Fermentation Sciences

APPALACHIAN STATE UNIVERSITY

ASU Fermentation Sciences: STEM-based learning and research

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Background:

BS Chemical Engineering, University of Rhode Island

MS and PhD in Food Science and Technology, Oregon State University

Wine Chemistry and Grape Metabolomics; Focus on secondary metabolites (phenolic biosynthesis).

Winding road between academics, research assistantship, wine production, and back to academics.

Interests in chemistry, process engineering, agriculture, fermented beverages and culinary arts.

Interest in pursuing graduate work in chemistry and wine lead me down this path.

Fortunate to have many great mentors along the way and the opportunity to work with some professional heroes.

While doing research in Montpellier, France, I was notified of a position at Appalachian State University - to resurrect a BS degree program proposal in Oenology and Natural Products Chemistry.

After some discussion, the value of a focused program in Fermentation Sciences was agreed upon (2010).

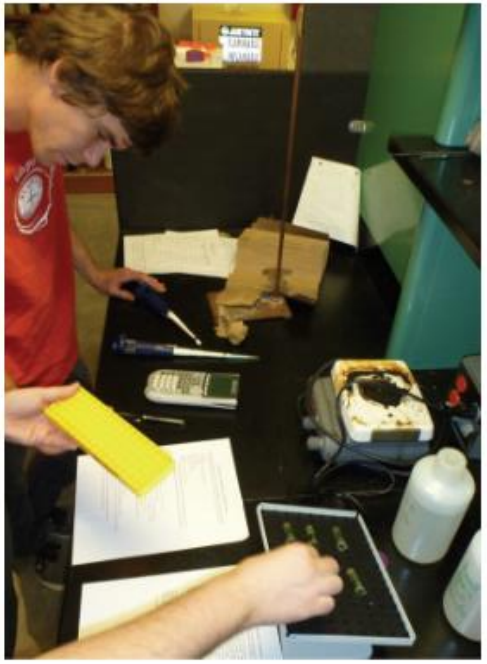
By 2012, our proposal to the UNC GA was reviewed and approved with students enrolled and classes underway.

Fermentation Sciences Core

FER 1000 Principles of Fermentation Sciences
FER 2000 Social Implications of Fermented Beverages
FER 3000 Viticulture
FER 3200 Facility Design and Operations
FER 3530 Biofuels and Bioprocessing[a]
FER 4100 Wine Production and Analysis[b]
FER 4200 Brewing Production and Analysis[b]
FER 4300 Sensory Analysis
FER 4530 Distillation Technology[a]

[a] *Planned as permanent course*

[b] *Currently being split across two semesters*



Multi-disciplinary degree and program of study; high level of cooperation and collaboration on- and off-campus.

Rapid program enrollment and very high level of in-state Community College transfers.

55+ currently enrolled, 12-15 incoming freshman and 12-15 expected to declare.

High number of 2nd degree seeking students from STEM disciplines currently.

High number of student requests for graduate degree.

BS/BA degrees, career professionals.

Attrition is becoming visible as some students realize the science core (CHE and BIO) is to intensive for them to keep up with.

NC Community Colleges establishing Brewing, Distillation, and Fermentation.

Established 2+2 programs, developing focus of western NC.

Faculty and Staff Support

Two FER faculty (**Cohen and Sommer**)

One staff instructor (**Hutchins (MS)**, ***externally funded position for 3+ years***)

One full-time staff technician (**Kuhfeld**, **receipt- funded position via Service Lab**)

One CHE faculty dedicated ½ time to FER (**Taubman**)

Several collaborating and contributing faculty from CHE and BIO.

*Tremendous potential for collaboration / cooperation within **COB and MKT/COM**.*

Plan to bring in an MS-level instructor and research/administrative assistant in the next six months.

Hope to have an additional faculty member in the next 2 years (potentially CHE/FER or BIO/FER split).

COMMUNITY COLLEGE BREWING COURSE WAIVER

SECTION 17.(a) Article 11 of Chapter 18B of the General Statutes is amended by adding a new section to read:

"**§ 18B-1114.6. Brewing, Distillation, and Fermentation course authorization.**

Potential to re-instate license for the sale of beverages could help generate thousands of dollars to fund student research, laboratory work, and lab upkeep. Provides opportunity for campus-wide collaborations and intensive student engagement.

How does fermentation science fit in exactly?

Multi-disciplinary program of study-

Utilizing our knowledge of microbes to help transform substrate into valuable products; the core of biotechnology.

As scientists, we aim to manipulate the environment to achieve a desired output.

This leaves a fascinating area of education, discovery, and consumable research projects.

The role of fermentation science throughout history.

Critical in the transition from hunter-gatherer lifestyles to the development of communities and centralization of dwellings (for good or bad).

How do me sustain communities on food and water without chasing them across seasons and migratory routes?

Unintentional fermentation processes lead to the discovery that some of these resulted in a durable food product; primitive food preservation and increased health safety.

With little knowledge on *why* and *how* these processes occur, humans propagated these methods over time and developed reliable methods for processing.

Students are introduced to the history of microbiology, chemistry, and biochemistry through the discussions of numerous food products and the pioneers of the scientific community.

Mulder- Early work with 'albuminoid' substances

von Liebig- Concept of cells comprised of proteins

Denis- Fractionation of proteins

Fischer- Work with peptides and amino acids

Leeuwenhoek- Microscope observations of organisms

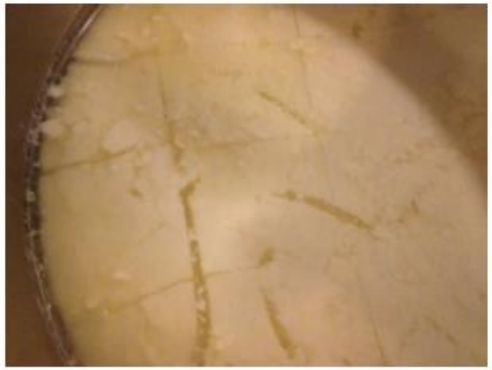
Gay-Lussac- Glucose → EtOH + CO_2

Cagniard-Latour, Schwann, and Kutzig – Revisited Leewenhoek observation that sugar conversion was not a strictly chemical process.

Pasteur- experiments drawing from previous work, heat inactivation of cells.

Much of this discovery and many others (e.g. Student's t-test by Gosset at Guinness and the concept of pH by Sorensen at Carlsberg) were tied to observations and curiosities aroused by fermenting juice and sugars.

The fascination: How do we transform something as simple as milk into any of the hundreds of cheeses we eat?



Microbial metabolism of sugars/carbohydrates, fats/lipids, proteins, organic acids. Generates the myriad of colors, tastes, aromas, flavors, and textures that we grow to appreciate – or not.

This is where the fascination and connection comes in- developing our understanding of the process and how we can take that knowledge and apply it in innovative or novel ways; to continue this tradition.


Beakers
Flasks
Large


White wine
#0
Rose
#1
INACTIVE
Bitter Faculty 2
#3
INACTIVE
INACTIVE
IVORY TOWER BREWERY
INACTIVE
INACTIVE
INACTIVE
INACTIVE
INACTIVE
INACTIVE
SAMSUNG


40
30
20
10
0
-10
-20
#0
20:02:33
10.00°C
SETUP
DETAIL
40
30
20
10
0
-10
-20
#1
17:05:56
10.00°C
SETUP
DETAIL


333


GERSTEL
MPS


1.400
1979
R-B1
30.00
40.0

Beer

During the 15th century German purity law, Reinheitsgebot, dictated that beer could be comprised of only three ingredients: Barley, Water, and Hops.

Anything missing here?

Yeast- *and why?*

This was about 400 years prior to the discovery that yeast exists and converted the sugars in barley into ethanol, CO_2, and some *other stuff*.

How do we make beer?

Barley and most grains contain relatively low levels of sugars small enough to be assimilated and metabolized by yeast (mono- to tetra-saccharides).

We malt barley in order to activate endogenous enzymes (glucosidase, amylase, dextrinase, protease) that mediate the degradation of starches into sugars and degrade proteins.

Malted barley is steeped in water at temperatures for optimum enzyme activity (150-165F) resulting in the liberation of yeast fermentable sugars.

