



Agronomic and Technological Practices to Increase the Content in Bioactive Constituents

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Zaragoza, 5 Abril 2006

Strategies for functional food production

- Improve the content or profile
- Increase bioavailability
- Increase or modulate bioactivity

Interventions to increase the content

- Genetics
- Agronomics
- Livestock
- Fish farming
- Technological
- Biotechnological
- Formulation and presentation

Livestock and Fish Farming

- Milk
- Meat
- Eggs
- Fish
- Lipids
- Proteins
- Metabolites (carotenoids, etc.)



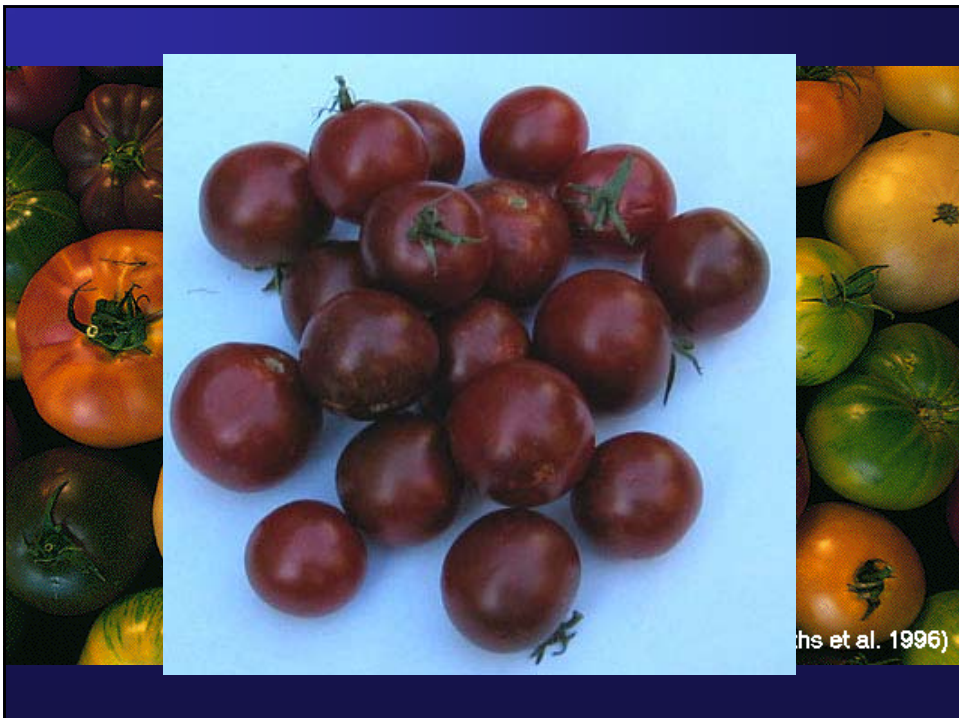


Factors affecting the plant bioactive compounds content

- 1-Internal (genetic)
- 2-Agronomic (environmental)
- 3-Postharvest treatments
- 4-Technological treatments
- 5-Biotechnological treatments

1) Genetic factors

- Flavonoid content
- Flavonoid quality
- Polyphenol oxidases



Phenolic content of different tomato types (mg/100g f.w.)

	HCA	Flavonols	Flavanones	total
cherry	170.97	1042.41	729.86	1943.24
cluster	31.02	12.94	42.4	86.36
Specialities	5.76	13.48	2.68	21.92
Marmande	9.34	80.19	47.13	136.66
medium	12.95	21.05	37.27	71.27
large GB	23.83	20.48	44.81	89.12
Large beef	7.39	18.71	15.72	41.82

2- Agronomic factors

- Irrigation
- Soil (nutrients)
- Temperature
- Irradiation (sun)
- Pests

Irrigation

- Water quality
- Water quantity



Soil (nutrients)

