

The Kombucha Core 4

Outline

- What is the Core 4?
- Why does the Core 4 matter?
- Parameters and Methods
- Kombucha Kup Acidity Results
- Fermentation Curve Examples
- What does this mean for you?

Our Company

- Our Team:

- John Richardson, Ph.D.
- Lauren Shearer, B.S.
- Jeb Kegerreis, Ph.D.

- What we do:

- Chemical testing
- Shelf-life testing
- Consulting
- Laboratory Set-up
- Research & Development



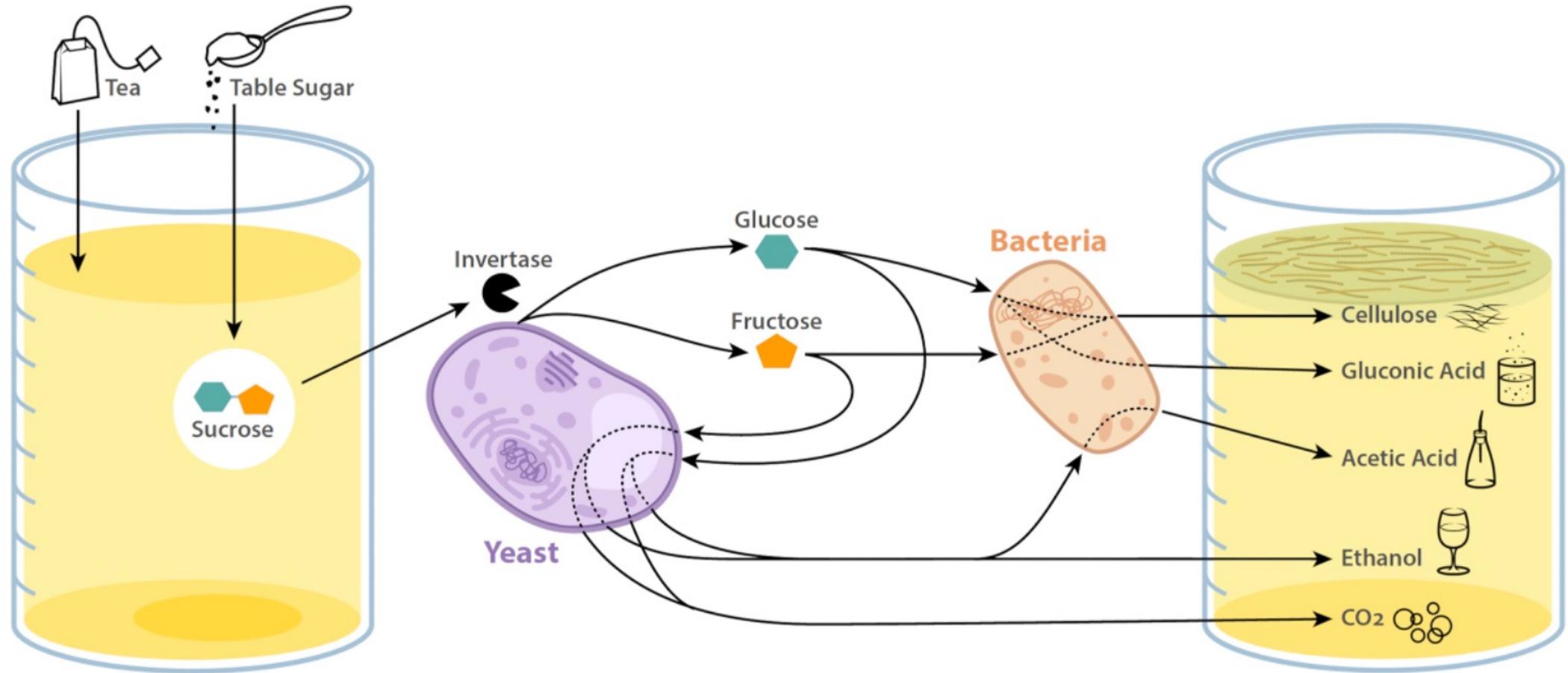
What is the Core 4?

Background of the Core 4

- Four key parameters that can be used to monitor and assess your kombucha fermentation
 - pH
 - Titratable Acidity (TA)
 - Sugar
 - ABV
- Understanding the fermentation process helps you produce a higher-quality and more consistent product
- These measurements should be incorporated into your process to build a historical database
 - In-house – as frequently as possible
 - External lab – monthly or quarterly

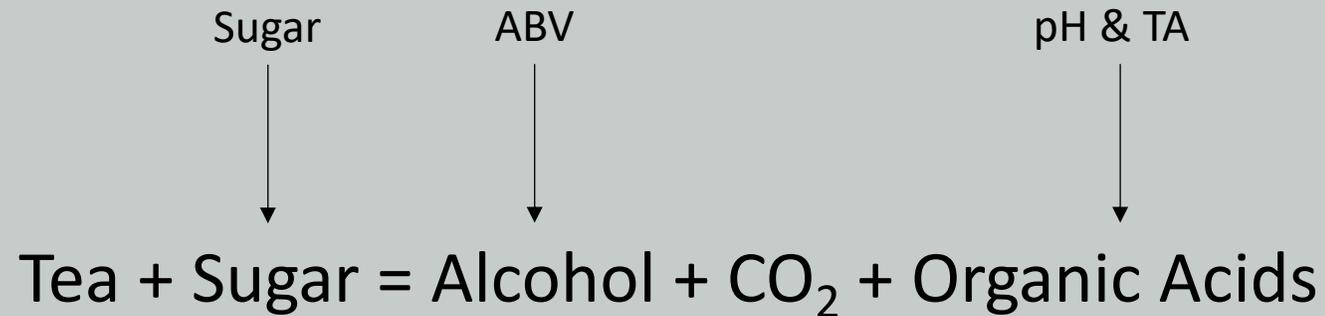
Why does the Core 4 Matter?

Kombucha Fermentation



Why the Core 4 Matter

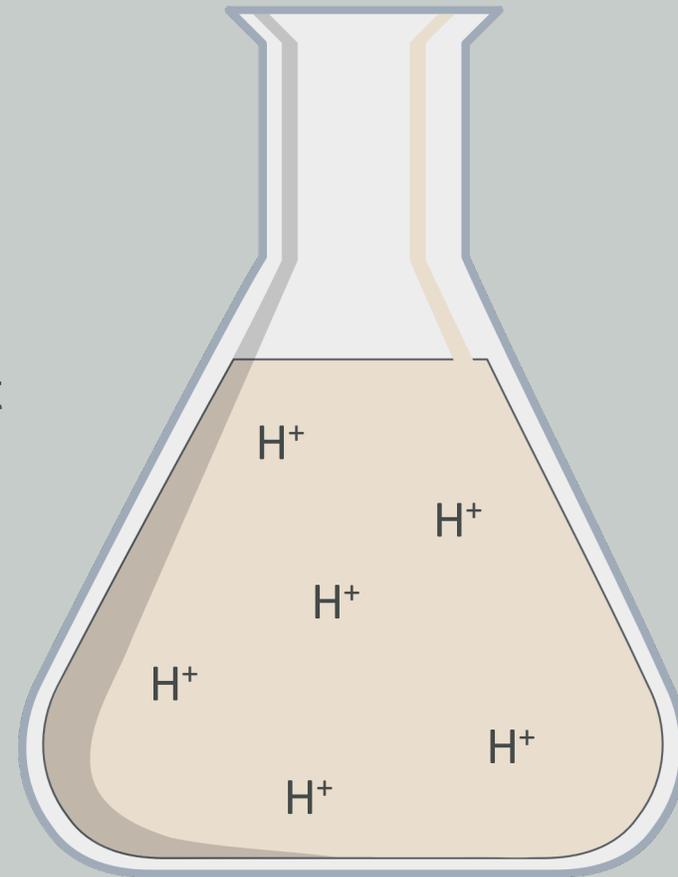
- Using the Core 4, we can measure the important inputs and outputs of the process



pH

pH

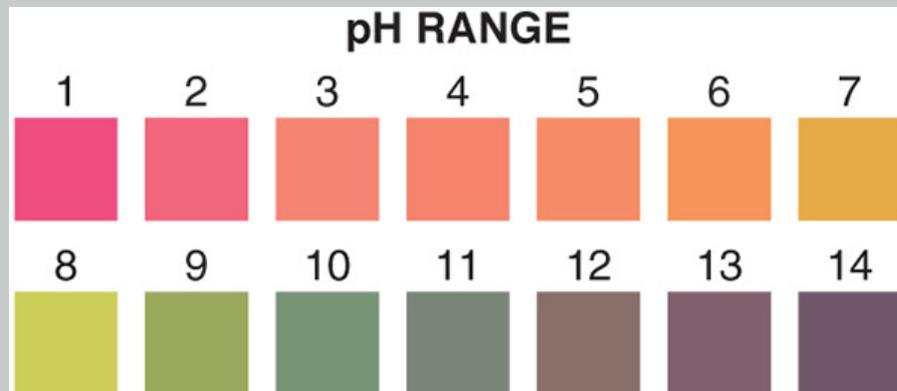
- Acid: a molecule that donates a proton (H^+)
- Measure of free protons (H^+) in solution
- pH should be below 4.6 for the product to be considered safe for consumption
- pH measures the environment which can give insight into the fermentation progress