Beer

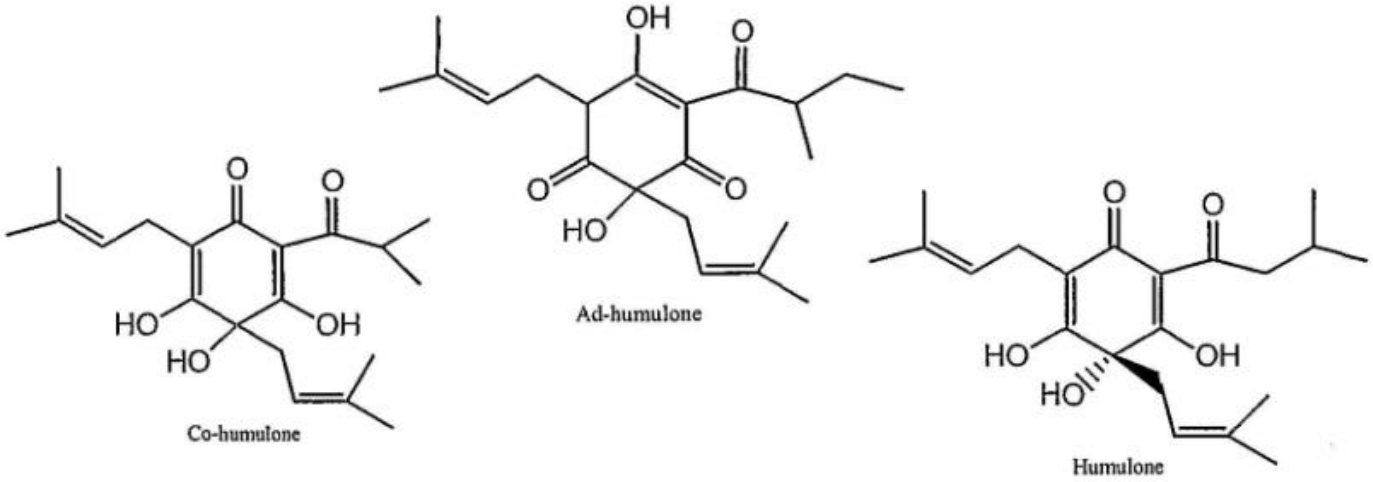
Hops are typically added to beer; these provide bitterness, aroma, and some antimicrobial activity.

Primary compounds of interest are **hop acids** and **essential oils**.

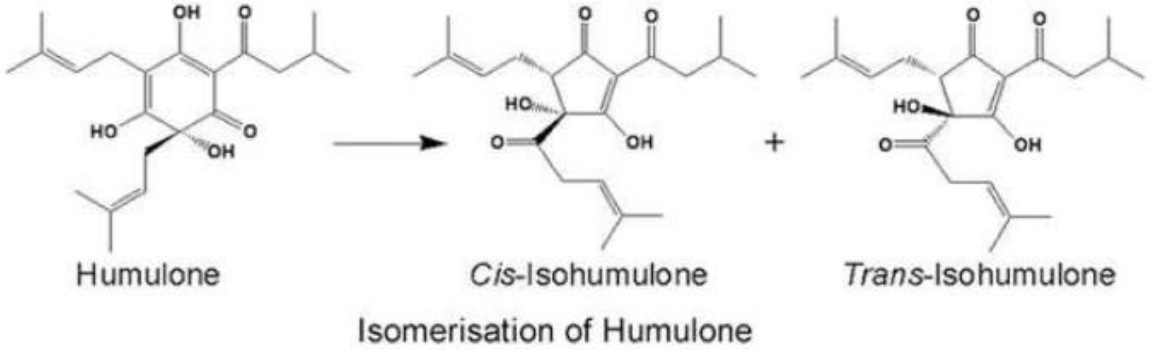
Hop aroma compounds include Humulene, Myrcene, Linalool, and 3-methylbutanoic acid (isovaleric acid), Caryophyllene, Farnesene, and geranyl-esters (geraniol based)

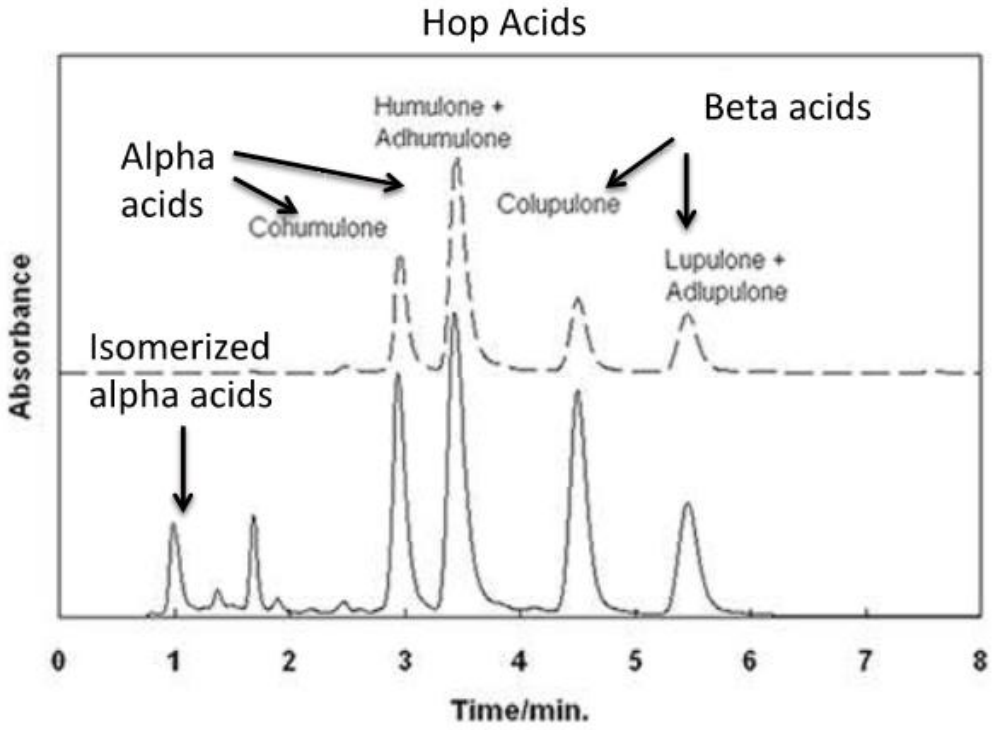
However, over 400 volatile compounds have been identified in hops.

Alpha Acids: primary driver for hop-bitterness.
Typically 3.0-5.0% or greater preferred (dry weight).



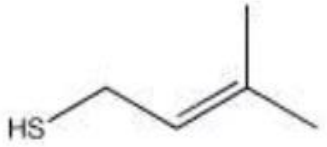
Isomerization of alpha acids during boil results in increased bitterness of hop acid



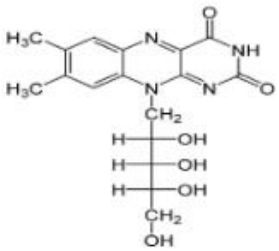


Skunky Beer Aroma- "Light Struck"

