Characterizing the impact of packaging type on beer stability using advanced analytical tools

ASBC
THE SCIENCE OF BEER

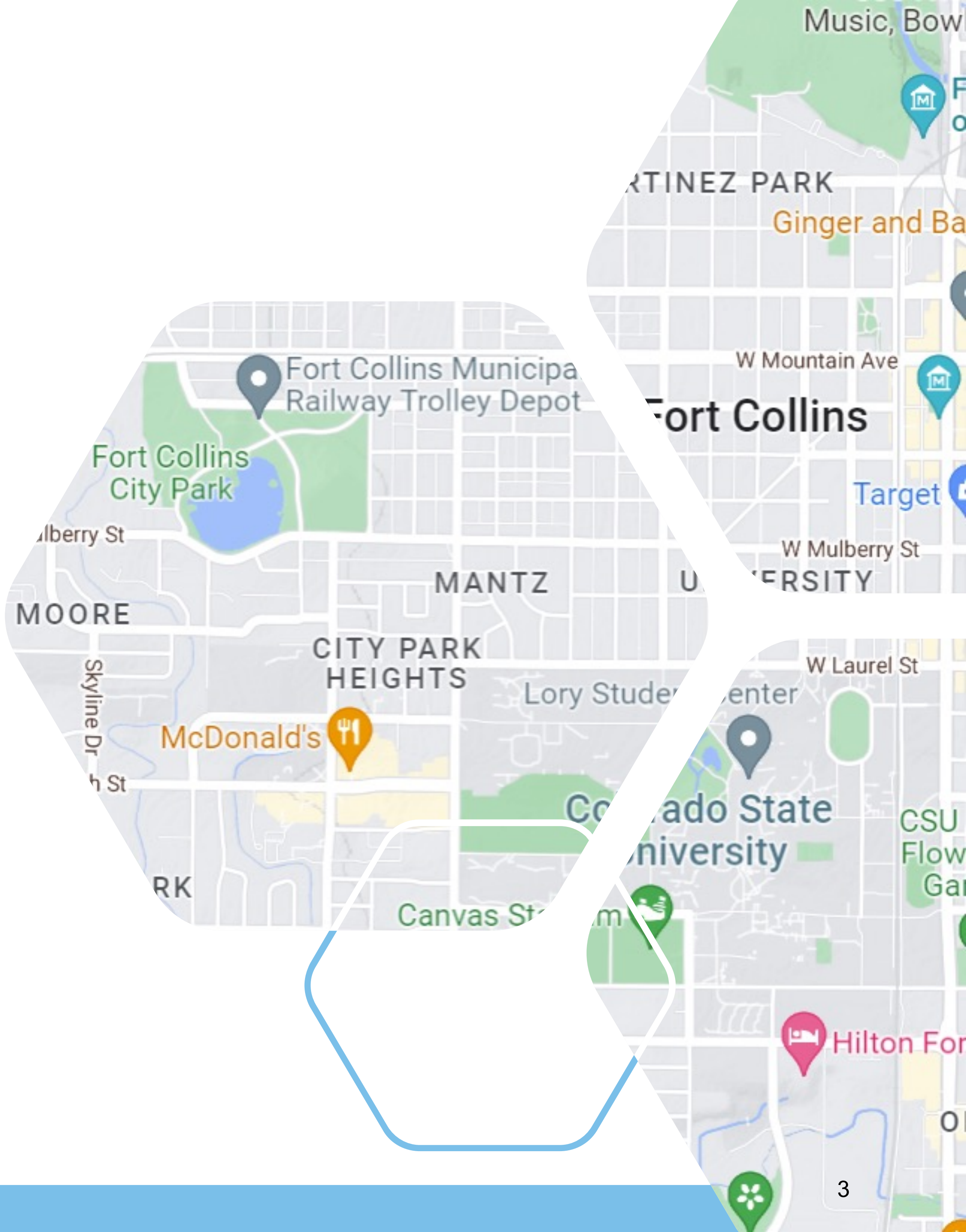
BREWING SUMMIT 2022
Providence, Rhode Island | August 14–16

That's me!---------------------------->

- Technical Brewing Projects Coordinator
  Brewers Association (2022)
- Quality Laboratory Director
  Colorado State University/FST
- Graduate Student @
  CSU (2019-2022)
  - **Presenting on thesis work**
- Quality Lab Manager
  Oskar Blues Brewery, Longmont, CO
  - **"First craft beer in a can"**

Presentation Roadmap

1. Background
2. Research Questions
3. Methods
4. Results
5. Q&A



Background

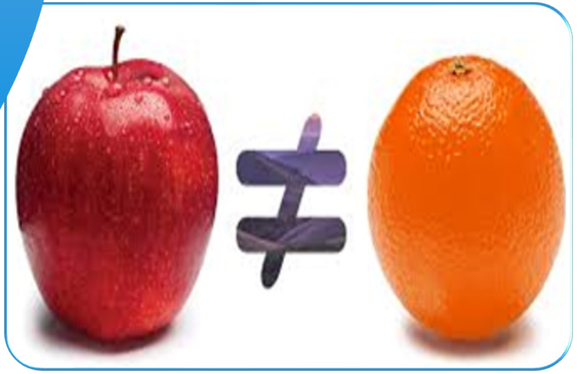
STABILITY MATTERS!