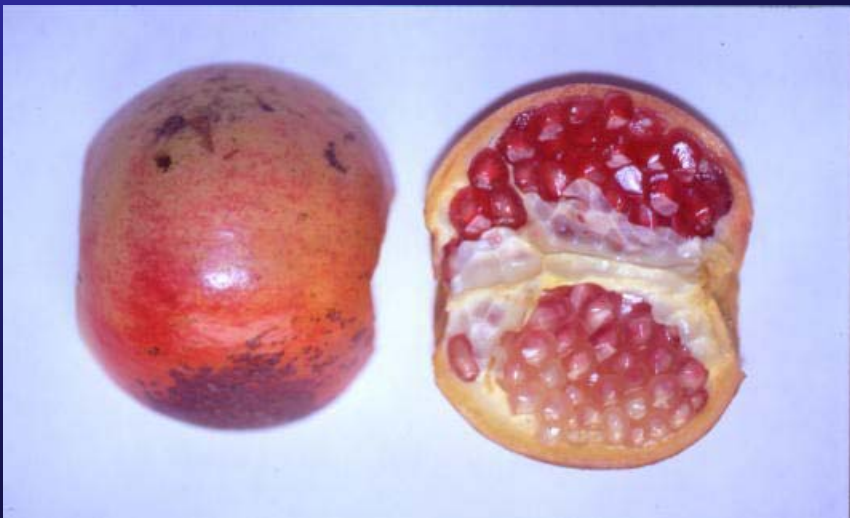
- Sulfur nutrients
- 15-30 kg/Hect (2-3 fold increase in glucosinolates)
- 50-90 kg/Hect (nutritional imbalance, the effects are lost)



Temperature

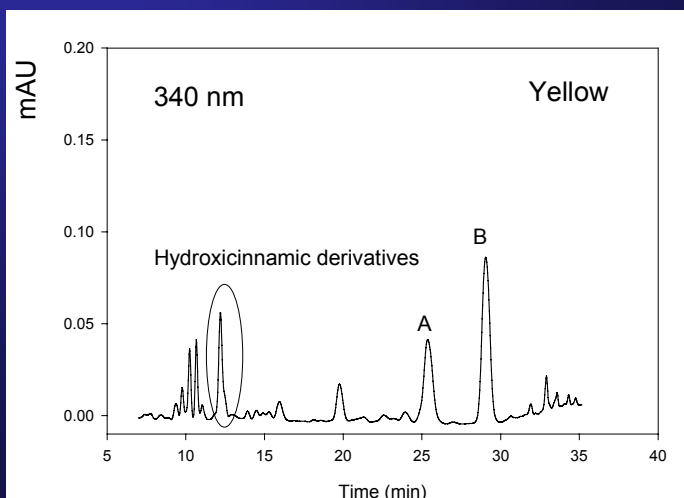


Sun irradiation





Sweet Pepper flavonoids California type (Cv Luzon)

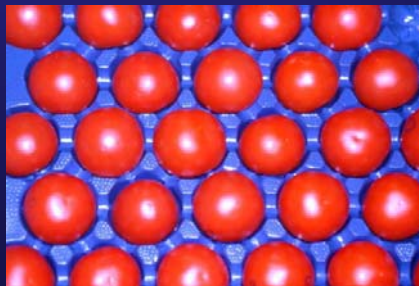


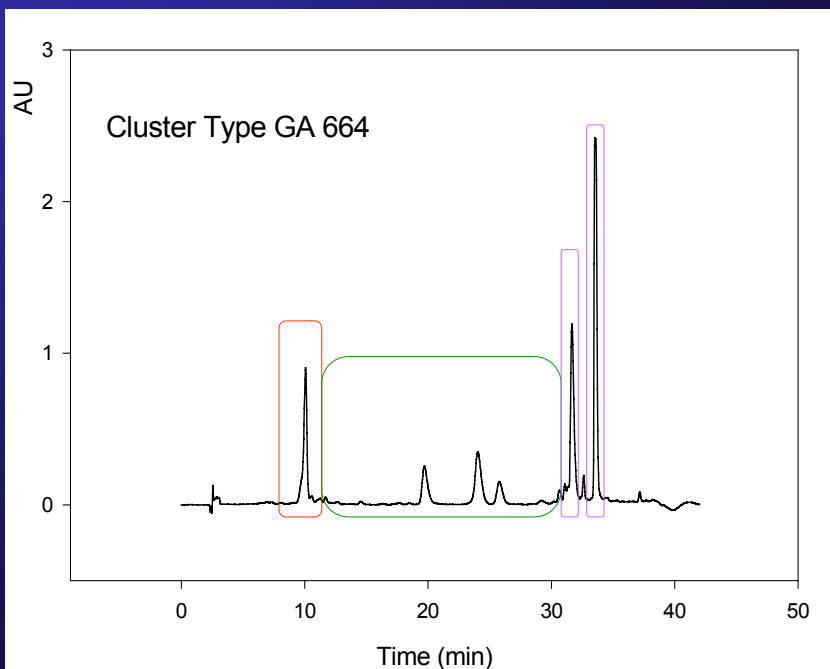
Phenolic content (mg/100 g f.w.)

