

Hop is an exceptional source of resveratrol for brewers



Dr. Vesna Jerkovic

Prof. Sonia Collin

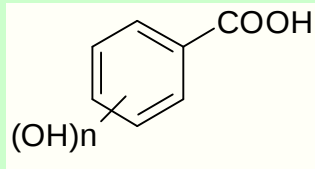
9 September 2008

Unité de brasserie et des industries alimentaires, UCL

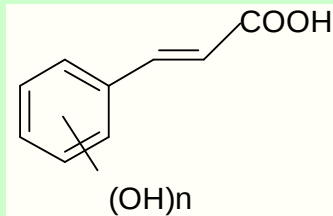
Polyphenols

Phenolic acids

→ Benzoic acid

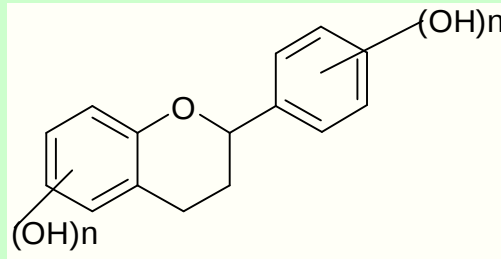


→ Cinnamic acid



Antioxidant, aroma precursors

Flavonoids



→ Flavonols
→ Flavanoids

→ Prenylchalcones

→ Flavanones

→ 3,4-Flavanediols

→ Flavan-3-ols

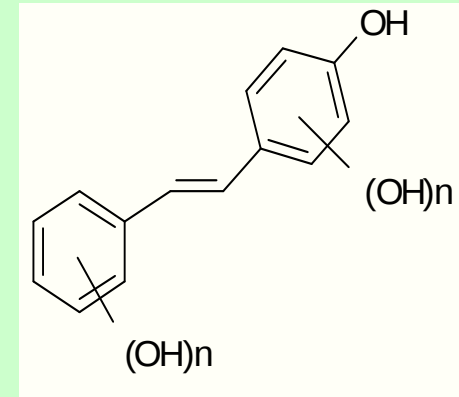
→ Flavones

→ Isoflavones

→ Anthocyanidins

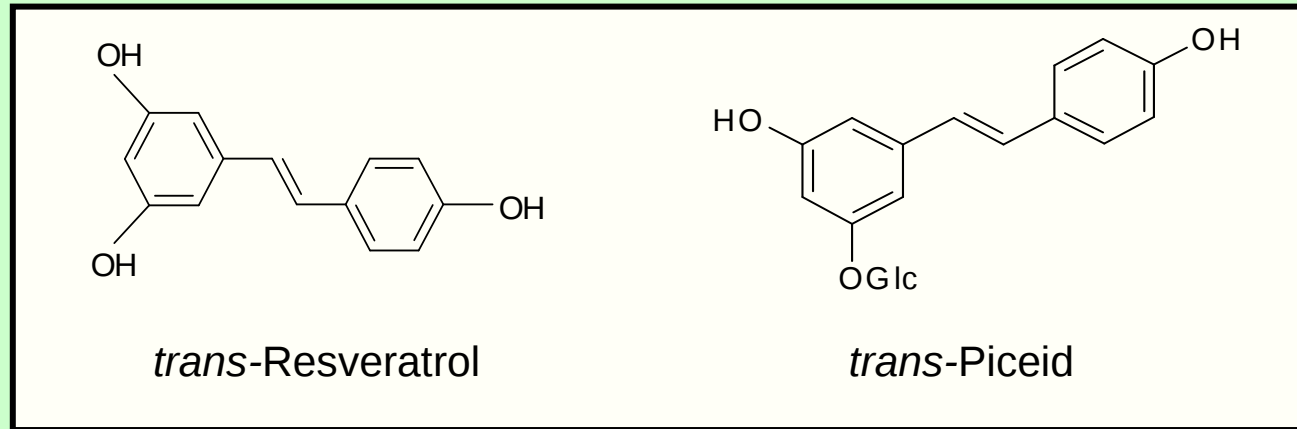
Antioxidant, astringency, color, health benefits (cardioprotector, estrogenic activities,...)

Stilbenes



Health benefits (cardioprotector, anti-carcinogenic, anti-inflammatory,...)

Resveratrol and glycoside



❑ Implication in the « French paradox »

In grapes : 0.5 to 39 ppm

In red wines : up to 20 ppm

Low compared to
flavonoids but

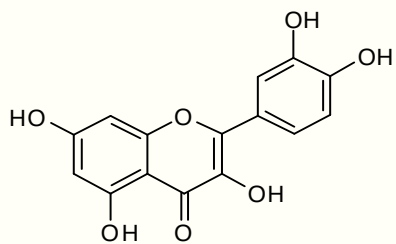
❑ Anti-carcinogenic, anti-viral, anti-oxidant,
anti-inflammatory and estrogenic activities

Hop polyphenols

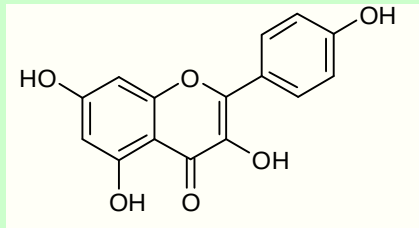
❑ Flavonoids (10000 – 50000 ppm) :

- flavonols (300 – 2000 ppm) :

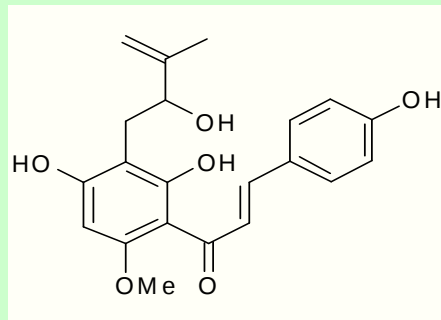
quercetin,



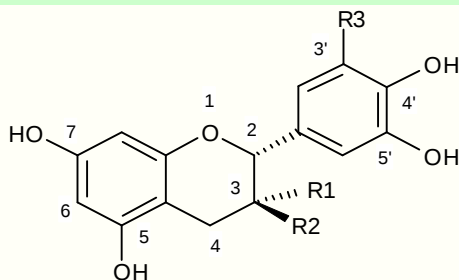
kaempferol,...



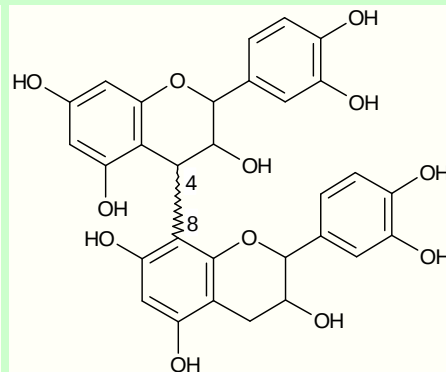
- prenylchalcones (up to 6000 ppm) :
xanthohumol,...



- flavanols (10000 → 50000 ppm) :
catechin, *epicatechin*, *dimers*, *trimers* → *oligomers*



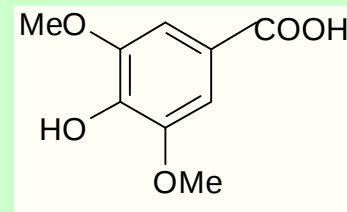
(+)-catéchine: R1=H R2=OH R3=H
(-)-épicatéchine: R1=OH R2=H R3=H
(+)-gallocatéchine: R1=H R2=OH R3=OH
(-)-galloépicatéchine: R1=OH R2=H R3=OH



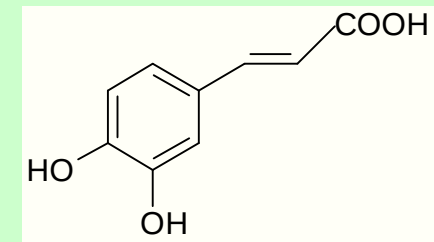
1:500

❑ Phenolic acids (10 – 60 ppm) :

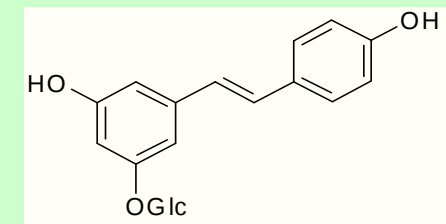
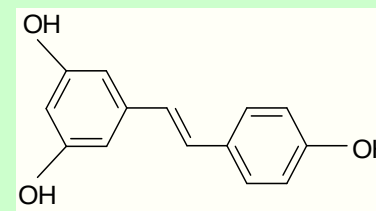
syringic acid,



cafeic acid,...



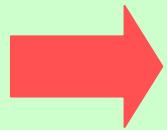
❑ Stilbenes (1-20 ppm):
resveratrol (1 - 5 ppm) *piceid* (1 - 15 ppm)



Context

2003 : Discovery of *trans*-resveratrol in hop

Callemien, Jerkovic, Rozenberg & Collin, *Journal of Agricultural and Food Chemistry* , 53, 2005, 424-429



Best cultivars ?

Impact of the harvest year ?

Impact of conditioning (pelletisation, ...) ?

Fate of stilbenes through the brewing process ?

Hop processing

Hop vines



Harvest
Drying



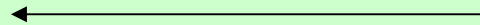
Hop cones



Pelletisation



CO2 extraction
Solvent extraction



Hop extract



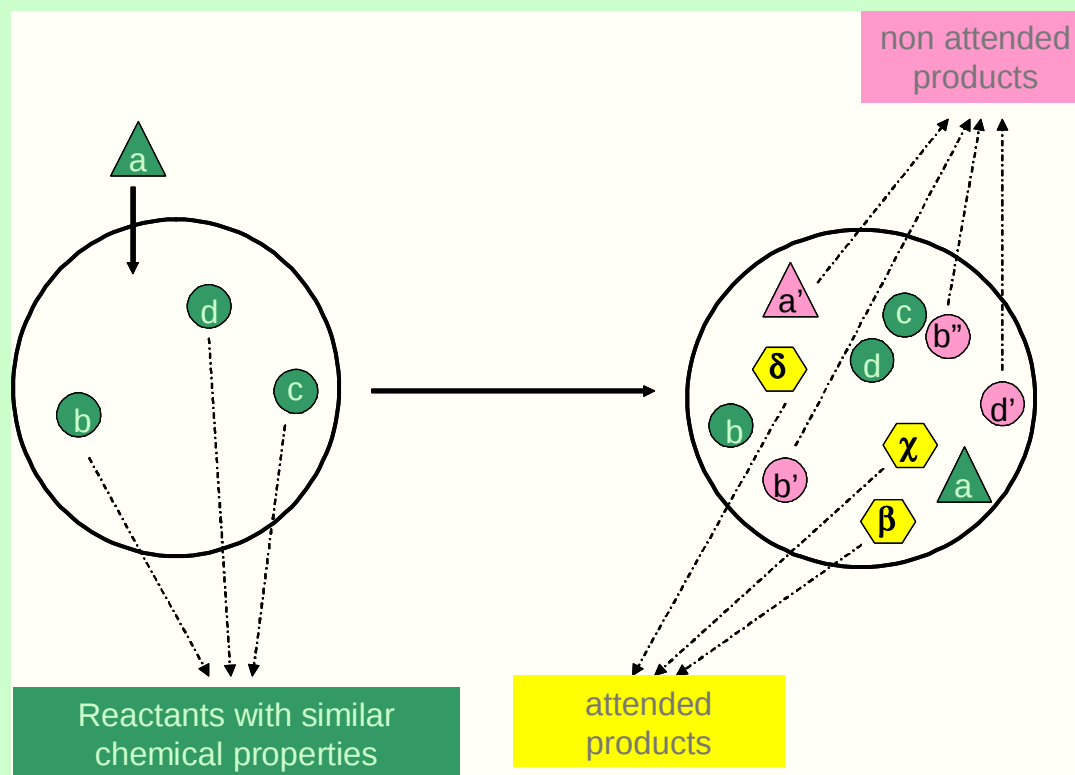
Hop pellets

1. New methodologies

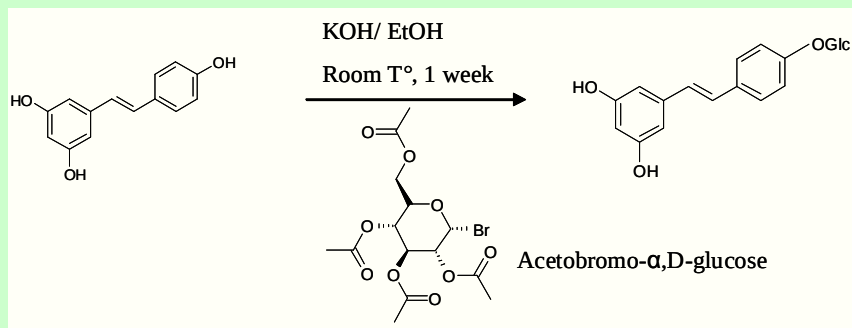
1.1. Standard synthesis

Jerkovic, Nguyen, Nizet & Collin, *Rapid Communication in Mass spectrometry*, 21, 2007, 2456-2466