Market is global & competitive

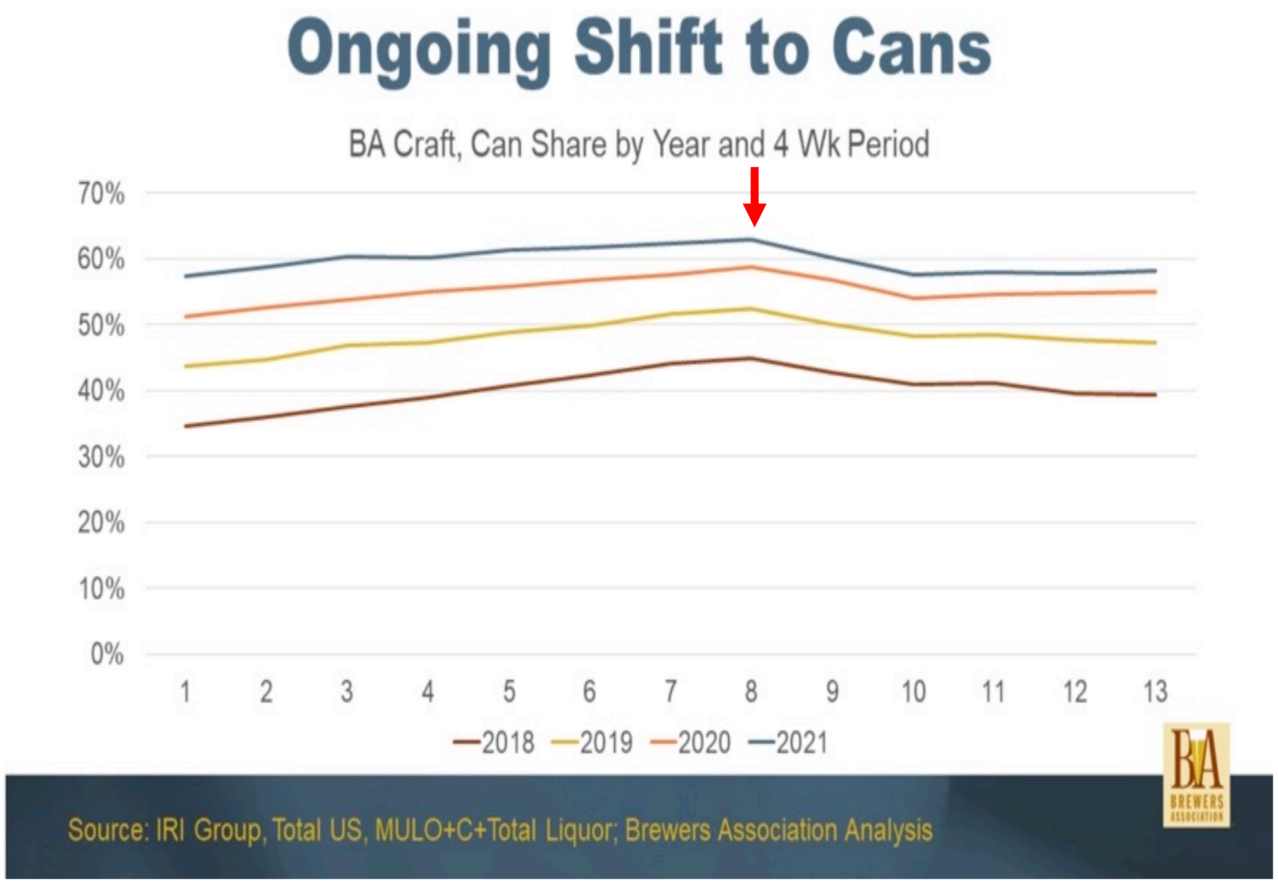
As beer changes in package

Most stability work performed on light lagers

True to brand keeps customers



Current literature lacks thorough understanding of beer stability for today's brewing industry.



www.brewersassociation.org/insights/craft-beer-packaging-trends-recap-2021/

- Stability research is not relevant to **modern beer matrix**
  - Light lager
  - **Style innovation** = changing matrix
- Shift in shares to **cans**
- No **direct** can vs. bottle stability comparison
  - Known package differences
  - **Anecdotal** claims

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Research Questions

Question 1

Are there differences in the chemical profiles of beer aged in cans vs. bottles?

Question 2

If so, does style impact the differences observed?

Question 3

What mechanisms might be driving the differences?

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Methods

Study Design & Sampling

“Complex” styles

Cans and Bottles

6-month aging

4ºC, then 20ºC

Sampling frequency

3 replicates per treatment/time

Aliquots for all research Qs

-80°C until analysis

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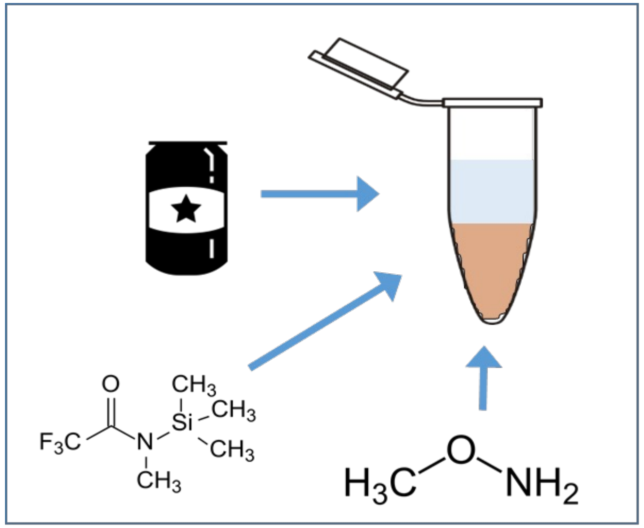
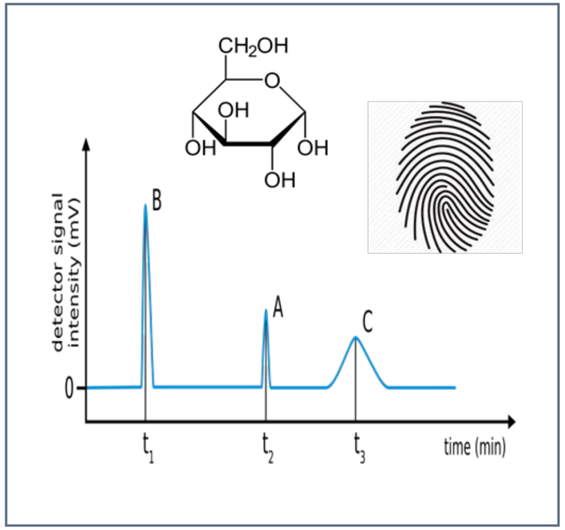
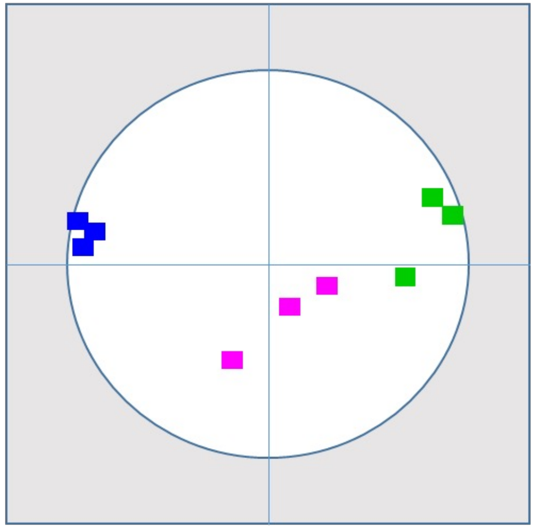
Non-Targeted GC-MS Approach for Chemical Detection

- Detect **metabolites =** chemical compound
- Headspace and direct injection GC-MS
  - Volatile and small polar non-volatile compounds
- **Non-targeted**
  - We don't know what we don't know
- Confidently identify **specific compounds**
- See how **chemical profiles** vary over time between package type and beer style
- Why?....to understand **mechanisms of beer aging** in different styles and package types

Use of metabolomics (small molecules) workflow







Frozen beer samples thawed and derivatized	Non-targeted metabolite profiling by GC-MS	Peak picking, data processing and metabolite annotation	Multivariate & Univariate statistics and predictive modeling
Methoxyamine & MSTFA	Volatile & Small polar non-volatile	XCMS/RAMClust for R & NIST/GOLM	SIMCA & R Programming

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Results

Detection
351
Molecular
Features

Annotation
73
Metabolites

Determined
17
Metabolites
of Interest

©

17 Metabolites of Interest

Subclass	Metabolite	Sensory Attribute
Amino acids	Glycine	NA
	Tyrosine	NA
	Asparagine	NA
Carboxylic acid ester	Ethyl Acetate	nail polish remover, solvent, fruity, sweet
	Isobutyl isobutyrate	grape skin, pineapple, tropical
Fatty acid ester	Ethyl decanoate	caprylic, soapy, estery
	Ethyl octanoate	apple, sweet, fruity, sour apple
	Ethyl hexanoate	apple, anise seed, citrus, solvent
	2-Methylbutyl butyrate	fruity, pear, apricot, tropical, spicy, apple
Monoterpene	Pinocarvone	minty
	β-myrcene	spicy, citrus, resinous, piney, lemon, woody
	β-pinene	woody, green, resinous, dry
Sesquiterpene	Humulene	spicy, herbal, grassy, woody, clove
	α-calacorene	citrus, spicy, woody
Alcohol	Isobutanol	malty, solvent
	myo-Inositol	NA
Carbonyl	2-Undecanone	varnish, bitter, green plants, geranium, fruity, citrus

