What Makes Kombucha Smell? Influences on the Olfactory Profile of Kombucha

Based on: Tran, T., Billet, K., Torres-Cobos, B., Vichi, S., Alexandre, H., Grandvalet, C., Tourdot-Maréchal, R., 2022. **Use of a minimal microbial consortium to determine the origin of kombucha flavor**. Frontiers in Microbiology; Under press.

Thierry Tran

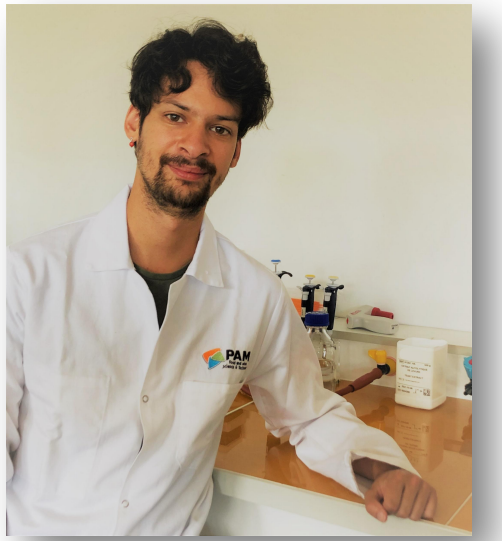
01/04/2022

KombuchaKon Conference & Expo

LEVELING UP — MARCH 30TH - APRIL 1ST, 2022 — LONG BEACH, CA

About the PhD project

Thierry Tran (PhD)



Food Engineer & Enologist

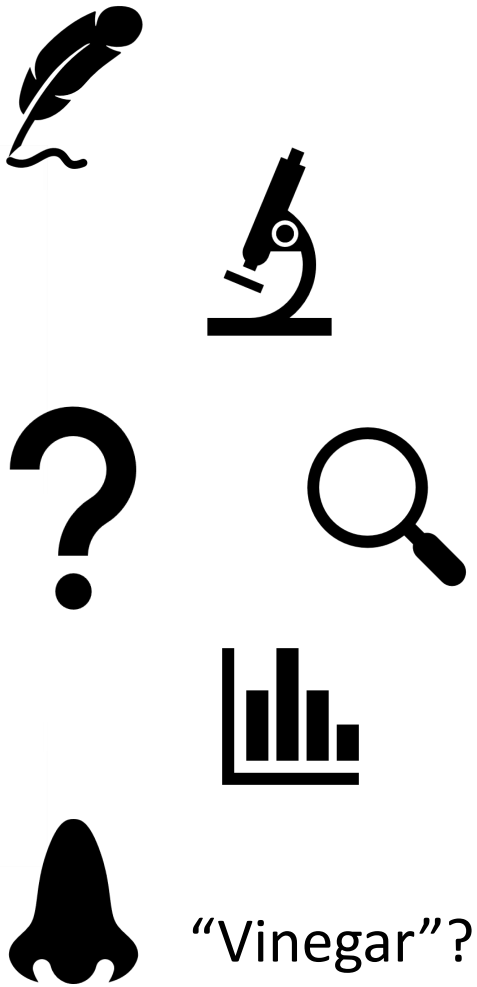
Raphaëlle Tourdot-Maréchal (Advisor)
Cosette Grandvalet (Advisor)

Antoine Martin
François Verdier
(Biomère / Jubiles co-funders)

PhD Project (defense 14/12/2021)

”Study of microbial dynamics for the control of kombucha production”

Defining Kombucha



“Vinegar”?
“Cider”?

Regulation

ABKOM Associação Brasileira de Kombucha

Food Safety and Quality Standard

KOMBUCHA BREWERS KB

Kombucha Code of Practice

Kombucha Brewers International Code of Practice

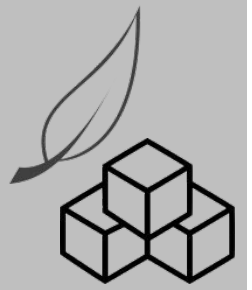
Definition of styles
Standization of vocabulary

March 28-29, 2022
KOMBUCHA KUP
AWARDS OF EXCELLENCE

Process

Parameters of kombucha production

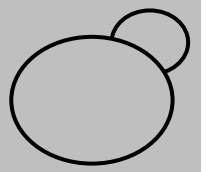
Tea infusion



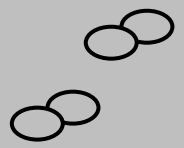
+ Sucrose

+ Starter

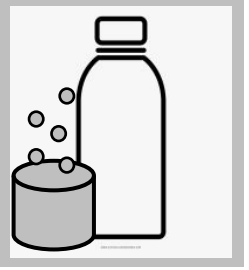
Yeast activity
(invertase, fermentation)



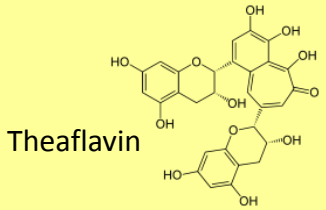
Acetic Acid Bacteria activity
(Oxidative metabolism, cellulose production)



Kombucha

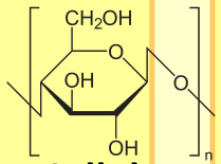


Clear & colored



Polyphenol pigments

Proteins
Polysaccharides

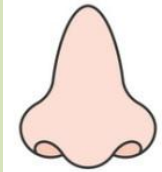


Cellulose
(from nanofibrils to pellicle)