How to Measure pH

Colorimetric

- Utilize an indicator molecule that changes color at different pH values



<https://www.preclaboratories.com/product/ph-1-14-test-strip/>

Potentiometric

- Utilize a pH meter



<https://hannainst.com/hi5221-research-grade-ph-orp-meter-with-cal-check.html>



Cultured
ANALYSIS

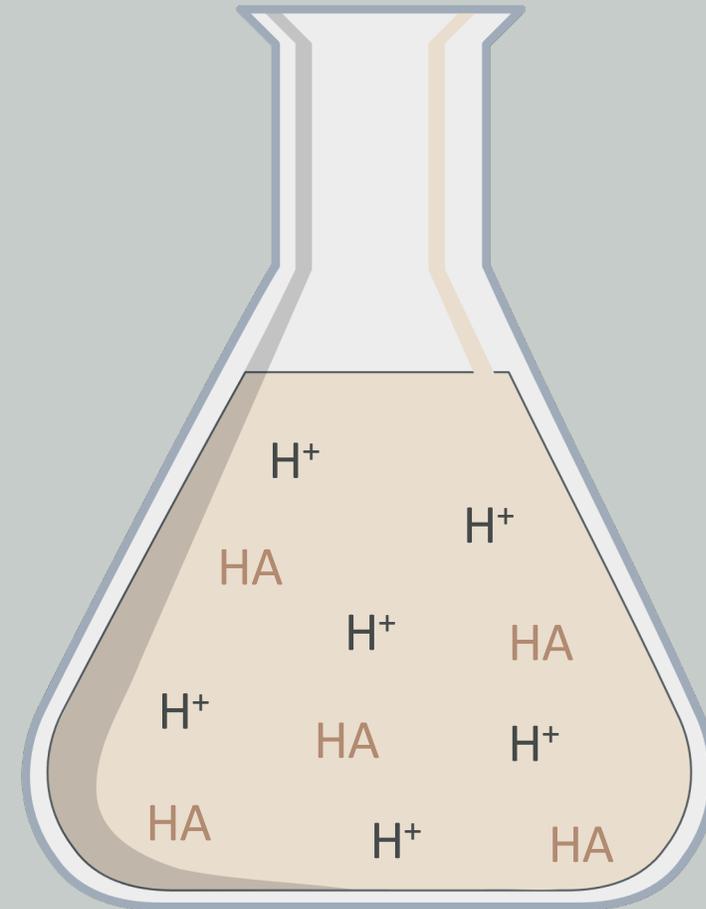
Titratable Acidity

Introduction to TA

- The organic acids in kombucha are weak acids

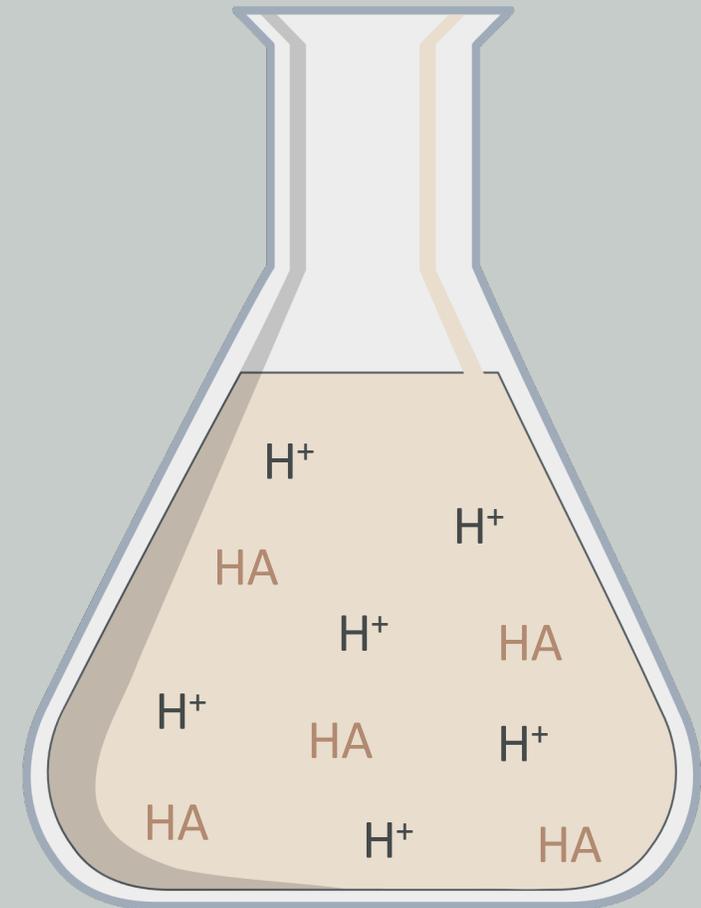


- Strong acid: goes 100% to product
- Weak acid: goes <100% to product
 - Not every acid molecule donates an H^+
 - Kombucha has a mixture of HA and H^+



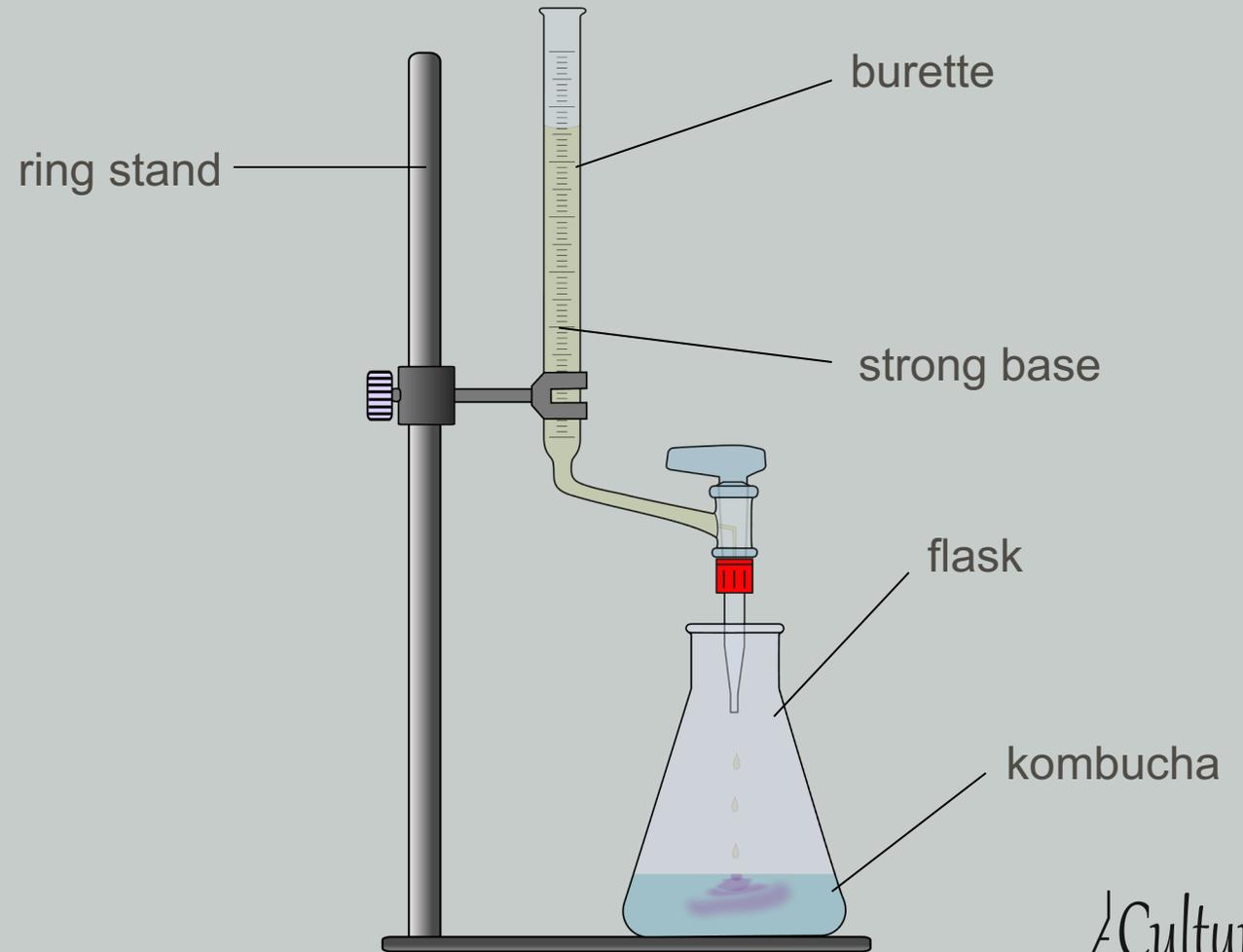
TA

- Measure of all acids in solution
- In a titration, a strong base with a known concentration is added to kombucha with an unknown acid concentration to determine the concentration of the acid
- Correlates better to taste perception than pH