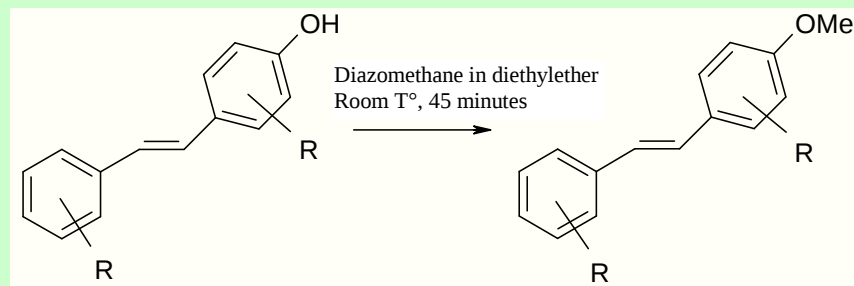
One-pot approach



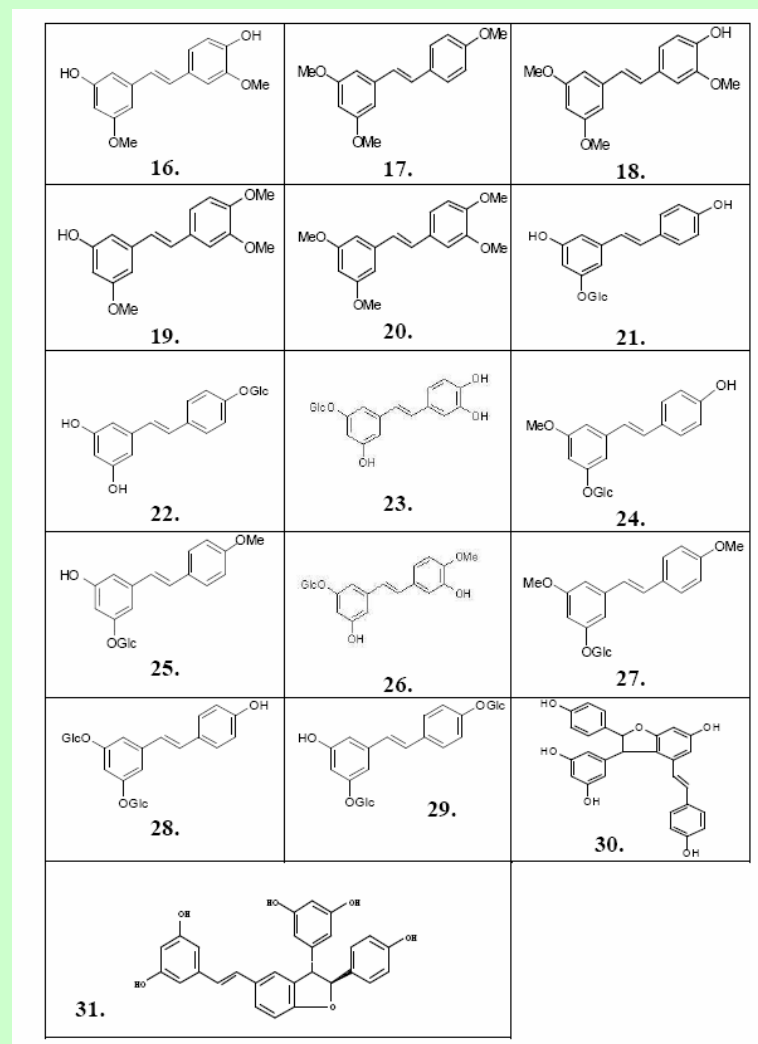
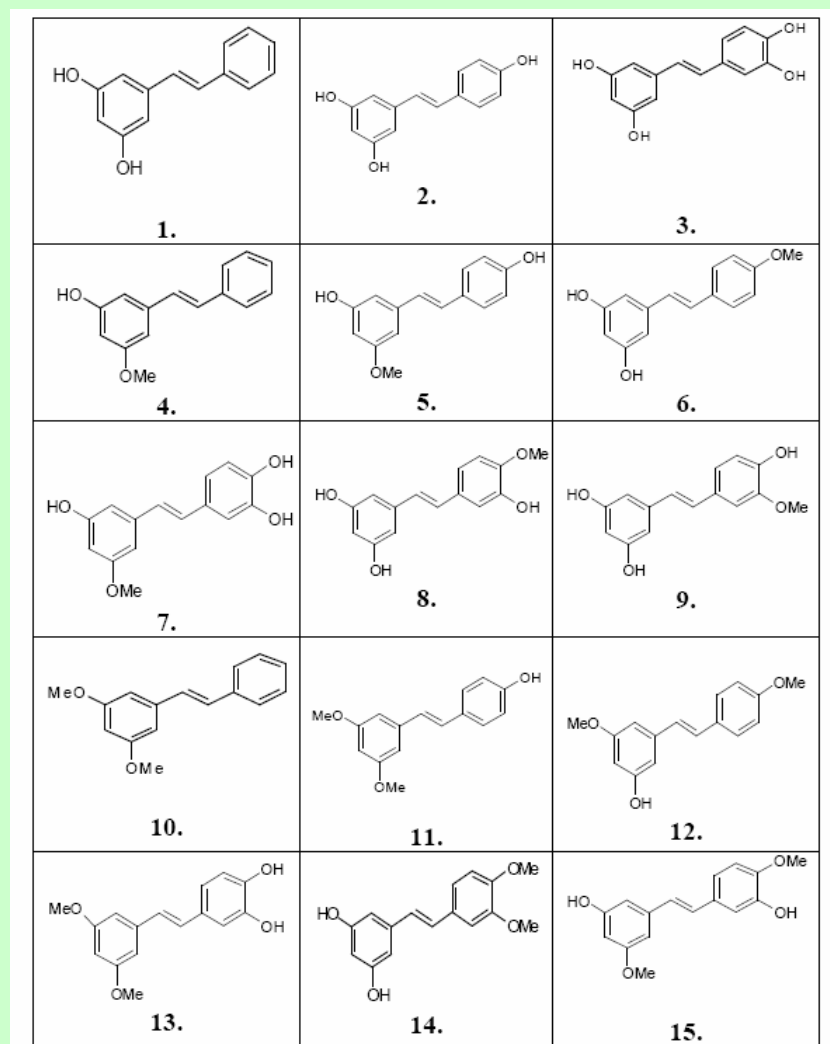
Synthesis of glycosylated stilbenes



Synthesis of methoxylated stilbenes



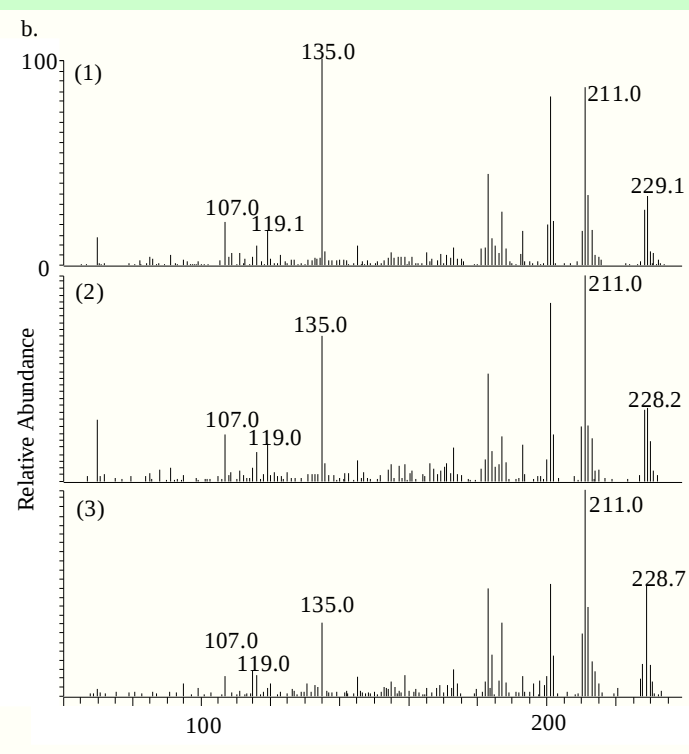
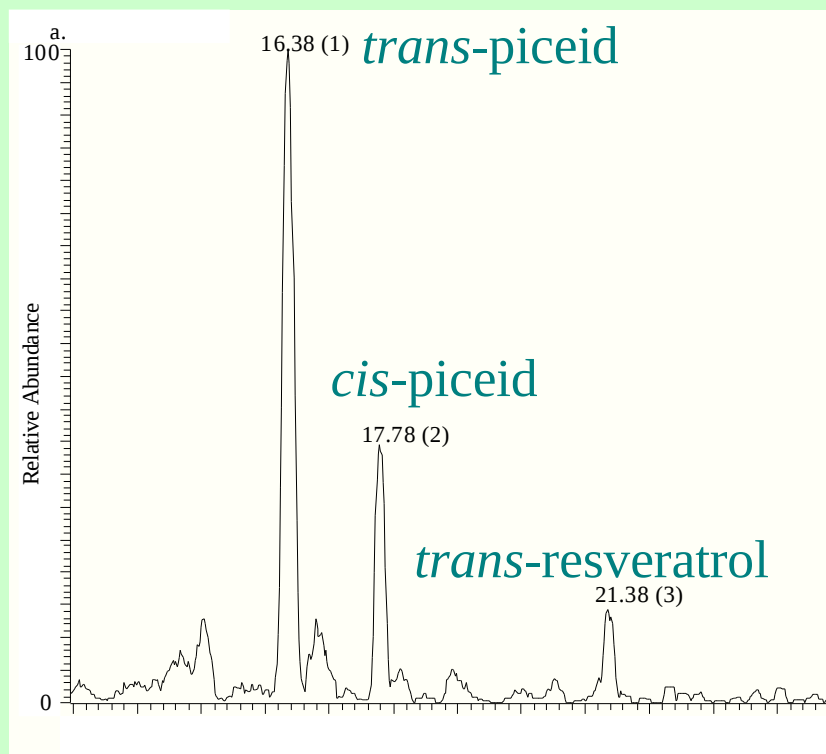
Synthesis and characterization of 22 analogs and 9 commercial standards



1.2. Optimization of analyses of *trans*-resveratrol and analogs in hop

Callemien, Jerkovic, Rozenberg & Collin, *Journal of Agricultural and Food Chemistry*, 53, 2005, 424-429

1. Elimination of hydrophobic compounds by toluene and cyclohexane
2. Stilbene extraction by ethanol:water (80:20), 60°C
3. Concentration
4. Analysis by RP-HPLC-MS/MS-APCI(+)



Polar

Apolar

Prevail C18 (150 x 2.1 mm, 3 μ m)

Temp. 30°C, Inj. vol : 10 ml, Flow 0,2 ml/min.