• California	• HCA	Flavonoids
– <i>Aifos</i>	– 0.181	1.976
• Lamuyo		
– <i>Duque</i>	– 1.008	5.033
• Dulce Italiano		
– <i>Italico</i>	– 0.584	7.702

Tomato flavonoids

- Greenhouse
- Out doors 2-10 times more flavonoids



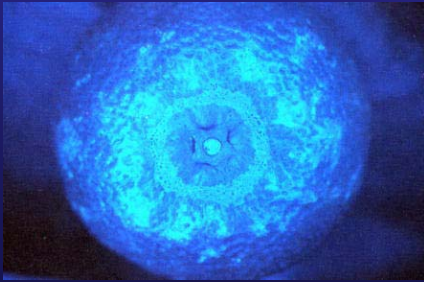


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Pests

- Phytoalexins



3- Postharvest factors

Storage Conditions

Temperature

Atmosphere

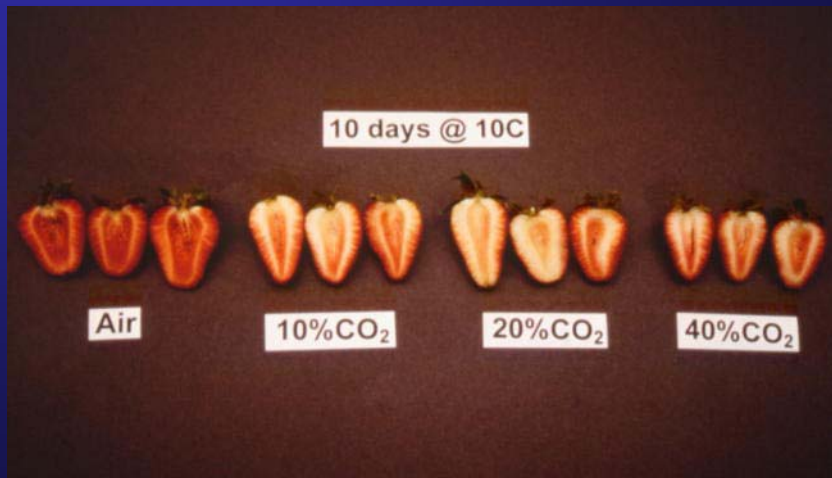
Plant Hormones

Technological treatments

– Cutting

– Irradiations

3- Factores Postrecolección



Improve profile

The diagram illustrates the process of improving the profile of grapes. On the left, a cluster of red grapes is shown. An arrow points from the grapes to the right, with 'UVC' and 'O₃' written above and below the arrow respectively. To the right of the arrow is the chemical structure of *trans*-Resveratrol, which consists of two phenolic rings connected by a double bond, with hydroxyl groups at the 3, 5, and 4' positions. Below the structure is the text '*trans*-Resveratrol'. To the right of the chemical structure is a photograph of a cluster of dark grapes inside a treatment chamber. The chamber is lined with reflective material and has a bright blue light source at the top, illuminating the grapes.

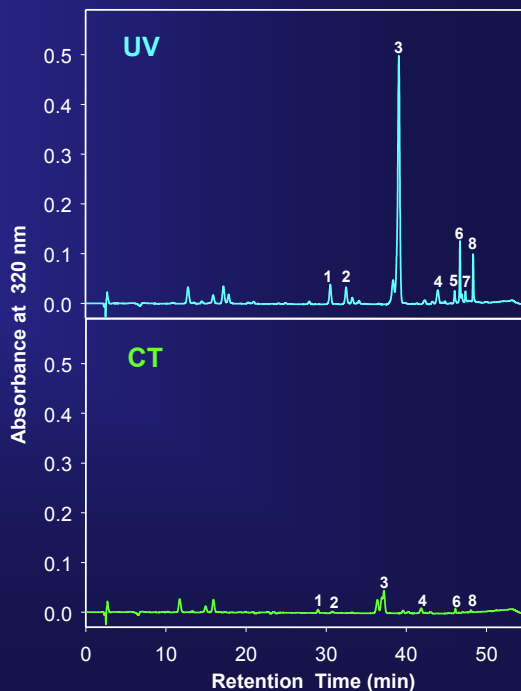
HPLC Analysis

Irradiation conditions:

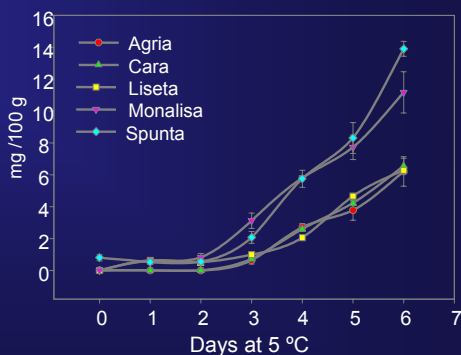
- 510 W
- 60 seconds
- 40 cm

Peaks:

1. Piceid
2. Piceatannol
3. Resveratrol
- 4-8. Viniferins



Induce flavonoid biosynthesis



Tudela, J.A., Cantos, E., Espín, J.C., Tomás-Barberán, F.A., Gil, M.I. 2002. Induction of antioxidant flavonols biosynthesis in fresh-cut potatoes. Effect of domestic cooking. J. Agric. Food Chem. 50: 5925-5931.

4. Effect of Processing

- Tissue disruption
- Thermal treatments
- Fermentation
- Freezing
- Drying
- Addition of other ingredients

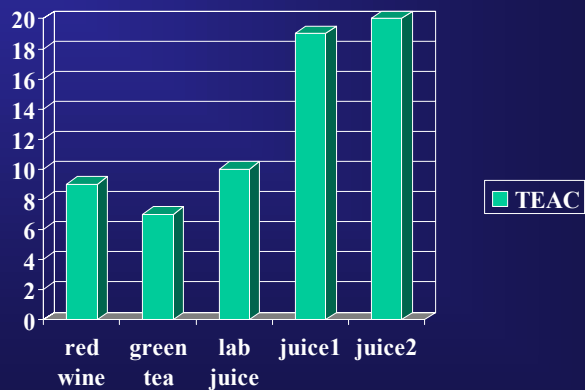
Processing and bioavailability



Juice preparation



Antioxidant activity of pomegranate juice

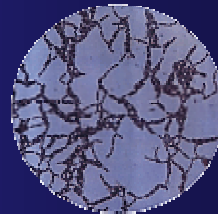


ENZYMATIC TREATMENT - SUPERIOR



Biotechnological treatments

- Use microorganisms
- Use Animal Cells
- Use enzymes
- Improve bioavailability
- Improve biological activity





CONVERSION OF PHENOLIC-RUTINOSIDES TO PHENOLIC-GLUCOSIDES

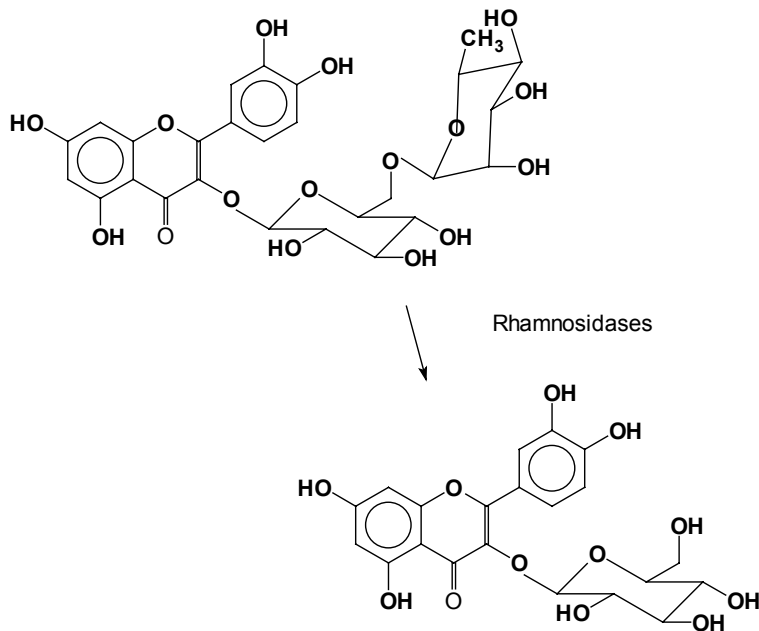
Background:

- Rhamnosidases from *Aspergillus aculeatus* can convert, in model solutions, phenolic-rutinosides to their phenolic-glucosides counterparts.
- Phenolic-rutinosides are less bioavailable than their phenolic-glucosides counterparts.