Basics of Titratable Acidity Measurement

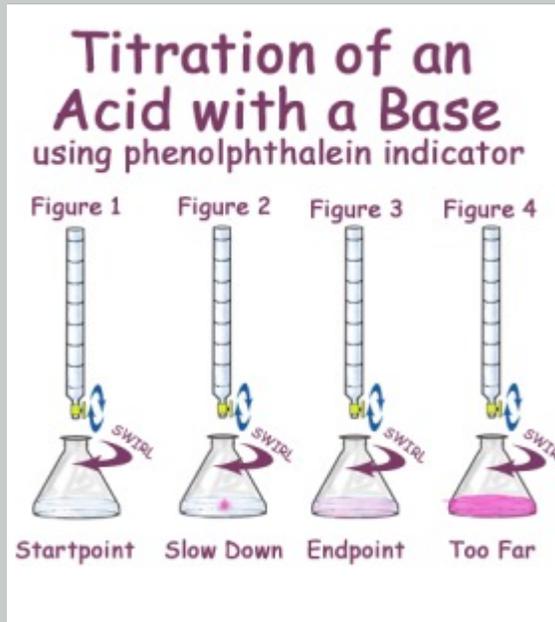
- Equipment
 - Standardized base (NaOH)
 - Sample in a flask
 - Burette and ring stand



Titratable Acidity Setups

Colorimetric

- Utilize an indicator molecule that changes color at specific pH
- Phenolphthalein - pH 8.2

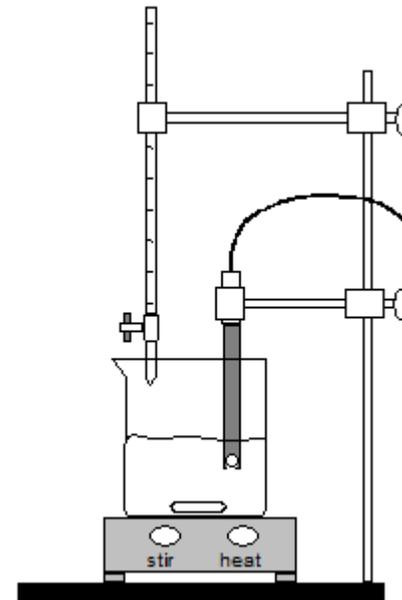


<https://i.pinimg.com/originals/15/f2/86/15f286058968458f9d177b5642156916.jpg>

Potentiometric

- Utilize pH meter to hit target pH 8.2

Burette + pH meter



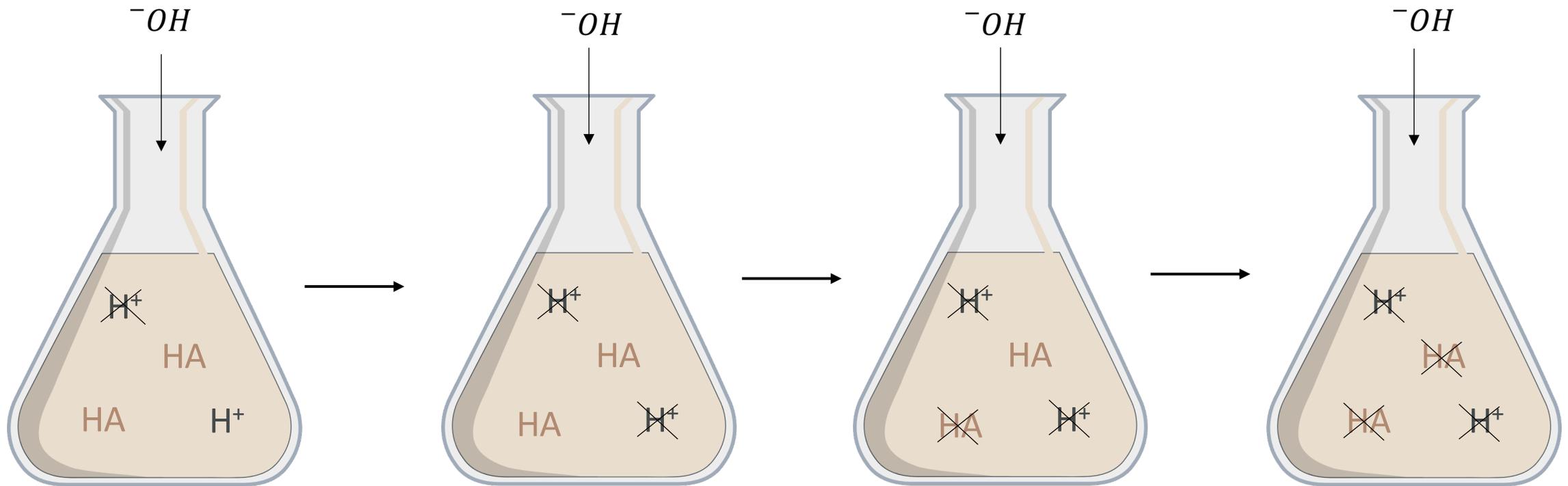
http://www.webassign.net/question_assets/ncsugenchem102labv1/lab_9/manual.html

Auto titrator



<https://www.hannaservice.eu/titratable-acidity-titrator-and-ph-meter-hi84534-02-product>

Calculations for Titratable Acidity



Calculations for Titratable Acidity

$$\text{TA} = \frac{\text{counts acidic species}}{\text{volume of kombucha titrated}}$$

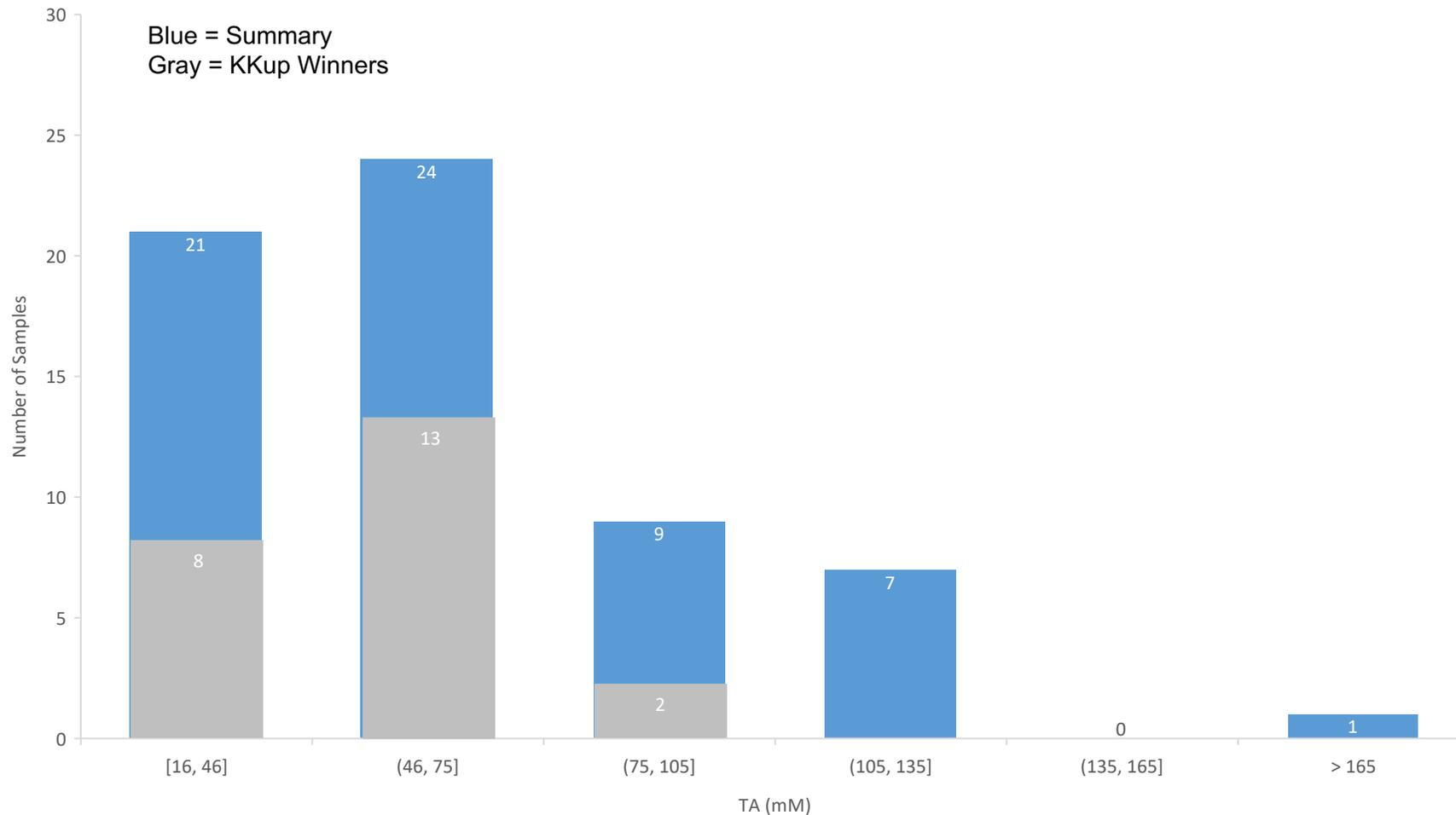
*Calculated concentration is in millimolar (mM)

- We count in a unit called moles (mol)
- When we consider the volume of kombucha that was titrated, we can calculate TA in a concentration unit called millimolar (mM)
- When comparing two kombucha TAs, the higher TA indicates a more acidic kombucha

Strengths of Titratable Acidity

- Monitor all weak organic acids in kombucha sample
- Can help tie analytical data to sensory data
- Allows evaluation of batch-to-batch consistency for a brew
 - Great QC parameter