Linear gradient from 95 % water (0,1% ACF +1% ACN) / 5 %ACN to 100% ACN

APCI (+)

1.3. Quantification of *trans*-resveratrol and *trans*-piceid in beer

Jerkovic, Nguyen, Timmermans & Collin, *submitted*

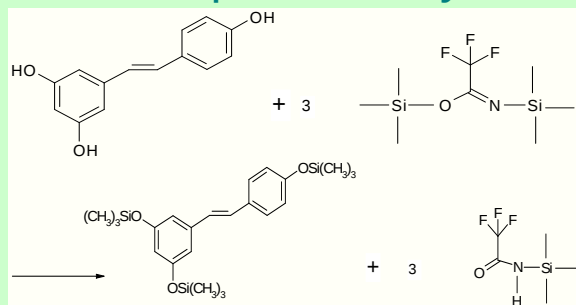
LOD : 5 ppb/ LOQ : 15 ppb

SPME - DIRECT ANALYSIS

Polyacrylate fiber

1. Extraction
2. Derivatization (BSTFA)
3. GC-MS

- + Avoid pre-cleaning
- + Easy and rapid (1.5 h)
- + *trans*-resveratrol ok
- *trans*-piceid N.D.
- Bad reproducibility



RP- HPLC ANALYSIS AFTER EXTRACTION

Elimination of hydrophobic compounds
(Toluene/Cyclohexane)

Liquid-liquid extraction

1. Extraction (Ethyl acetate)
2. Concentration

RP-HPLC-MS/MS-APCI(+)

- + *trans*-resveratrol
- + *trans*-piceid
- Bad recovery

Elimination of hydrophobic compounds
(Toluene/Cyclohexane)

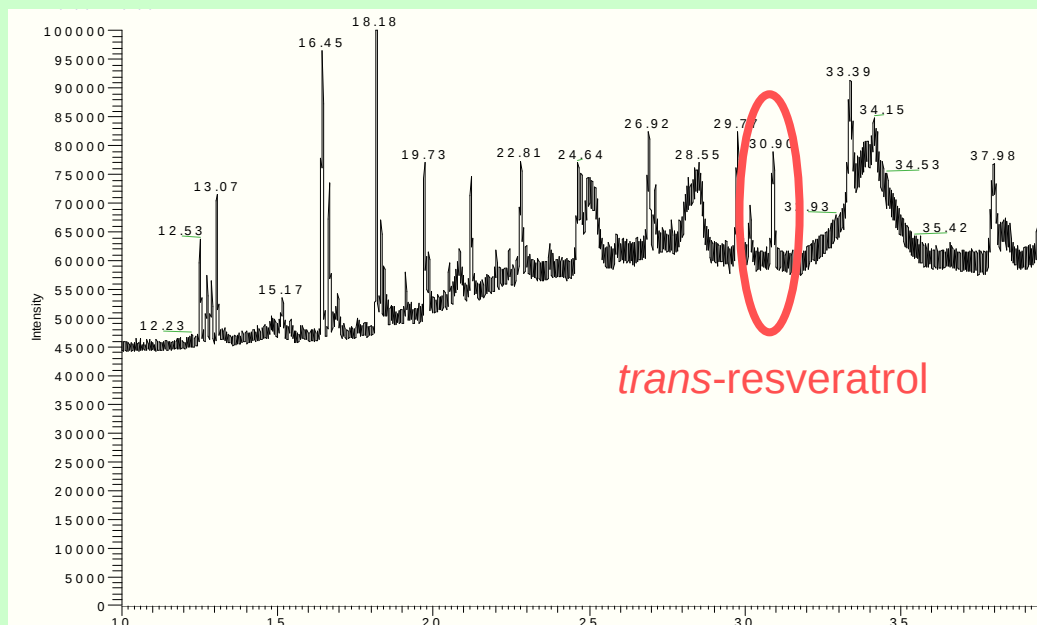
Solid-phase extraction

1. Conditioning
2. Loading of beer
3. Elution (Ethanol)
4. Concentration

RP-HPLC-MS/MS-APCI(+)

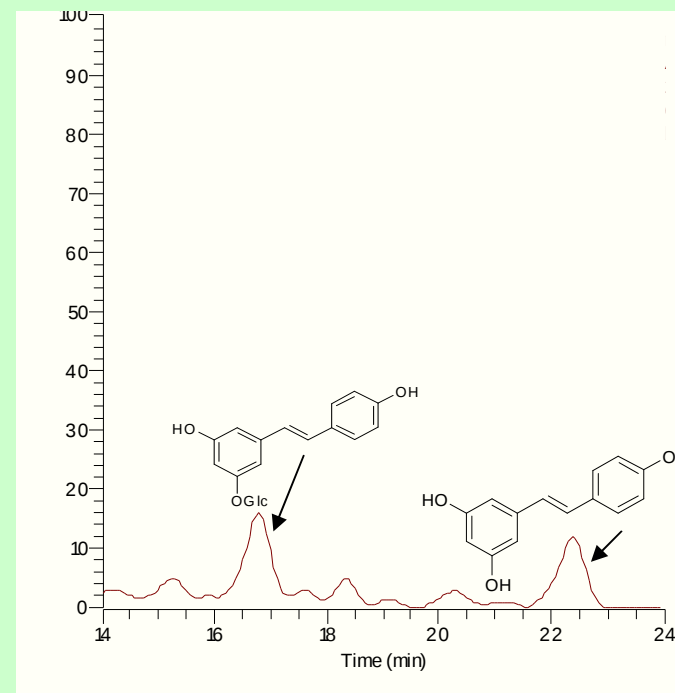
- + *trans*-resveratrol
- + *trans*-piceid
- + High recovery (76%)
- Long (8 h)

SPME-DIRECT ANALYSIS



Lager beer without adjunct
 Polyacrylate fiber
 CP-Sil 5 CB column
 Desorption 7 min at 280°C
 100 → 250°C at 10°C/min, 250°C for 30 min
 SIM m/z = 444
 Helium = 1 ml/min

RP-HPLC ANALYSIS



Polar

Apolar

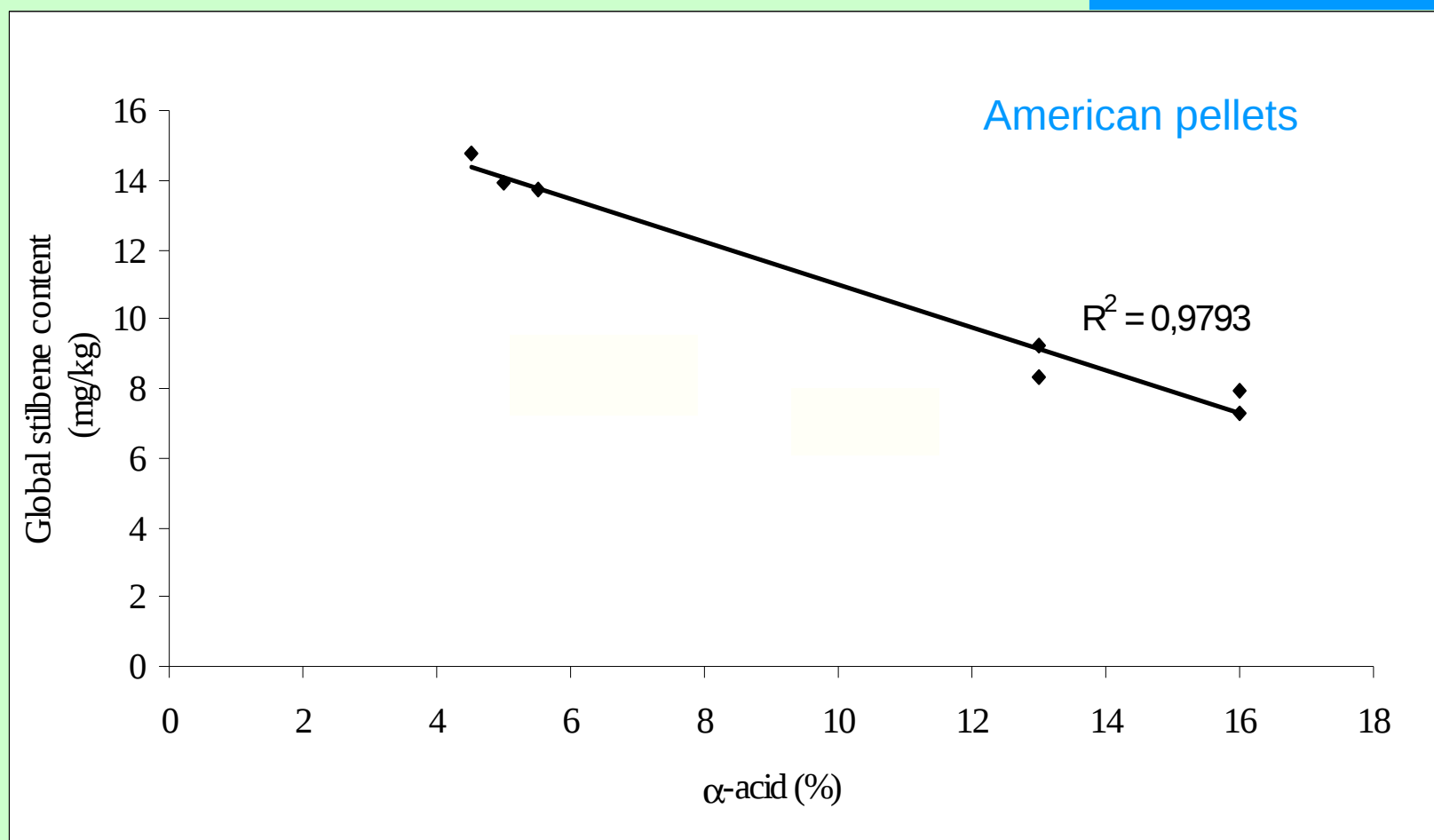
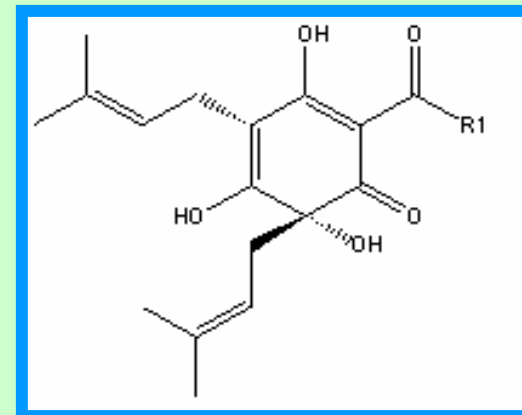
Lager beer without adjunct
 Prevail C18 (150 x 2.1 mm, 3 μm)
 Temp. 30°C, Inj. vol : 10 ml, Flow 0,2 ml/min
 Linear gradient from 95 % water (0,1% ACF +1% ACN) / 5 %ACN to 100% ACN
 APCI (+)

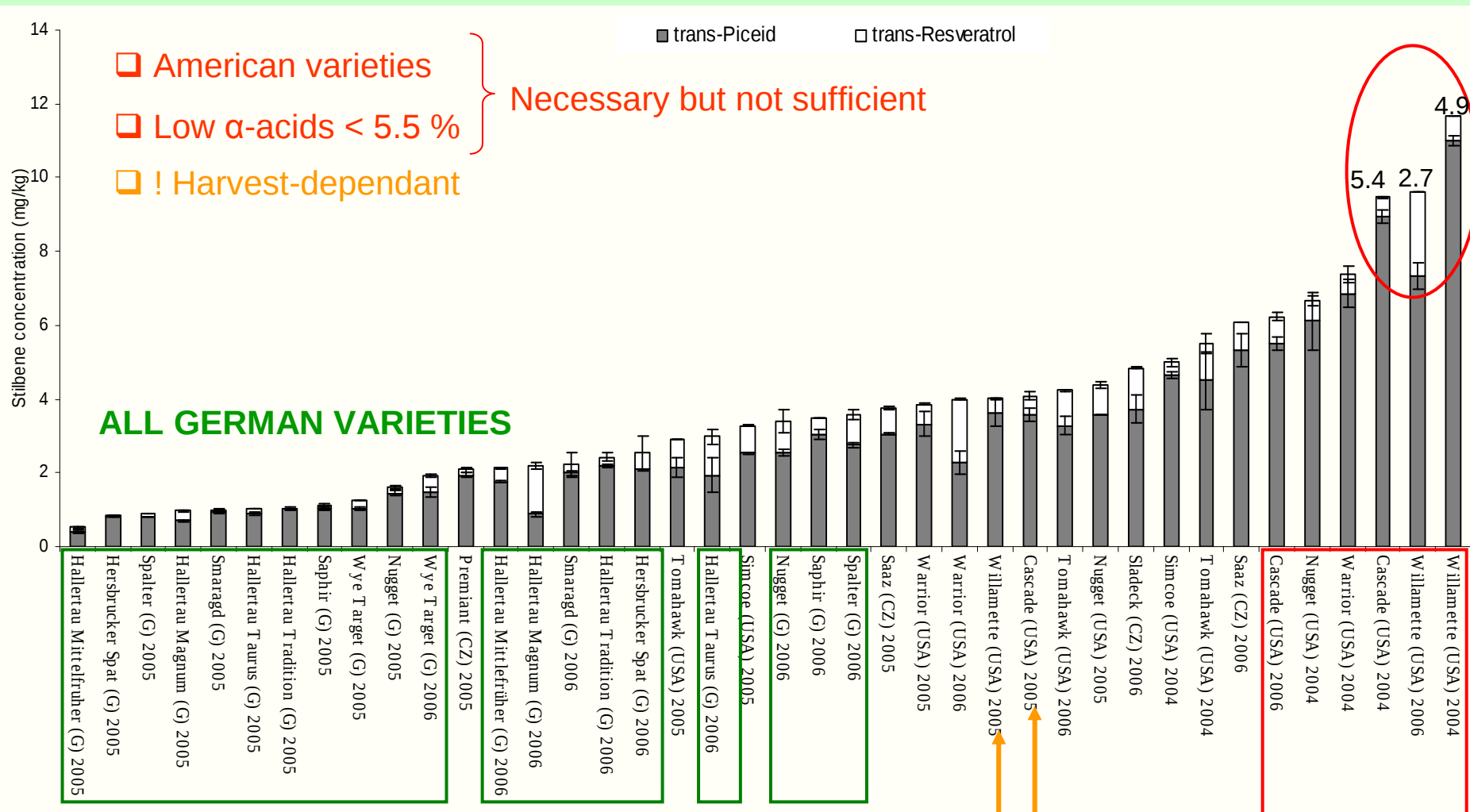
2. Stilbenes in hop and other raw materials