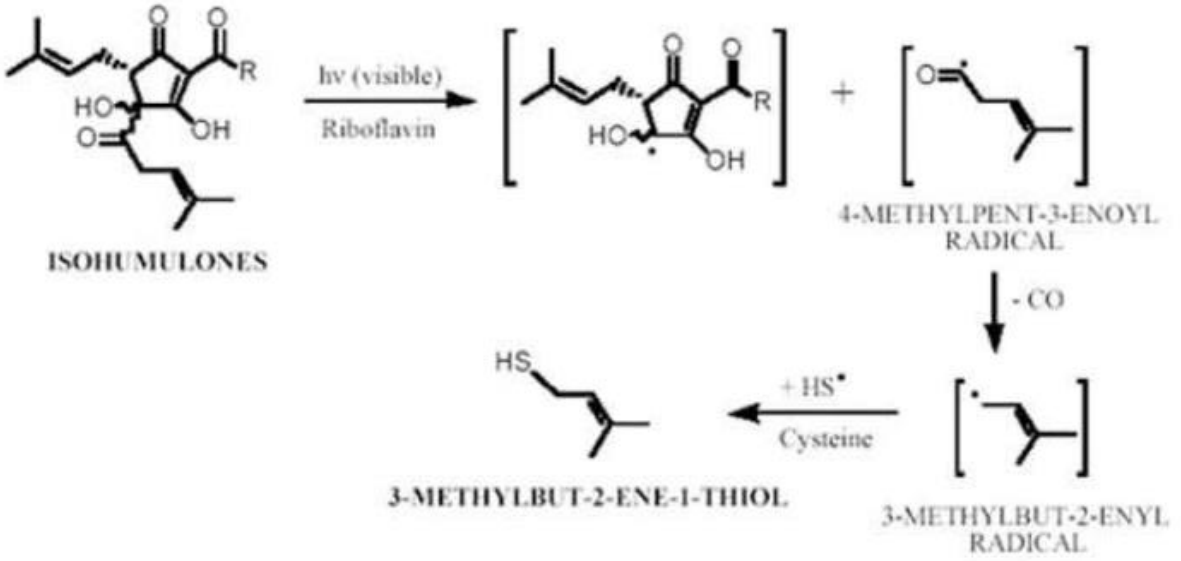
3-methyl-2-butene-1-thiol



MBT

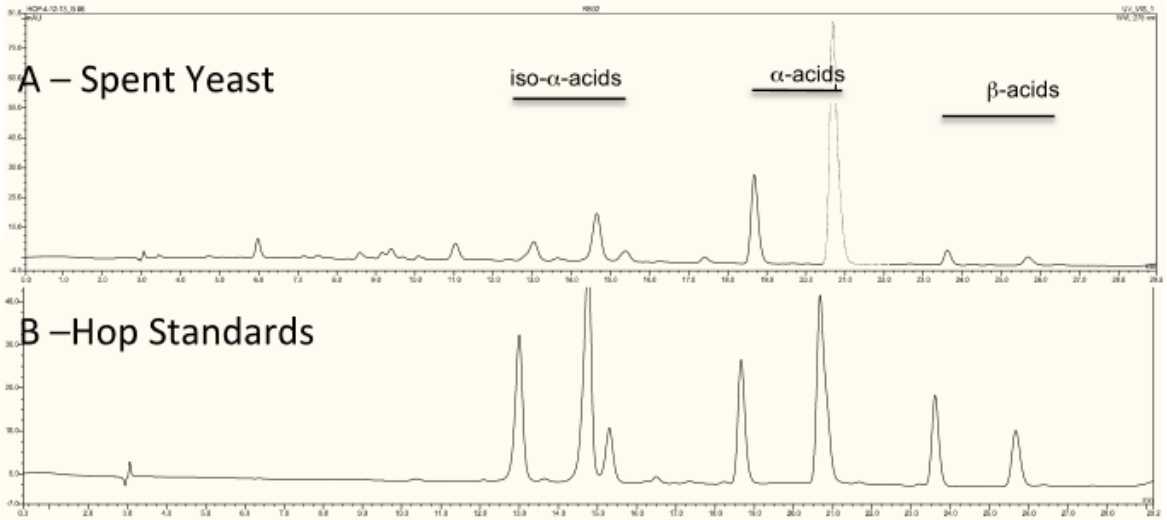


Riboflavin or
Vitamin B2



Scheme 2. Formal Kuroiwa-mechanism for formation of 3-methylbut-2-ene-1-thiol from photodecomposition of isohumulones in the presence of riboflavin.

With the amount of spent yeast being produced by breweries, is there a potential to harvest a value-added food ingredient from an empty tank?



Hop Acid Levels in Spent Yeast Obtained From Several Craft Beer Styles vs. Yeast from Several Multinational Brewing Styles

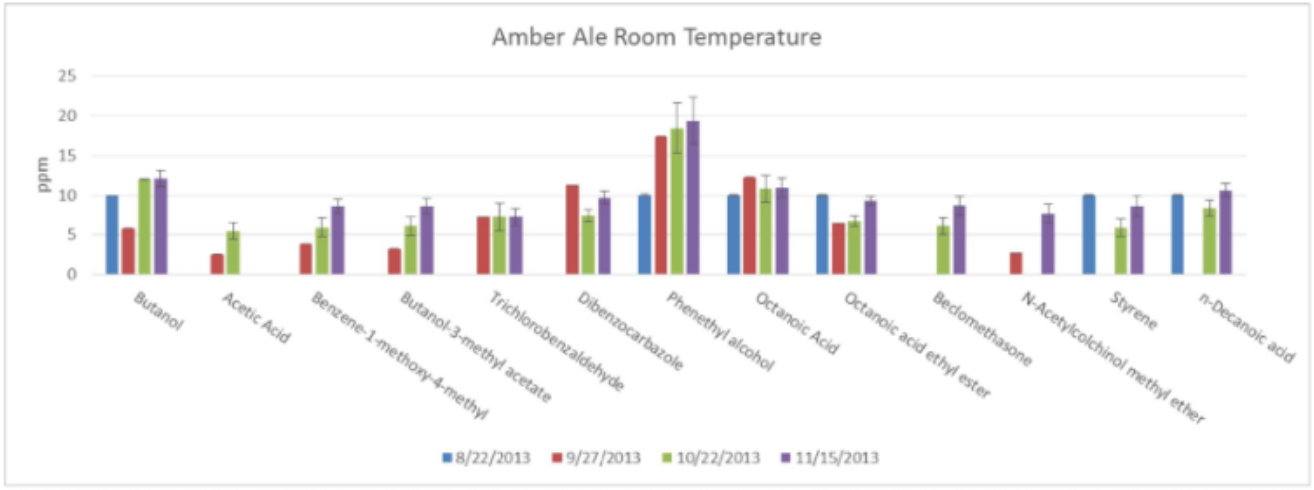
	Hop Acid Content (mg/g)			
Spent Yeast Source	**Total Iso-Alpha-Acids**	**Total Alpha-Acids**	**Total Beta-Acids**	**Combined Hop Acids**
Craft Brewing[a]	269 ± 77	2074 ± 531*	213 ± 42	2542 ± 546*
Multinational Breweries[b]	95 ± 82	167 ± 177	131 ± 216	426 ± 99

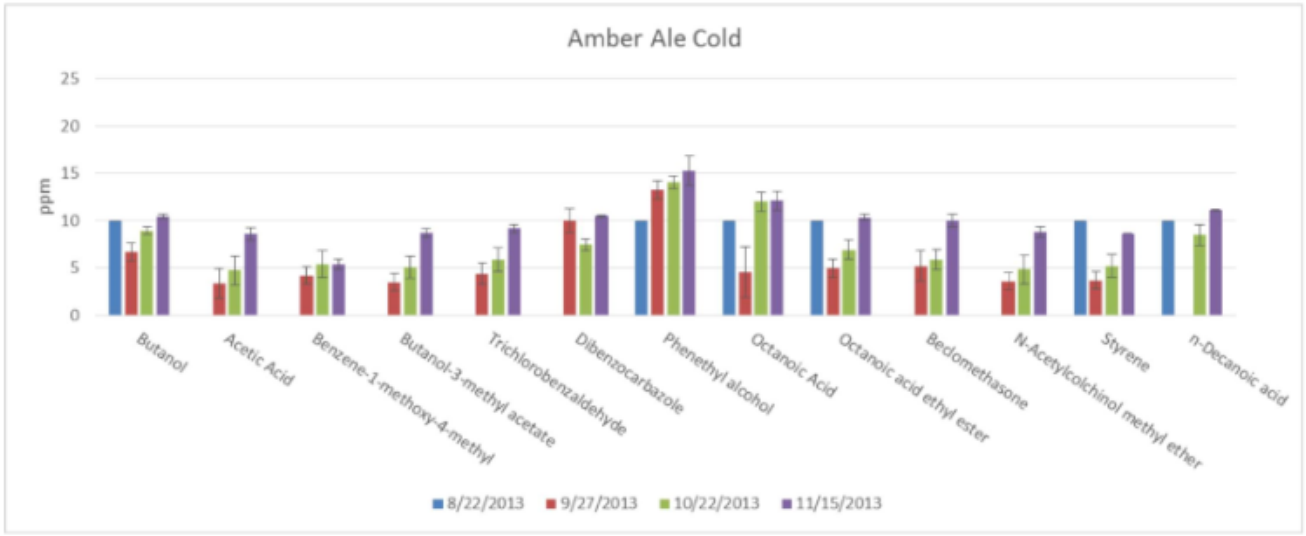
[a] Average of 4 samples and SEM in Tables II and III