Kombucha Kup TA Summary

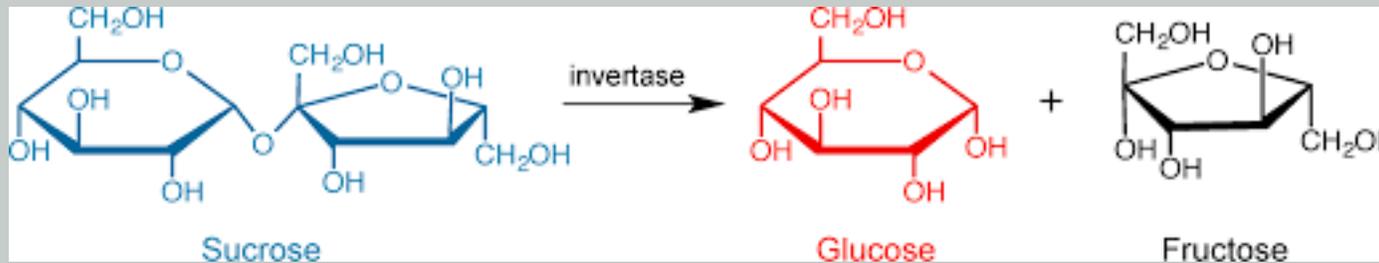


- 62 total samples
- Range: 16-187mM
- Average TA: 65mM
- 73% fell in the range of 16-75mM

Sugar

Sugar

- Most common sugars for kombucha are sucrose, glucose, and fructose
 - Sucrose contains one molecule of glucose and one molecule of fructose

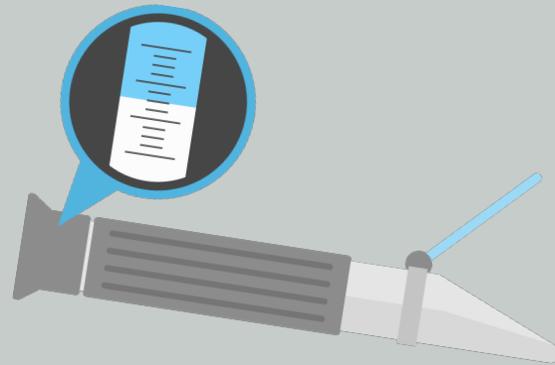


<https://pubs.acs.org/doi/pdf/10.1021/ed300889s>

- Honey is normally 50% glucose/50% fructose

Methods for Monitoring Sugar

- High Performance Liquid Chromatography (HPLC)
 - Most accurate values
- Enzyme kits
 - Pretty accurate values
- Density/Brix/Extract and Refractometry
 - Good for QC and relative comparison but inaccurate



Why is Brix Inaccurate?

- Density and refractometry methods are calibrated for pure solutions of sugar (sucrose) in water with no other chemicals present
- Kombucha contains components such as acids that can interfere with the measurement
- These measurements should not be used to determine residual sugars for a nutrition facts panel

ABV

Alcohol by Volume

- Concern for non-alcoholic kombucha producers
 - Common brewing practices can lead to %ABV up to 3.0%
- Ethanol is the alcohol produced in kombucha
 - Produced primarily by the yeast
- Without testing, you won't know the ABV of your kombucha

Methods for Monitoring ABV

- Gas Chromatography
 - e.g. Headspace GC-FID
- Near-Infrared Spectrophotometry
 - Anton-Paar AlcoLyzer
- OptiEnz Alcohol Sensor
- Rare Combinations Alcohol Detector

Problems with Measuring ABV

- Any instrument or method is only as good as the operator running it
 - Training, accurate standards, and quality control checks are required to achieve reliable measurements
- Certain methods are preferable over others with respect to accuracy
- Hydrometers and refractometers do not work well in native kombucha
 - High sugar and acid levels lead to interferences
 - Underreports alcohol content

Using Core 4 Data

QC Data

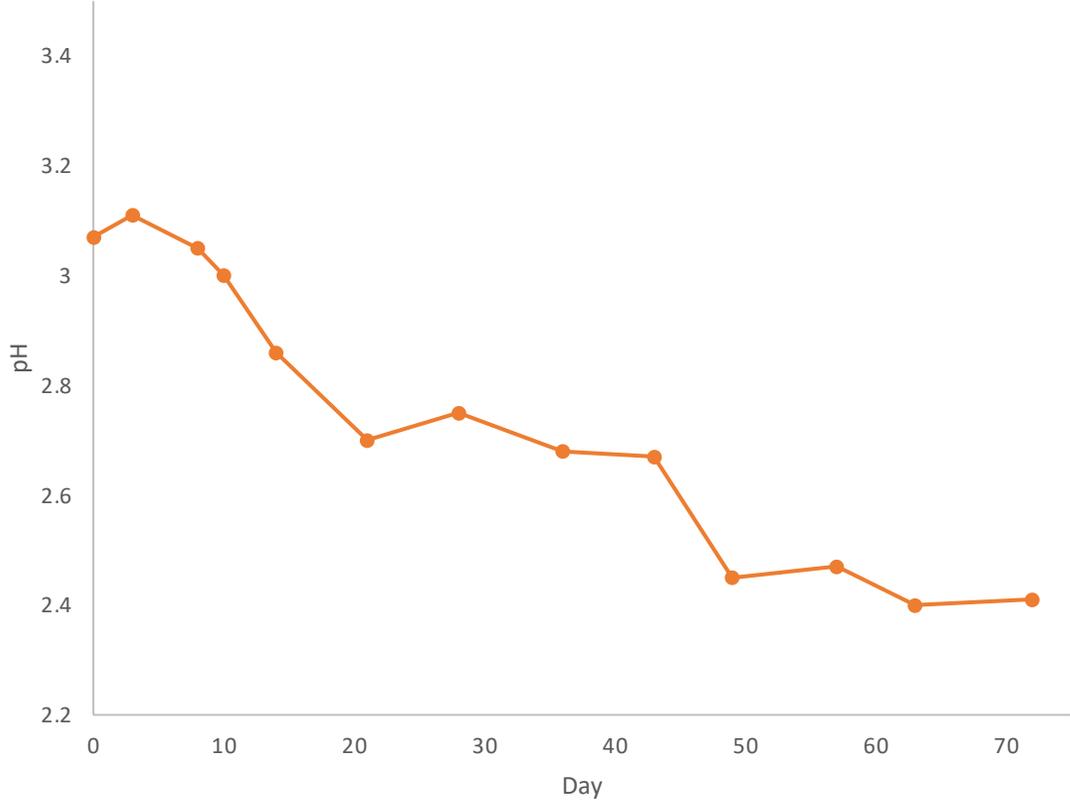
- It is impossible to trouble shoot without having baseline data
- Take good notes and use brewing sheets
- Track Core 4 in starter culture
- Track Core 4 in kombucha
 - Data points should be taken at reasonable time intervals
- Collected data is worthless if it is not understood or used
 - Build fermentation curves
 - Hit ideal pH, ABV, sugar, and TA targets

CA Example Fermentation Curve Data

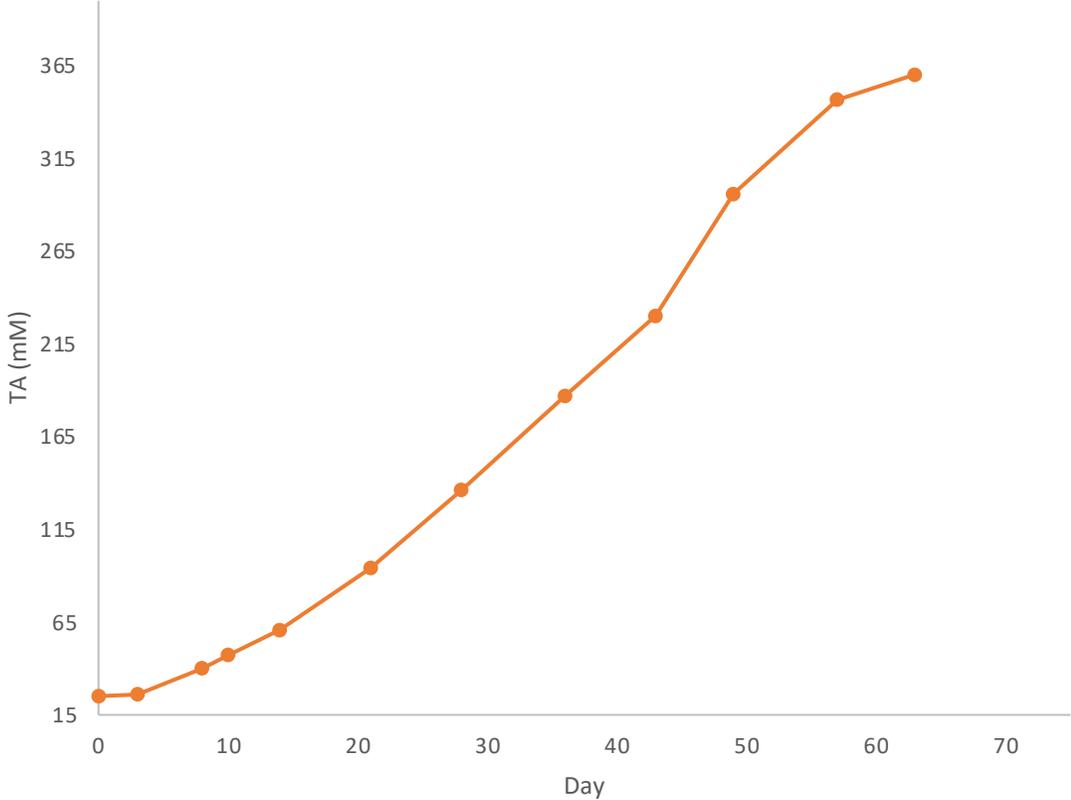
- Analyzed a starter over approximately 60 days
- Measured the Core 4
 - pH
 - TA
 - Sugar
 - ABV
- Measured acetic acid and gluconic acid production individually
 - Using HPLC or CA method