2.1. Secondary metabolite

→ relationship stilbenes/ α -acids (hop bitterness)

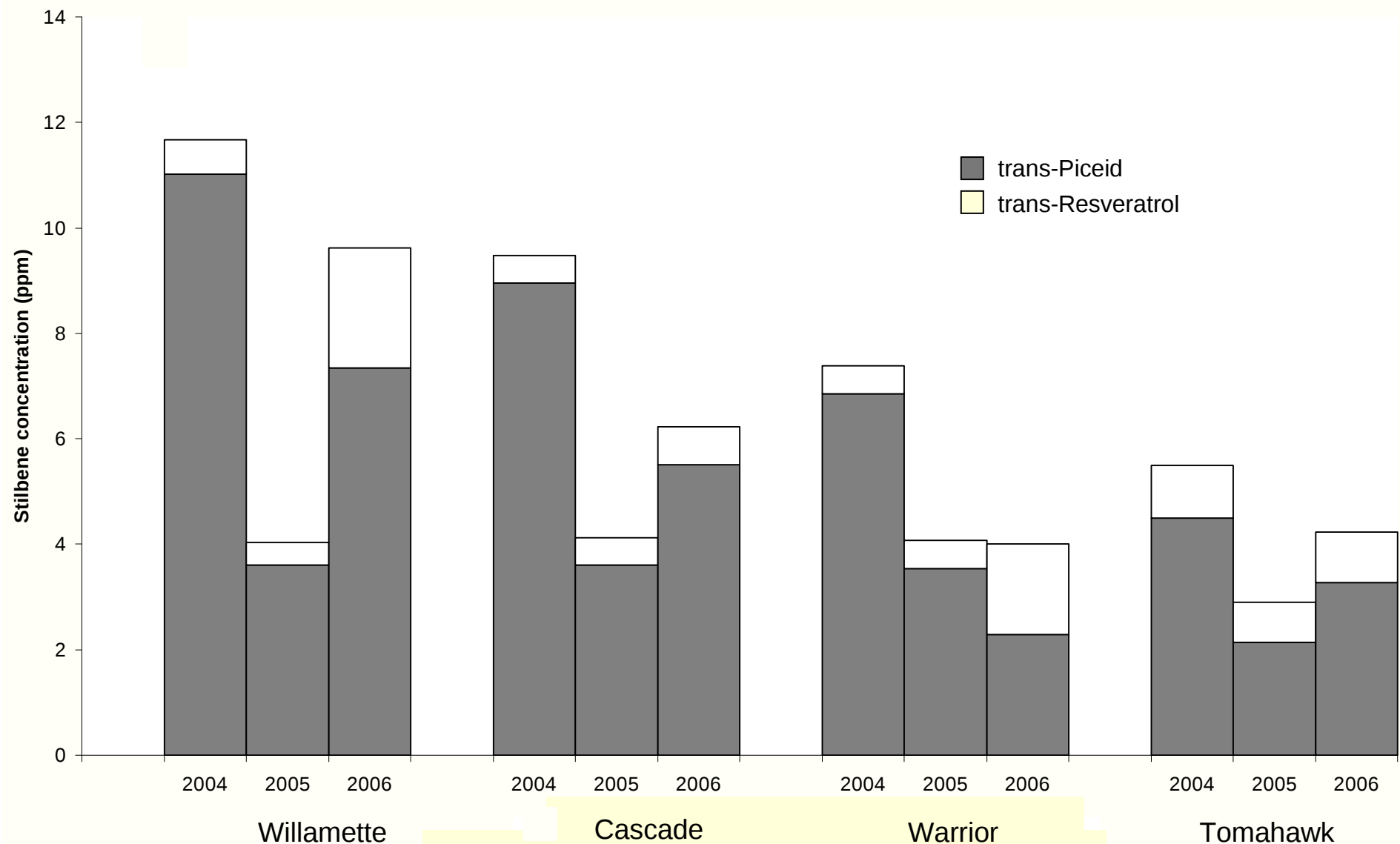
Jerkovic, Callemien & Collin, *Journal of Agricultural and Food Chemistry*, 53, 2005, 4202 – 4206





2.2. Phytoalexin → strong influence of the harvest year

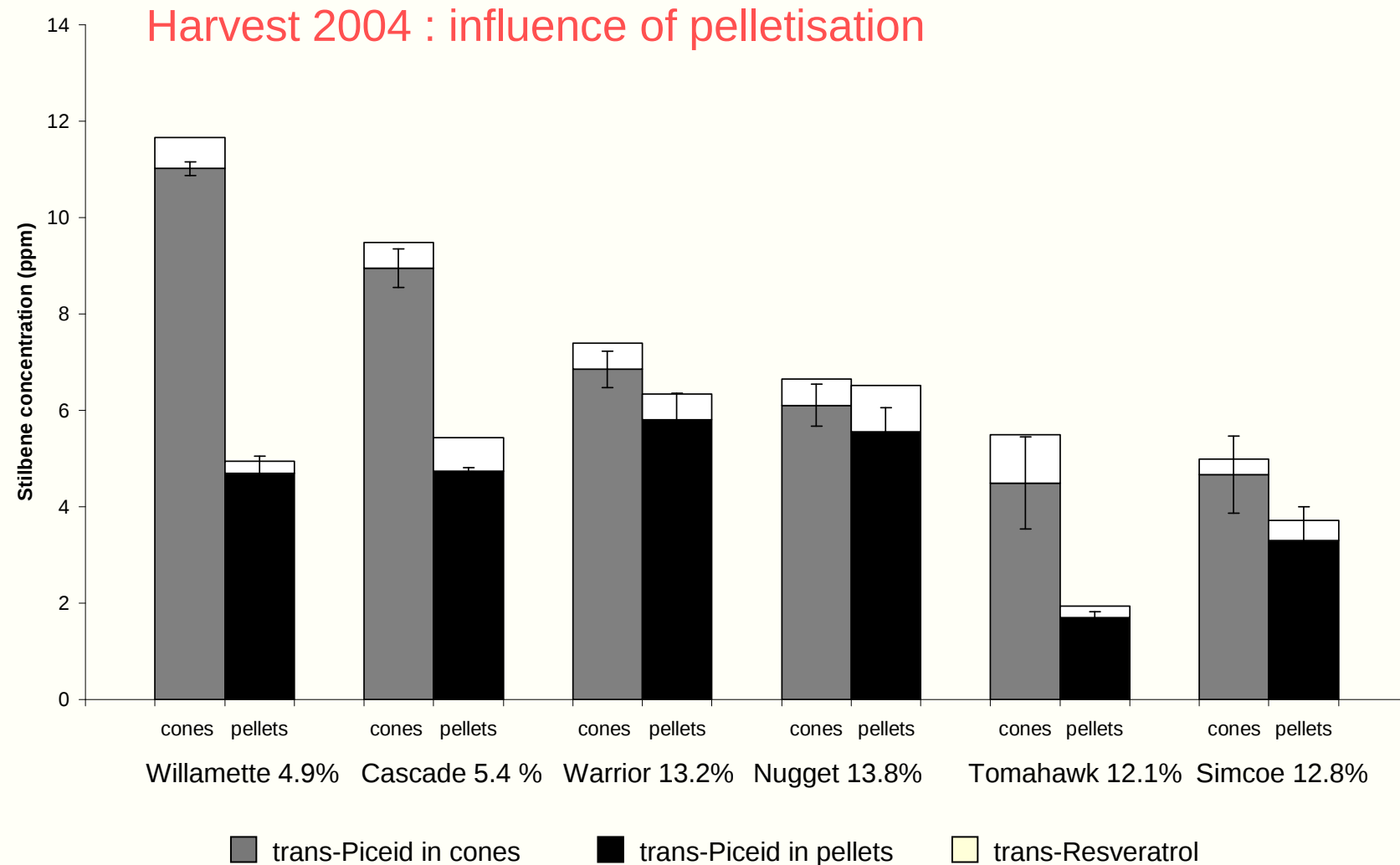
Jerkovic & Collin, *Journal of Agricultural and Food Chemistry*, 55, 2007, 8754-8758



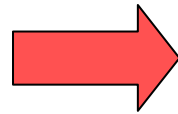
2.3. Influence of hop processing and storage

Stilbene → light sensitive → strong influence of hop processing

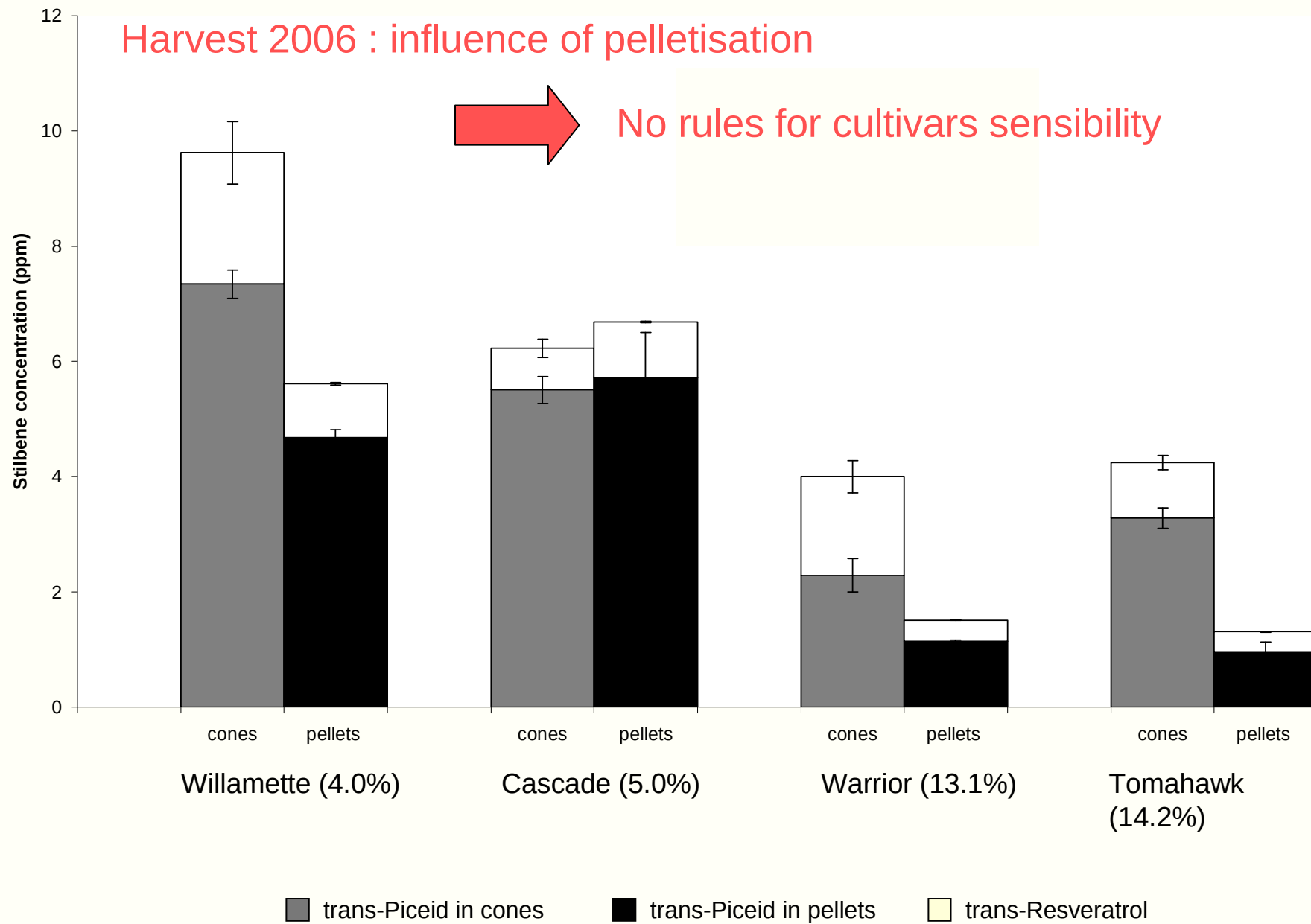
Jerkovic & Collin, *Journal of Agricultural and Food Chemistry*, 2007, accepted



