

Quantitative Analysis of Bioflavonoids in *Cannabis* using HPLC

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Presentation Agenda

1. Flavonoid Definitions

- Secondary metabolite biosynthesis
- The Cannflavins

2. Optimal Extractions of Flavonoids


- Techniques and factors to consider
- Challenges of HPLC Analyses
- Ultrasonic enzyme-assisted extractions

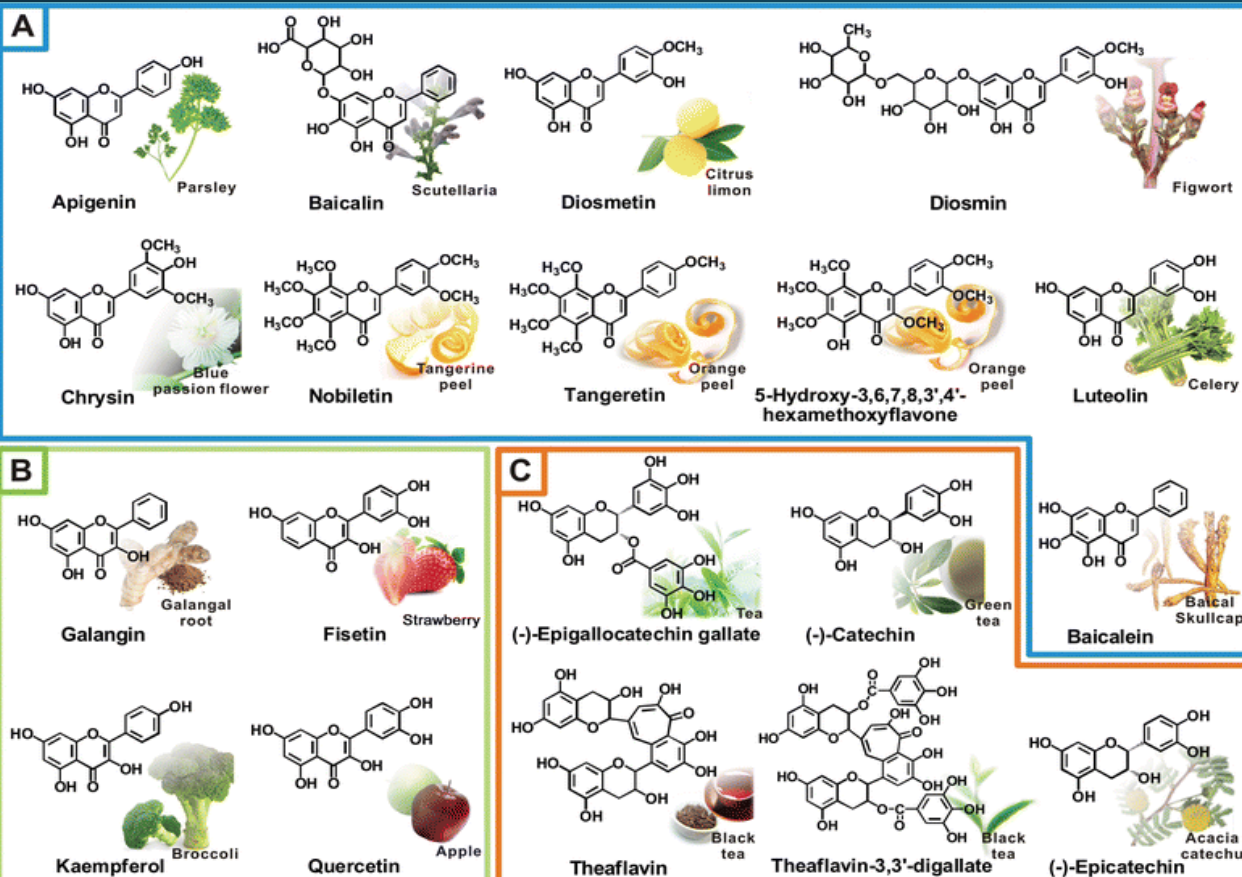
3. Metabolomics / 'Flavolomics'

- Flavonoid presence, content, and variance
- Predictive breeding through metabolomics



Flavonoid intake is associated with lower mortality in the Danish Diet Cancer and Health Cohort

[Nicola P. Bondonno](#) , [Frederik Dalgaard](#), [Cecilie Kyrø](#), [Kevin Murray](#), [Catherine P. Bondonno](#), [Joshua R. Lewis](#), [Kevin D. Croft](#), [Gunnar Gislason](#), [Augustin Scalbert](#), [Aedin Cassidy](#), [Anne Tjønneland](#), [Kim Overvad](#) & [Jonathan M. Hodgson](#)