Fermentation Curve on Starter

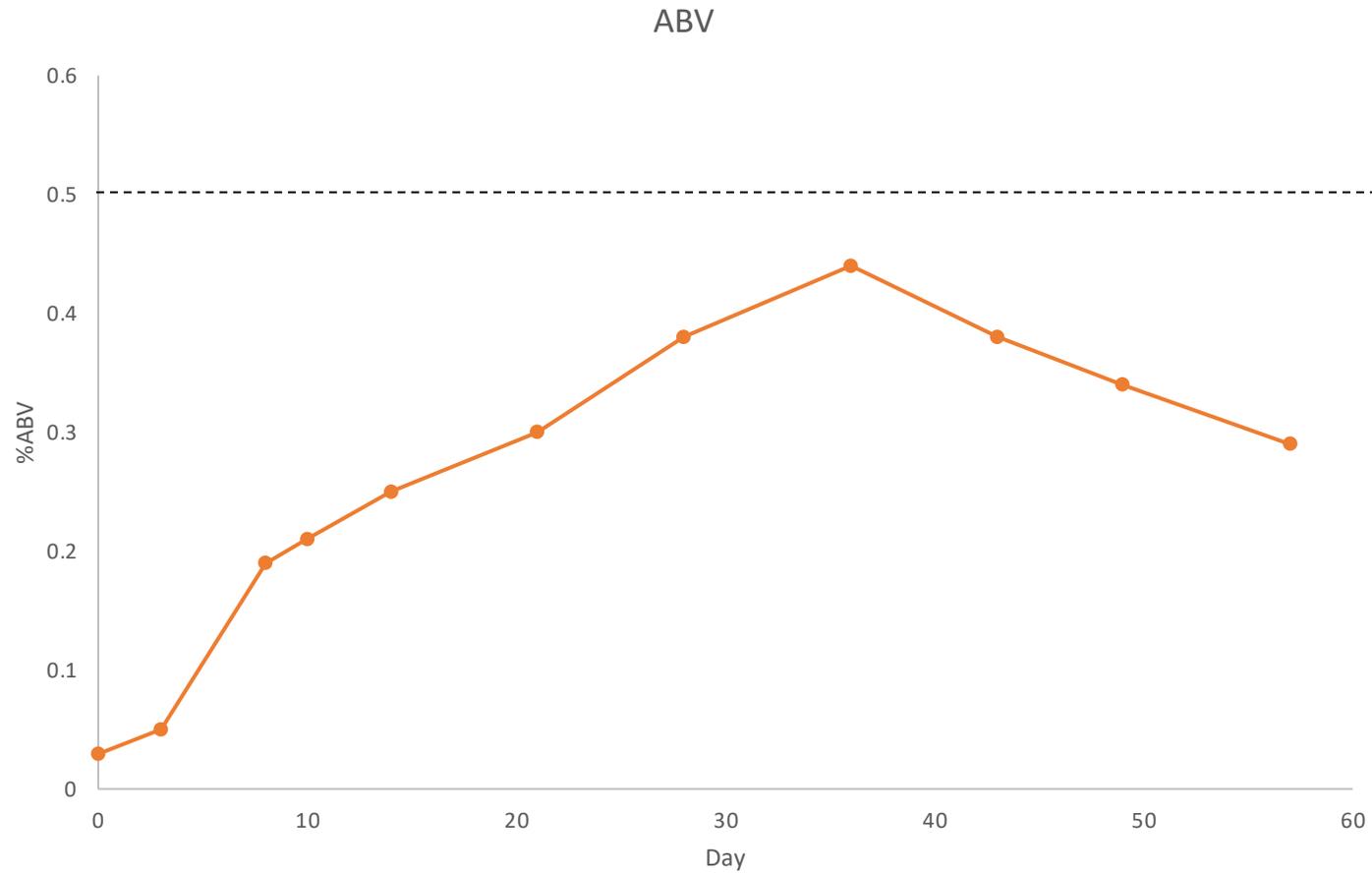
pH



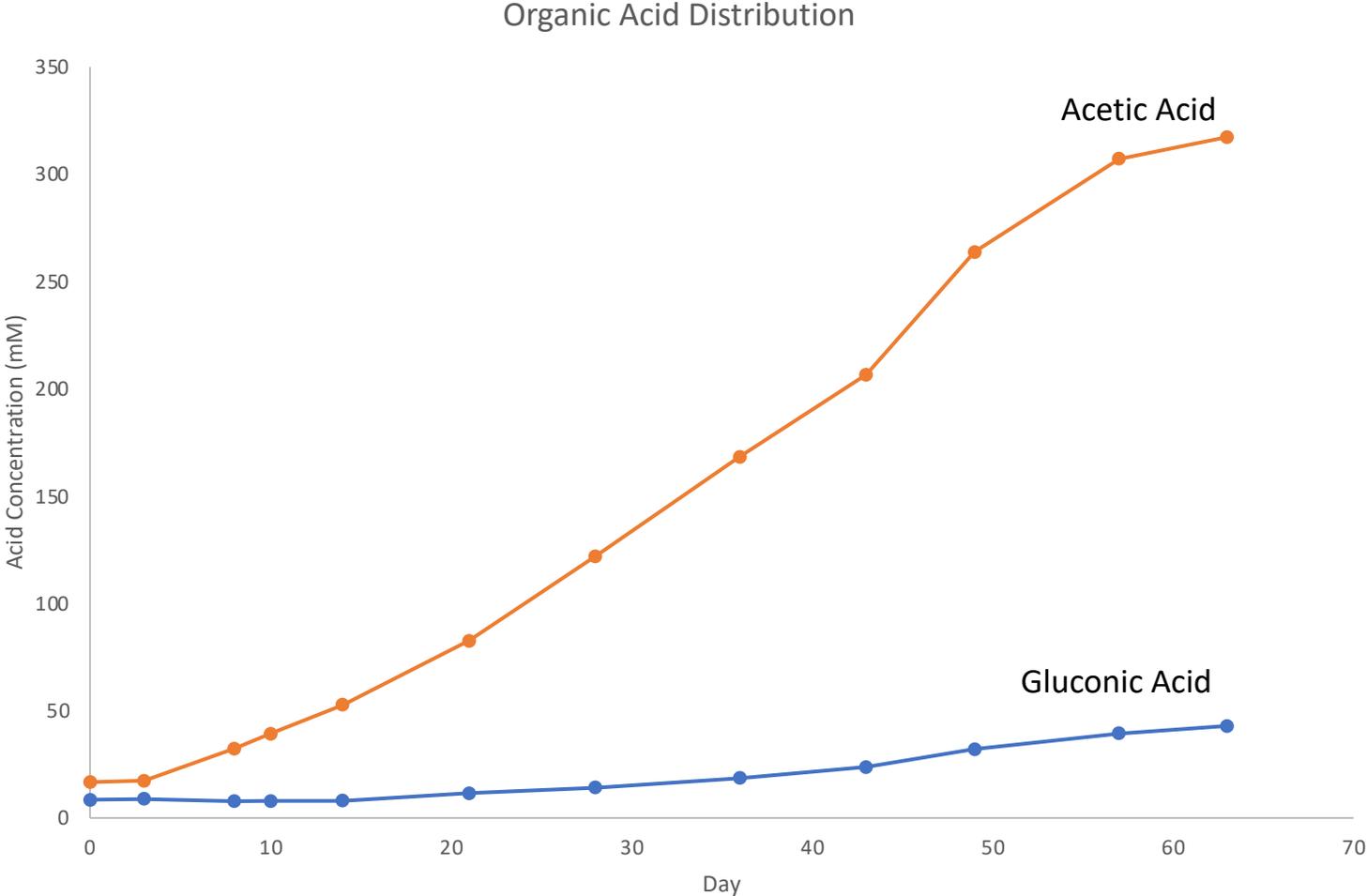
TA



Fermentation Curve Examples

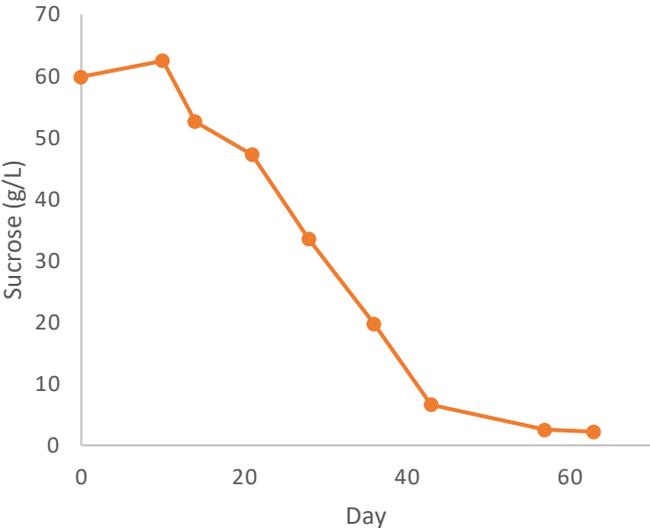


Fermentation Curve Examples

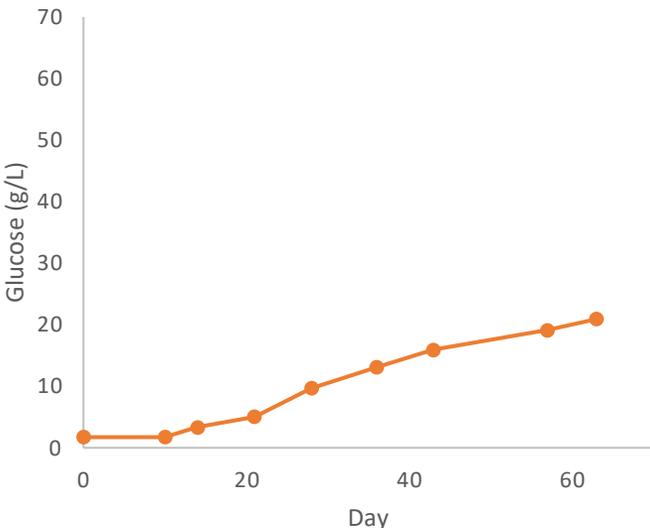


Fermentation Curve Examples

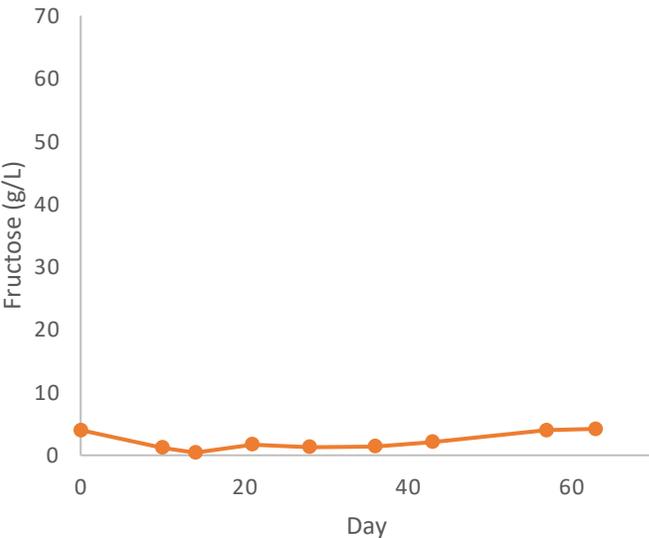
Sucrose