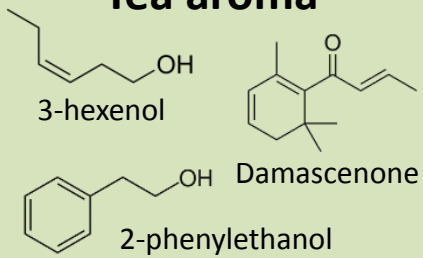
Color loss

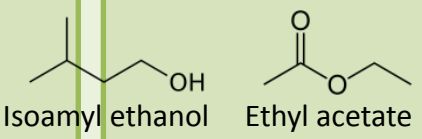
Colloids

Light colored, turbid & sparkling



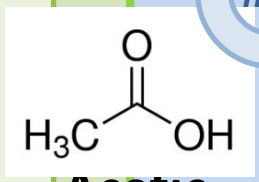
Tea aroma





Fermentation aroma ?

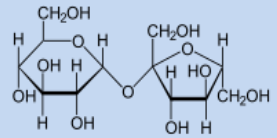
?



Acetic acid

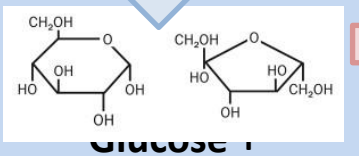
Vinegary & cidery smells

Sweet & bitter



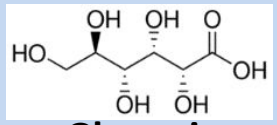
Sucrose

invertase



Glucose + Fructose

oxidation



Gluconic acid

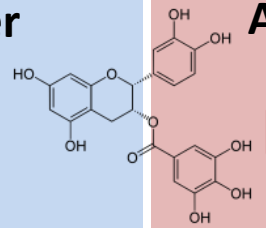
Organic acids

oxidation

Sweet / Sour balance

+ fruitiness

Astringent

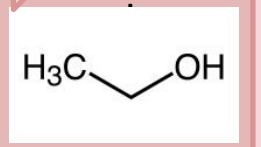


Epicatechin gallate

Polyphenols

CO_2

alcoholic fermentation



Structure change?

Tinglin & Astringency

+ sourness

Origin of volatile aroma compounds

Process

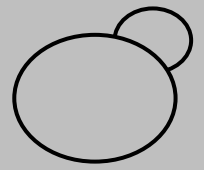
Parameters of kombucha production

Tea infusion

+ Sucrose

+ Starter

Yeast activity
(invertase, fermentation)



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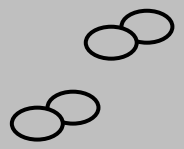
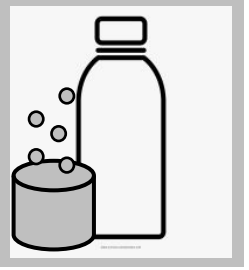
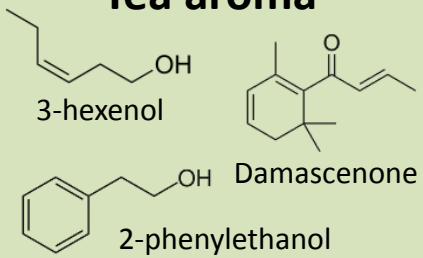
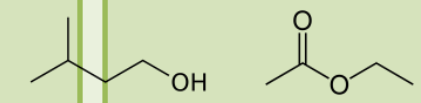


Kombucha

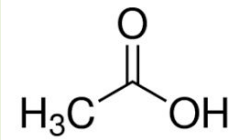


Tea aroma





Fermentation aroma ?



Acetic acid

?

Vinegary & cidery smells

Varietal aroma compounds
From the raw material (e.g., tea)

Fermentative aroma compounds
From microbial activity (yeasts, bacteria)

In wine, beer …

The Ehrlich Pathway
Amino acids -> Fusel Alcohols

Esterification
Alcohol + Fatty Acid -> Ester (fruity)