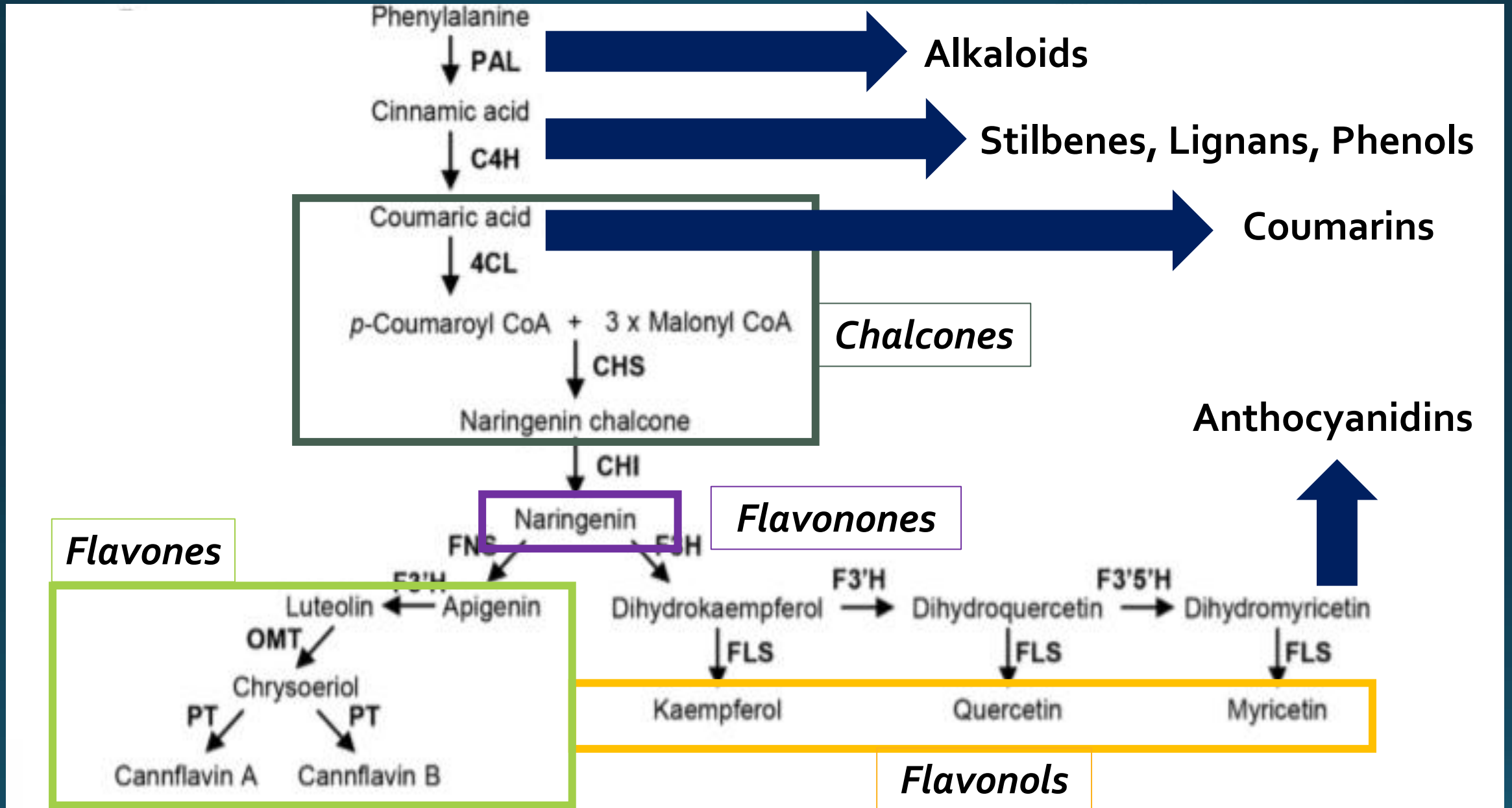


Flavonoid Human Health Potential

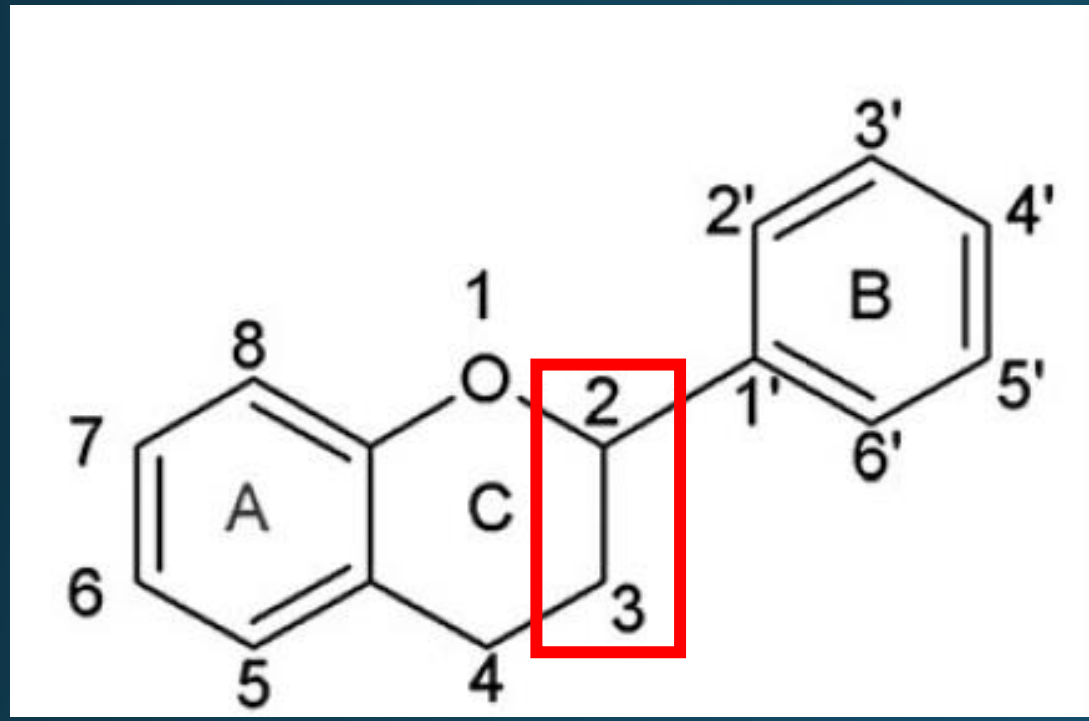
- Anti-mutagenic
- Antimicrobial
- Anti-inflammatory
- Antioxidant
- Anti-carcinogenic
- Anti-thrombogenic
- Antiplatelet
- Enzyme inhibition

> 6,000 naturally occurring plant flavonoids

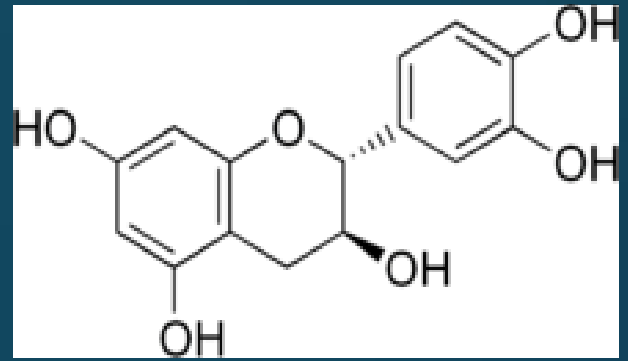
Non-Terpenoid Plant Secondary Metabolite Biosynthesis