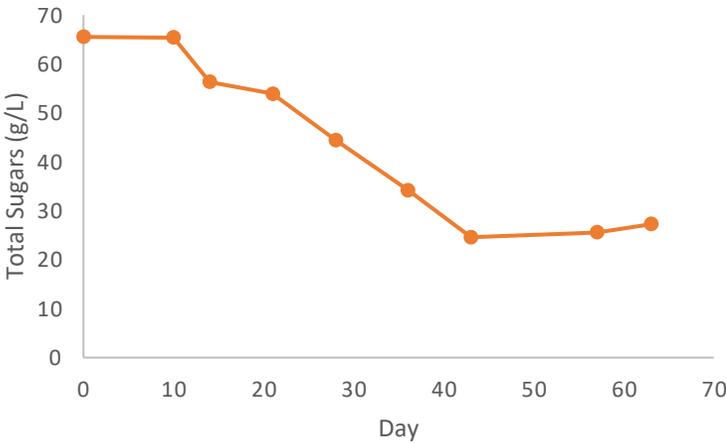
Glucose



Fructose



Total



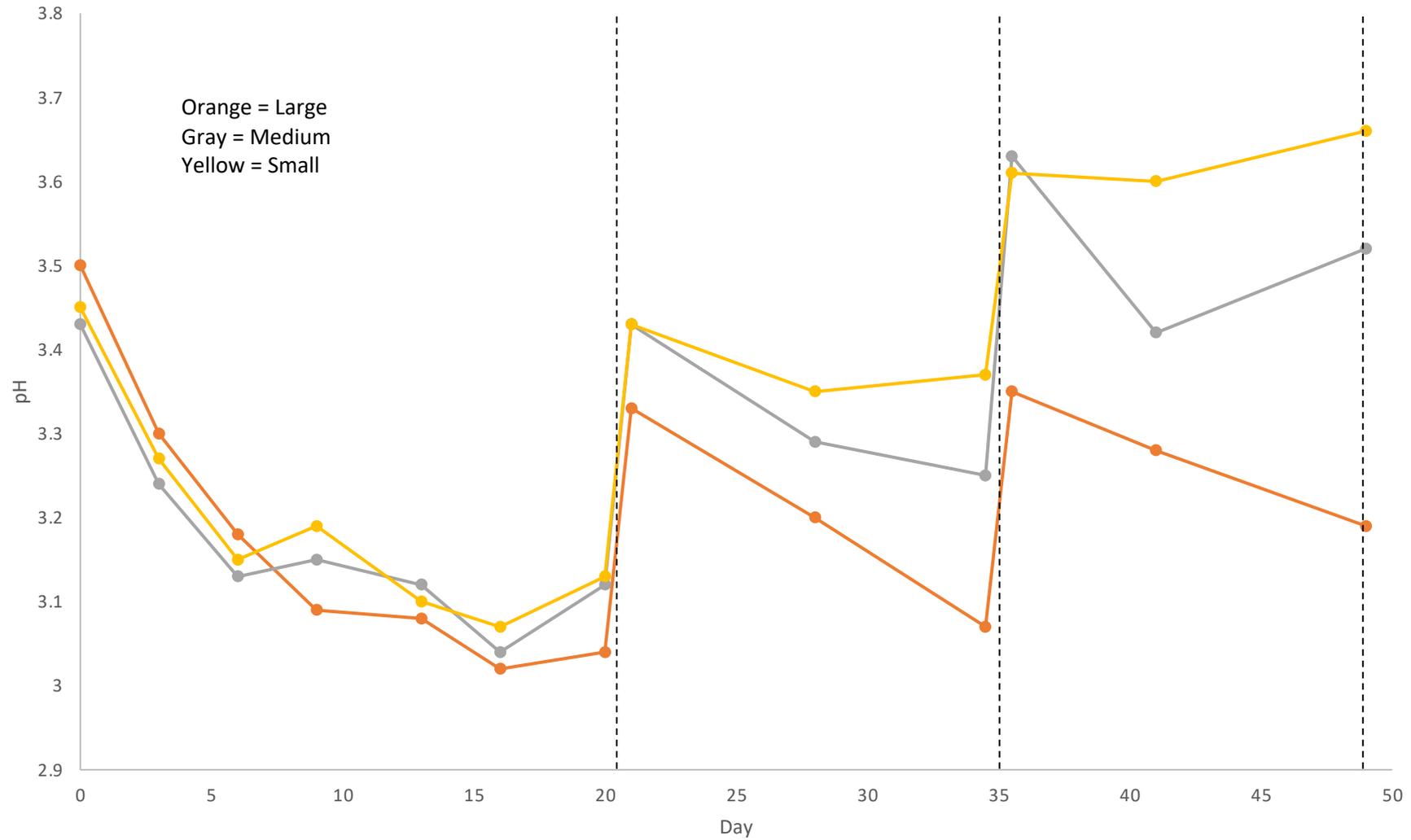
What does this tell us?

- By day 60, our TA was 360 mM
 - More acidic than we intended
- ABV had peaked and rolled over by day 60
- pH was a good level for starter
- Sugar levels were low
- Overall trends followed our expectation – successful starter fermentation