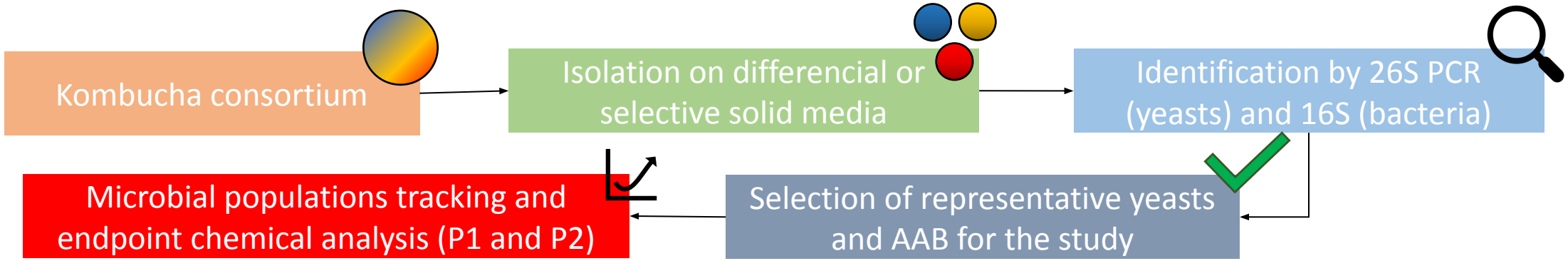
Objectives of the study

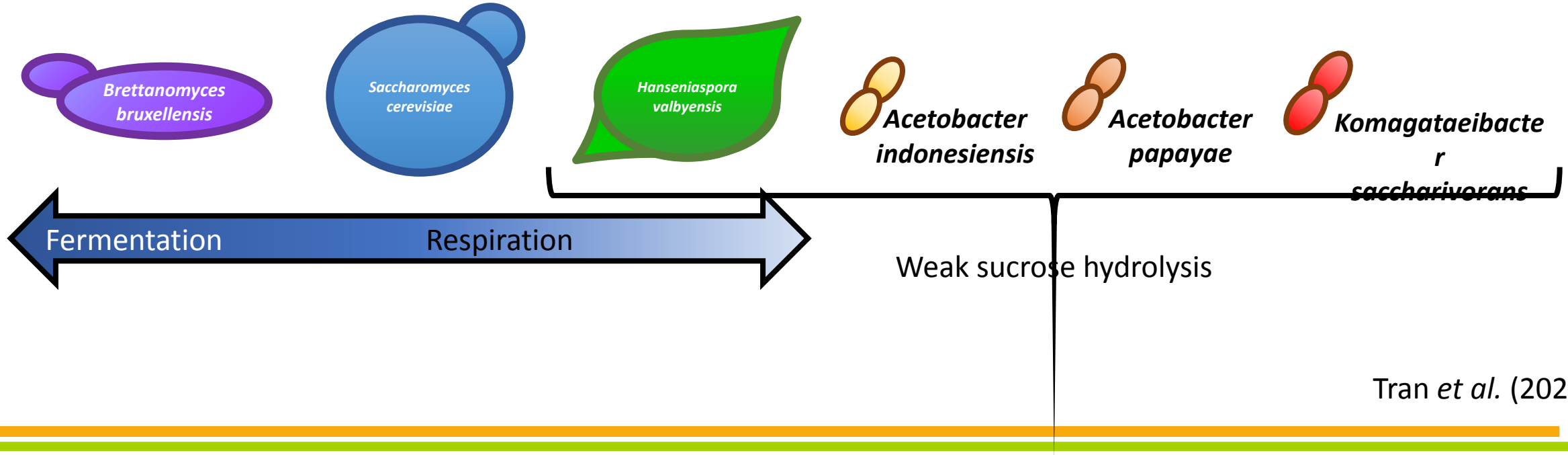
- Understand the origins of sensory compounds and flavor of kombucha
  - From the tea ?
  - From the microorganisms (SCOBY)?
    - Specifically from yeasts or bacteria?

- Determine the impact of fermentation phases and tea types
  - What is produced during F1 , F2?
  - Differences between black tea and green tea?

- **Focus on volatile compounds and the smell**

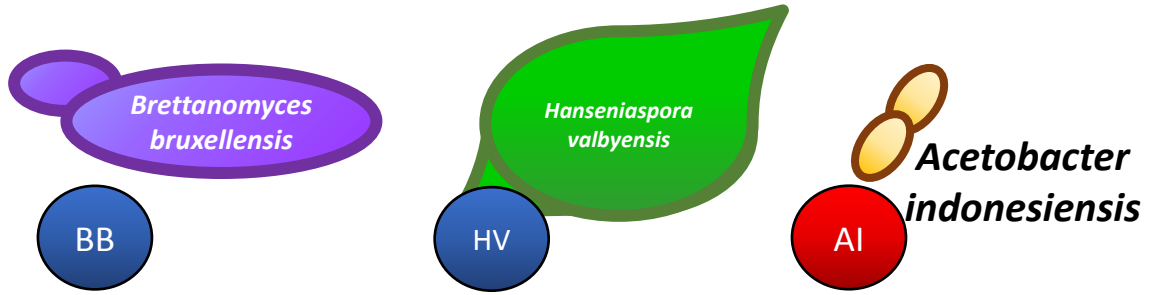
Experimental procedure

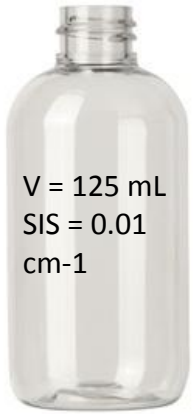




Tran *et al.* (2020)

Experimental procedure





General parameters:

1 g/L Black tea
50 g/L Sucrose

7days open (D7)
then
5 jours closed (D12)

26°C / 5 log/mL inoculation

Sugared Teas

Sugared Black Tea (**SBT**)

Sugared Green Tea (**SGT**)

3 Monocultures

BB
HV
AI

9 Cocultures

BBAI
HVAI
BBHV
T (BBHVAI)

Original Kombuchas

Black Tea Kombucha (**BTK**)

Green Tea Kombucha (**GTK**)