Bioflavonoid Nomenclature



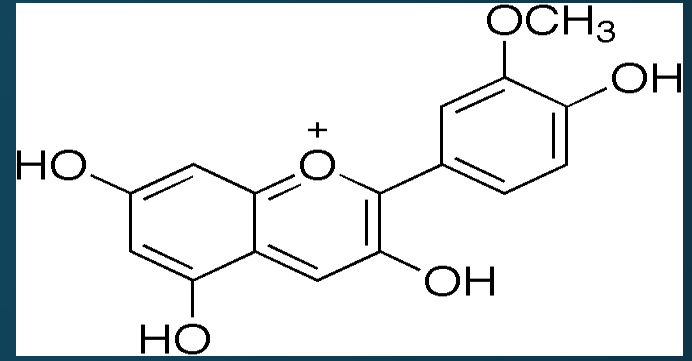
Catechins



(+)-Epicatechin



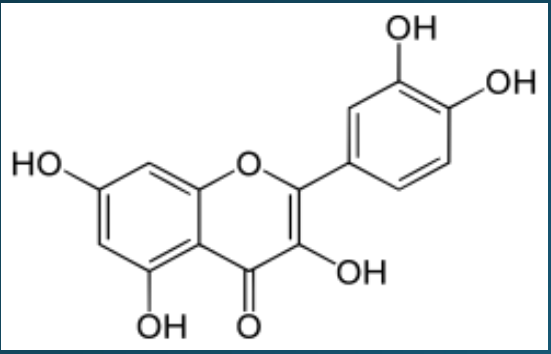
Anthocyanidins



Peonidin

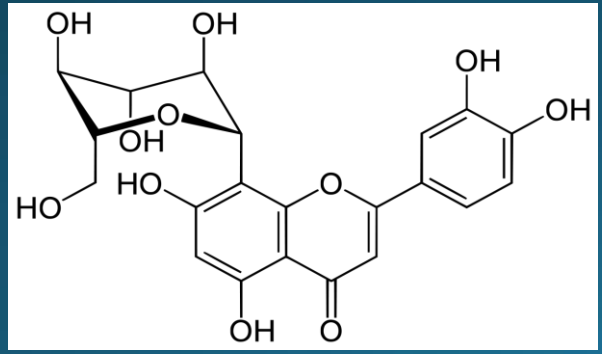


Flavonols



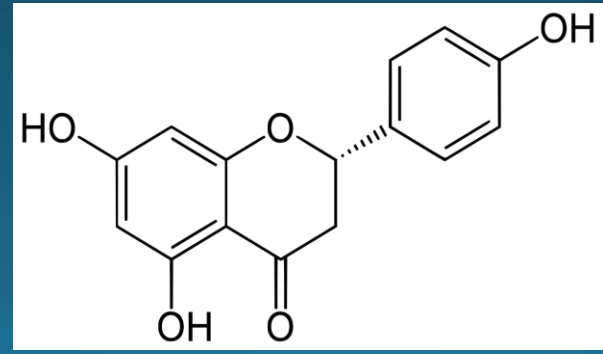
Quercetin

Flavones



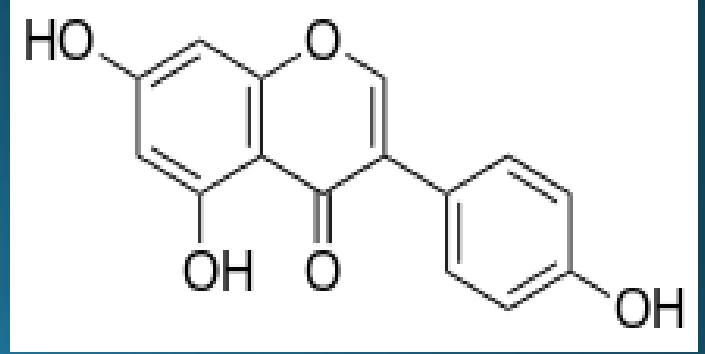
Orientin

Flavanones



Naringenin

Isoflavones

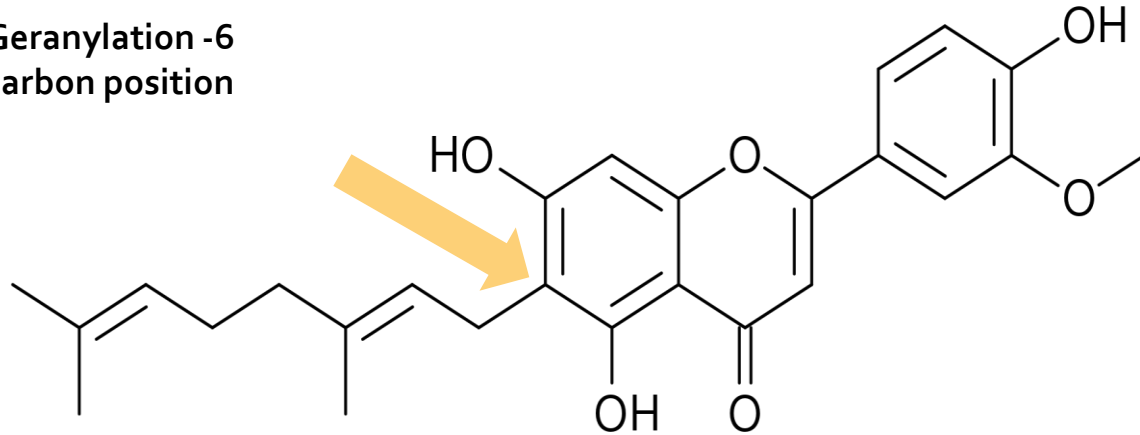


Genistein

Flavones of Cannabis

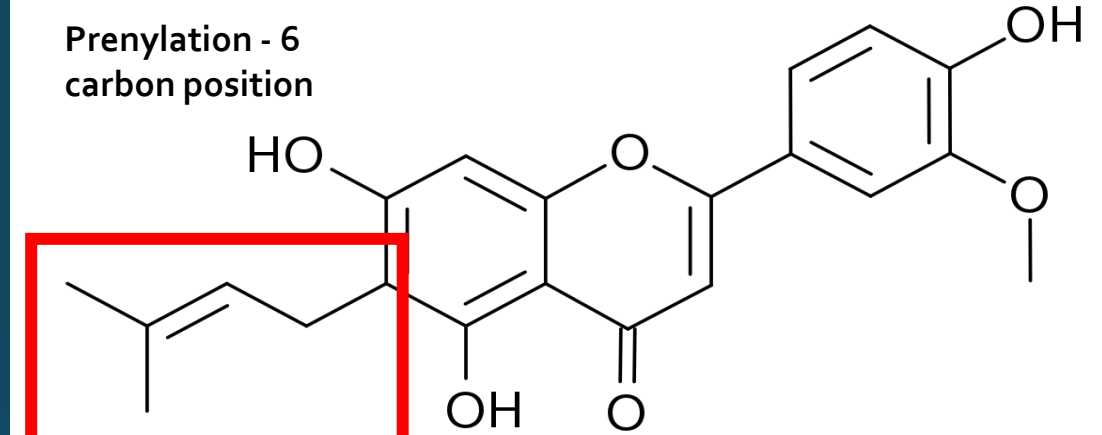
Cannflavin A

Geranylation - 6
carbon position



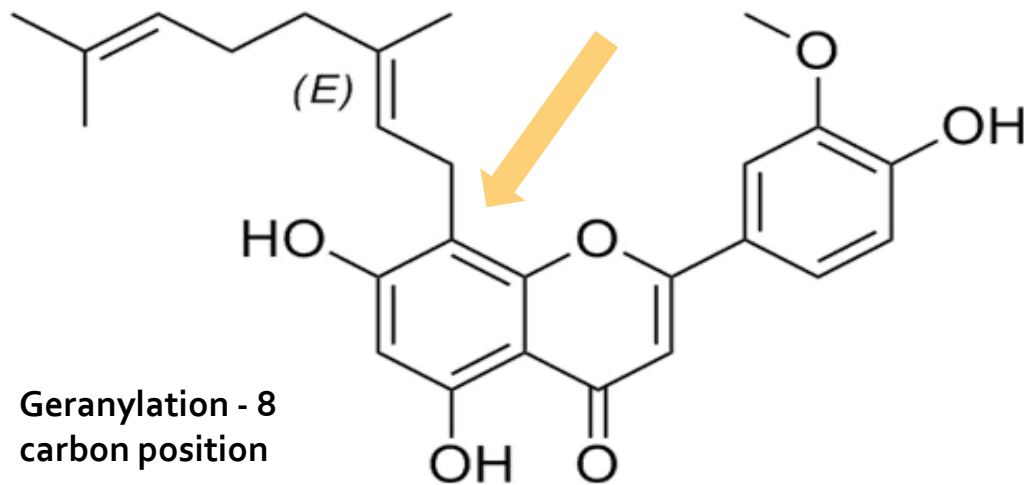
Cannflavin B

Prenylation - 6