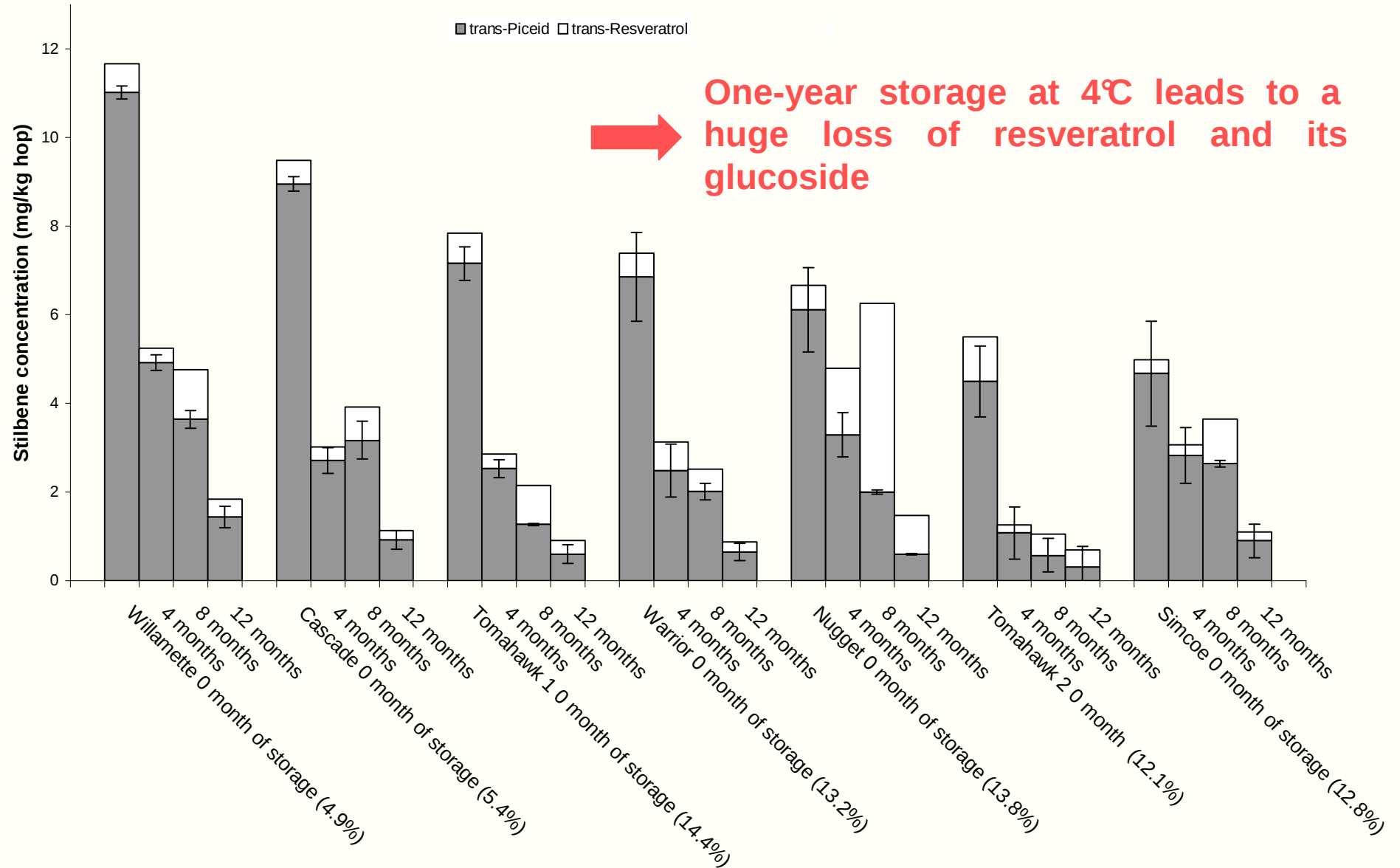
Harvest 2006 : influence of pelletisation