[b] Average of 3 samples and SEM in Tables II

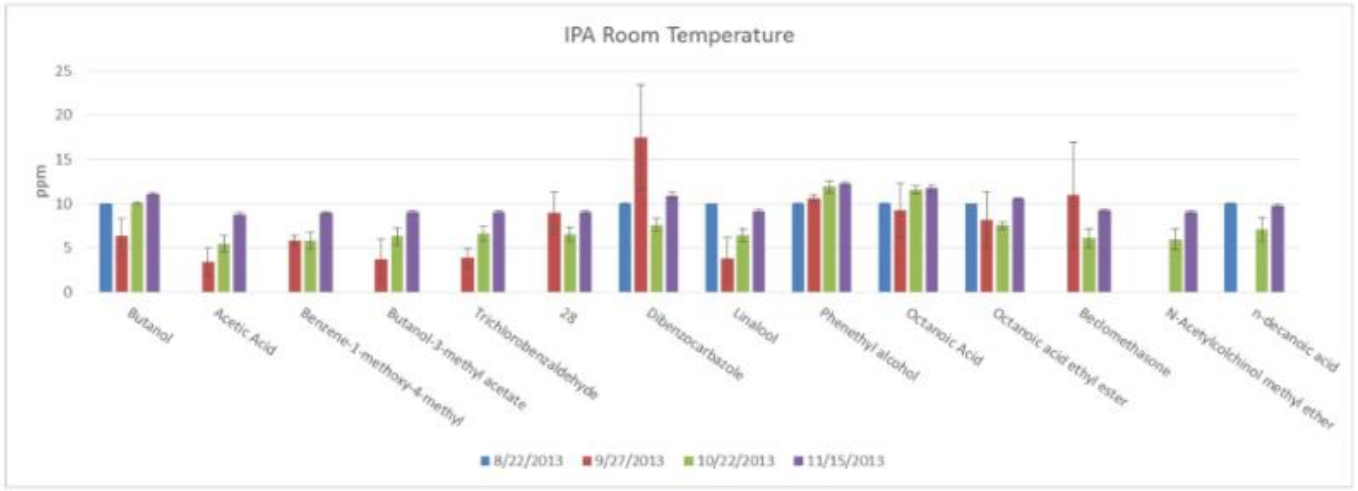
* $p<0.02$ by two-tailed Students t Test

Storage conditions of beer (Amber ale): Room Temperature vs Cold Storage (ca. 38F) over 90 days shelf life – Identifying markers of aging.





Storage conditions of beer (IPA): Room Temperature vs. Cold Storage (ca. 38F) over 90 days shelf life – Identifying markers of aging.



Wine

If we account for a glass of wine we may have 14% ethanol, less than 0.5% sugar, around 0.6% organic acids, and water.

What we don't account for may total another 0.5%.

This is the fraction that differentiates a red from a white wine and any number of varieties from each other.

A very simple process

The key to wine production is manipulating the extraction of wanted compounds out of the fruit, developing aroma and flavor during fermentation, and developing additional character during aging.

The tricky part: this process is not the same each year.

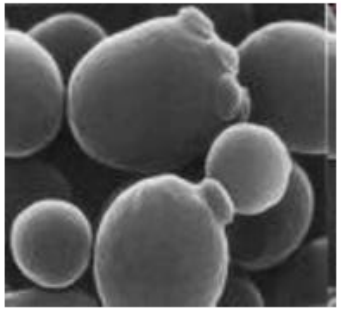
Much of the research in grape and wine chemistry has been geared towards understanding the connection between the environment, the grape, and the composition of a finished wine.

Wine Production- Players, Factors and Influences

1° Grapes - Variety, age, season, climate, H2O, integrity, nutrition, yield, micro-organisms / flora, cultural practices, butterfly flapping its wings in China…

2° Yeast and Bacteria (enzymes) – Species, population, growth conditions (pH, temp., H2O etc.), substrates, time…

3° Aging – Time and conditions, oxygen, wood / aging vessel, yeast and bacteria, substrates…



Major Classes of Aroma Compounds

There are **hundreds** of detectable aromas compounds **in wine**.

The aroma complexity of **grape berries is not quite so diverse on its own.**

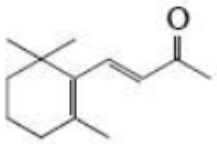
-**Terpenoids**

(Linalool)

Linalool

-**Norisoprenoids**

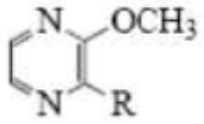
(β-Ionone)



(I)

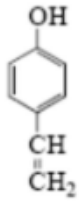
-**Pyrazines**

(i.e. 2-ethyl-3-methoxypyrazine)



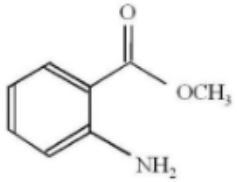
-**Phenols**

(4-vinyl-phenol)



-**Esters**

(methyl anthranilate)



-Other compounds (alcohols, aldehydes, acids via enzymatic or chemical modifications) and **aroma-glycosides**.

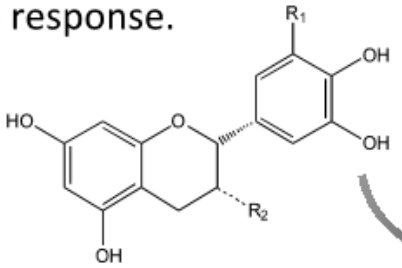
Much of my work and interests have involved grape phenolics and their role in wine qualities.

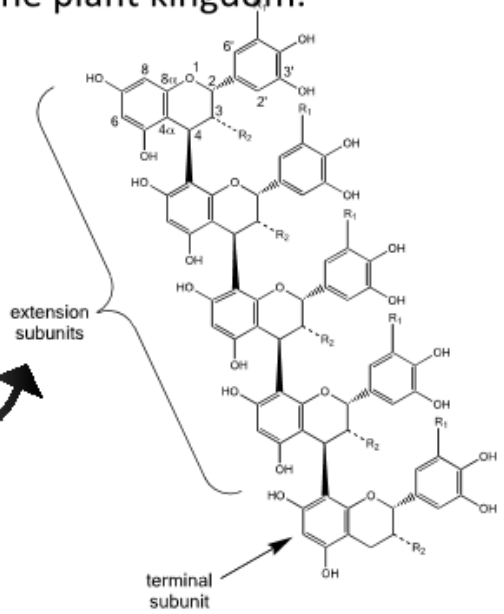
These are a ubiquitous class of compounds throughout the plant kingdom.

<u>Role of Flavonoids / Phenolics</u>

Proanthocyanidins (PAs) *(Tannins / Flavan-3-ols)*

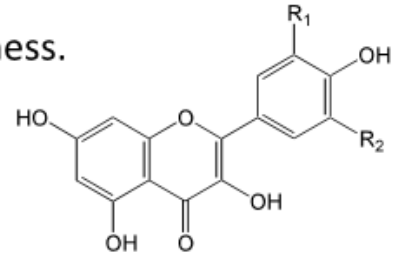
- Astringency, bitterness; tactile response.
- Color / Pigmentation.





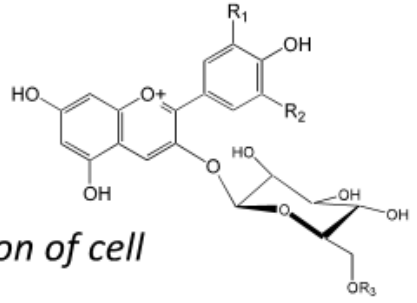
Flavonols

- Color / colloid stability, bitterness.