carbon position



Cannflavin C

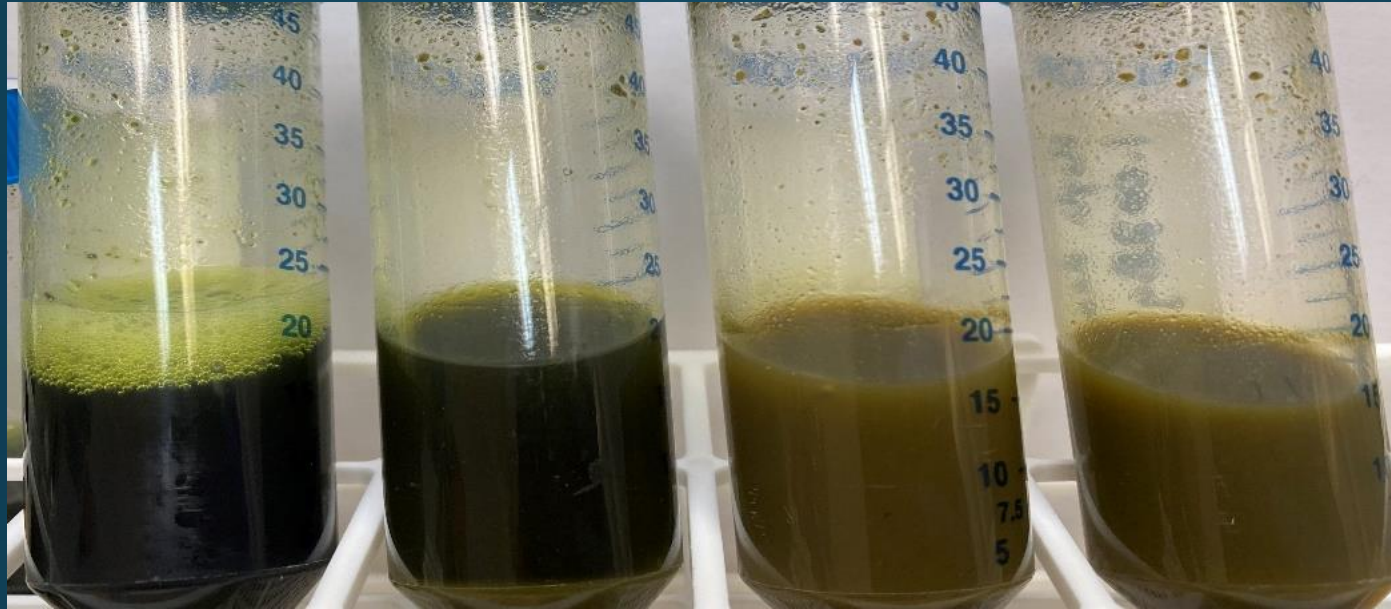
Geranylation - 8
carbon position



- Cannflavin A & B specific to *Cannabis* spp.
- Anti-inflammatory properties **30 X > aspirin** (prostaglandin and leukotriene modulated).
- Young vegetative tissue < 0.1% by weight

Extraction Techniques

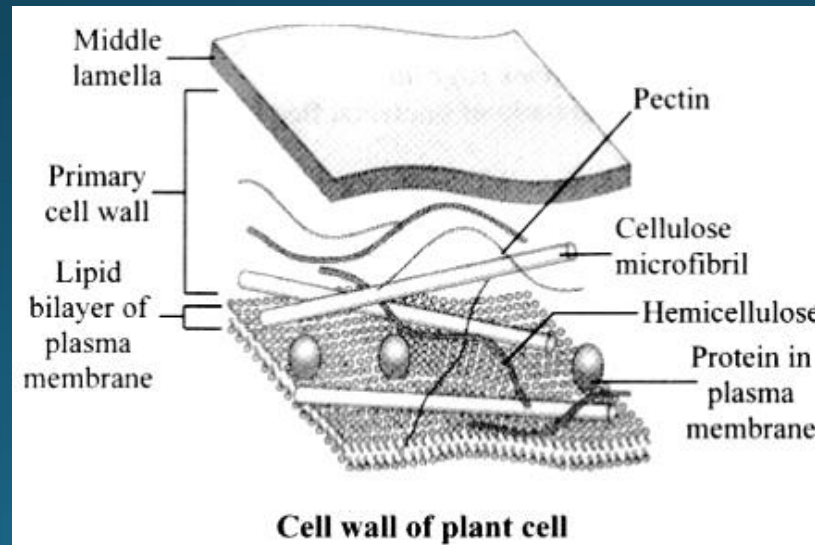
DMSO



H₂O/EtOH

Endless Choices

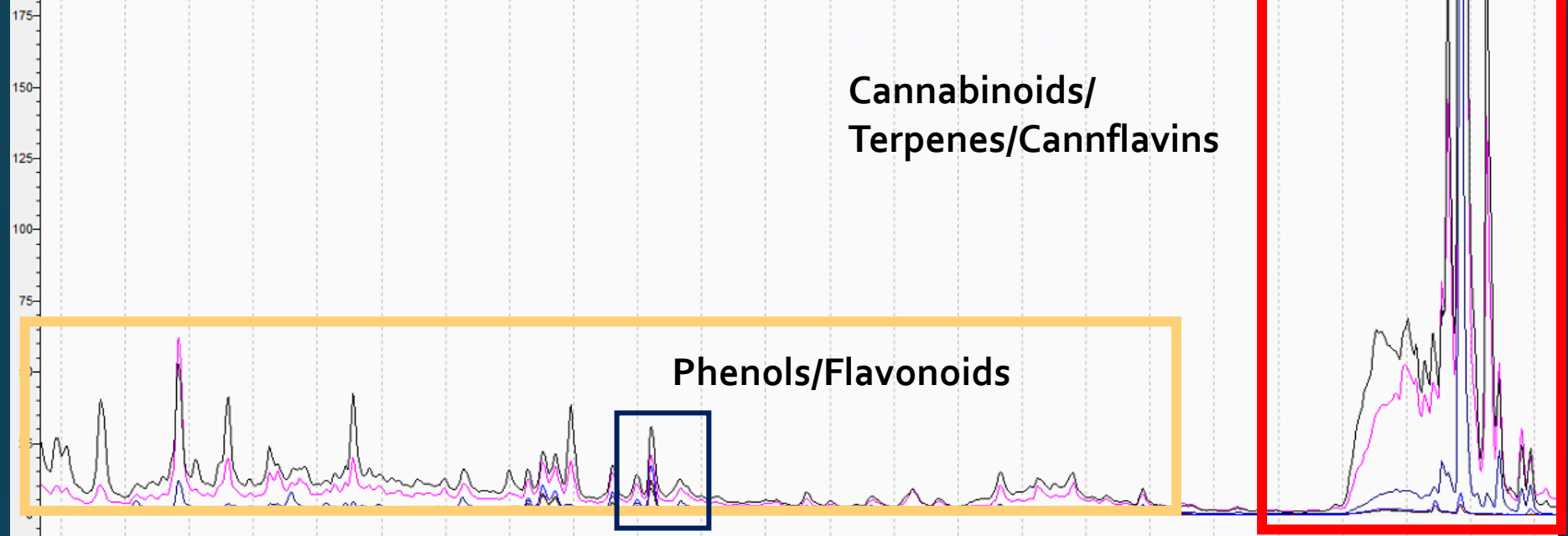
- Soxhlet Extraction
- **Enzymatic Assisted Extraction**
- Acid Leaching
- Ultrasonic Extraction
- Supercritical Fluid Extraction (SFE)
- Microwave-assisted Extraction
- Pulsed Electric Field (PEF)