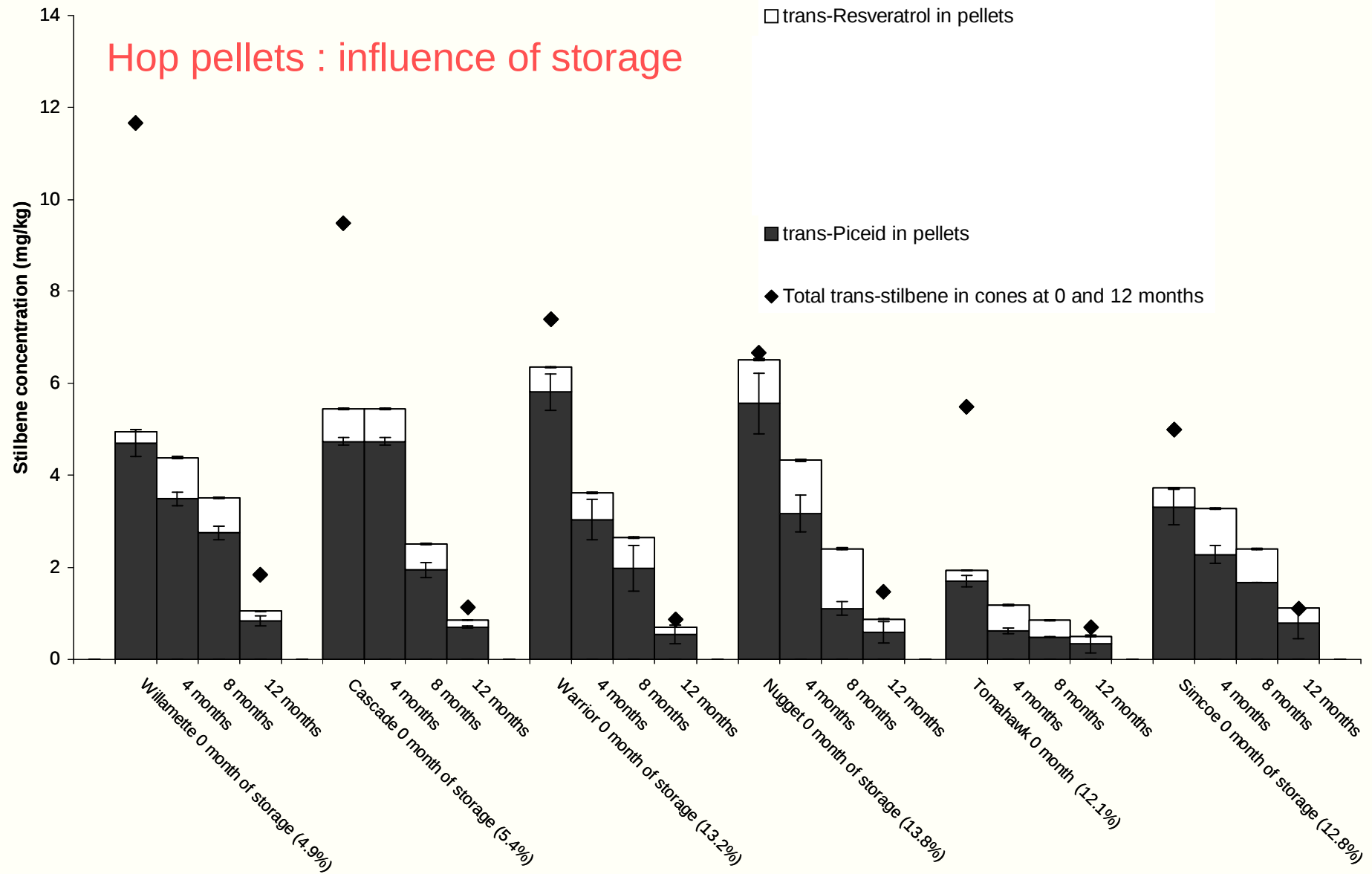
No rules for cultivars sensibility



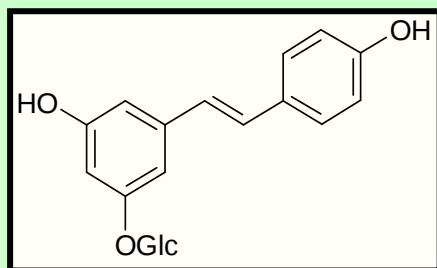
Hop cones : influence of storage



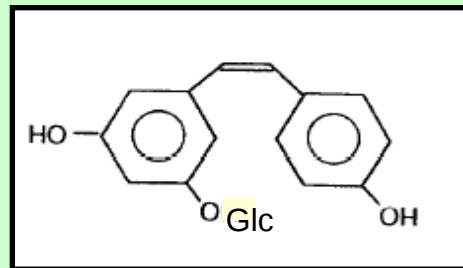
Hop pellets : influence of storage



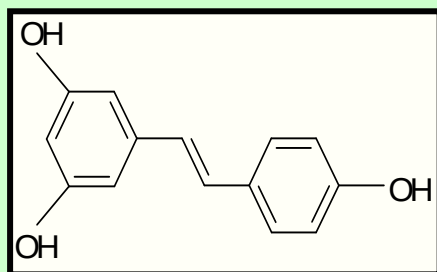
trans-Piceid



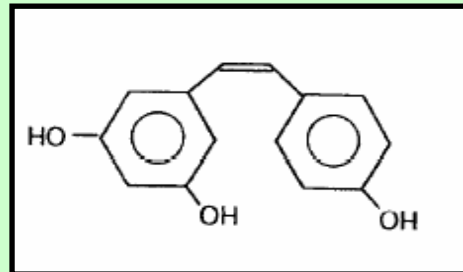
cis-Piceid



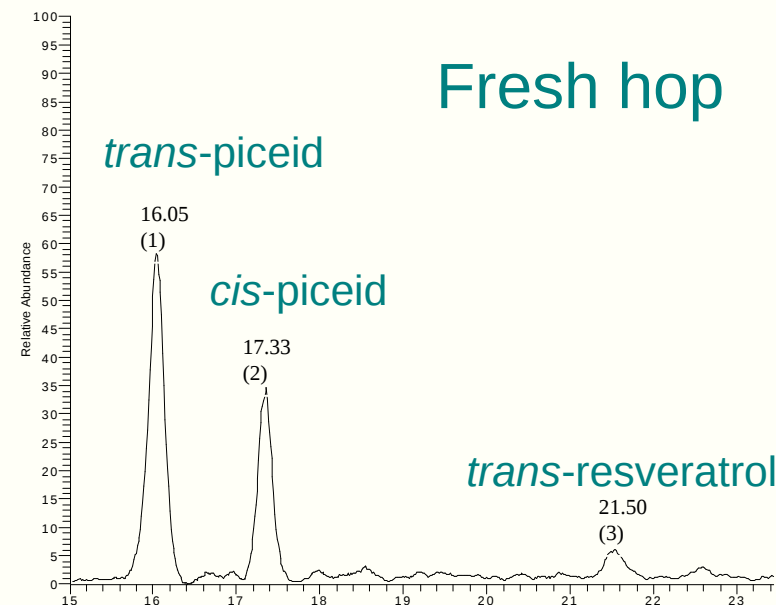
trans-Resveratrol



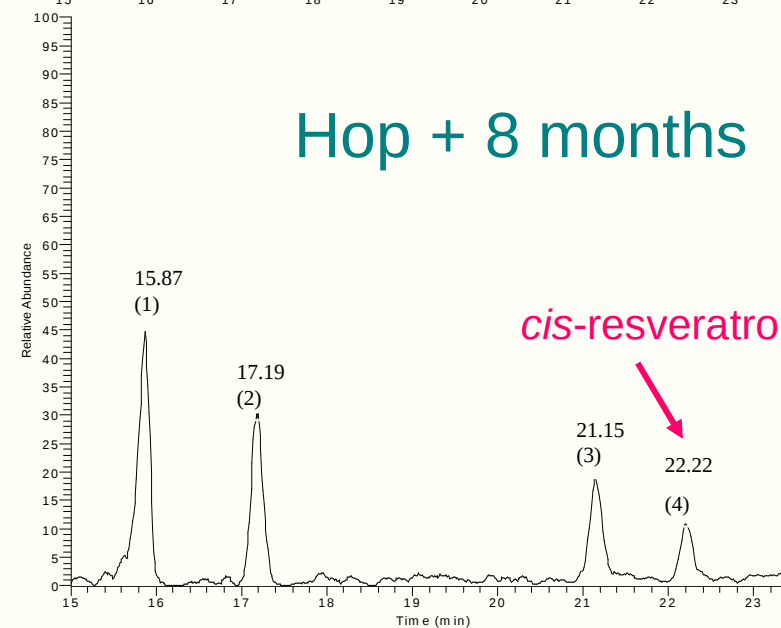
cis-Resveratrol



Fresh hop

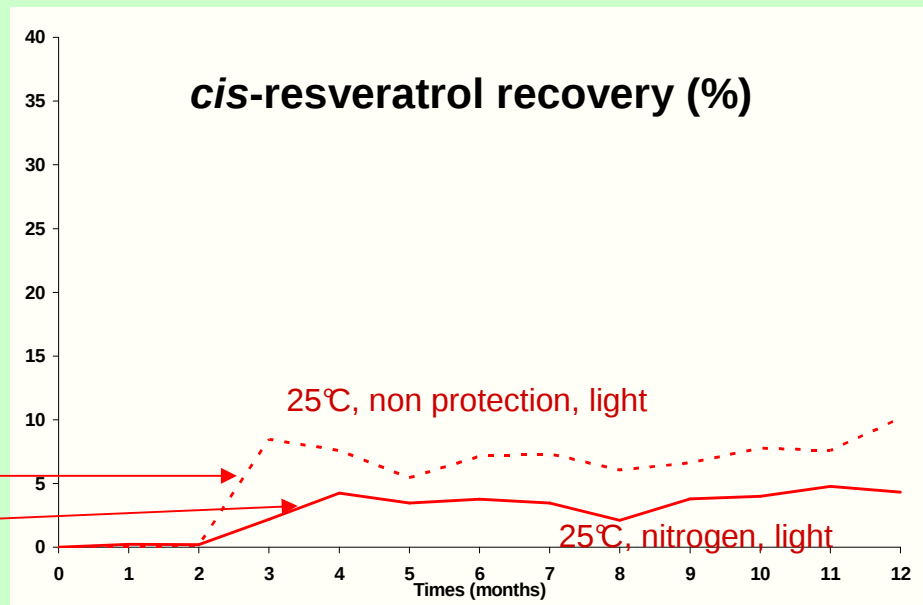
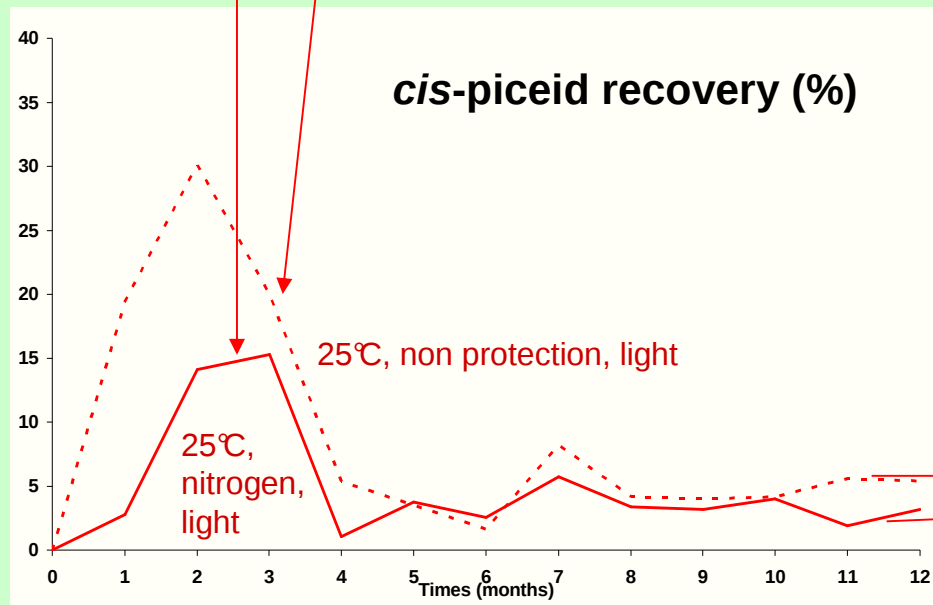
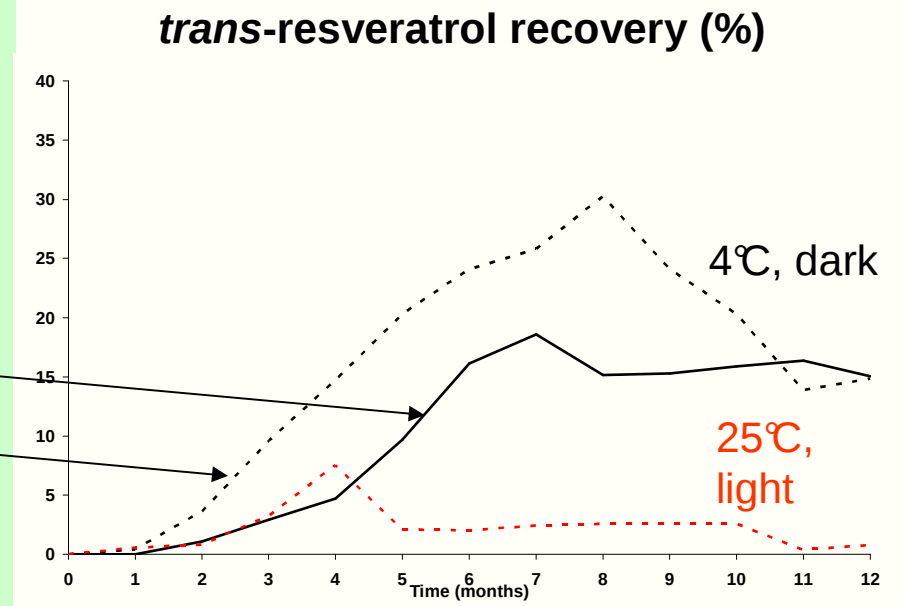
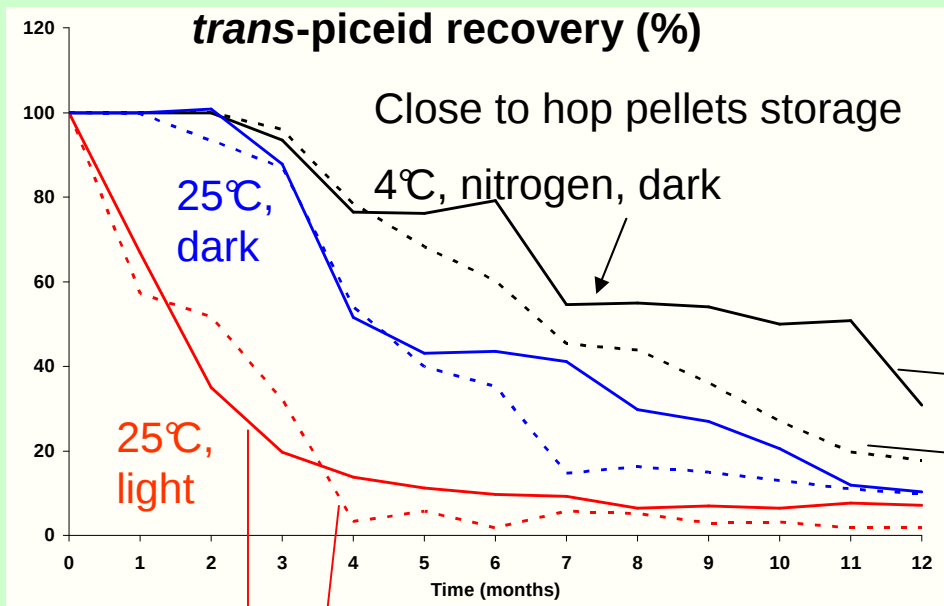


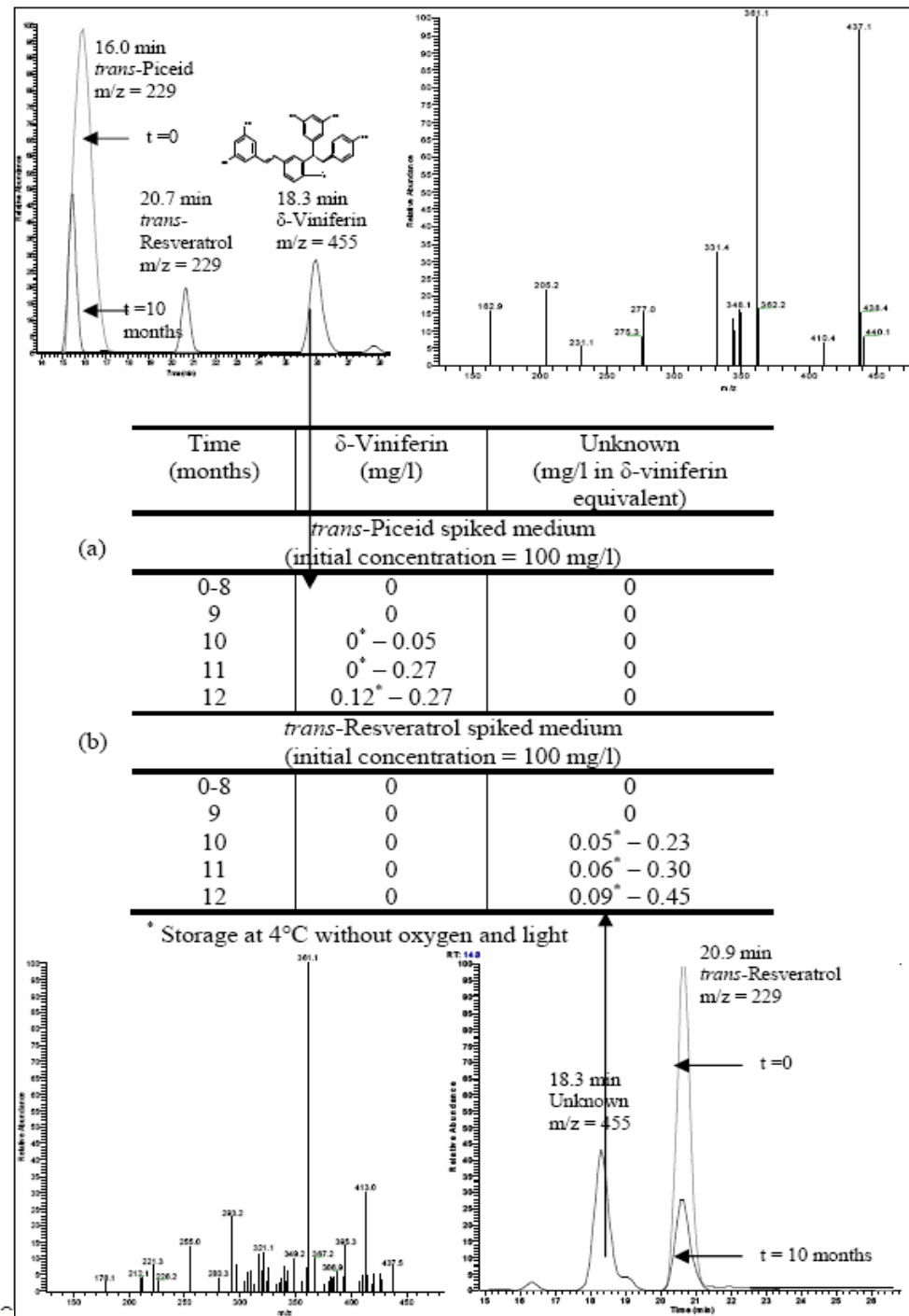
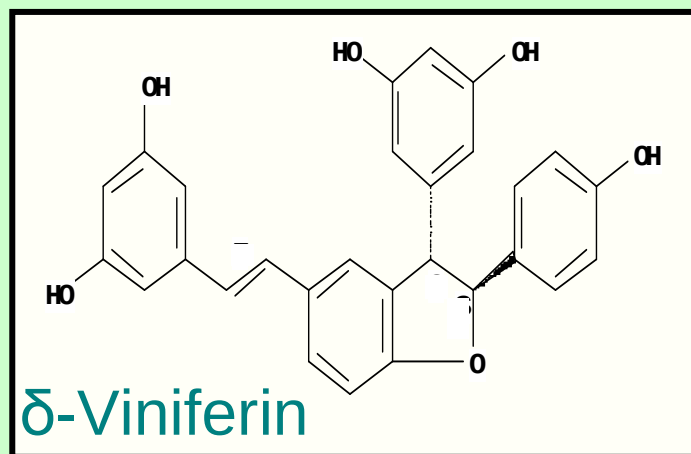
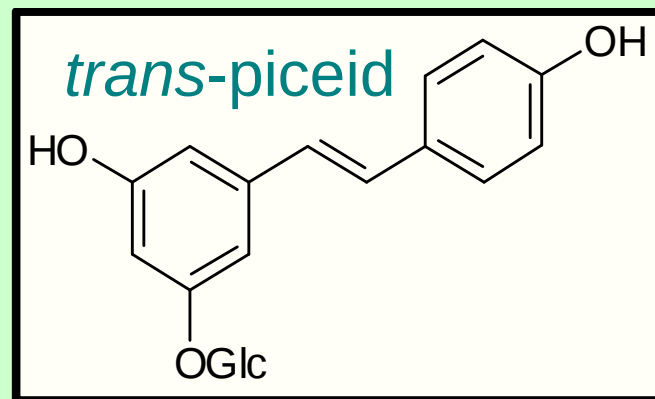
Hop + 8 months



