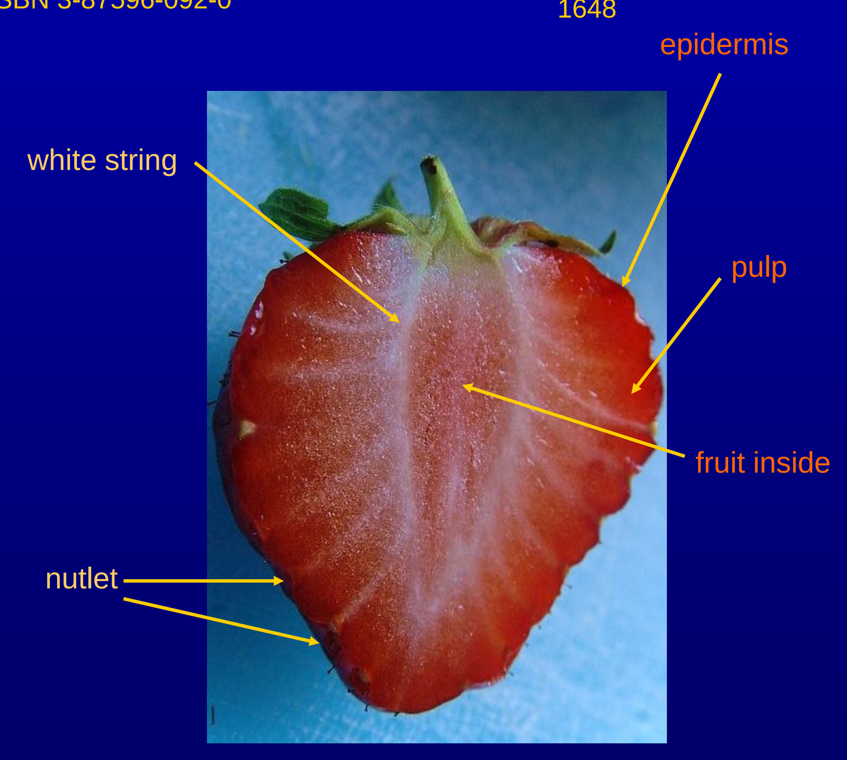
Feucht and Treutter 1989
Phenolische Naturstoffe
ISBN 3-87596-092-0

Wellmann et al. 2006
FEBS Letters 580: 1642-1648

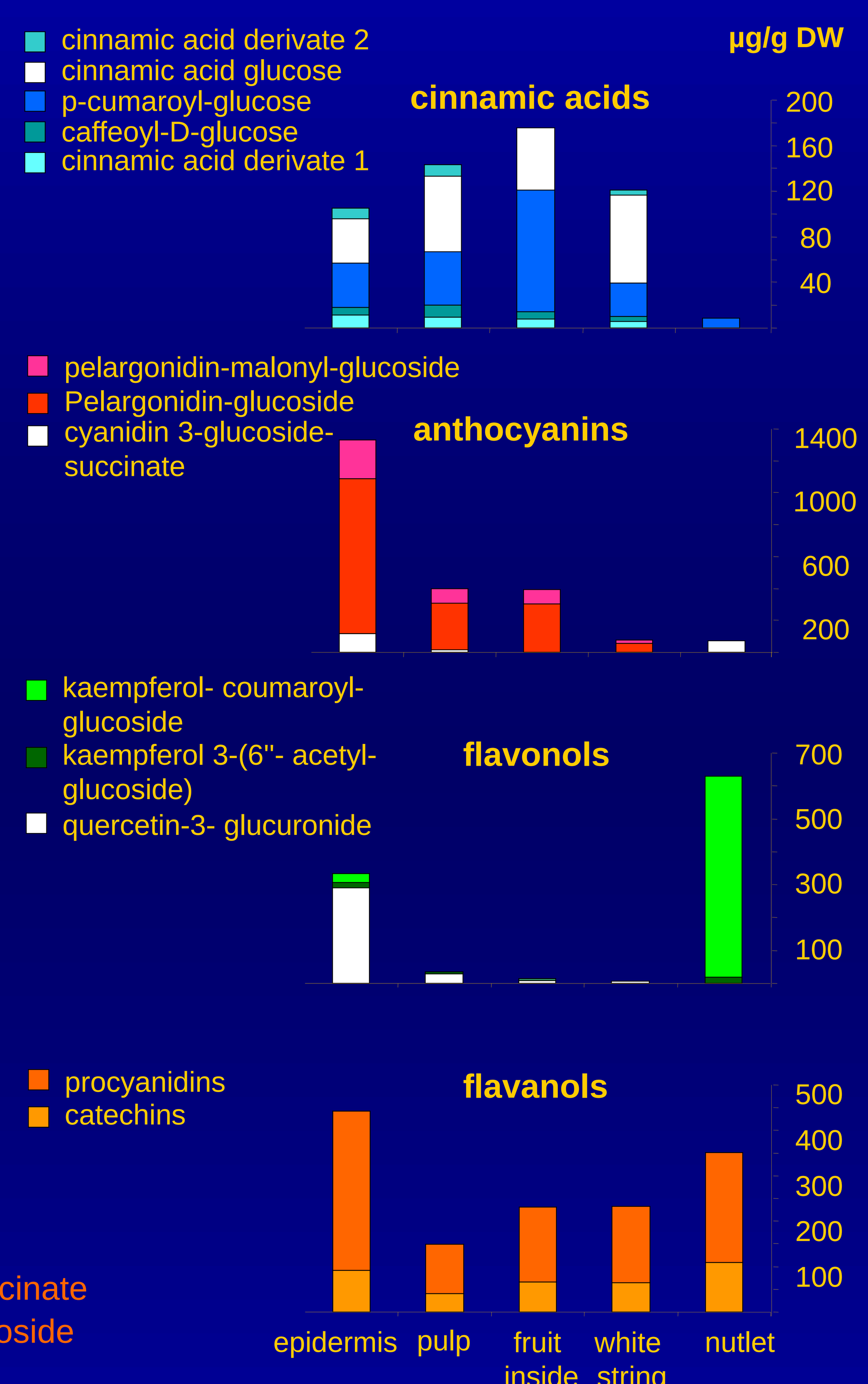
MATERIALS AND METHODS:

The phenolic compounds were separated on a *Nucleosil 120-3 C18* column, following a stepwise gradient using mixtures of solvent A (formic acid 5% in water) and solvent B (methanol) from 95:5, v/v to 10:90, v/v with a flow rate of 0.5mL per min (Treutter et al. 1994). Hydroxycinnamic acids and flavonols were detected at 280nm, anthocyanidins at 540nm, whereas flavanols were estimated at 640nm after post-column derivatization with DMAZA (4-di-methoxyaminocinnamic aldehyde) (Treutter, 1989). Identification of compounds was achieved by comparing their retention time values and *UV* spectra with those of standards if available. MS/MS data were compared with literature. The p-coumaroyl-glucose identified and purified at the Technical University Munich (Römmelt, 2001). Cinnamoyl-glucose was kindly provided by W. Schwab (TUM).





t_R (min)	λ_{max} (nm)	Av m/z [M-H]-	MS/MS fragments	Identification
Benzoic acids				
7,8	287			galloyl-ester
Cinnamic acids				
19,2	286	341		caffeoyl-glucose
37,0	314	325	117, 145, 119	p-coumaroyl-glucose
66,6	284	355 [M +COOH]	103, 71, 146	cinnamoyl glucose
Flavanols				
23,1	278			procyanidin B3
24,1	277			procyanidin B1
25,7	278			catechin
80,4	278			epicatechingallate
Anthocyanins				
109,4	272, 329sh, 501		268, 223, 146	pelargonidin-glucoside
141,7	278, 518			Cyanidin 3-glucoside-succinate
148,4	271, 330sh, 502	519		pelargonidin-malonylglucoside
Flavonols				
147,8	264, 341			quercetin-glucoside
147,3	264, 346			Kämpferol 3-glucuronide
162,5	264, 346	489	283, 255, 227, 285	Kaempferol 3-(6''-acetyl- glucoside)
169,7	268, 314	593	284, 285	kaempferol-coumaroyl-glucoside
Unknown compounds				
5,1	248	603	279	simple phenolic
17,1	275	399		cinnamic acid-derivate



Dynamic of flavonoids during fruit maturation

In this study, the accumulation of cinnamic acids showed the same dynamics as the anthocyanins. The phenylpropanoid metabolism was probably activated to deliver precursors for the biosynthesis of anthocyanidins. The increasing concentration of cinnamoyl glucose seems to be a good marker for fruit maturity.

The concentration of flavanols (catechins and procyanidins) decreased during fruit maturation. There is an metabolic shift from the biosynthetic path of the catechins to the anthocyanidins. Both groups are therefore good markers for defining the stage of maturation which was already described by Halbwirth et al. (2006). Noteworthy is the decrease of the cyanidin-derivate in the mature fruit.

Whereas quercetin 3-glucunoride decreased during ripening the flavonols with a mono-hydroxylated B-ring kaemperol-3-glucunoride and kaempferol 3-(6''-acetyl-glucoside) increased.