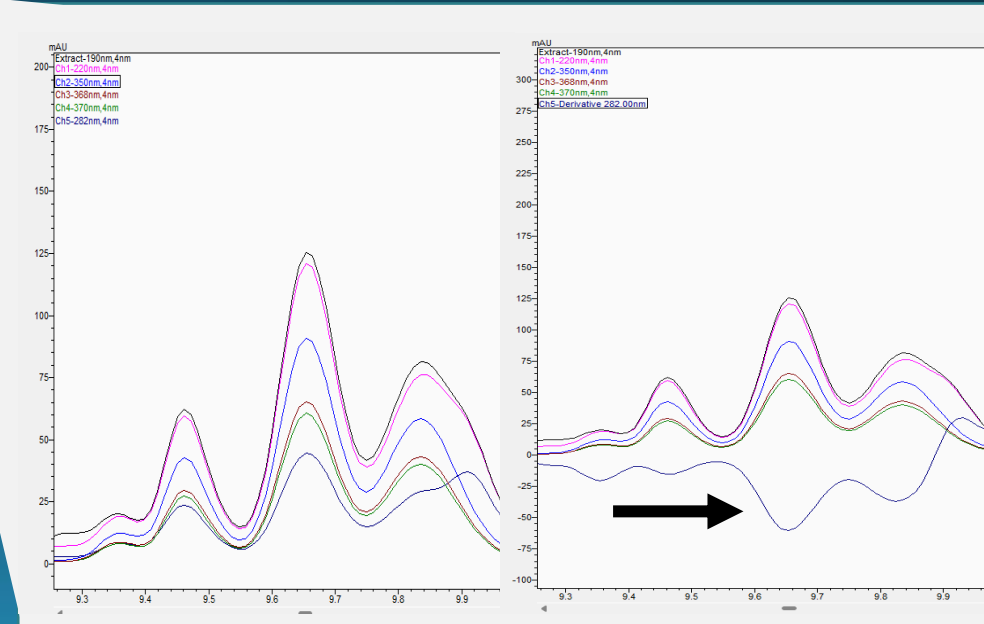
Factors To Consider

- Time
- Temperature
- Solvent Choice
- Sample Moisture Content
- Hydrolysis of Glycosides
- Solvent to Sample Ratio
- Flavonoids of interest/the matrix

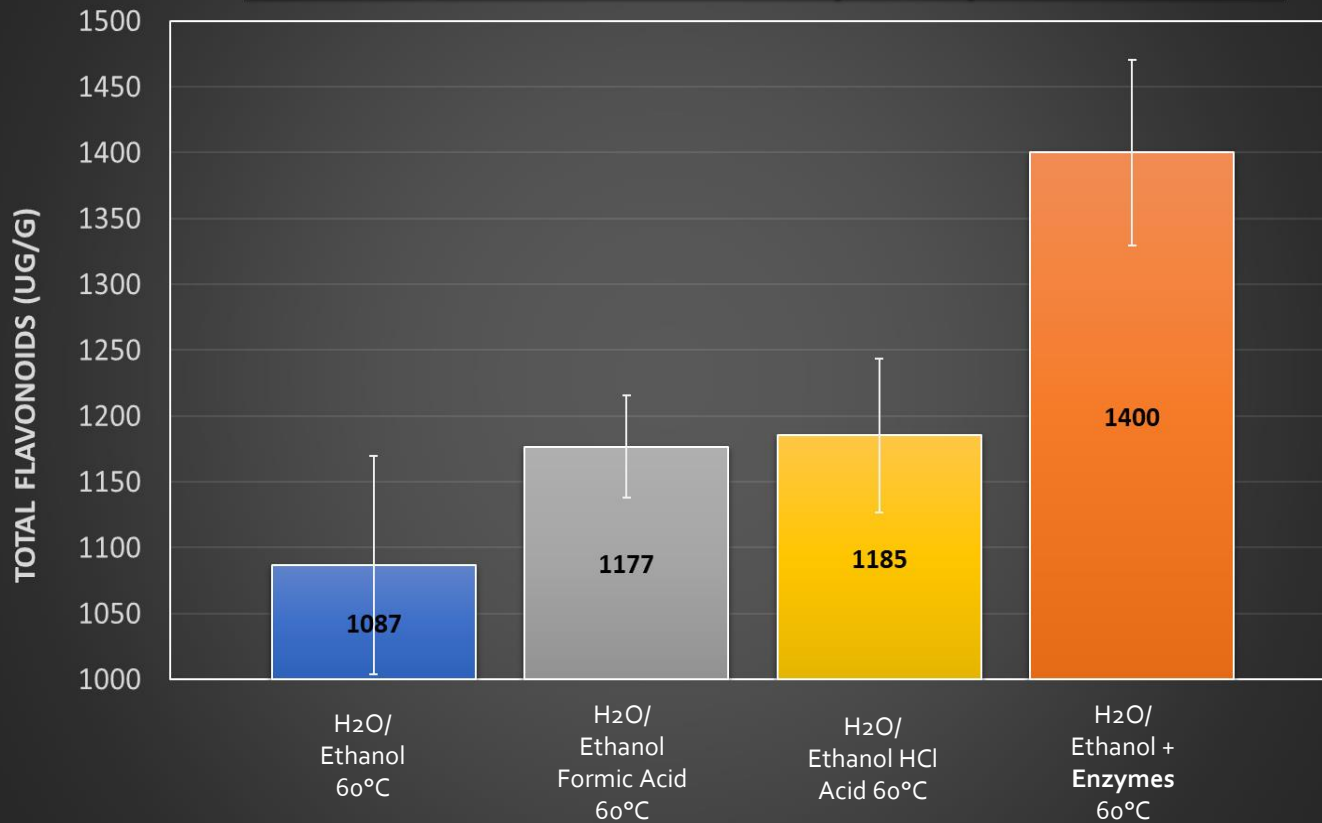
HPLC-PDA Flavonoid Quantitation



- Quantification of 21 Flavonoids in < 25 minutes
- Complex chromatograms!