2.3. Influence of hop processing and storage

Jerkovic & Collin, *Journal of Agricultural and Food Chemistry*, 2007, accepted.

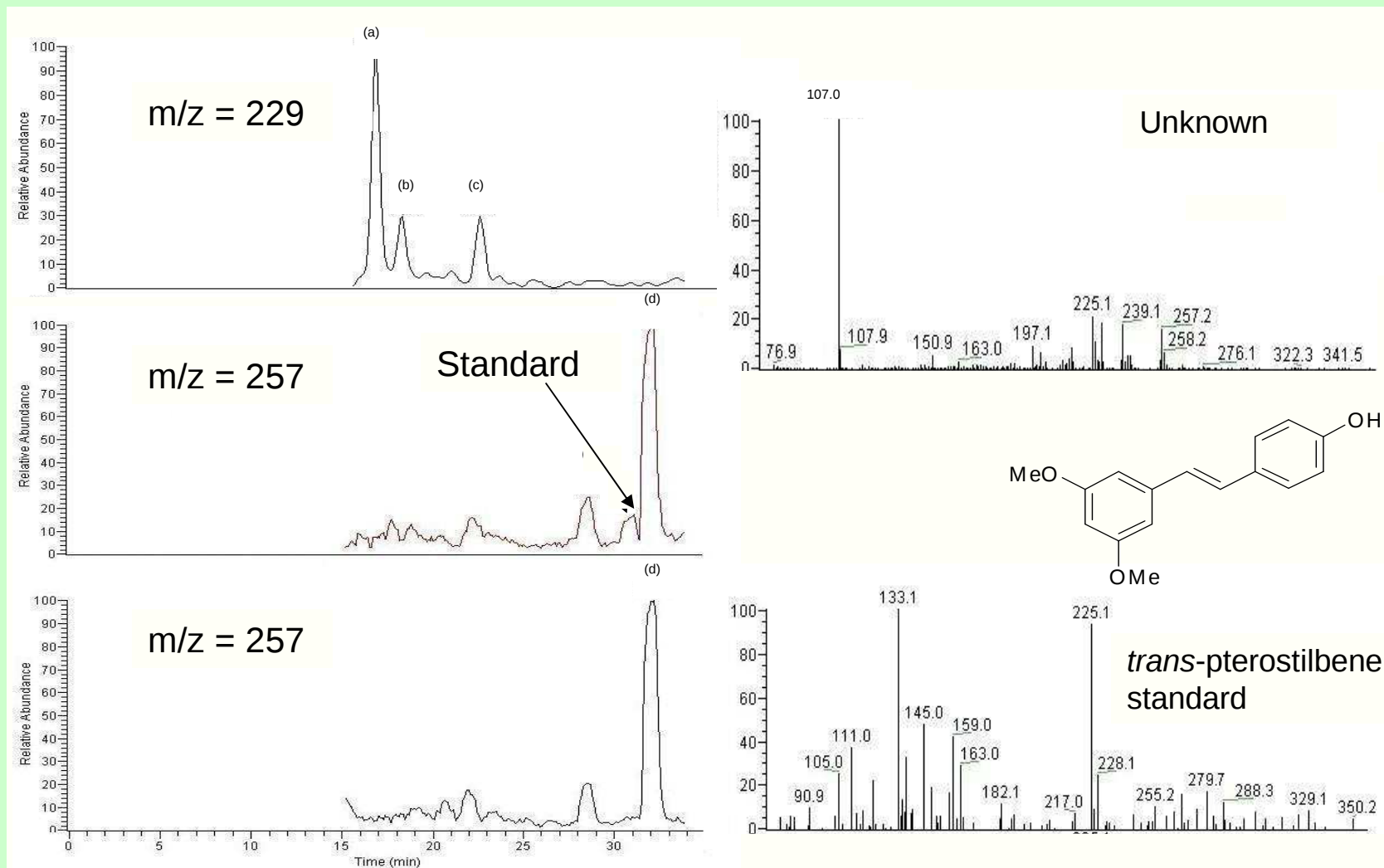




2.4. Search for resveratrol analogs in hop

Jerkovic & Collin, *EBC*, 2007, in press

Detection of a *trans*-pterostilbene analog (same $M+1 = 257$) : concentration close to 1 ppm in pterostilbene equivalents



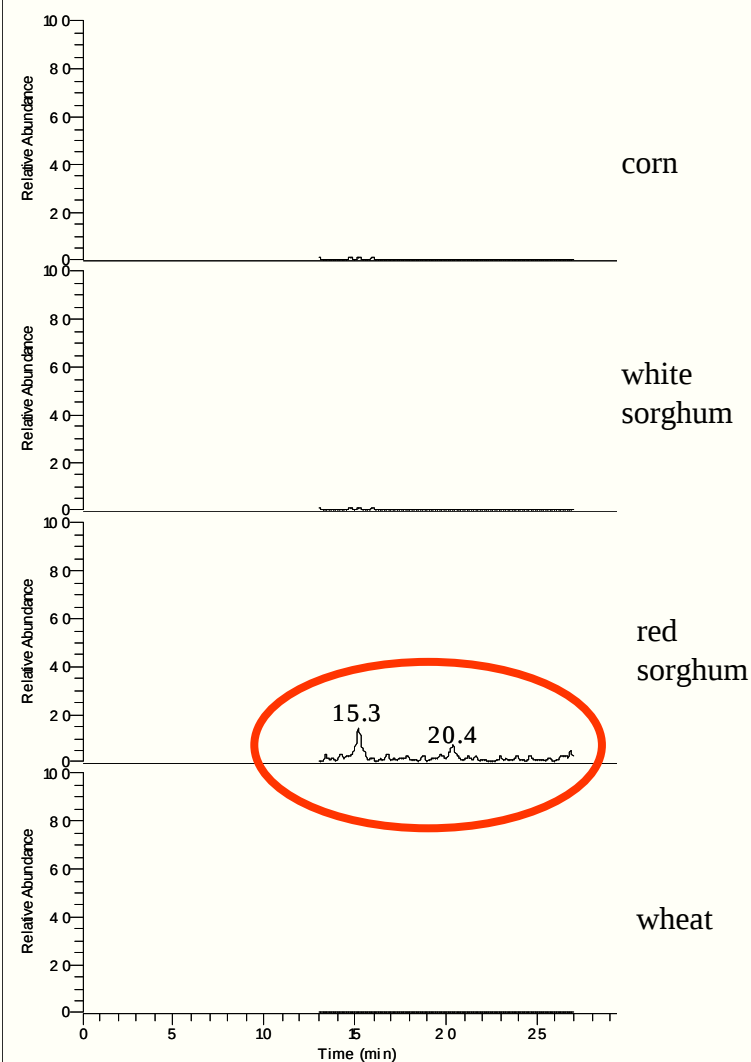
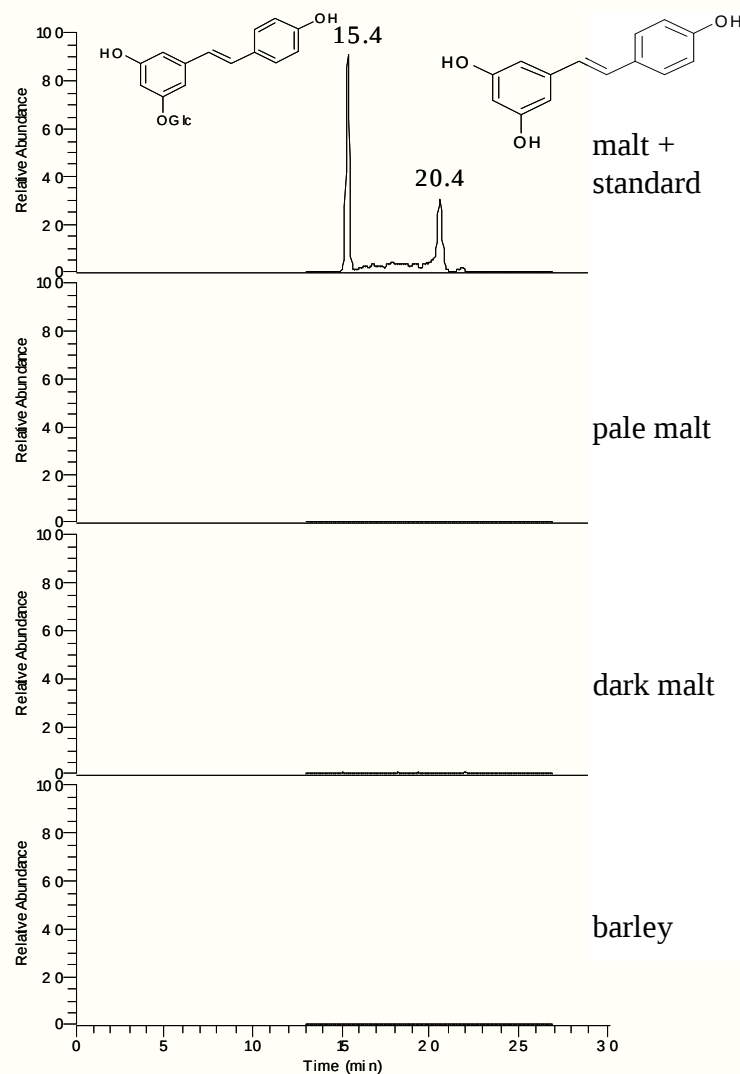
2.5. Other raw materials for brewers

Jerkovic & Collin, *EBC*, 2007, in press

Adaptation of hop extraction : cyclohexane + ethanol:water

For the first time, detection of traces of stilbenes in red sorghum : 0.5 – 1 ppm !