Question: Is possible to convert phenolic-rutinosides to phenolic-glucosides in juices?

Enzyme: Rhamnosidases from *A. aculeatus* 'A and B' (provided by Leo de Graaf's group, WAU).

Juices: Black currant and orange (commercial and fresh).

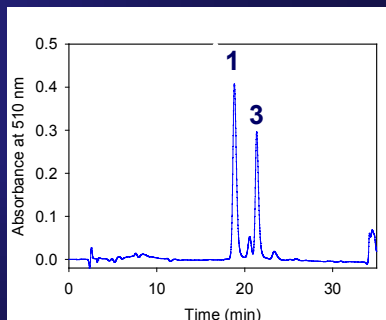
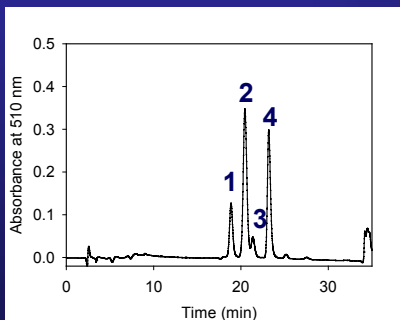




CONVERSION OF PHENOLIC-RUTINOSIDES TO PHENOLIC-GLUCOSIDES

Black currant

(R-A, 2 $\mu\text{g/mL}$)



- 1 = Delphinidin-3-glucoside
- 2 = Delphinidin-3-rutinoside
- 3 = Cyanidin-3-glucoside
- 4 = Cyanidin-3-rutinoside

Conversion: 2 $\rightarrow$ 1 and 4 $\rightarrow$ 3



CONVERSION OF PHENOLIC-RUTINOSIDES TO PHENOLIC-GLUCOSIDES

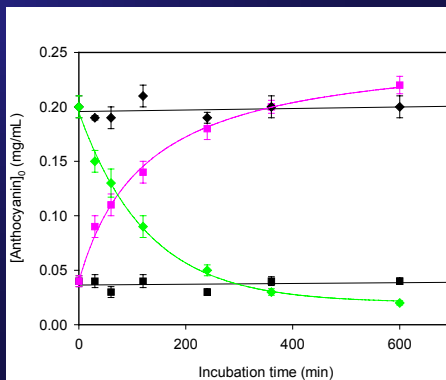
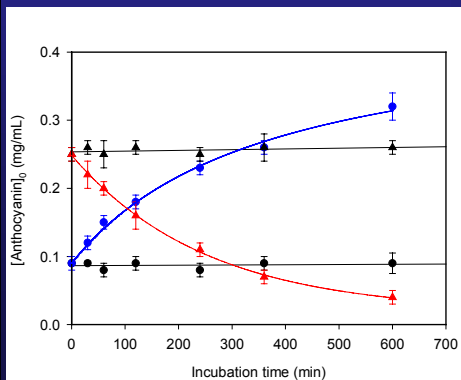
Kinetics

Black currant

(R-A, 2 $\mu\text{g/mL}$)

- CONTROL Dpn-3-glucoside
- R-A, Dpn-3-glucoside
- ▲ CONTROL Dpn-3-rut
- ▲ R-A, Dpn-3-rut