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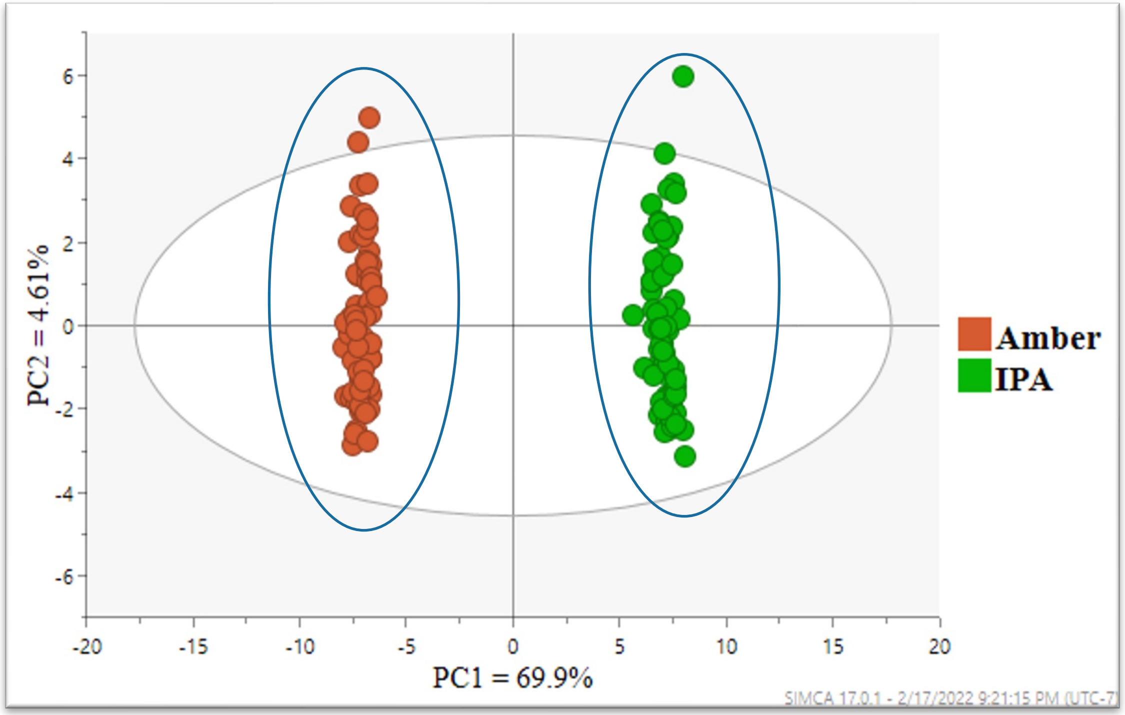
PCA model shows classification of samples by beer style



$R^2 = 0.867$

MULTIVARIATE ANALYSES

- Overall variation in 2D
- Points close together = more similar

- **PCA** - Principal components analysis
- **R^2** = Model fit

- **PLS-DA** - Partial least squares discriminant analysis
- **Q^2** = Model predictability

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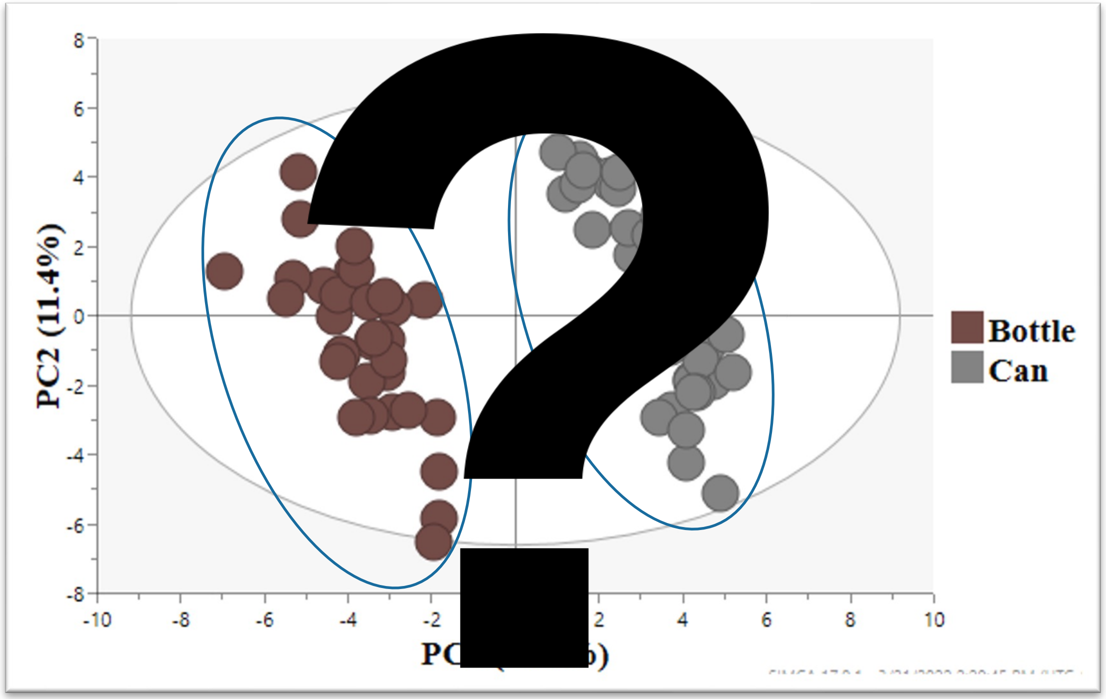
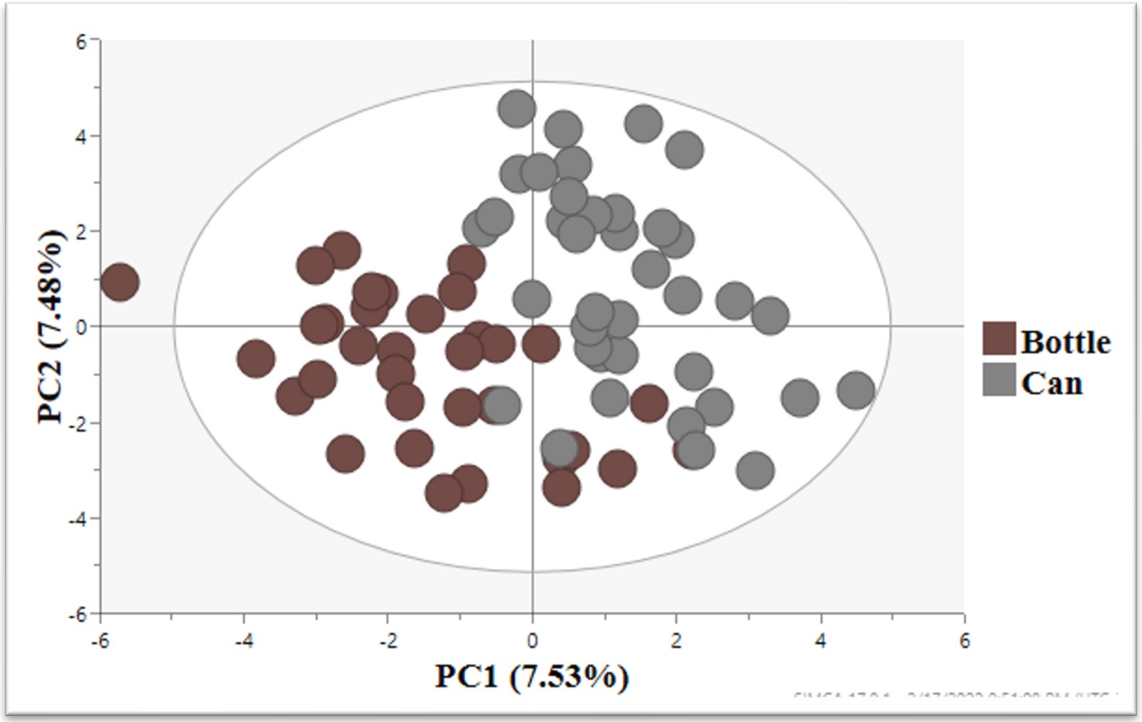
PLS-DA models show package predictability is style dependent