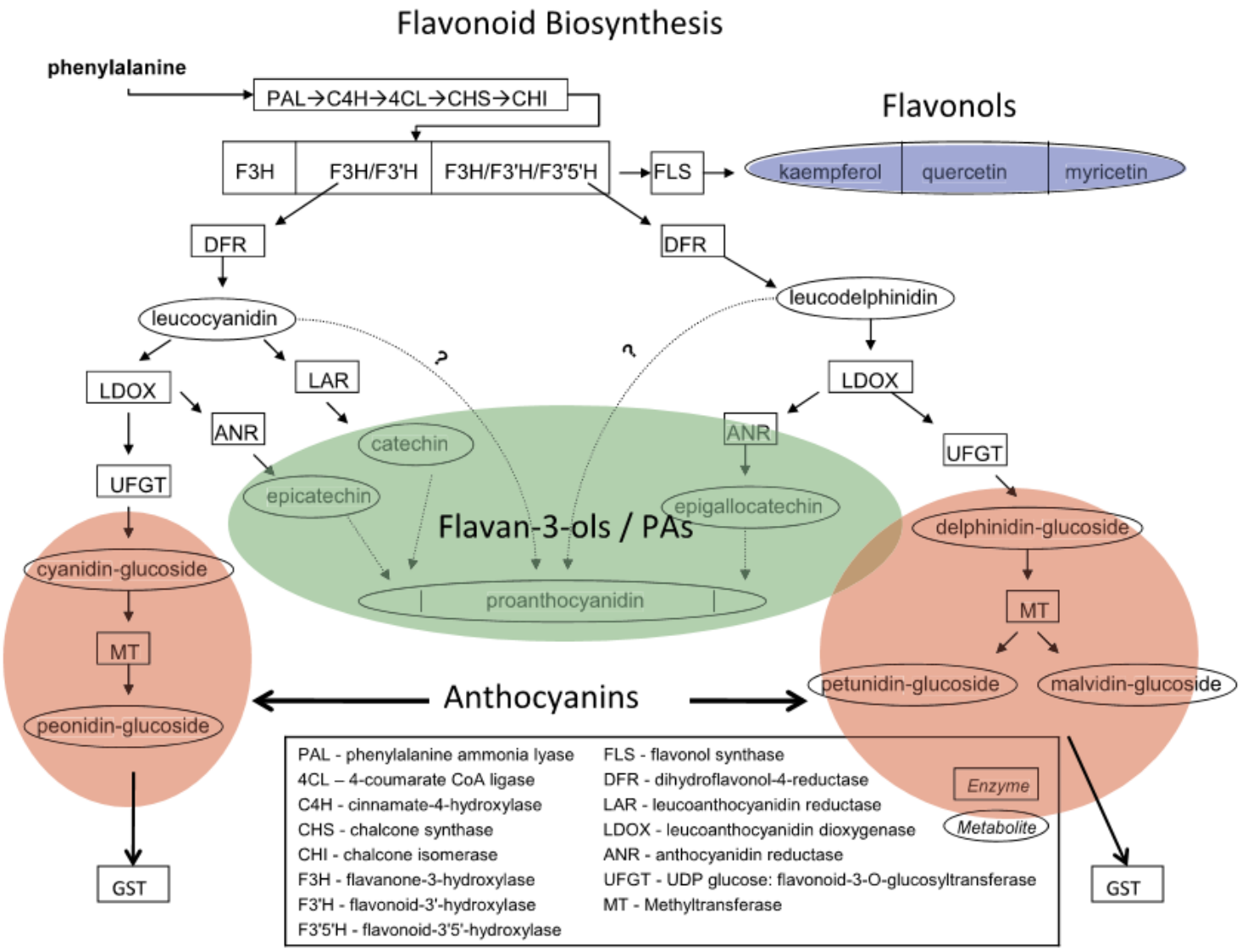


Anthocyanins

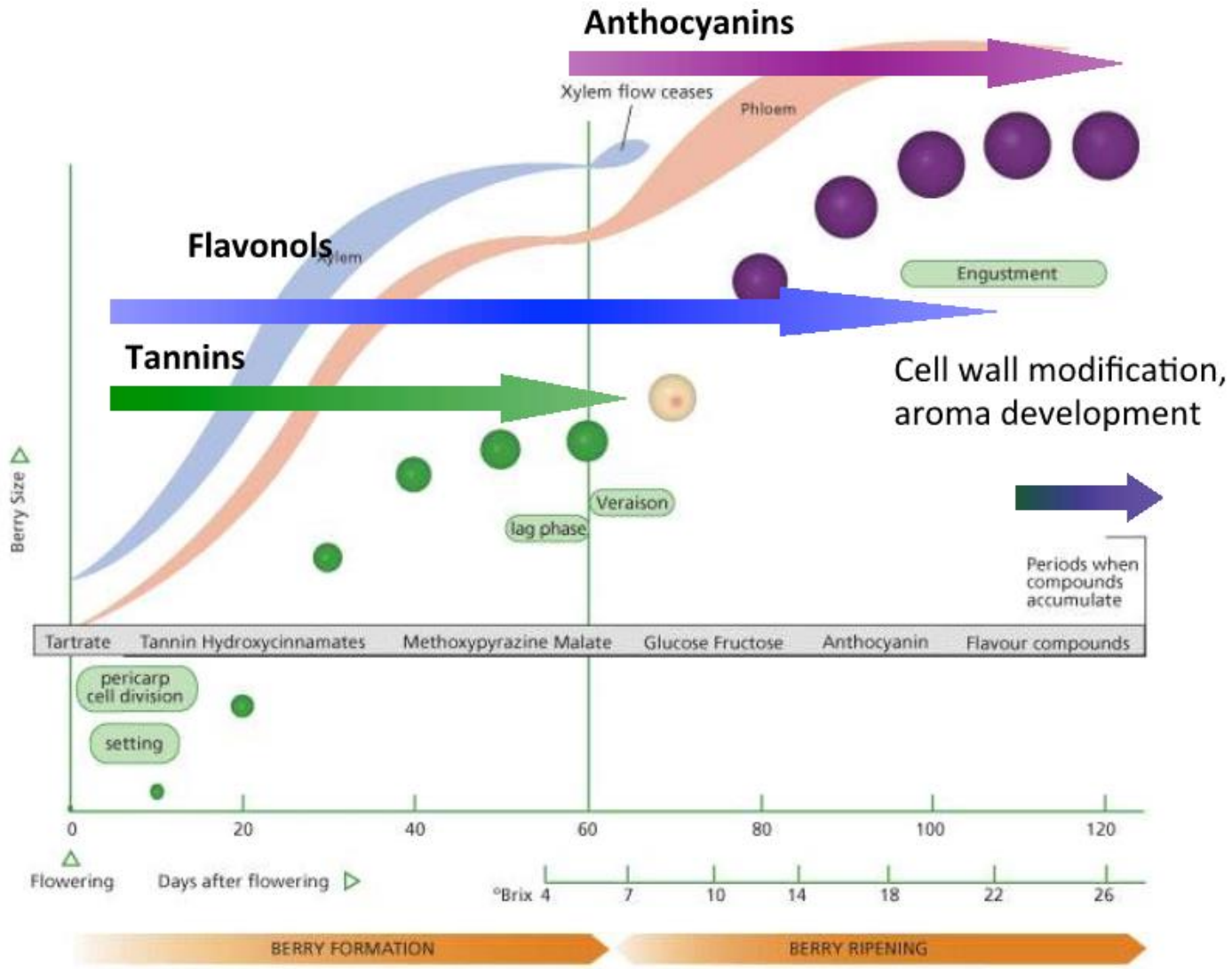
- Color, colloid / pigment incorporation, tactile response.



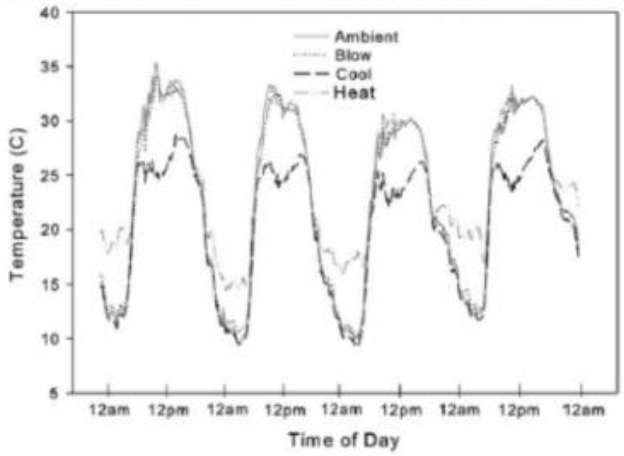
Implicated in human health via. antioxidant, anti-carcinogen (inhibition of cell division), and anti-inflammatory activities.