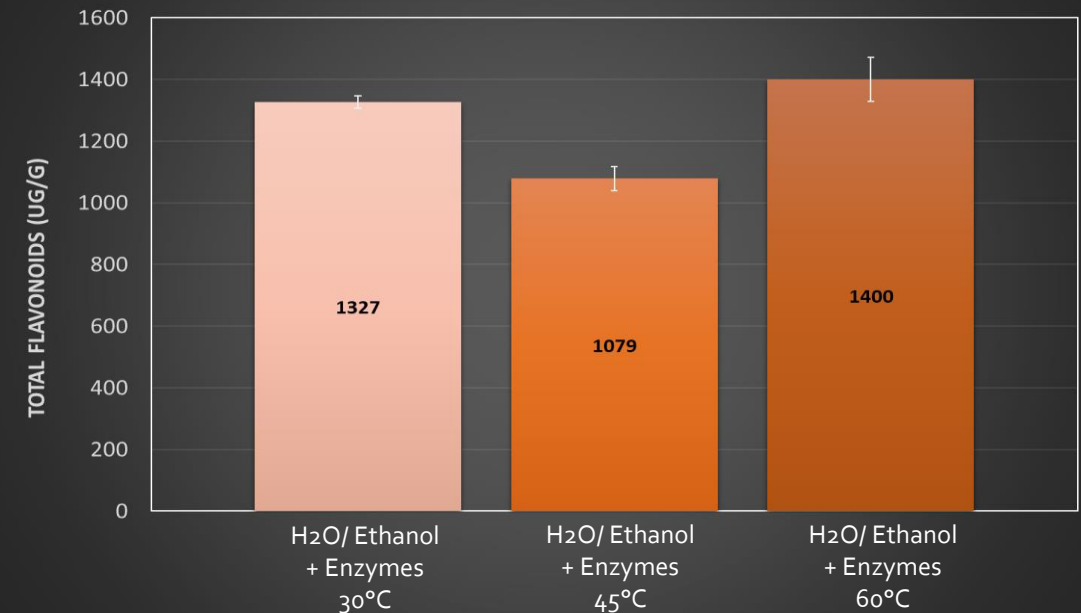


Total Flavonoid Content By Sample Treatment

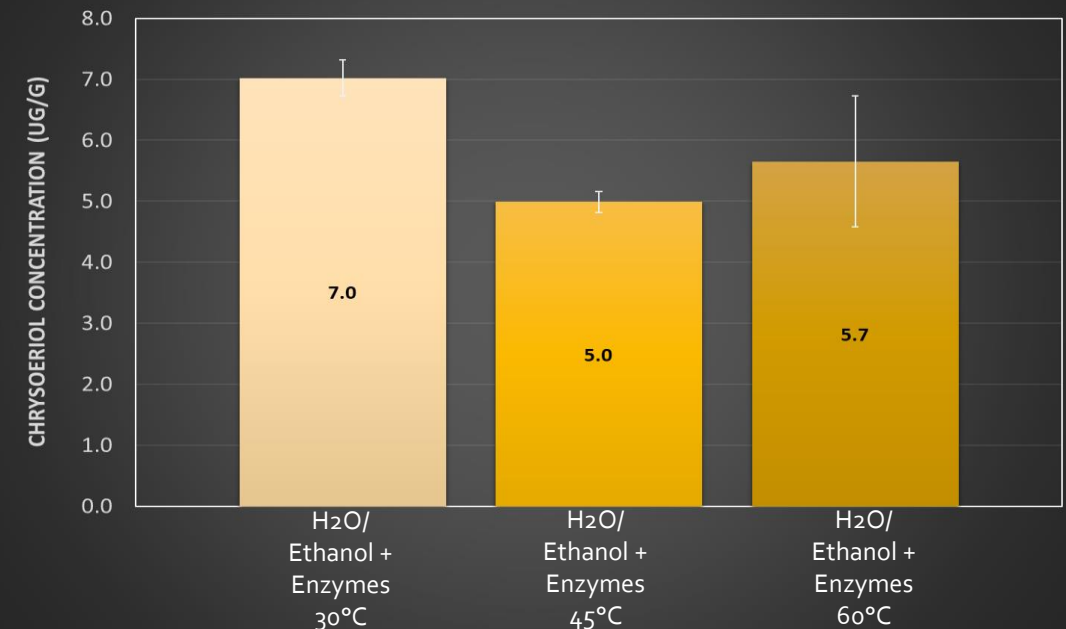


- Enzyme hydrolysis of flavonoid glycosides effective. Most effective within narrow pH and temperature window.
- Medium/low temperature, mixed polarity solvent, ultrasonic enzyme-assisted extraction technique determined.

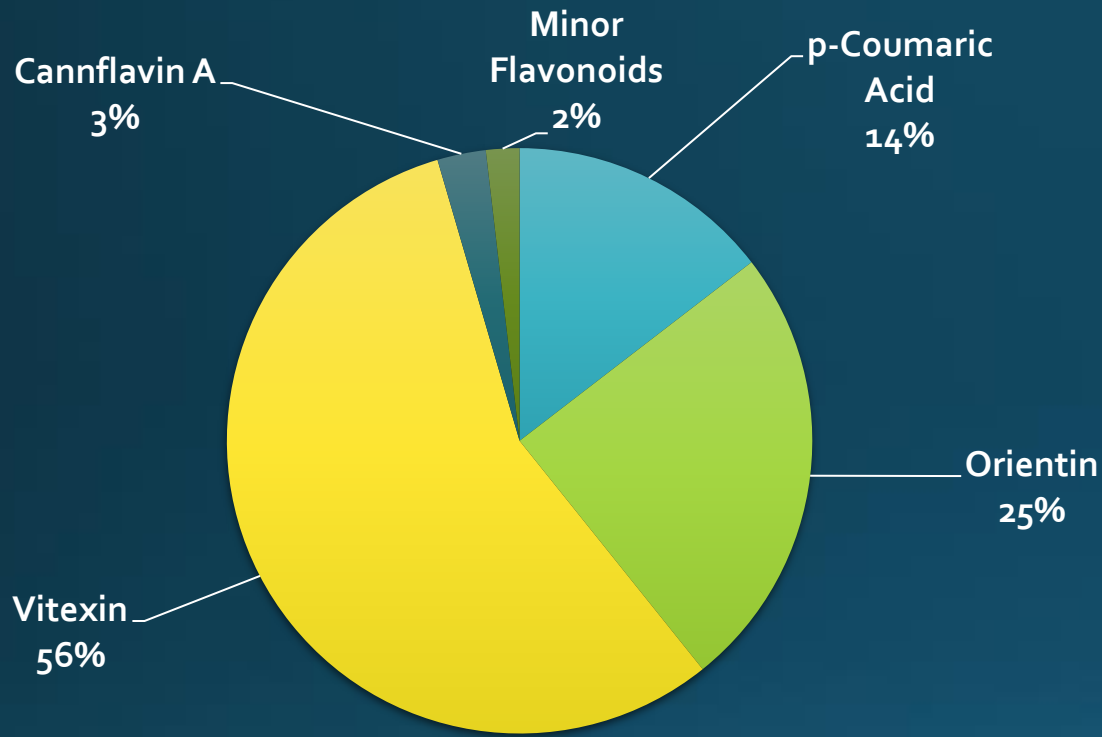
Total Flavonoid Content By Temperature Treatment