Gas Chromatography / Mass Spectrometry

Non-targeted analysis of **volatile co**

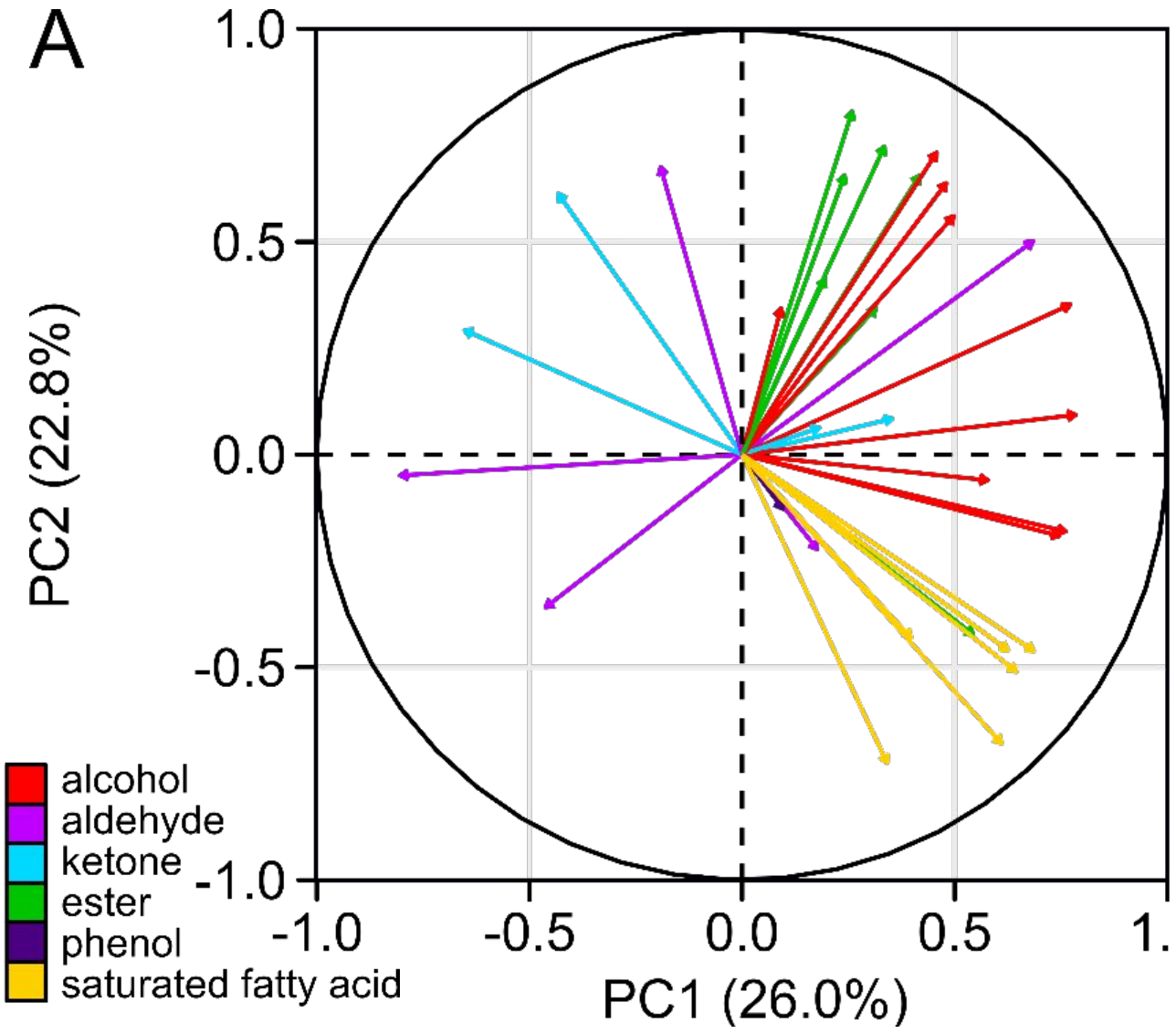
Collaboration with LiBiFood Lab

UNIVERSITAT DE BARCELONA

Descriptive Sensory Analysis

Evaluation of **descriptors** using **intensity scales** by a **trained panel (12)**

Results: Volatile compounds



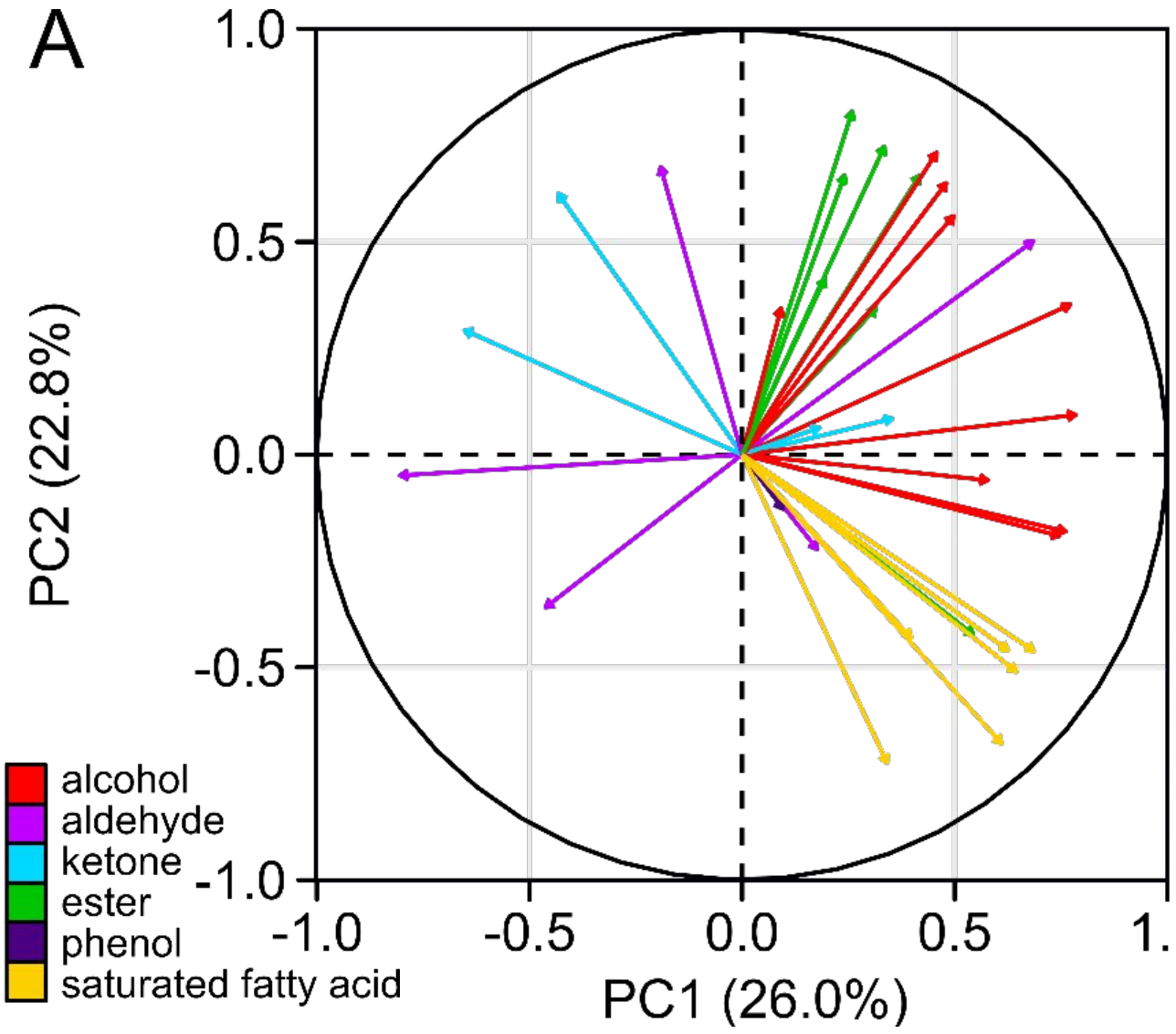
Examples of main metabolites :
(besides ethanol and acetic acid)