Flavonoid Biosynthesis
phenylalanine
PAL→C4H→4CL→CHS→CHI
F3H
F3H/F3'H
F3H/F3'H/F3'5'H
FLS
Flavonols
kaempferol
quercetin
myricetin
DFR
DFR
leucocyanidin
leucodelphinidin
?
?
LDOX
LAR
LDOX
ANR
catechin
ANR
UFGT
UFGT
epicatechin
epigallocatechin
Flavan-3-ols / PAs
delphinidin-glucoside
cyanidin-glucoside
proanthocyanidin
MT
MT
petunidin-glucoside
malvidin-glucoside
peonidin-glucoside
Anthocyanins
GST
GST
PAL - phenylalanine ammonia lyase
4CL – 4-coumarate CoA ligase
C4H - cinnamate-4-hydroxylase
CHS - chalcone synthase
CHI - chalcone isomerase
F3H - flavanone-3-hydroxylase
F3'H - flavonoid-3'-hydroxylase
F3'5'H - flavonoid-3'5'-hydroxylase
FLS - flavonol synthase
DFR - dihydroflavonol-4-reductase
LAR - leucoanthocyanidin reductase
LDOX - leucoanthocyanidin dioxygenase
ANR - anthocyanidin reductase
UFGT - UDP glucose: flavonoid-3-O-glucosyltransferase
MT - Methyltransferase
Enzyme
Metabolite

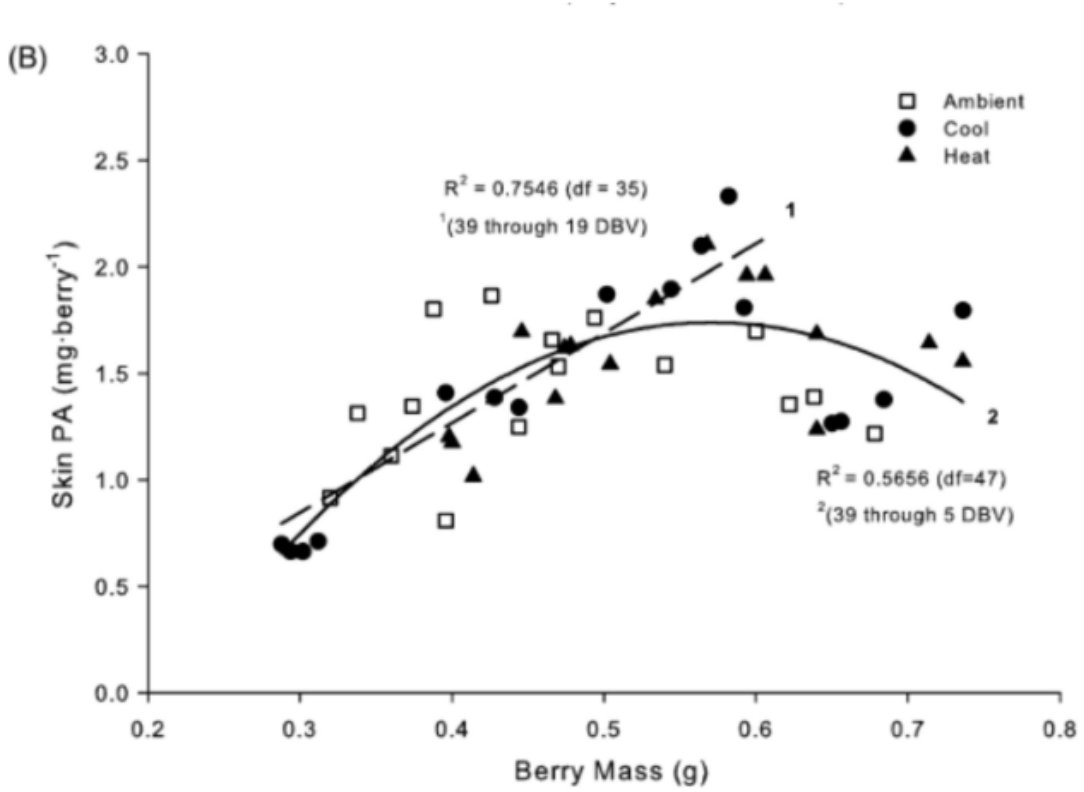
Temporal Development of the Grape Berry



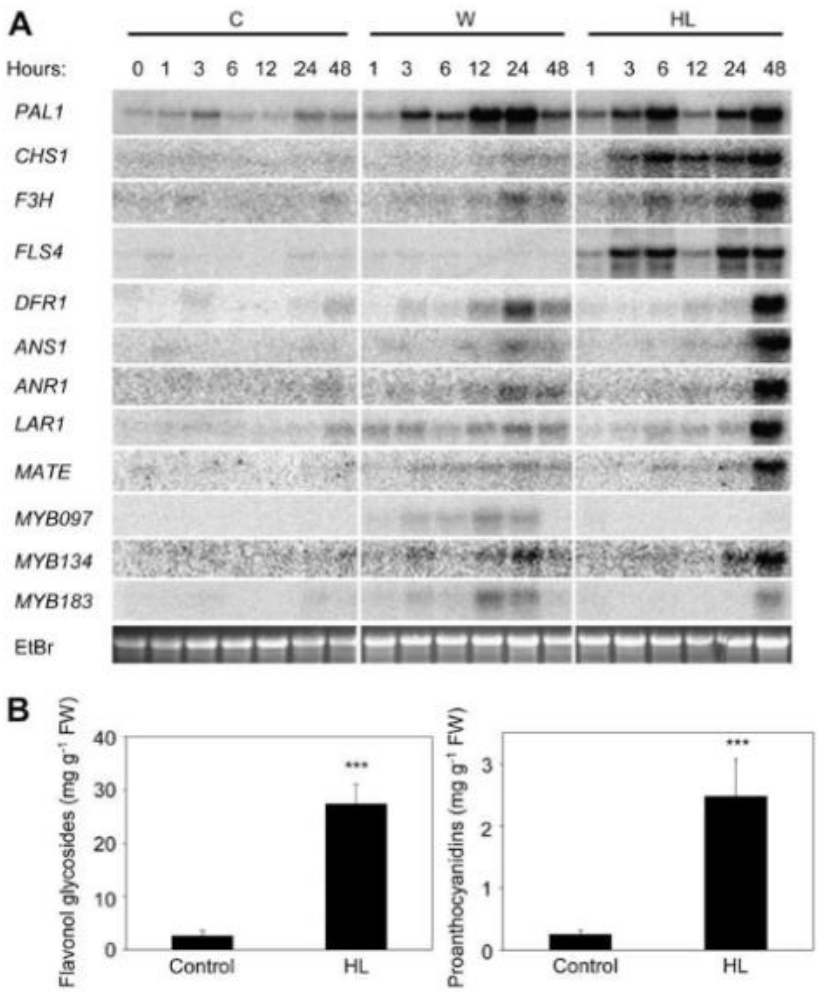
Temperature and phenolic metabolism



Temperature and phenolic metabolism



Expression analysis of key pathway genes and metabolites showed minor shifts in metabolism due to temperature (partitioning) but not major effect with regard to PA accumulation.