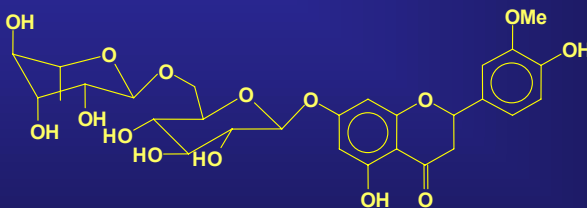
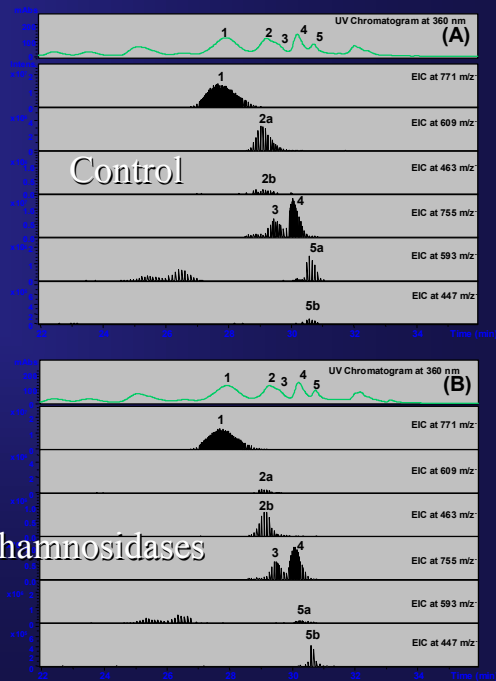
- CONTROL Cyn-3-glucoside
- R-A, Cyn-3-glucoside
- ◆ CONTROL Cyn-3-rut
- ◆ R-A, Cyn-3-rut



Green Tea

Hplc MS-MS

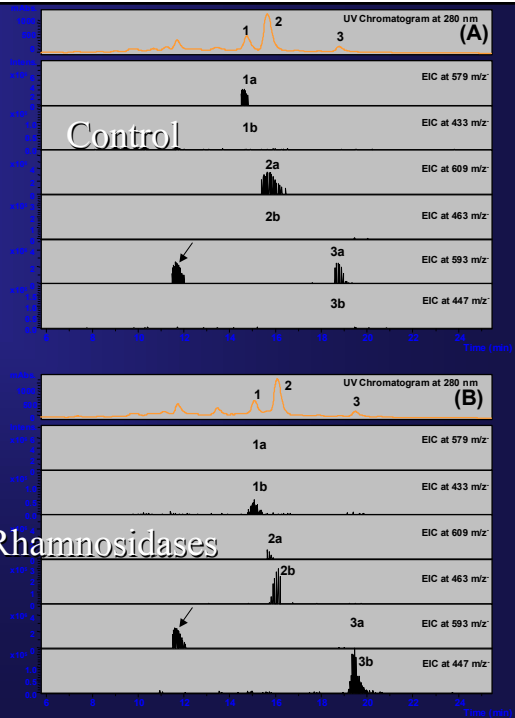
- 1 Q-glc-rha-glc
- 2 Q-glc + Q-glc-rha
- 4 K-glc-rha-glc
- 5 K-glc + K-glc-rha



Citrus

Hplc MS-MS

- 1 N-glc-rha + N-glc
- 2 H-glc-rha + H-glc
- 3 D-glc-rha + D-glc



BIOAVAILABILITY IN VIVO



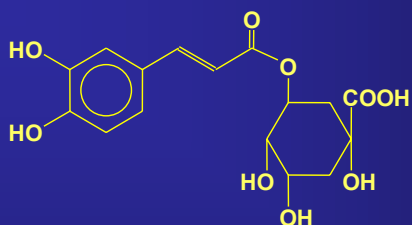
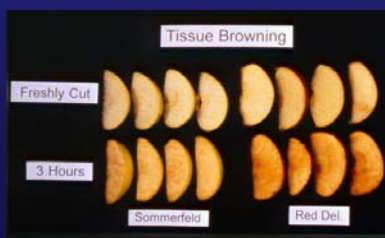
Gary Williamson et al., Nestlé Research Centre

16 healthy volunteers

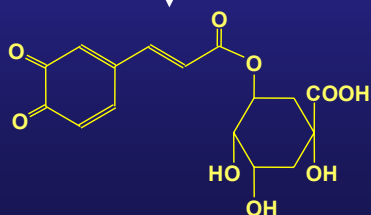
5.4-fold higher bioavailability for hesperetin-glucoside

Bioavailability of hesperidin can be modulated by the enzymatic conversion to the glucoside.