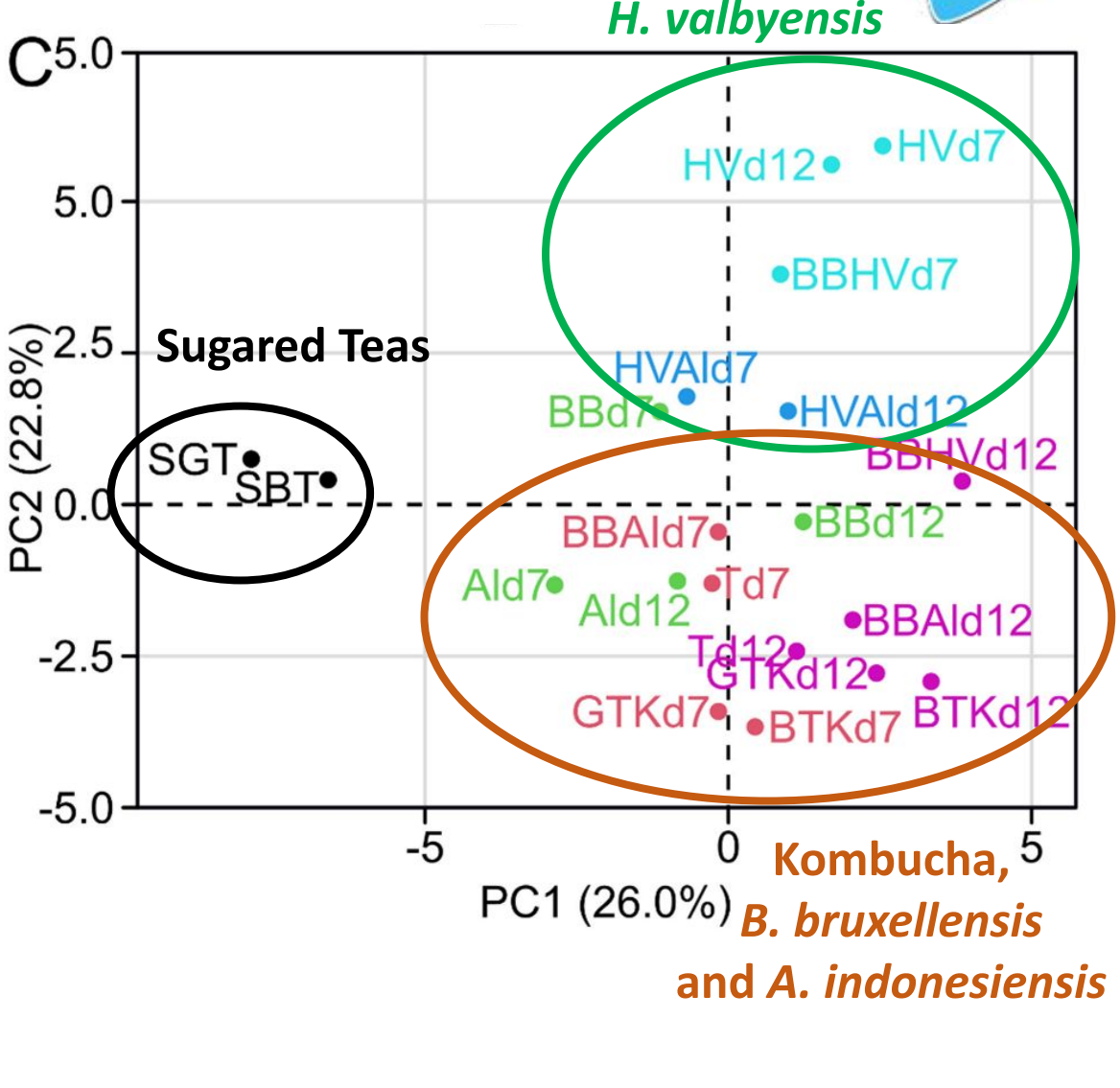
- Isoamyl alcohol (fruity)
- Isovaleric acid (cheesy)
- Ethyl acetate (fruity)
- Diacetyl (buttery)
- Hexanal (grass)