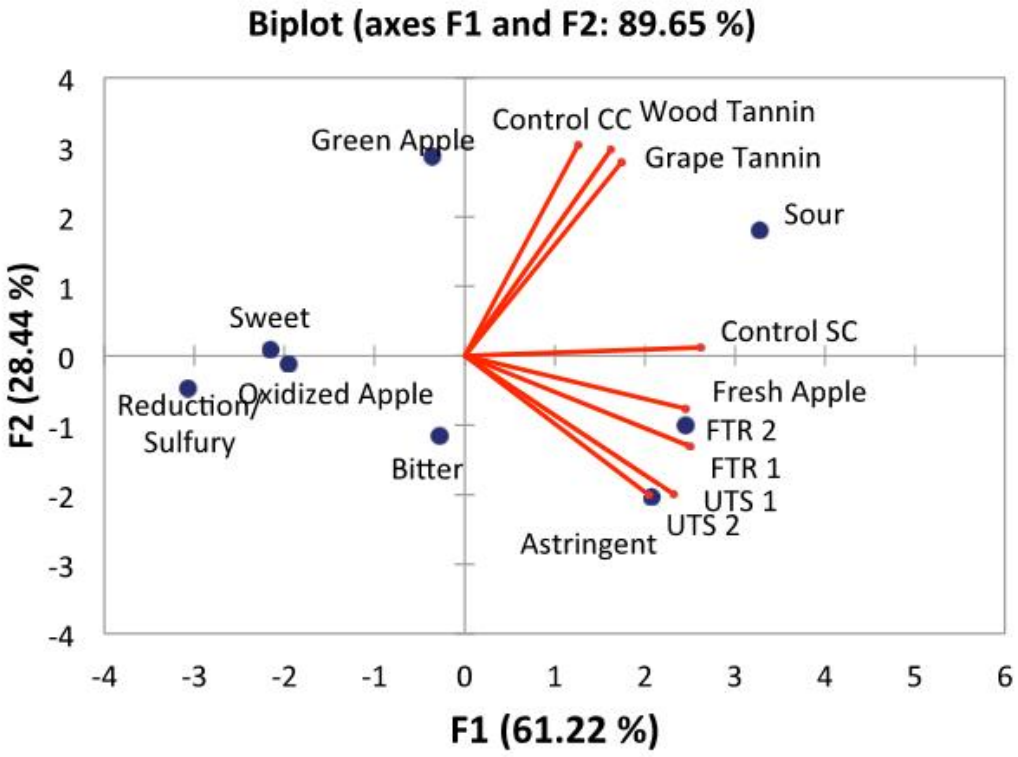
Mellway et al., Plant Phys., June 2009; vol. 150

Work around this same time demonstrated a large effect of light on PA biosynthesis in Poplar; we have been investigating this in *Vitis vinifera* using shade cloth in the vineyard.

Cider

Addition of exogenous tannins to hard apple cider- effect of type of tannin and timing of addition.

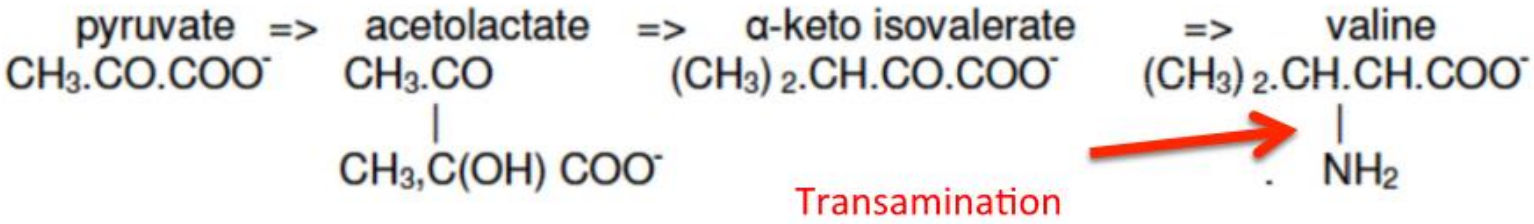
Considering the impact on aroma (fresh and during aging), mouthfeel, and stability.

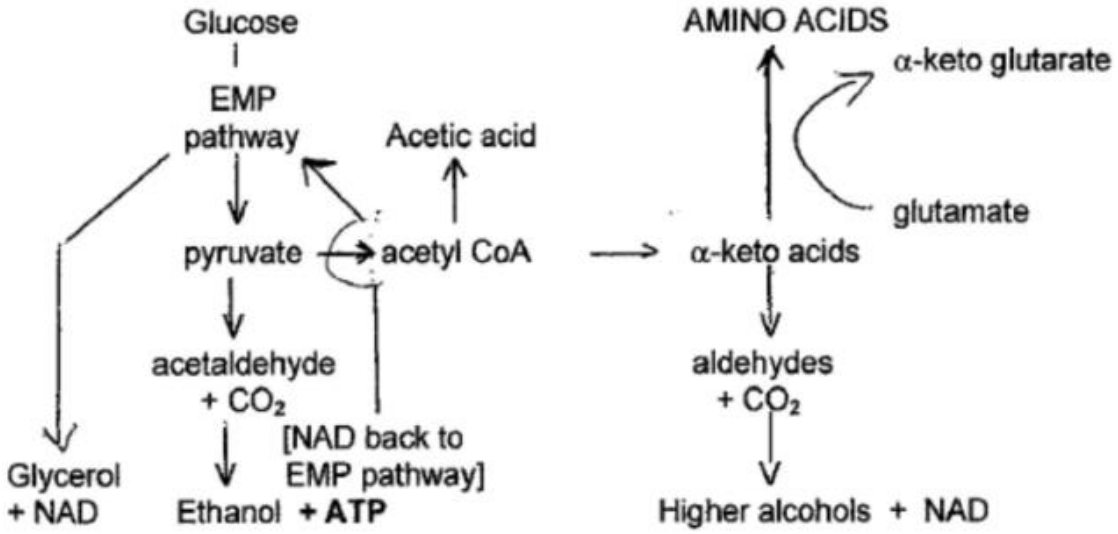


Yeast Aromas- the last component

Nitrogen and Amino Acid Metabolism

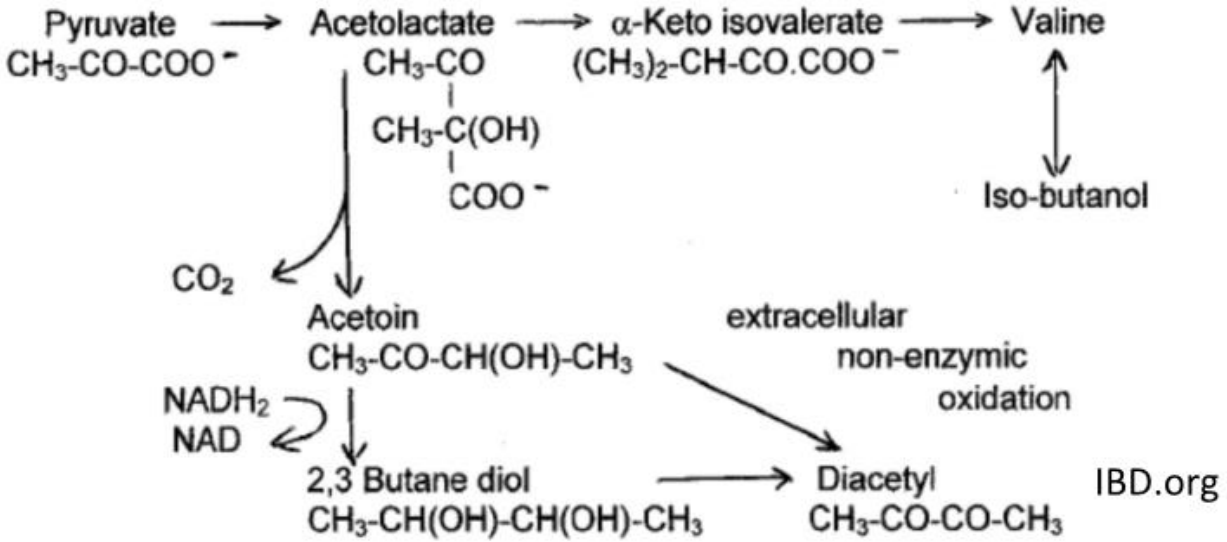
Production of amino acids is coupled to EMP-Glycolysis; for example- the production of valine from acetolactate:





Without available N; acid will be decarboxylated and then reduced to corresponding alcohol.

Diacetyl metabolism- regeneration of NAD, production of acetoin and 2,3 Butane diol.
Exported from cell, can be oxidized easily to 2,3 Butanedione.
Difficult to remove during distillation.