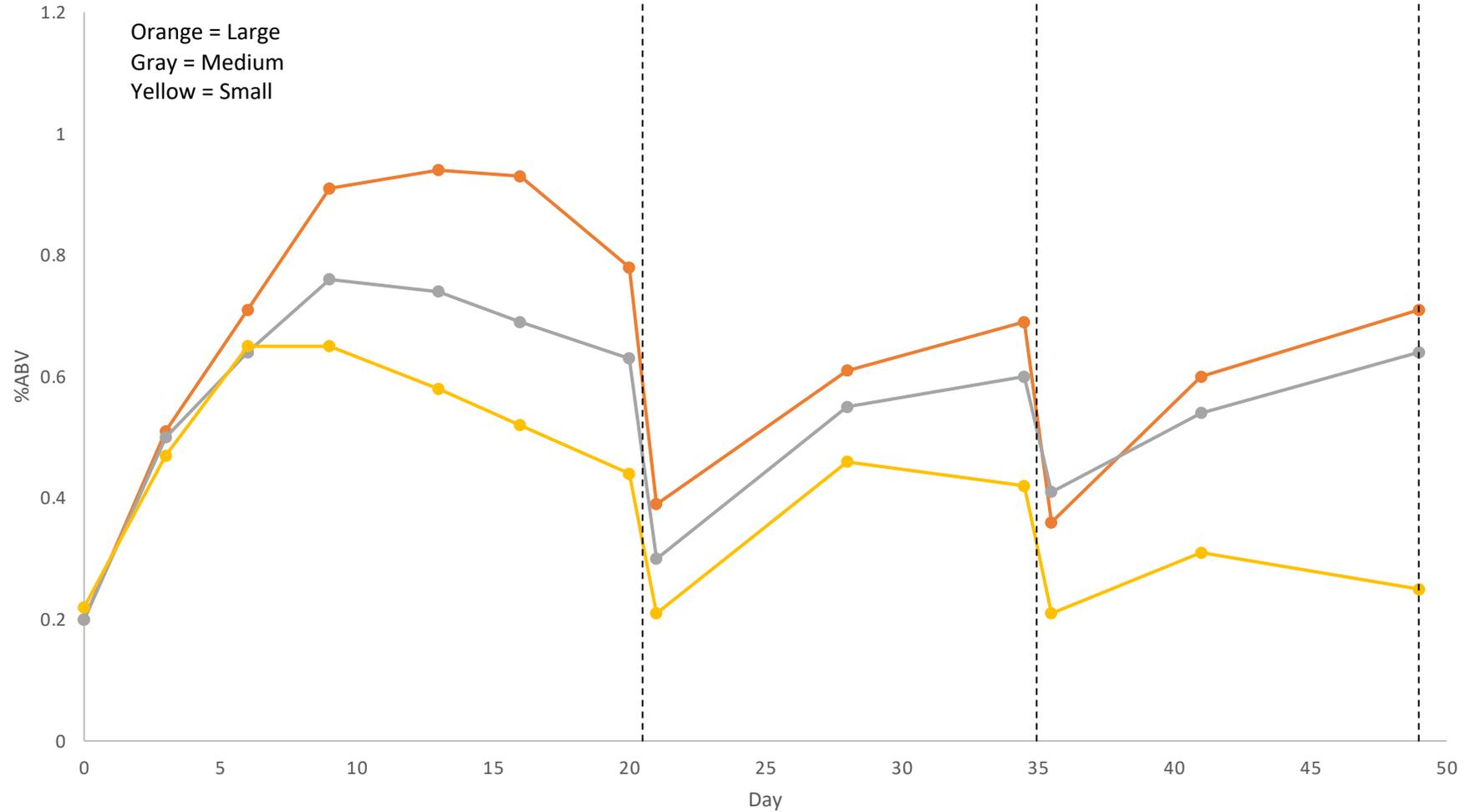
Using Historical Data to Improve Consistency

- Varied initial sugar concentration in 3 kombucha ferments
 - Large
 - Medium
 - Small
- Tracked three consecutive cycles for each vessel
- Using Core 4 data, we could draw conclusions about the effect of sugar concentration on the ferments