PLS-DA of Amber Ale



$R^2 = 0.981$
$Q^2 = 0.964$

PLS-DA of India Pale Ale



$R^2 = 0.667$
$Q^2 = 0.115$

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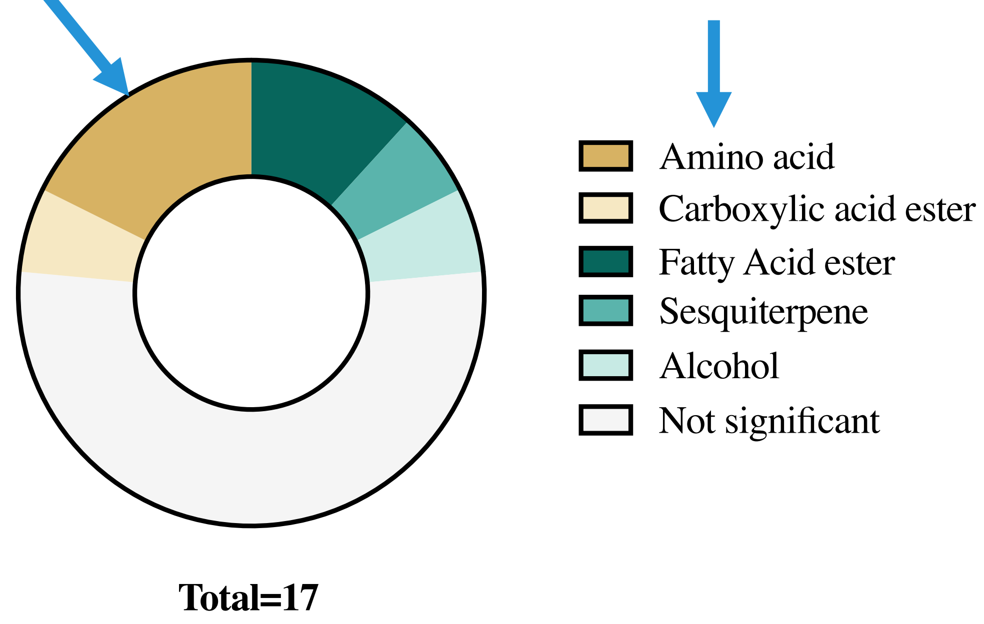
Baseline differences explain part of amber ale variation

Amber Ale Baseline Differences



Metabolite	Baseline*
Glycine	<0.001
Tyrosine	<0.001
Asparagine	<0.001
Ethyl acetate	0.21
Isobutyl isobutyrate	<0.001
Ethyl decanoate	<0.001
Ethyl octanoate	0.06
Ethyl hexanoate	0.74
2-Methylbutyl butyrate	<0.001
Pinocarvone	0.33
β-myrcene	0.76
β-pinene	0.41
Humulene	<0.001
a-calacorene	0.47
Isobutanol	0.43
myo-Inositol	<0.001
2-Undecanone	0.50

**P-values derived from emmeans()*

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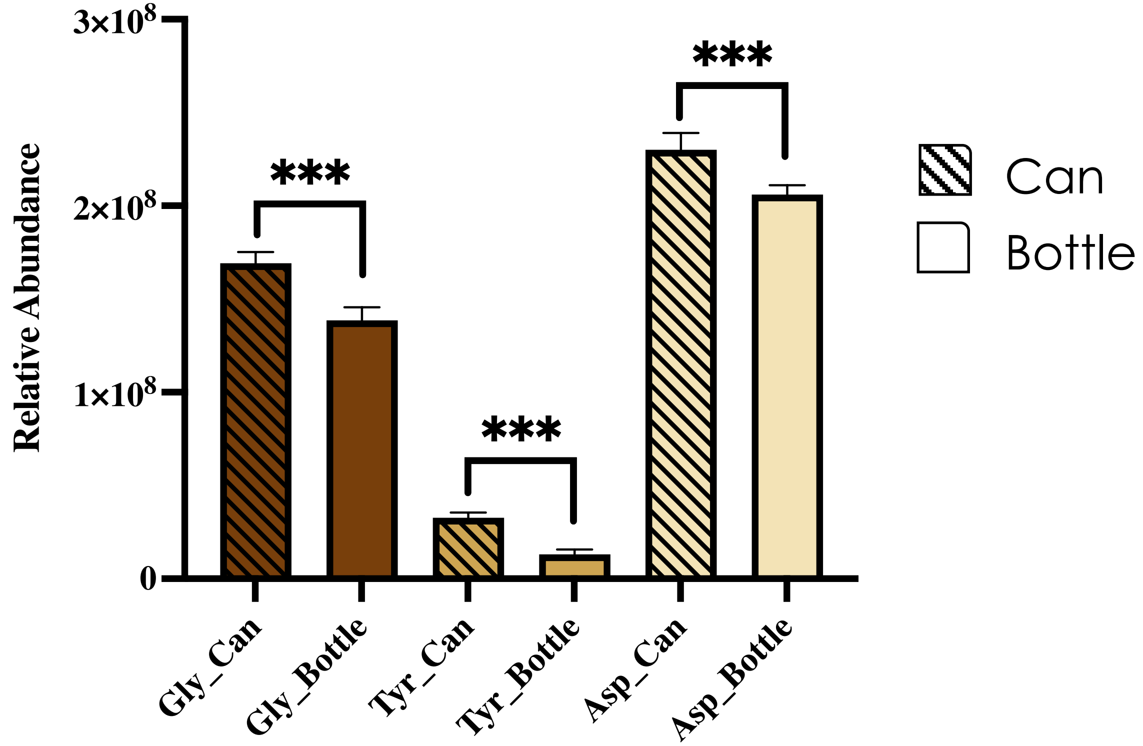
Amino acid baseline levels are lower in AA bottles



Gly = glycine, Try = tyrosine, Asp = asparagine

*** indicates *P* <0.001

- Amino acids can **adsorb** to glass

- Amino acids are a substrate for **staling compounds** (Strecker aldehydes)

Amber ales packaged in cans could be more susceptible to increased staling compounds