➡ Matrix rich in flavonoids = potential source of stilbenes



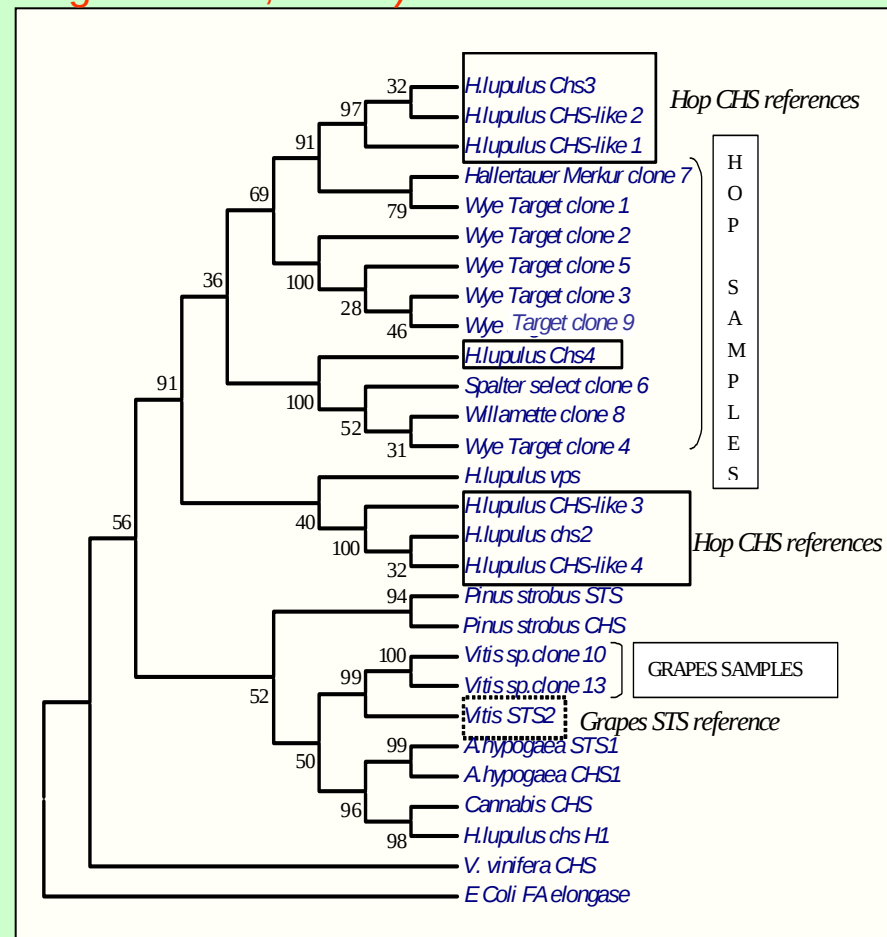
2.6. Search for stilbene synthase in hop

Jerkovic & Collin, *EBC*, 2007, in press

Method : PCR and DNA sequencing

Comparison with Stilbene synthase extracted from grapes

No stilbene synthase was found in hop → chalcone synthase might be involved in stilbene biosynthesis (Yamaguchi *et al*, 1999)



3. Stilbenes in beer

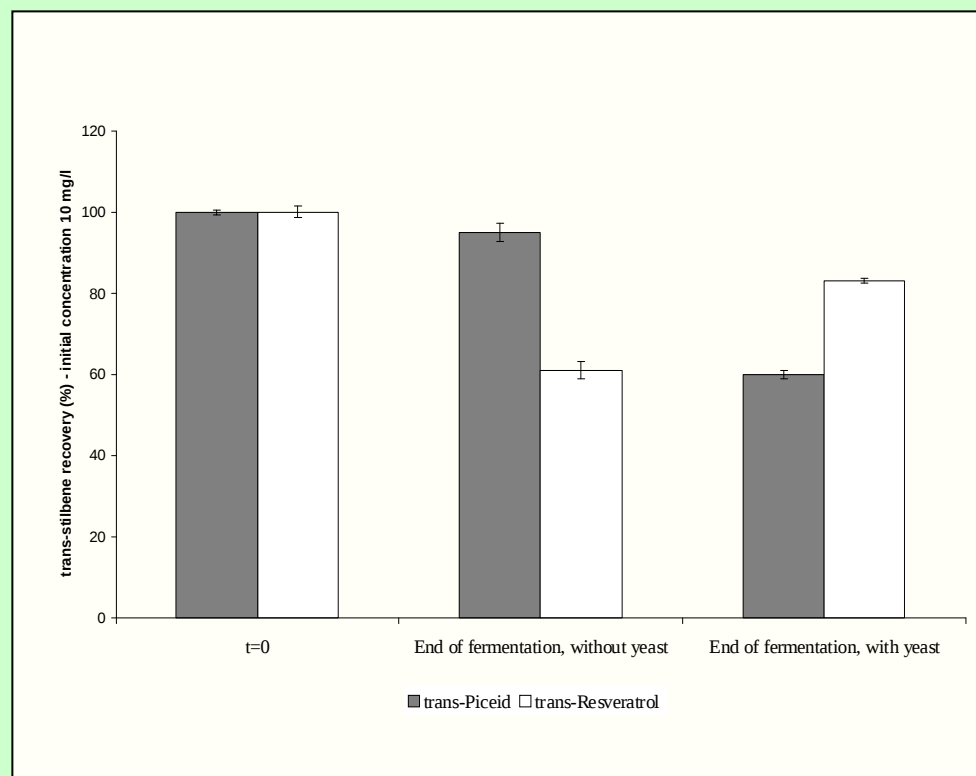
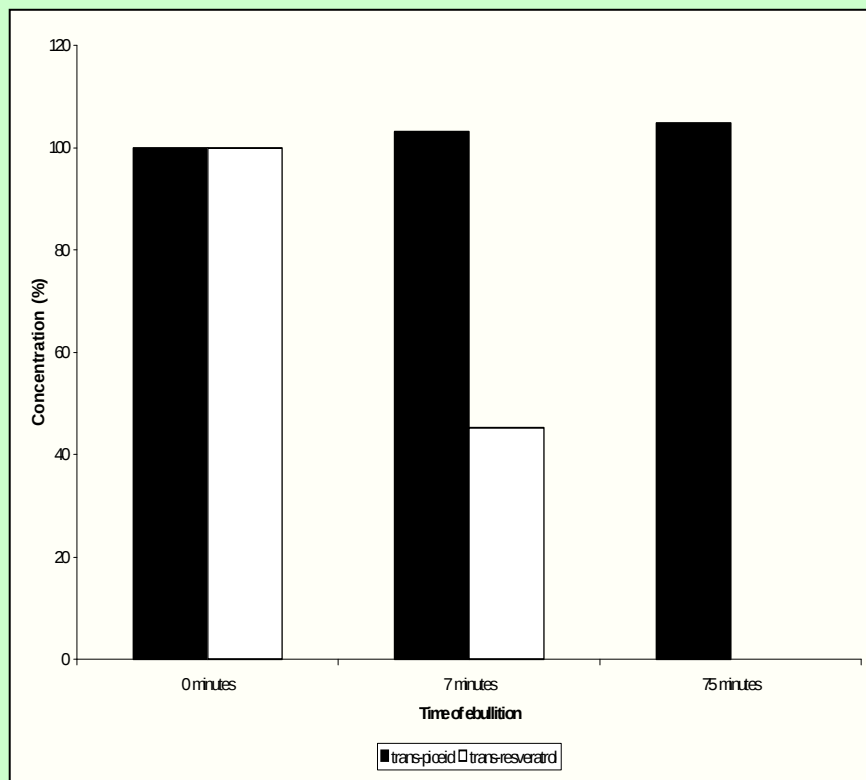
3.1 Stilbenes commercial beers

Jerkovic, Nguyen, Timmermans & Collin, *Journal of the Institute of brewing*, 2008, in press.

Beers	<i>trans</i> -Resveratrol ($\mu\text{g.L}^{-1}$)	<i>trans</i> -Piceid ($\mu\text{g.L}^{-1}$)
LG1	>5*	>5*
LG2	>5*	>5*
LG3	<5	>5*
LG4	<5	<5
TFW	>5*	>5*
TFA	>5*	>5*
TFP	<5	<5
TFL	<5	<5

**Resveratrol in commercial
beers ~ 5 $\mu\text{g/l}$**

* Under the quantification limit ($15 \mu\text{g.L}^{-1}$) of the SPE procedure.



Ebullition = critical step

Fermentation = release from the glucoside

How to increase resveratrol content ????

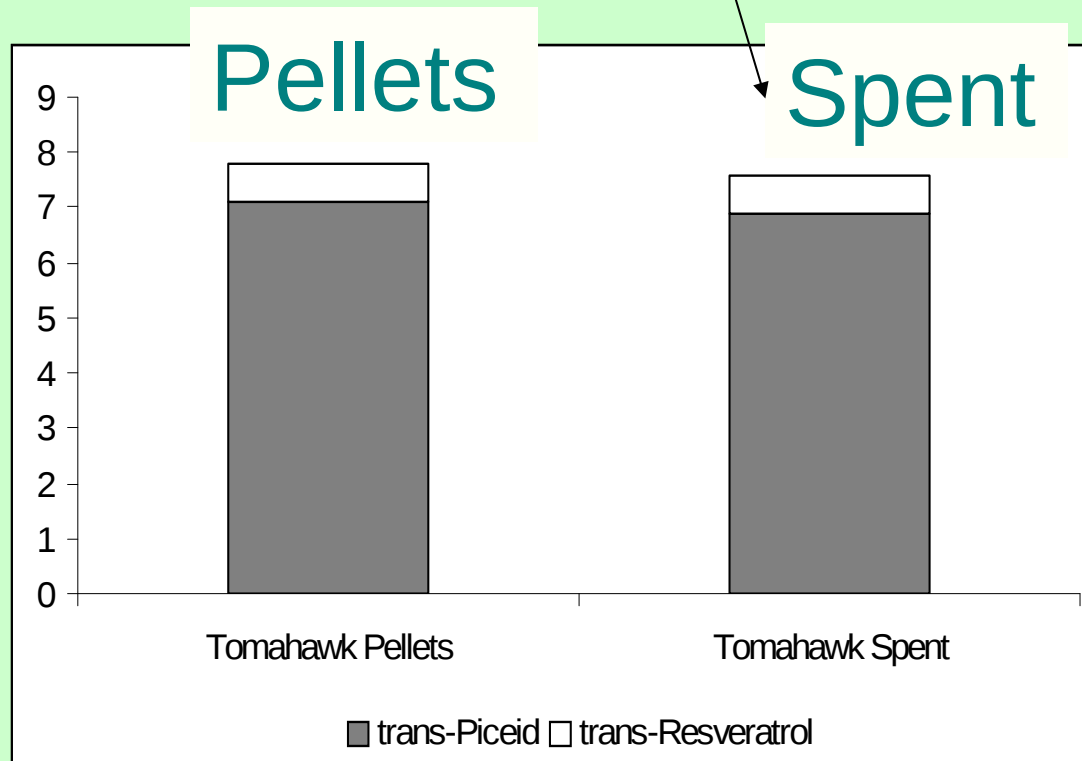
→ Preparation of stilbene-rich hop extracts

? Use of spent hops

Jerkovic, Callemien & Collin, *Journal of Agricultural and Food Chemistry*, 53, 2005, 4202 – 4206

3.2 Enriched-hop extract

CO₂ extracts



CONCLUSIONS

Methods have been optimized for stilbenes analysis in hop and beer

A new stilbene library is now available

A. Hop = exceptional source of polyphenols

B. For hop stilbenes :

- the American low- α -acid cultivars emerge as the most concentrated before pelletization;
- strong influence of the harvest year;
- pelletization induces strong stilbene degradation;
- one-year storage at 4°C leads to a huge loss of res veratrol and its glucoside, especially in the case of hop cones.

C. Beer :

- resveratrol in commercial beers ~ 5 $\mu\text{g/l}$;
- spent hop extracts could be very interesting to increase and standardize resveratrol level in beer.

D. No stilbene synthase in hop

- chalcone synthase could be involved in stilbene synthesis.

E. Red sorghum is another source of stilbenes for brewers

Thanks to...

- La fondation Inbev-Baillet Latour pour le soutien financier
- Prof. Sonia Collin
- L'équipe INBR : Fanny, Etienne,...
- Les mémorants qui ont participé au projet
- Monsieur Stéphane Meulemans de chez Yakima Chief pour son aide précieuse dans l'obtention d'échantillons
- Ma famille
- Mes amis : Béné, Alexis, Aurore, Sabine, Delphine, ...
- Michaël
- Toutes les personnes qui de près ou de loin m'ont permises de mener à bien ce travail