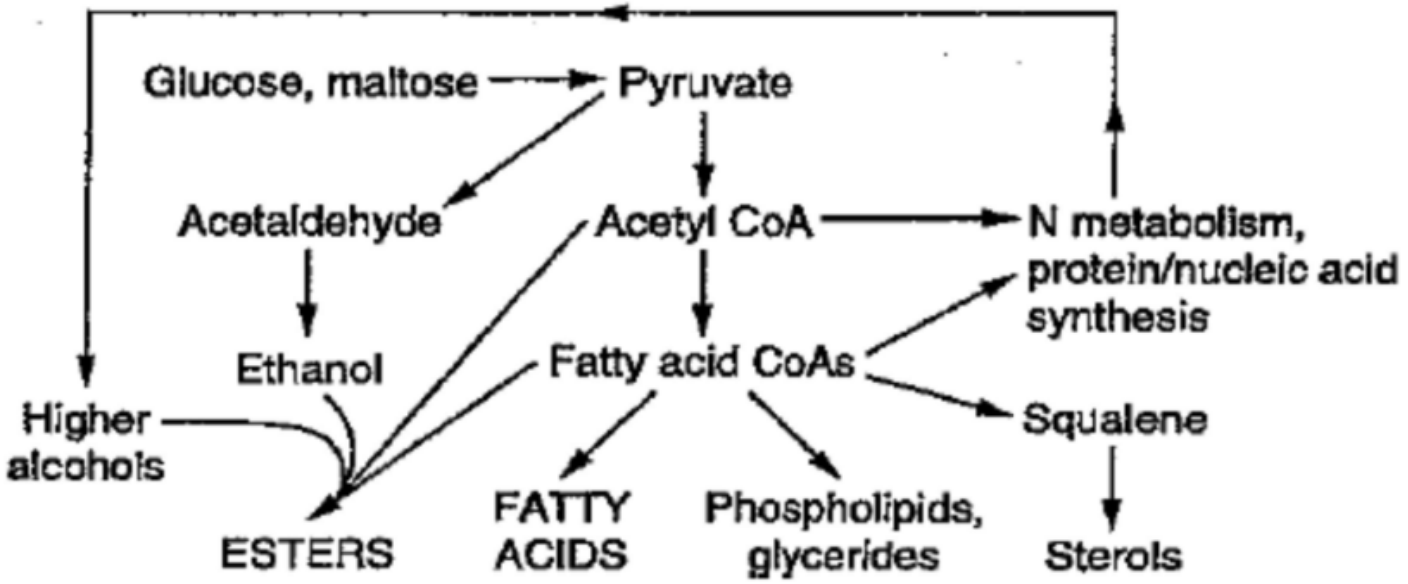
Relationship Among Metabolic Pathways

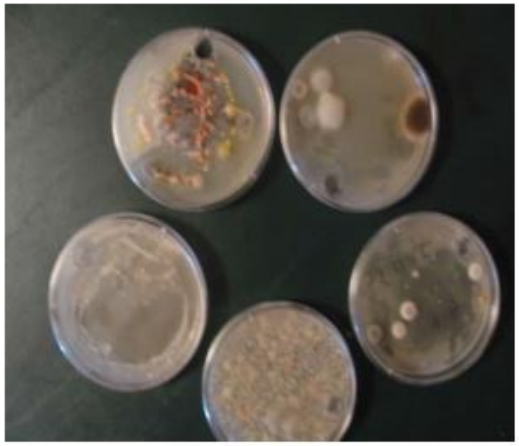
Many characteristic yeast derived aromas fall in to the category of esters.

Acetyl Coenzyme A (AcetylCoA) and larger homologs (R-CO~S-CoA) are critical for production of amino acids, proteins, RNA/DNA, lipids etc.

If the Acyl group is not required (or cannot be used) for synthesis, the energy rich bond is recycled and the acid group is stabilized (reduce cellular damage) via esterification with an available alcohol group.

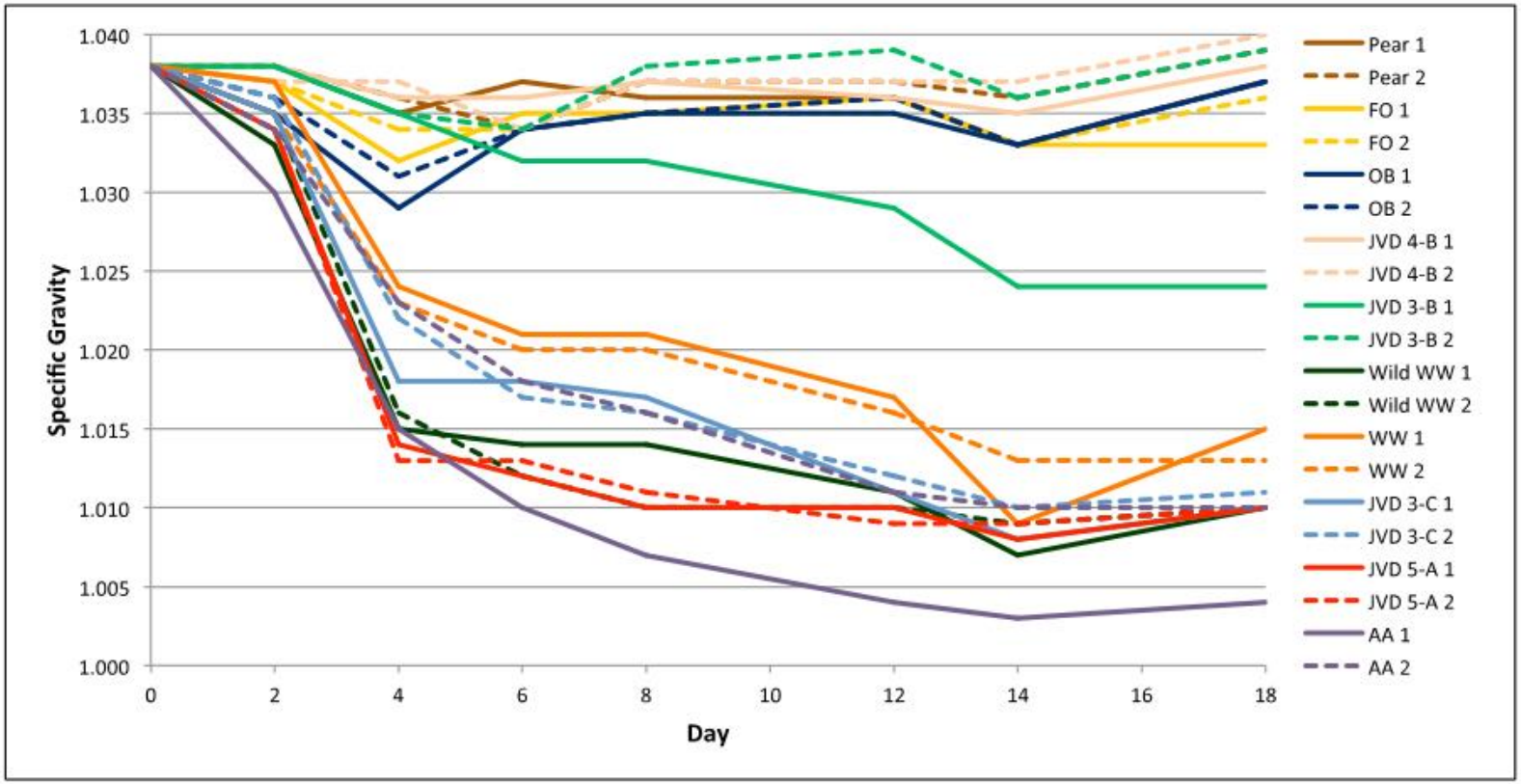
Yeast have been selected for and hybridized to produce high levels of esters and other sensory-active components.





Capturing and isolating 'wild' yeast

Samples taken from local plants, trees, fruit, vineyards etc. Yeast colonies purified and tentatively Identified using ITS sequencing.



	Glucose Limited	Glucose Limited + Hops	Maltose
FO	+	+	+
OB	+	+	+
Pear	+	+	+
JVD 3-B	+	+	+
JVD 4-B	+	+	+
WW	+	+	+
AA	+	+	+

Non-*Saccharomyces*	Aroma
Pear 1	fruity, lemon, nutty, bread
FO 1	pineapple, butter, rubber
OB 1	honey, sweet malt, fruity
JVD 4-B 1	fruity, cereal, sweaty, putrid
JVD 3-B 1	fruity, candy, sweet wort

Saccharomyces	Aroma
Wild WW	sweet, fruity, bitter, almond
WW	grainy, fruity, phenolic, bitter
JVD 3-C	fruity, sulfur, burnt
JVD 5-A	wine, apple, sulfur
AA	tart, honey, rubber, alcohol

Have used a few yeasts from collection; one Saccharomyces yeast has performed well in brewing applications.

Fermentation Sciences

APPALACHIAN STATE UNIVERSITY