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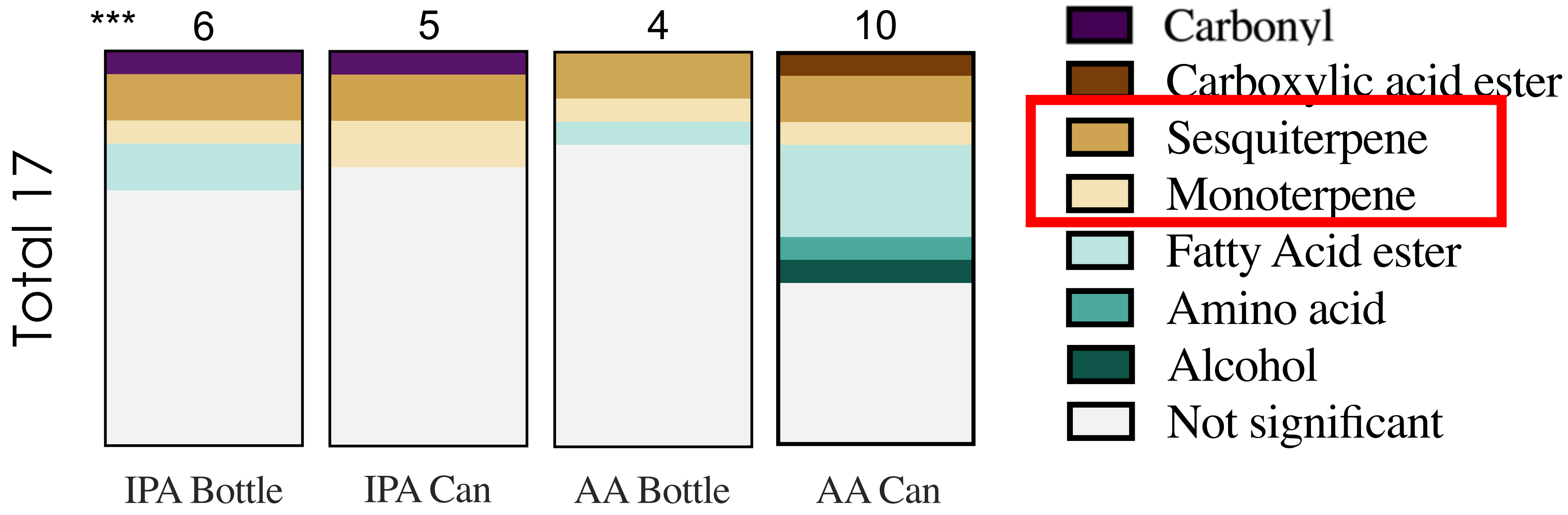
Significant changes of metabolites over time by sample treatment and subclass



*** Estimated marginal means of linear trends, $P \leq 0.05$

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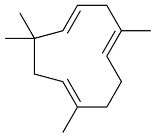
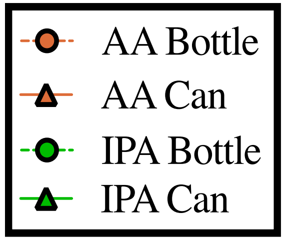
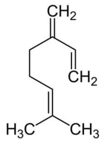
Humulene decreases significantly more in bottles than in cans in both styles