Fermentative

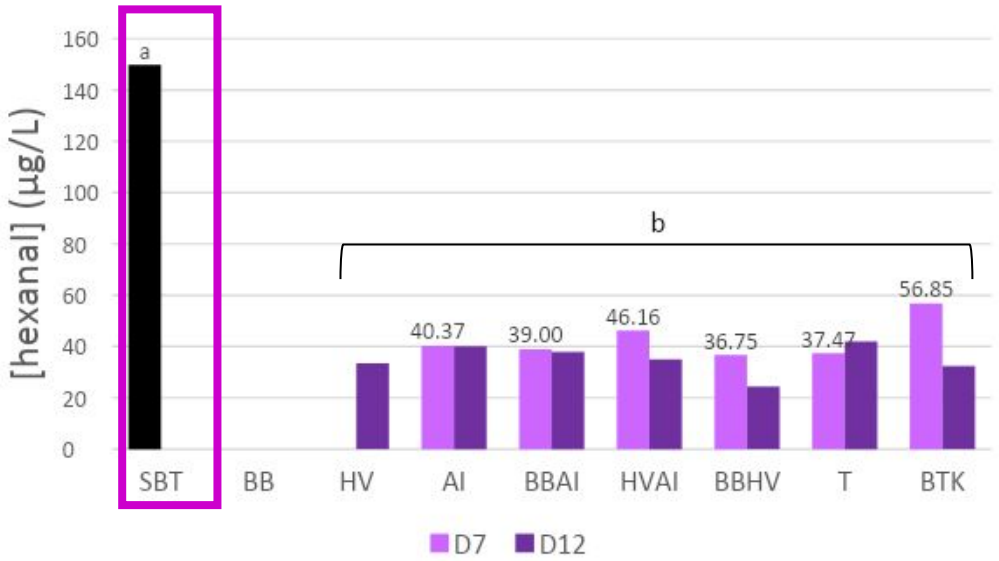
Varietal

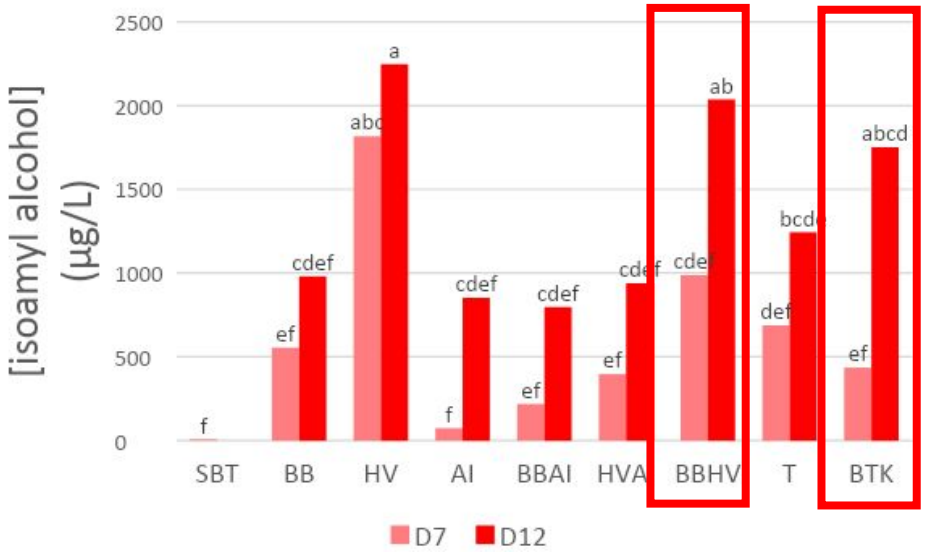
Results: Volatile compounds





Results: Volatile compounds (examples)