Identification of phenolics in strawberry (Fragaria X ananassa)

The major pigment detected in strawberry var "Elsanta" is the anthocyanin pelargonidin 3-glucoside. Minor amounts of cyanidin 3-glucoside-succinate have also been detected as already described by Lunkenbein et al. (2006) and pelargonidin-malonylglucoside was identified according to Määttä-Riihinen et al. (2004). Based on *MS/MS*-data ellagic acid and a methyl-ellagic acid-pentose were identified according to Seeram (2006) and Zheng (2005). The phenylpropanoids cinnamoyl glucose, p-coumaroyl-glucose and caffeoyl-glucose were identified based on comparison with literature (Lunkenbein et al. (2006; Määttä-Riihnen et al., 2004). In case of cinnamoyl-glucose co-chromatography was made with an authentic sample kindly provided by W. Schwab. The most important flavanol of "Elsanta" is the monomeric catechin followed by its oligomeric derivatives procyanidin B3 and procyanidin B1 identified by their reaction with DMACA and by comparing with reference compounds. Other flavanols were tentatively identified by their UV-spectra and their reaction with DMAZA. Furthermore, epicatechingallate was found. The flavonols found in the fruits were quercetin-glucoside, quercetin-glucuronide, kaempferol-glucuronide, kaempferol-acetyl-glucoside and kaempferol-coumaroyl-glucoside

Localization of flavonoids within the strawberry fruit

The concentration of cinnamic- and hydroxy-cinnamic acids increased from the outer tissue layer to the inner part of the fruit. The accumulation in the fruit interior is probably due to the weak synthesis of anthocyanins, flavonols and flavanols.

The epidermis exhibited the highest concentration of anthocyanins while their content decreased to the middle of the fruit. The apearence of anthocyanins in the white string of strawberries seems to be attributed to not enough accurate tissue preparation.

The content of flavonols exhibit the same localyzation within the fruit tissues as the anthocyanins. The flavanols show the highest content in the epidermis.

The nutlets contain high amounts of flavanols and a high content of kaempferol-coumaroyl-glucoside as well as a number of not yet identified compounds (data not shown).

Halbwirth H, Puhl I, Haas U, Jezik K, Treutter D, Stich K (2006) Two-Phase Flavonoid Formation in Developing Strawberry (Fragaria x ananassa) Fruit. J Agr Food Chem, 54, 1479-1485

Lunkenbein, S., Bellido, M., Aharoni, A., Salentijn, E.M.J., Kaldenhoff, R., Coiner, H.A., Mun˜ oz-Blanco, J., Schwab, W., 2006: Cinnamate Metabolism in Ripening Fruit. Characterization of a UDP-Glucose:Cinnamate Glucosyltransferase from Strawberry. Plant Physiology 140: 1047-1058

Määttä-Riihinen, K.R., Kamal-Eldin, A., Törrönen, A.R., 2004: Idenfication and Quantification of phenolic compounds in Berries of Fragaria and Rubus Species (Family Rosaceae). Journal of Agriculture and Food Chemistry 52: 6178-6187

Römmelt, S., 2001: Beteiligung phenolischer Verbindungen an der induzierten Resistenz von Apfel (Malus domestica) gegen Feuerbrand (Erwinia amylovora). Dissertation, Fachgebiet Obstbau, TU München-Weihenstephan

Seeram, N.P., Lee, R., Scheuller, S., Heber, D., 2006: Identification of phenolic compounds in strawberries by liquid chromatography electrospray ionization mass spectroscopy. Food Chemistry: 97: 1-11

Treutter, D.; Santos-Buelgy, C.; Gutmann, M.; Kolodziej, H. 1994: Identification of flavan-3-ols and procyanidins by HPLC and chemical reaction detection. Journal of Chromatography A 667, 290-297

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Phenolics and anthocyanins in strawberry

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Phenolic compounds are plant secondary metabolites with a large variability in their structure and occurrence, that can be divided into subgroups including anthocyanidins, flavonols, flavones, flavanols, flavanones, chalcones, dihydrochalcones and dihydroflavonols. Very simple phenolics such as hydroxybenzoic acids as well as large polymers such as condensed and hydrolysable tannins have relevance both in plant resistance reactions and in the quality of plant-derived food. The defence-related flavonoids can be divided into two groups: “preformed” and “induced” compounds. The “induced”compounds are synthesised by plants in response to physical injury, infection, or stress and may occur constitutively in plants or they may occur as so-called phytoalexins. The “preformed” flavonoids are innate compounds that are synthesised during the normal development of plant tissue and they may be involvemed in several host–pathogen interactions. Little differences in the chemical structure of defense-related compounds lead to an change in the defence-capactity against pathogens (LANGCAKE ET AL. 1979; JEANDET ET AL. 2002; PEZET und PONT 1988). Probably different compounds may induce various reactions in human. So it‘s important to comprise the exactly chemical structure of polyphenolics for estimating and comparing the anti-pathogenity and healthy effect after consumption. Strawberries involves various compounds associated to benzoic acids, hydroxy cinnamic acids, flavonols, flavanols, anthocyanins and combinations from different groups. Some compounds are associated with resistance against fungal pathogens on leaves (YAMAMOTO ET AL. 2000), against rooting pathogens (OKASHA 1967) or postharvest pathogens on fruits (ZHANG ET AL. 2006).

In this study the secondary compounds of the strawberry cultivar „Elsanta“ were investigated by HPLC-(highpressureliquidchromatography) and mass-spectroscopy at different degree of ripeness as foundation for comprising the dynamik of secondary methabolism and possible interaction between several compounds and fungal pathogens like *Botrytis cinerea*.

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