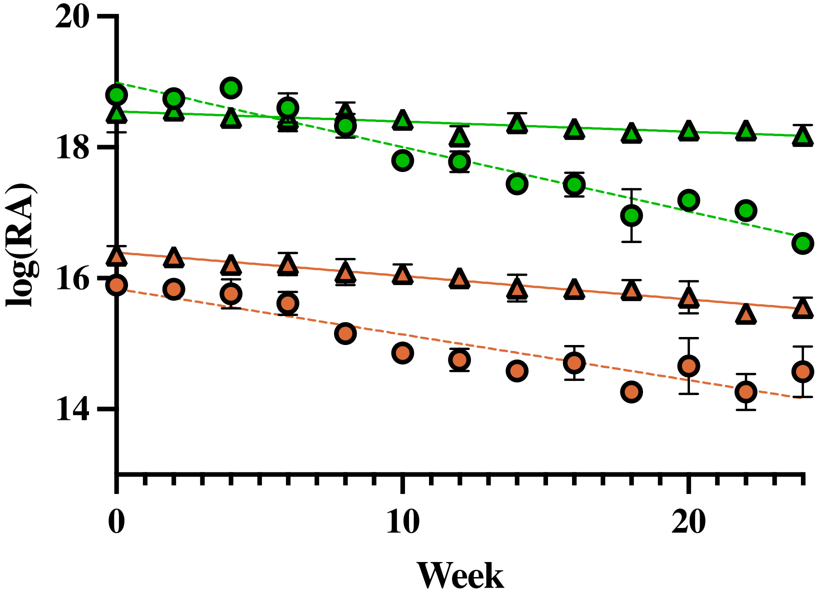


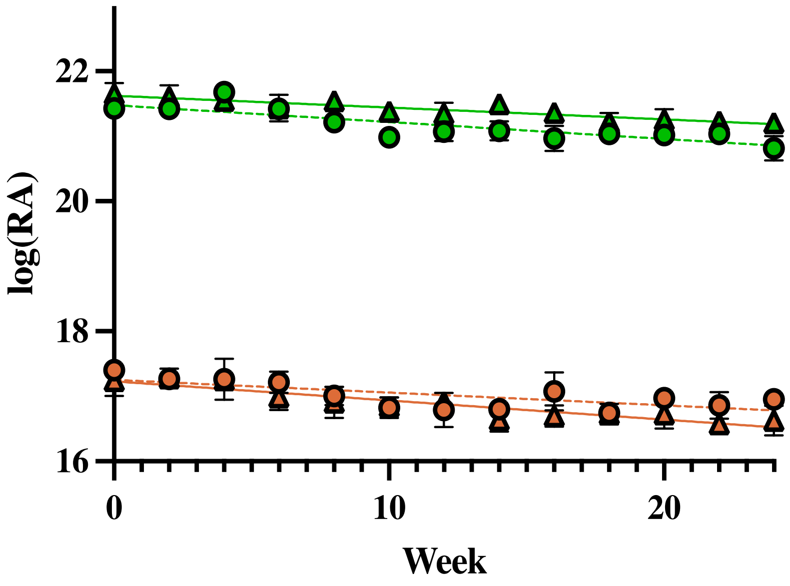
HUMULENE



B-MYRCENE







ANOVA P <0.001

ANOVA P = 0.74

Water Solubility = 0.011g/L

Water Solubility = 0.077g/L

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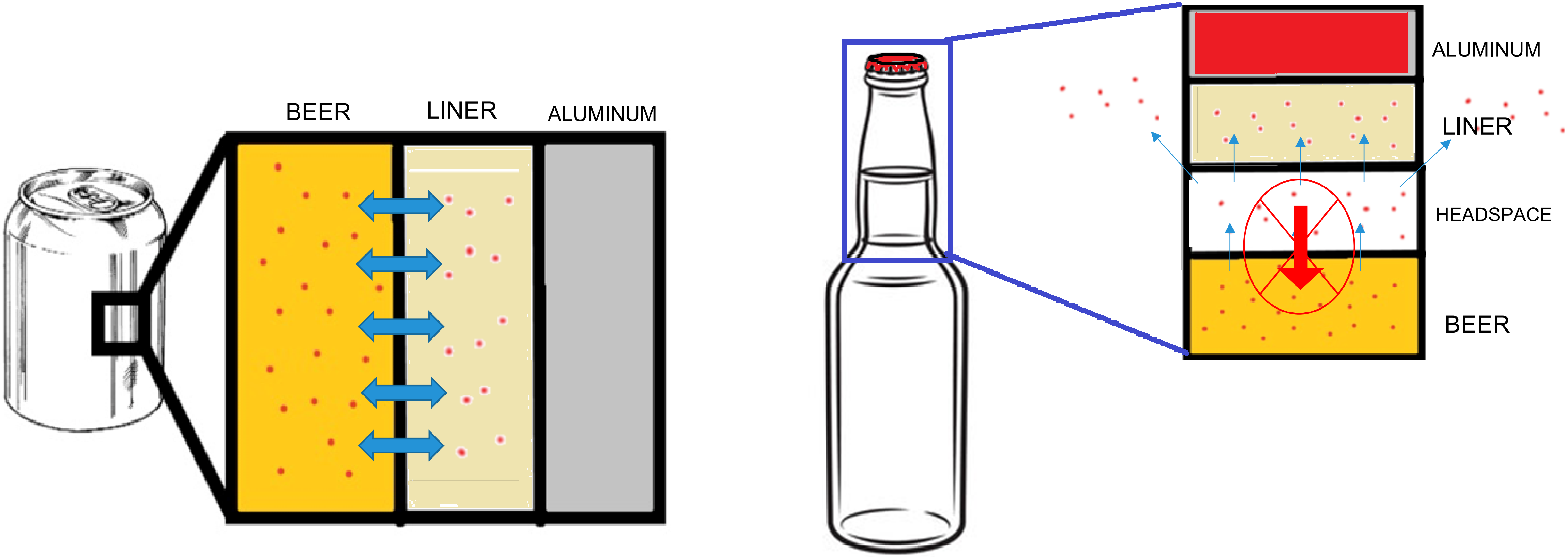
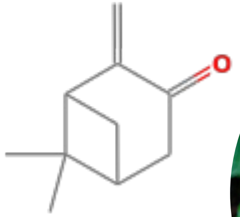
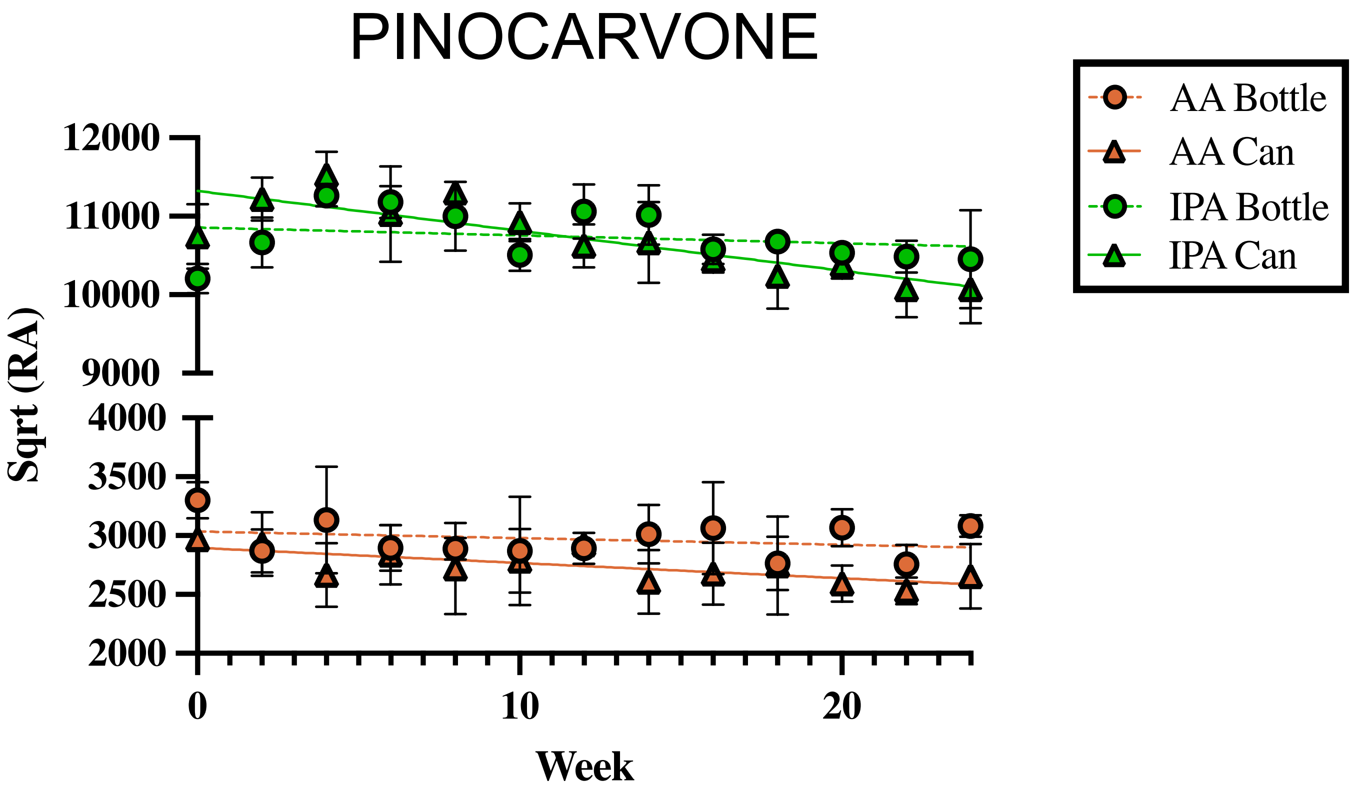
Proposed flavor scalping mechanisms



Metabolite interactions with packaging environment

Metabolite chemical properties influence package type effects over time





ANOVA P = 0.001

Water Solubility = 0.62g/L

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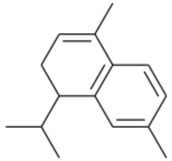
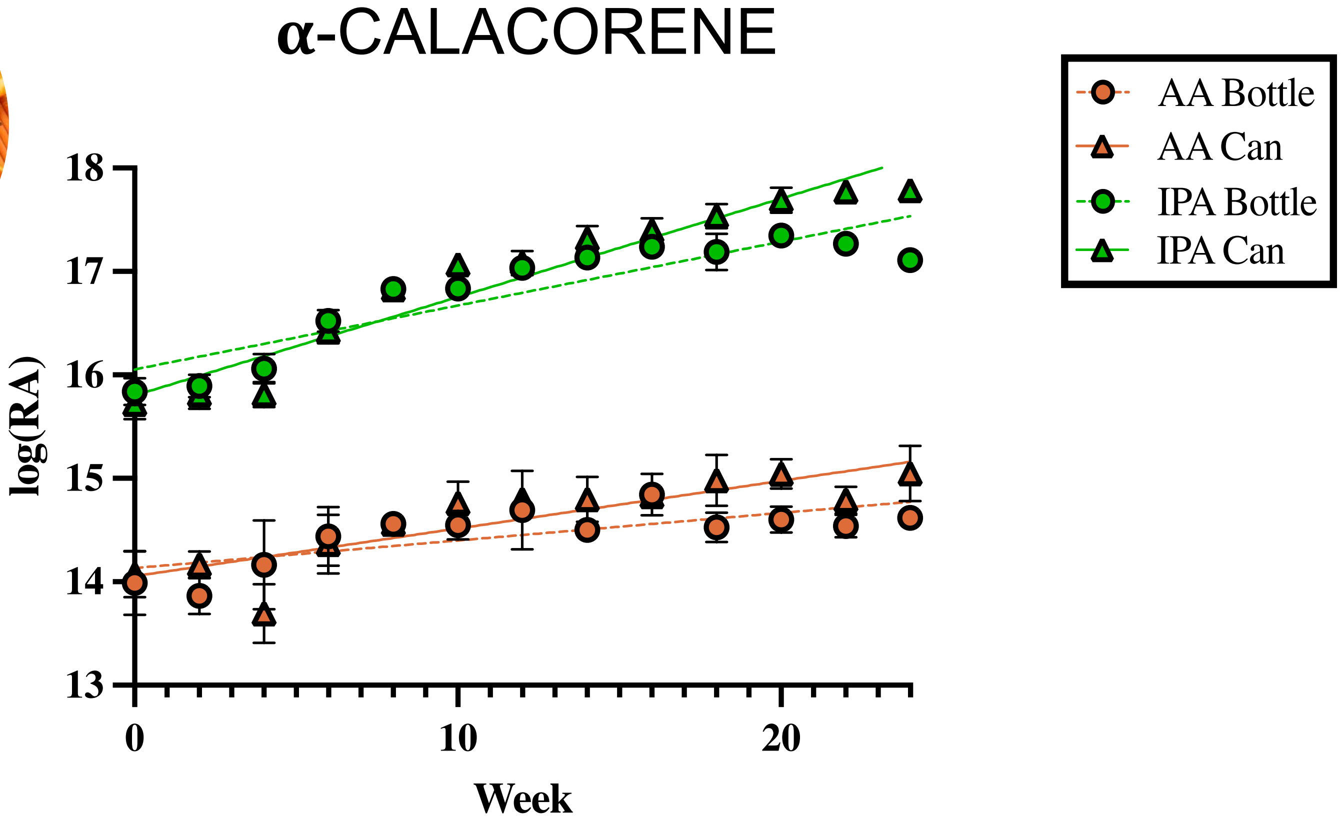
Evidence of hop terpene formation or release during beer storage depends on package type