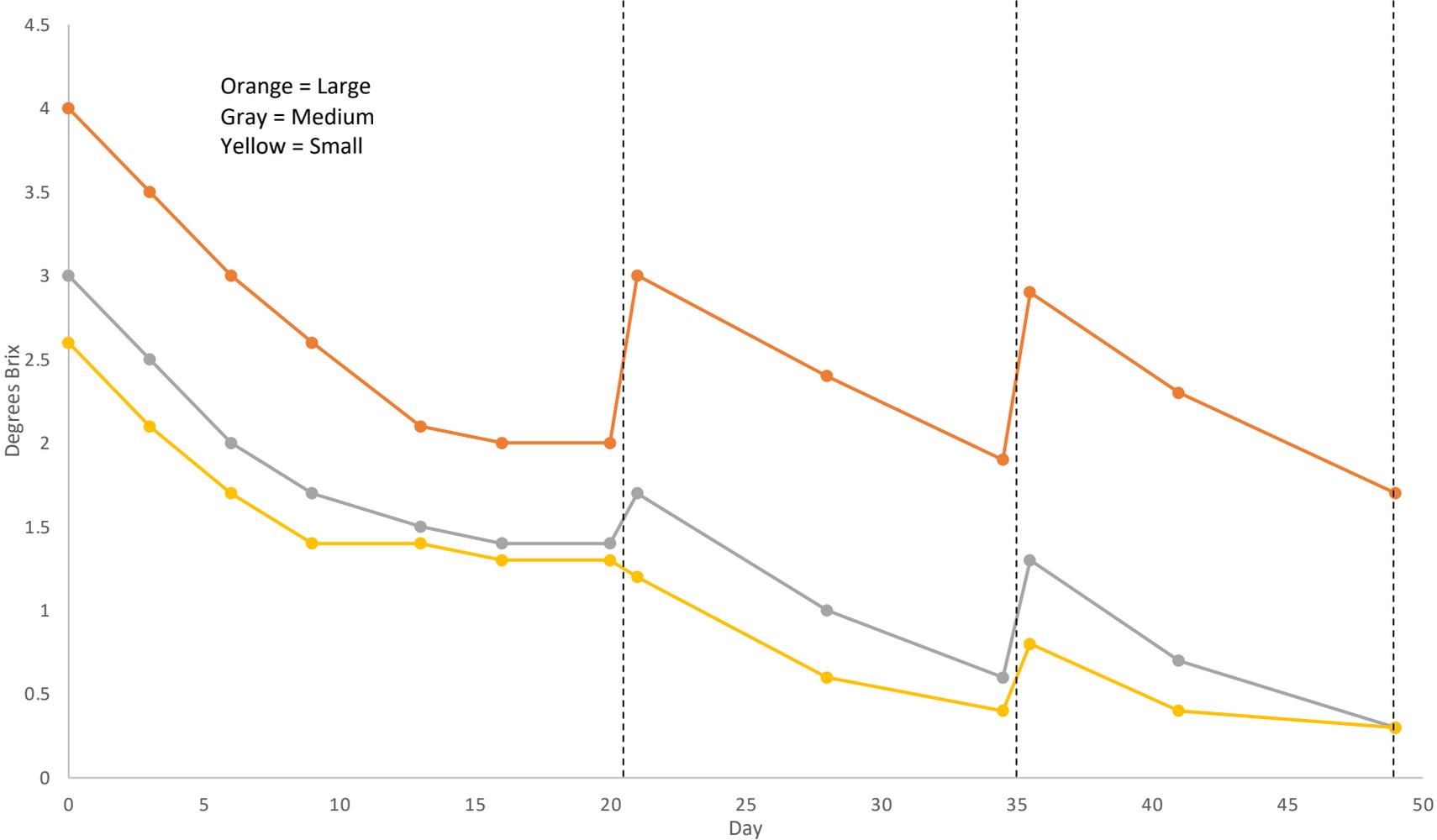
pH



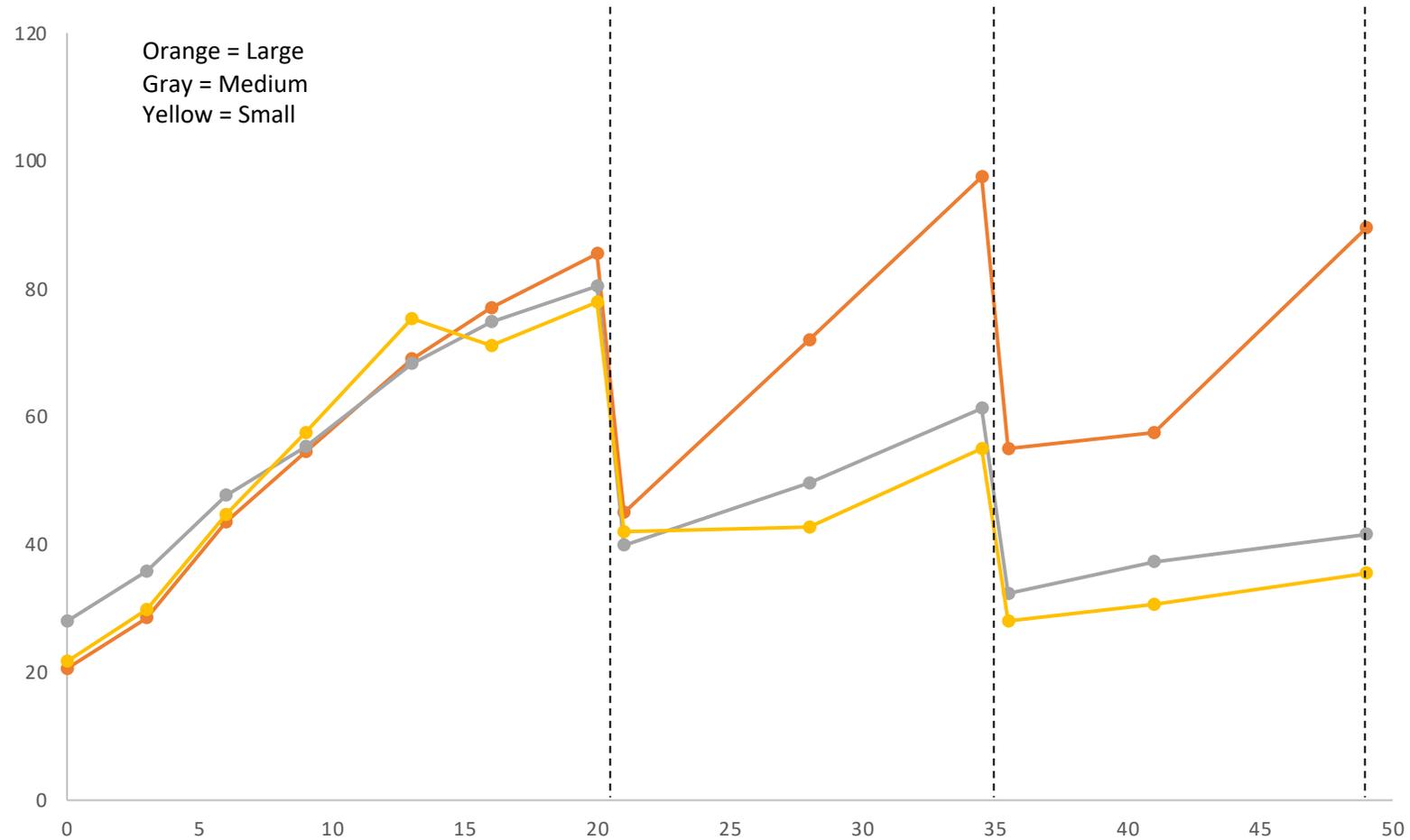
ABV



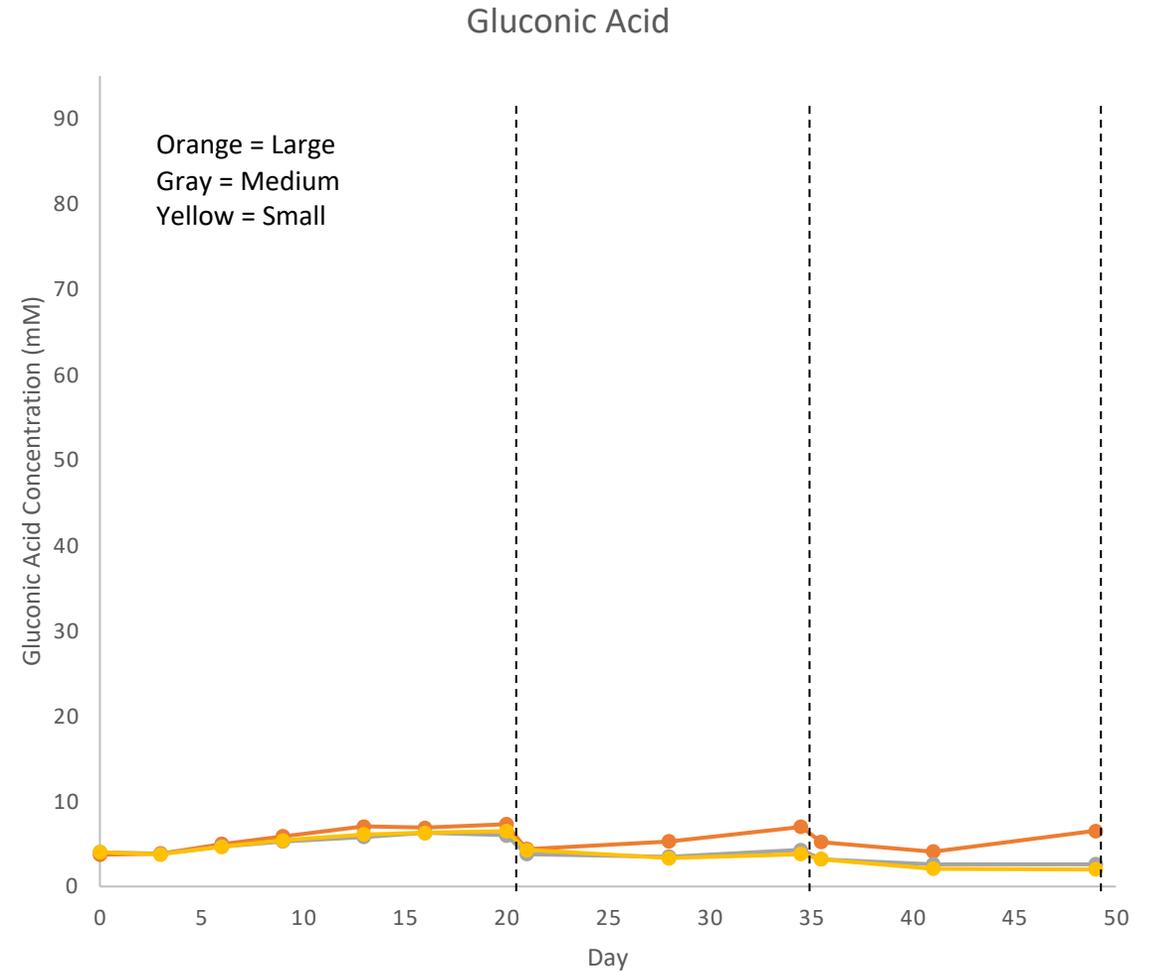
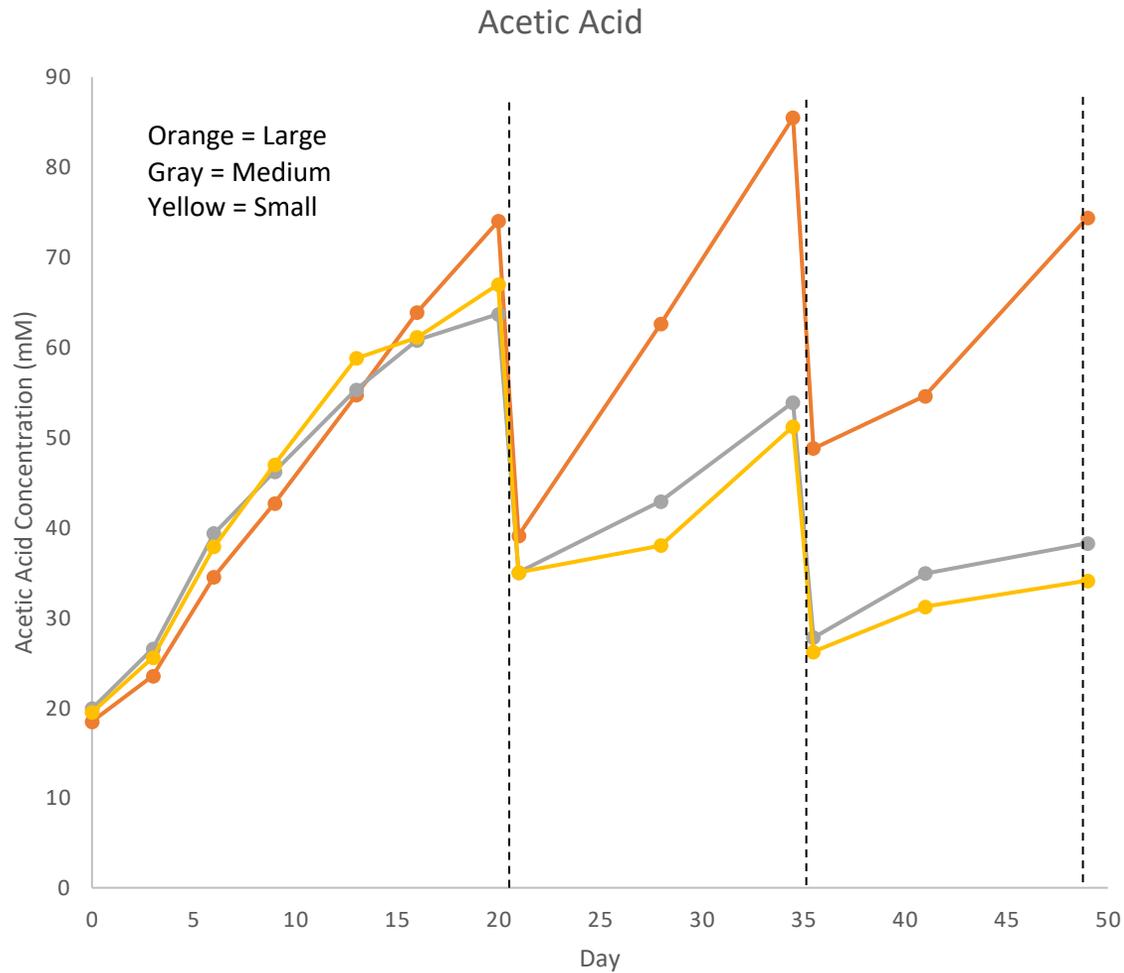
Degrees Brix



TA



Organic Acid Distribution



Data Analysis

- Large – consistently able to hit similar metrics in two-week brew cycle
- Small and Medium – activity slowly decreased over brew cycles
- Having the Core 4 data helped us determine that the sugar amount for Large was the best choice for consistency
 - ABV for Large was above 0.5% so other changes to the process could be explored
 - We now have a historical record of the kombucha's behavior, so when other variables are changed, we can have a better foundation to interpret the outcomes

Summary

- Always track Core 4 Parameters
 - pH, TA, sugar, and ABV
- Multiple different methods and instruments exist to monitor the parameters
 - Certain methods are more accurate and are normally only done by an external lab
 - Other methods are more applicable for in-house use, which are less accurate but suitable for internal QC
- Test frequently
 - It is impossible to troubleshoot without baseline data
- Use resources to help solve problems

Questions

Get 10% of Core 4 Analysis Package with discount code: **CORE4**

Contact us if you have any questions!

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