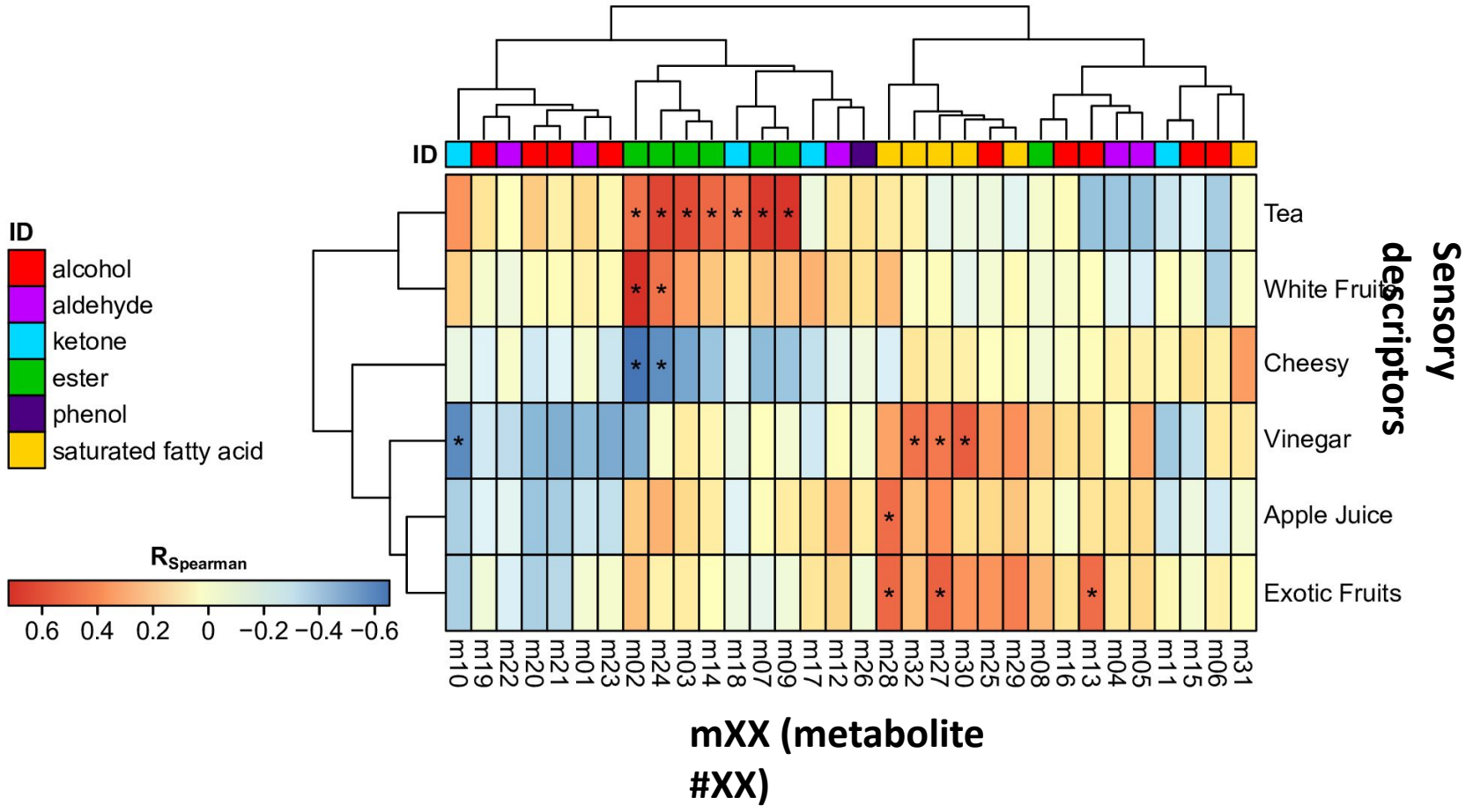


- Varietal hexanal decreased in concentration during the process
- *H. valbyensis* contributed in ethyl acetate. Inhibited by the presence of other microorganisms
- Increase of isoamyl alcohol during F2, probably produced by yeasts

Common letters = no significant difference according to ANOVA with 95% confidence

Results: Vol.Compounds vs Sensory Descriptors

- Significant correlations between volatile compounds and olfactive descriptors
  - **esters** x “tea” and “white fruits”
  - **fatty acids** x “vinegar”; “apple juice” and “exotic fruits”
- In detail, “apple juice” smell is more intense when *B. bruxellensis* and *A. indonesiensis* are both present



Results: Vol.Compounds vs Sensory Descriptors

Clustering comparison between

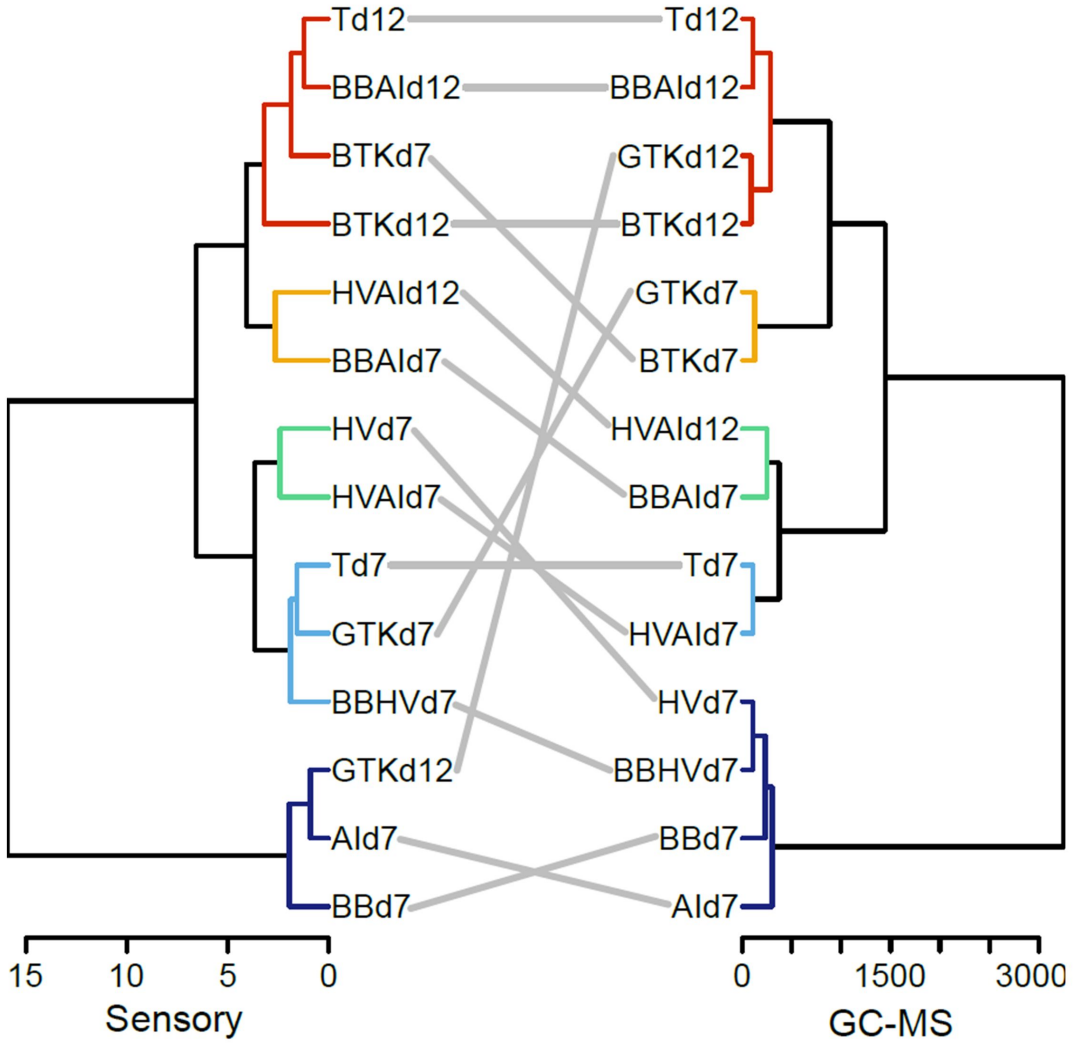
Volatile Compounds analysis (GC-MS)