ANOVA P <0.001

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Main Takeaways

“10,000-foot view”

What we learned…

1. Package type predictability was **style dependent.**

2. Baseline differences between cans and bottles drove variation in AA samples.

3. Evidence **amino acids** were lower in AA bottles due to **adsorption.**

4. Evidence **hop terpenes** are interacting with **packaging materials** and their chemical properties impact those effects.

5. This work **scratches the surface** of packaging effects on beer stability. **More work is needed** to fully understand the mechanisms.

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Industry Impacts & What Next?

- A **"best package"** for a particular style
  - Or not…
- **Understand mechanisms** and control those effects
- **Targeted analysis**
  - Amino acids, terpenes
  - Proposed mechanisms
- **Liner** composition
- **Non-volatile** fraction
- Expand range of **styles**
- Pair work with **sensory**

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Resources

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- You, X. and S.F. O'Keefe, *Binding of volatile aroma compounds to can linings with different polymeric characteristics.* Food Science & Nutrition, 2018. **6**(1): p. 54-61.
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Q&A

Cheers!

Email: katie@brewersassociation.org

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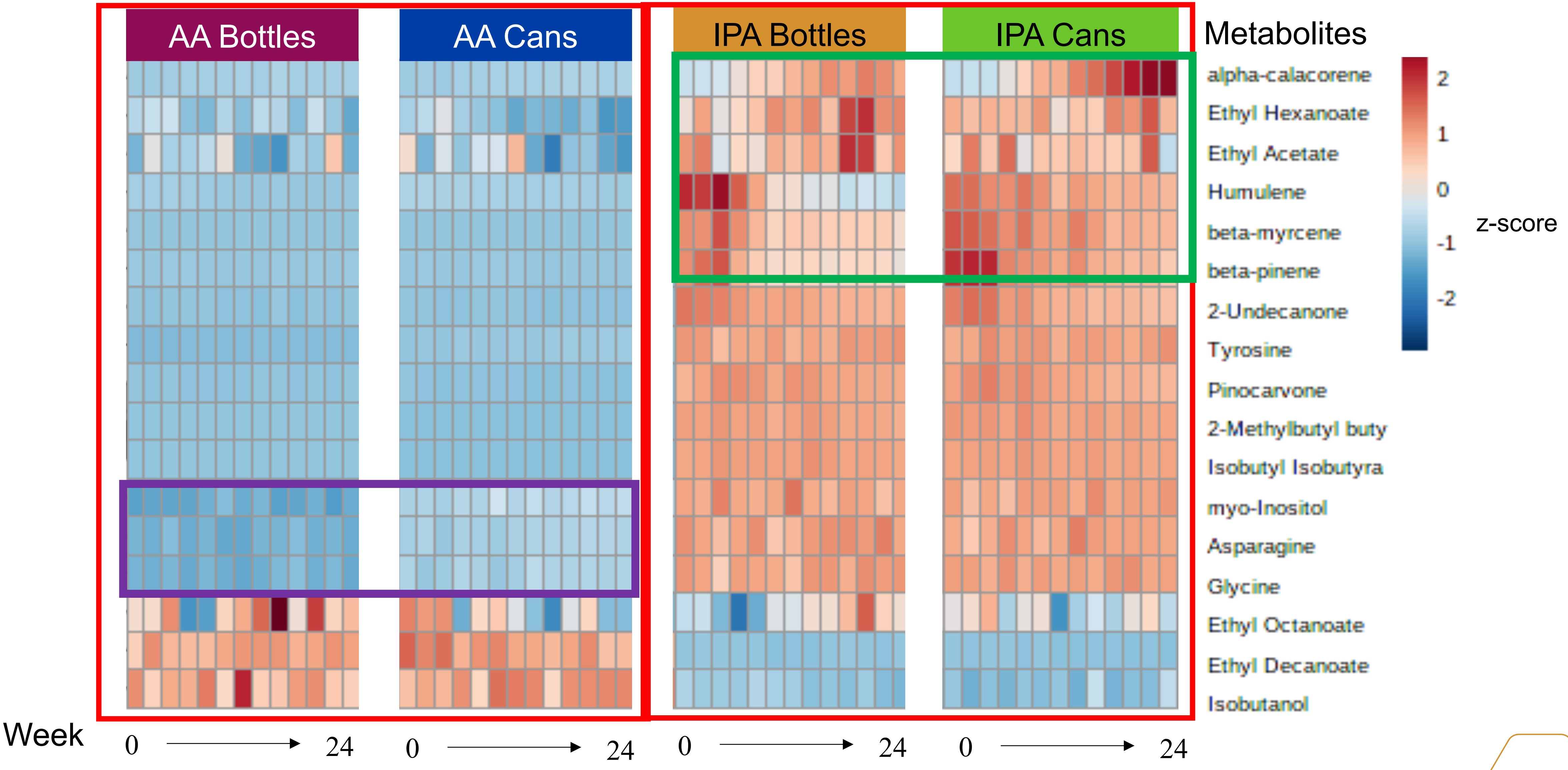
Heatmap visualizes big picture relationships between style, package, time