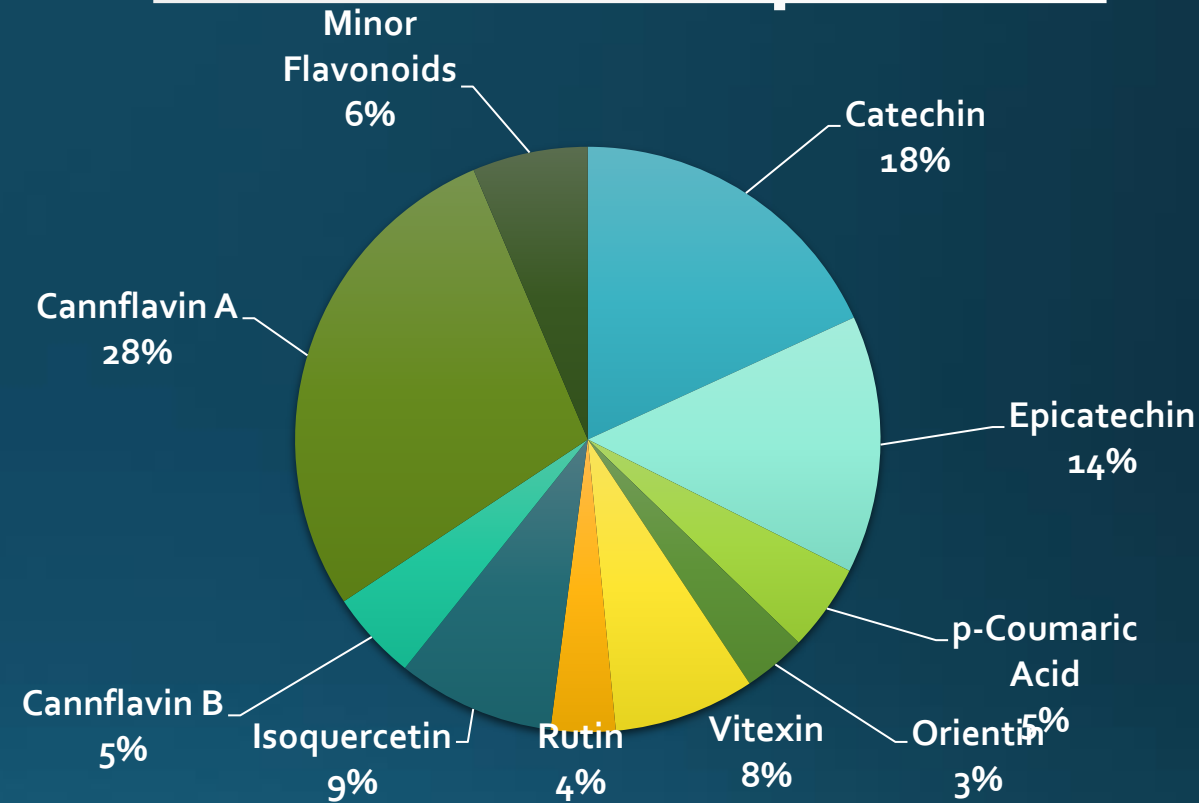
Chrysoeriol Content By Temperature Treatment