x

Sensory analysis

- One very conserved cluster including Original Kombucha (BTK) and at least *B. bruxellensis* and *A. indonesiensis* (BBAI and T)

- Impact of the tea type on sensory clustering was not reflected in the chemical analysis



Conclusions

Influence of tea on kombucha smell

- Varietal aroma compounds from tea include aldehydes, along with esters and ketones
- Pure varietal compounds tend to degrade during the process
- However, the tea type (black or green) can have an influence on the smell of kombucha
- The profile in fermentative compounds might be determined by the available substrates of the sugared tea influsion (rich in sugar, poor in nitrogen)

Conclusions

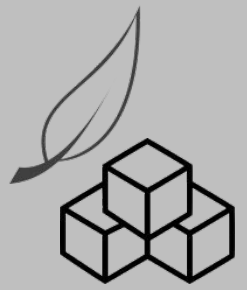
Influence of microbial composition on kombucha smell

- Core association of yeast and AAB induce kombucha's characteristic smell
  - Produced fatty acids and alcohols induce are together perceived as "apple juice", "vinegary" and "exotic fruits".
- The presence of certain yeasts can influence the smell, like the yeast *H. valbyensis*
  - Production of esters associated to "tea" and "white fruits" smells
- The F2 phase had a minor impact on sensory despite the production of alcohols

Process

Parameters of kombucha production

Tea infusion



+ Sucrose

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Yeast activity
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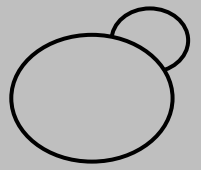

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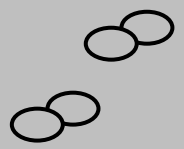
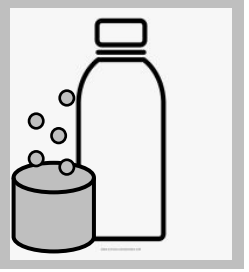
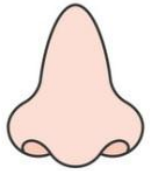
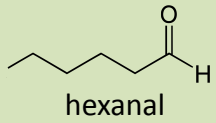


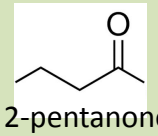
Kombucha



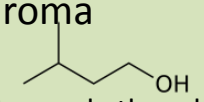


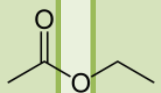
Tea aroma

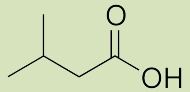




Varietal aroma



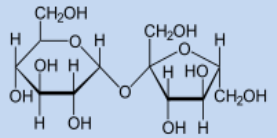




Fermentation aroma

+ varietal and fermentative compounds

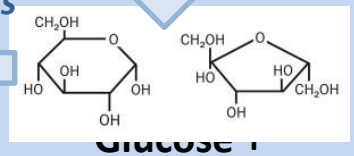
Sweet & bitter



Sucrose

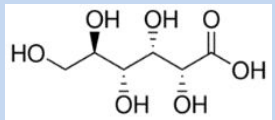
invertase

carbon metabolism

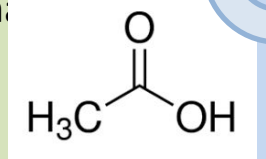


Glucose + Fructose

oxidation



Gluconic acid



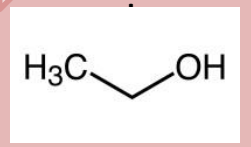
Acetic acid

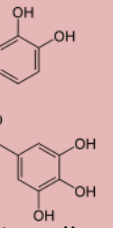
Organic acids

oxidation

alcoholic fermentation

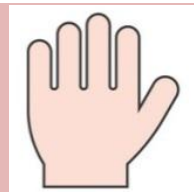
CO_2





Epicatechin gallate

Polyphenols



Astringent

Structure change

Vinegary, fruity & cidery smells

+ fruitiness

Sweet / Sour balance

+ sourness

Tinglin

Pschitt

Thank you for your attention

Please ask your questions or contact me at thierrytran01@gmail.com

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Suppl. Result: Analysis of Metabolic pathways

- Sugar availability : High
  Profile in volatile compounds includes mainly metabolites derived from **glucose**
- NH_4^+ / Amino acids availability : Very poor
  Absence of compounds found in media rich in diverse amino acids
- Sugared tea infusion seems to determine the olfactive profile of kombucha