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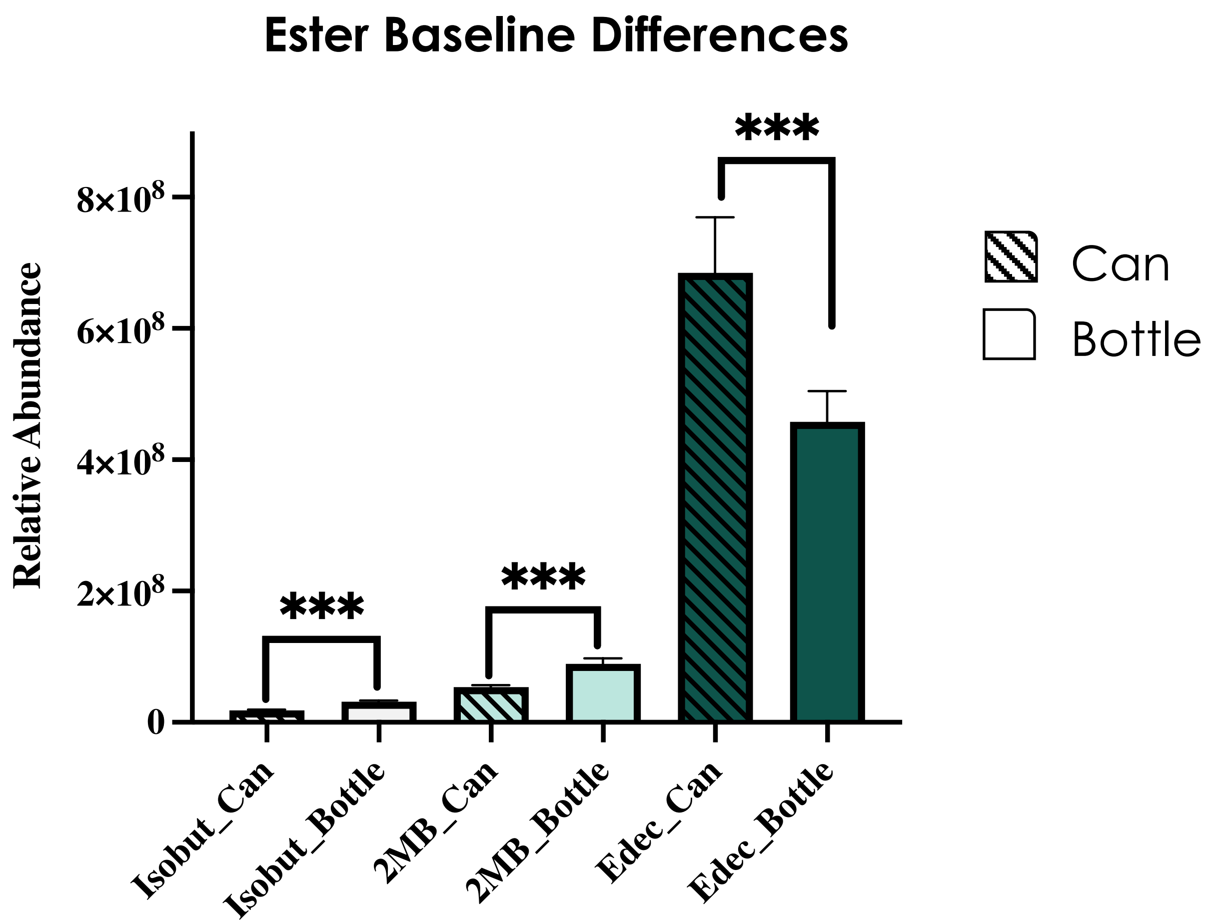
Canning process may cause more ester volatilization in AA cans compared to bottles



Isobutyl isobutyrate, 2-methylbutyl butyrate, Ethyl decanoate

*** indicates *P* <0.001

- Esters **driving variation** in AA
- Esters = **volatile, fruity aromas**
- Cans have larger opening
  - **Increased volatilization and oxygen pick up**
- **Polyphenols** from hops may be **protective**
  - Antioxidant

Amber ales packaged in cans may be more susceptible to dampened aromas.

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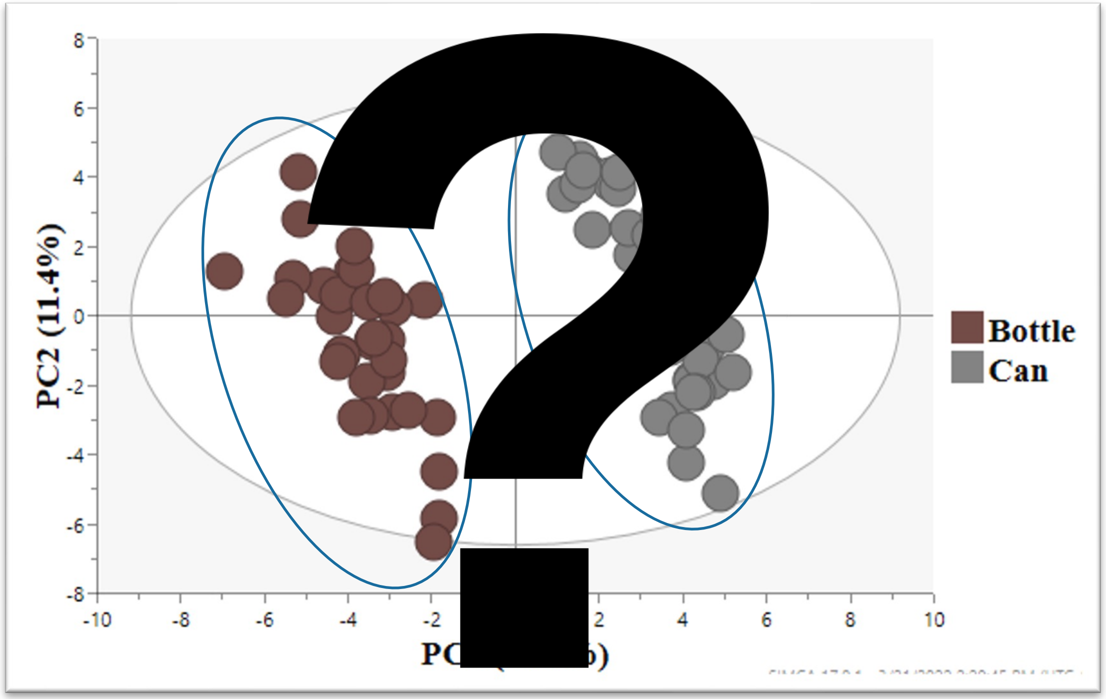
PLS-DA models show package predictability is style dependent

PLS-DA of Amber Ale



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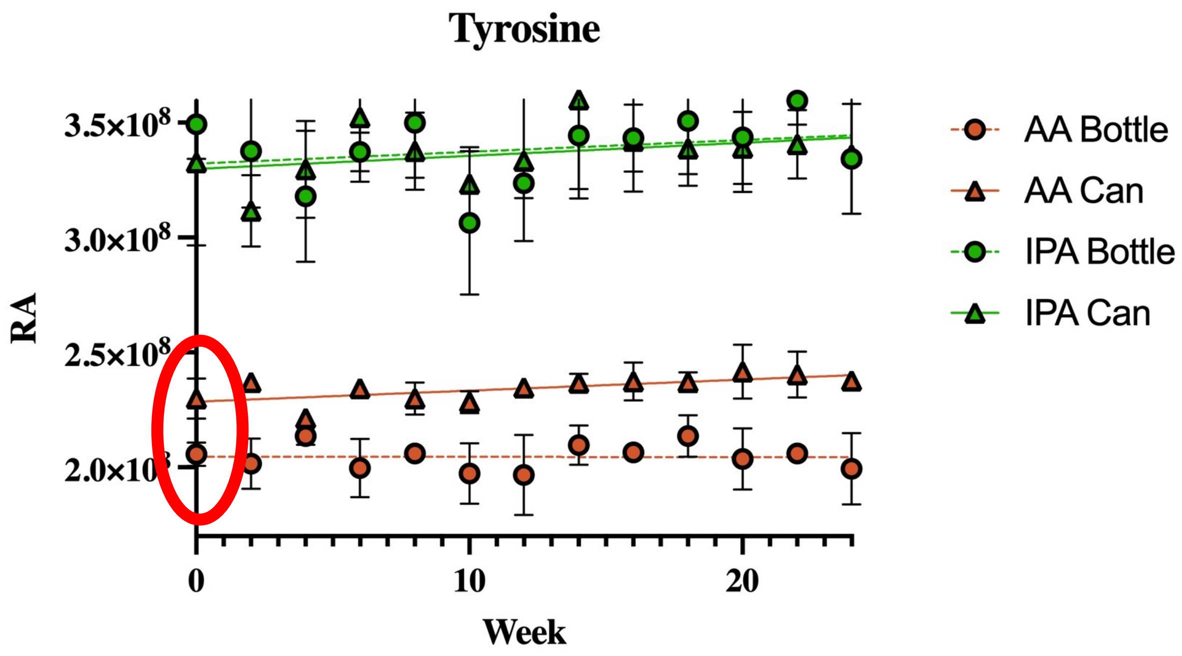

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