Hemp Leaf Flavonoid Components

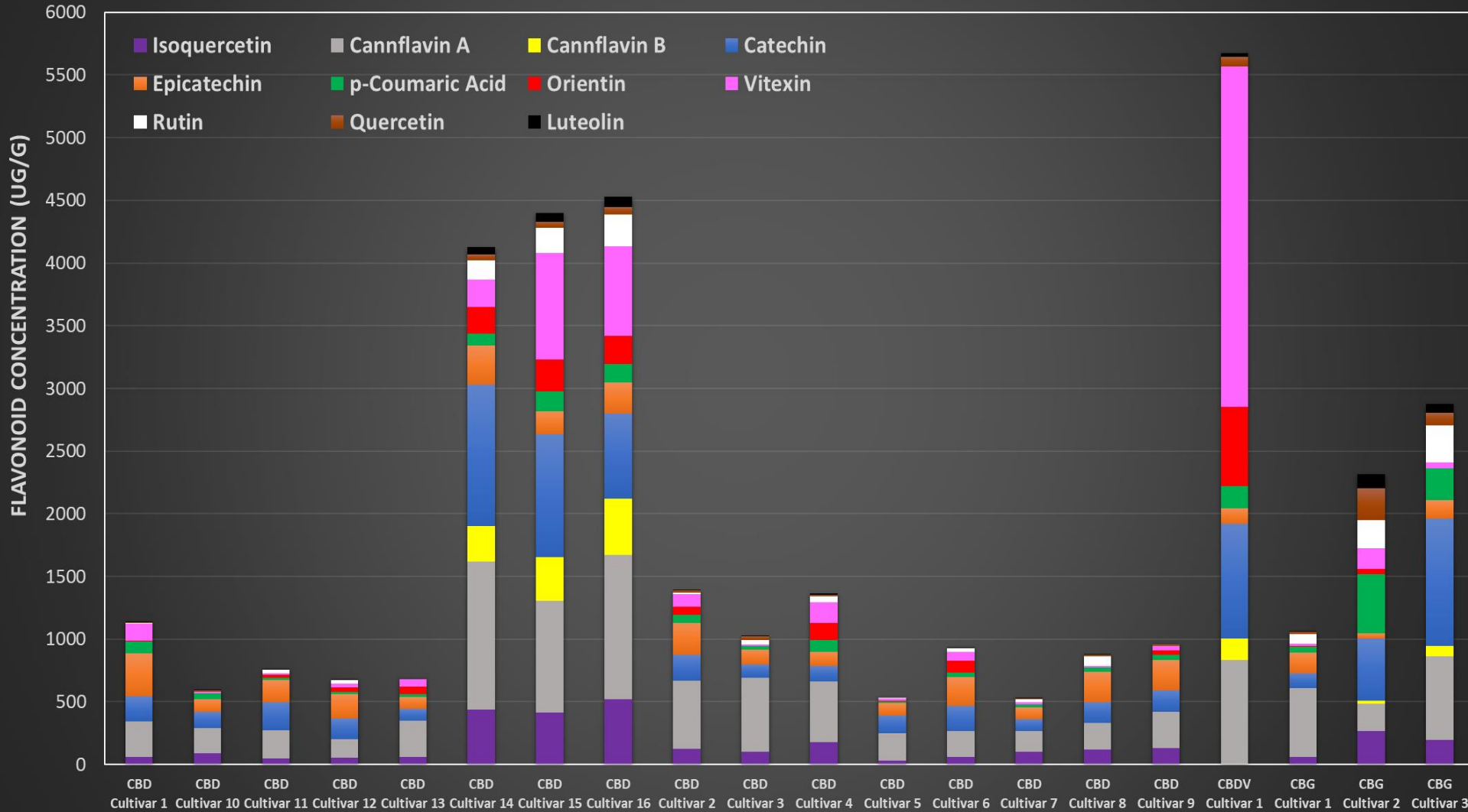


Hemp Flower Flavonoid Components

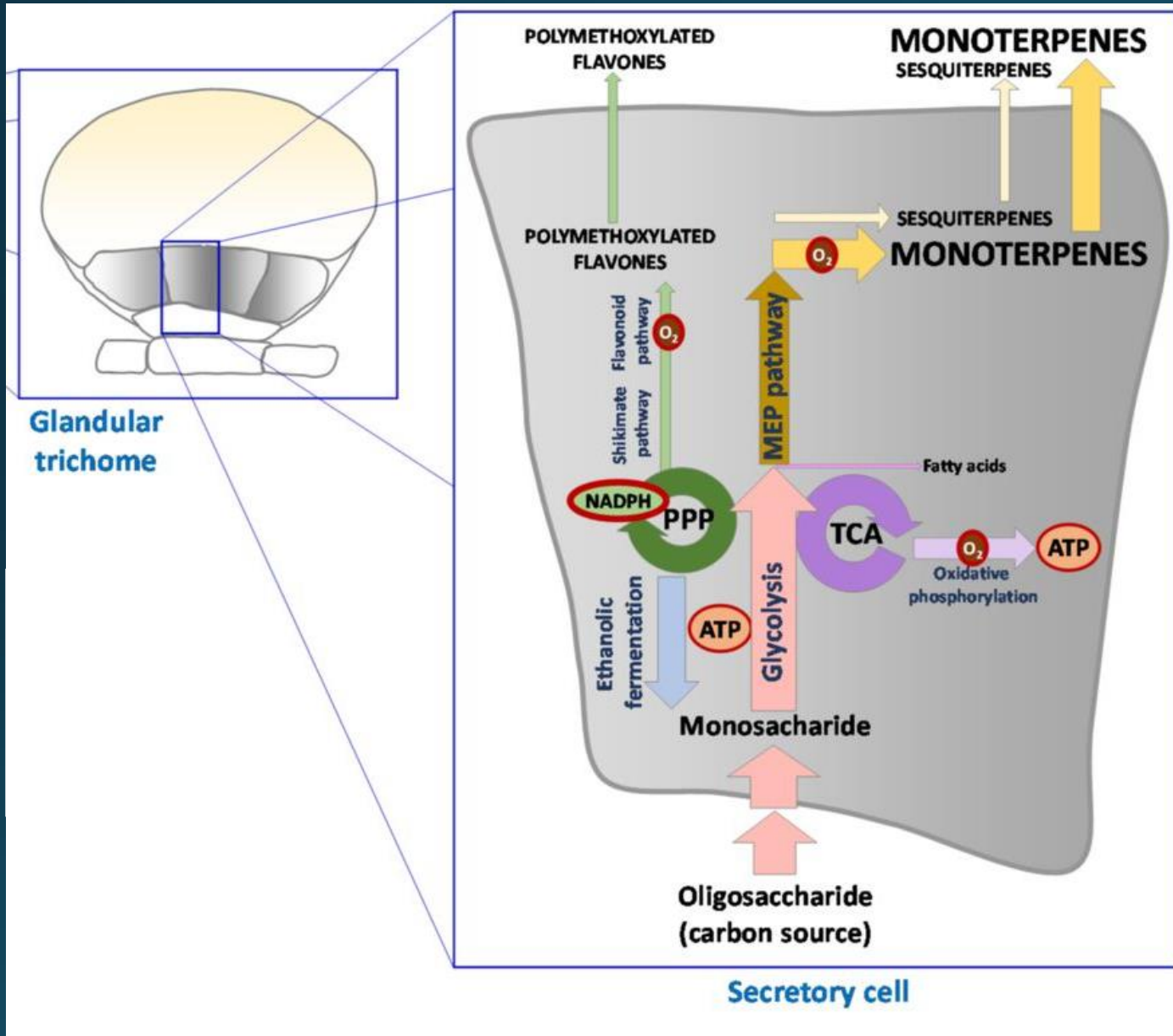


Hemp Flavonoid Variance

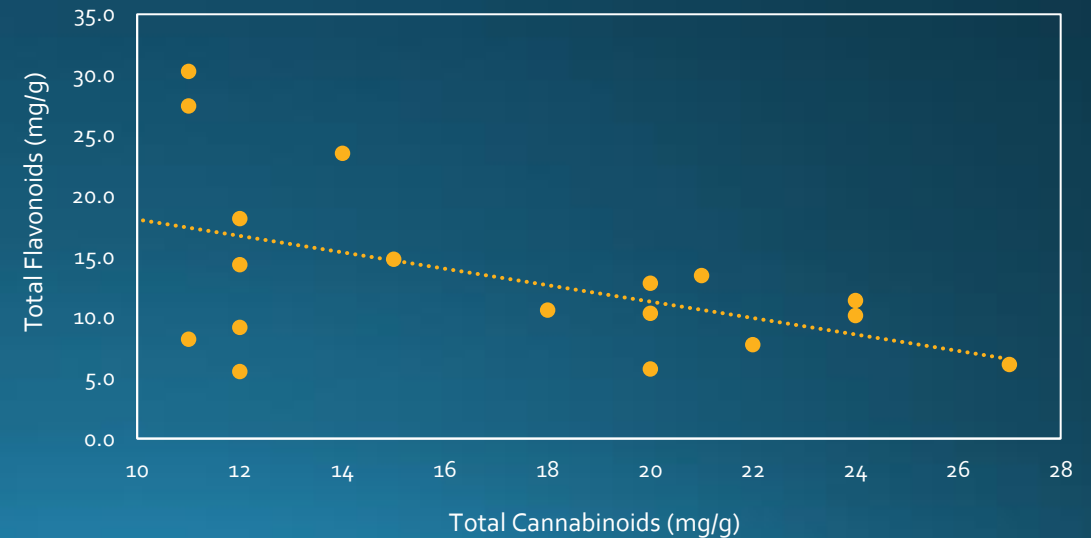
Flower Cultivar Flavonoid Concentrations



Cannabis Metabolite Fluxes - 'Flavolomics'



Total Leaf Flavonoids vs. Total Leaf Cannabinoids



Acknowledgements

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- Dr. Reggie Gaudino – Chief Science Officer
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