Enzymatic browning



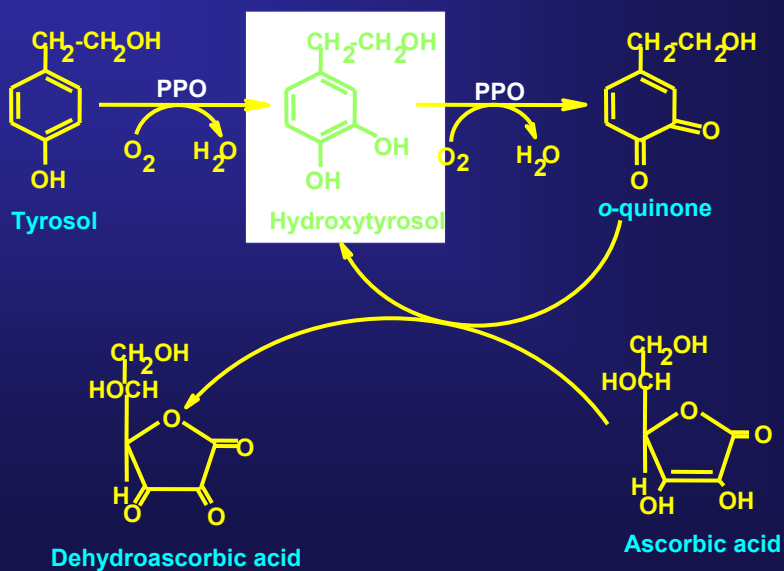
Polyphenol oxidase (PPO)



Brown Polymers

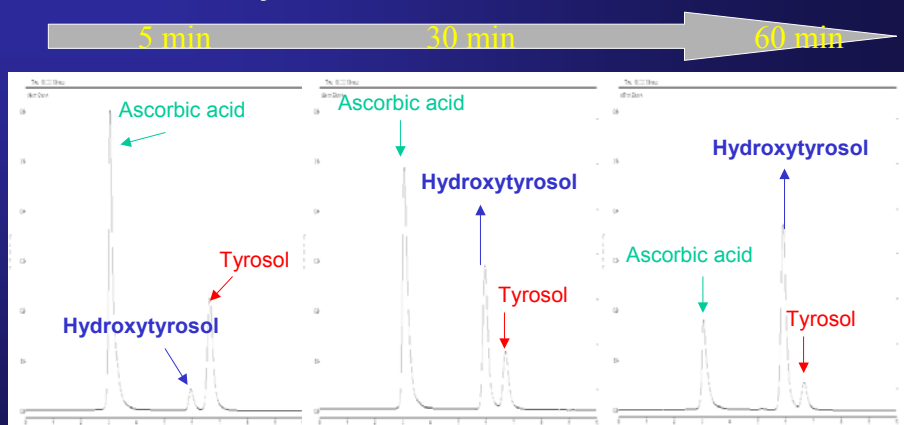
Enzymatic Browning

Enzymatic synthesis of hydroxytyrosol using tyrosinase as biocatalist



HPLC follow-up of the reaction

Synthesis evolution

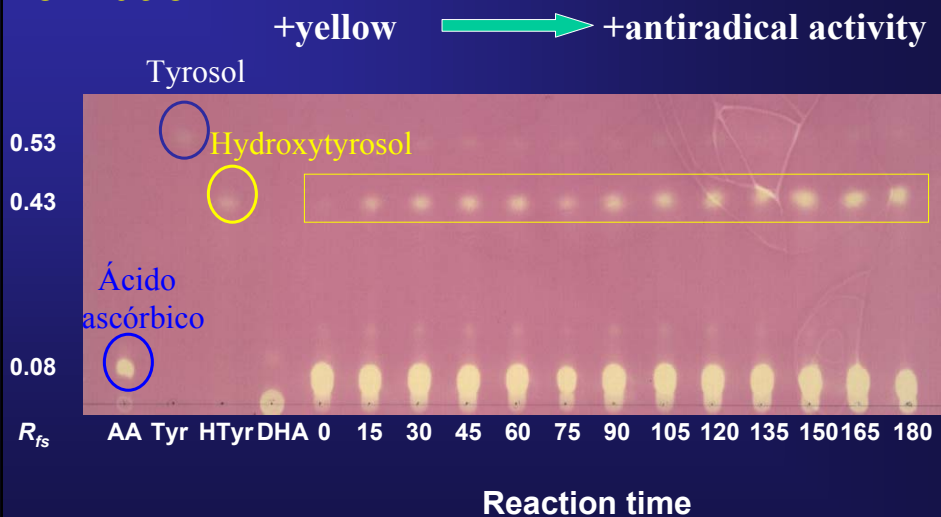


Process modelling



PATENT

TLC-DPPH Follow-up of hydroxytyrosol formation



Enzymatic synthesis of piceatannol

