Autor/s
Dr. Roger Bringué Tomàs
Departament d'Enginyeria Química i
Química Analítica

Grau en Enginyeria Química

Treball Final de Grau

Analysis on the elaboration of kombucha

Joana Majó Ylla-Català

June 2023

UNIVERSITAT DE BARCELONA

Omne quod dulce est cito satiat
(Tot el que és dolç embafa aviat)

Macrobi

Primer de tot donar les gràcies a en Roger per confiar en mi i acompanyar-me al llarg d'aquest projecte, sobretot per deixar-me tirar endavant una idea que em feia moltíssima il·lusió, ajudar-me a fer-la possible, i oferir la seva ajuda sempre que l'he necessitat. També agrair a totes les persones del departament d'Enginyeria Química, a l'Eliana i en Jordi, per mostrar interès en el projecte i ajudar-me a resoldre qualsevols dels dubtes que els hi he presentat. Gràcies a l'Olga i la Belén per oferir-me tot el material del laboratori que he necessitat i obrir-me cada dia la porta del seu despatx.

Per altra banda, agrair les companyes per haver fet aquest camí el més amè possible i donar-me suport durant tot aquest temps, tant amb el treball, com amb els anys de carrera, sempre mostrant interès. Màxima gratitud també, a les meves amigues per escoltar-me i donar-me un cop de mà quan jo no sabia ni que el necessitava.

Finalment, moltíssimes gracies als meus pares, per no deixar de confiar en mi i encoratjar-me a seguir sempre amb el cap ben alt, tot i els entrebancs.

Moltes gràcies,

Joana

CONTENTS

SUMMARY

Kombucha is a fizzy fermented beverage made from tea, sugar and microorganism (SCOBY, symbiotic culture of bacteria and yeast) that has been known worldwide for centuries, but nowadays is gaining more and more popularity for its health benefits. Consequently, the known fermented beverage that was homemade by a few, is now being produced by breweries at an industrial scale. Despite its increasing popularity, there is still a lack of understanding of the kombucha fermentation process and how it affects the quality and properties of the final product.

Furthermore, the industrial production of kombucha is still in its early stages, with little standardization of the production process and quality control measures. This thesis aims to provide a thorough review of the industrial elaboration of kombucha, combining theoretical bases with experimental data obtained in the laboratory. Moreover, the evolution of parameters such as pH (acid content), density, sugar and alcohol content are indicators of the accurate progress of the fermentation and form a tracking system for such process.

Experiments performed, following such parameters, confirmed a temperature effect influencing the microorganism's metabolism and an optimal temperature range of 21-28 ºC, being approximately two times faster at 28 ºC than 21 ºC. In addition, the initial substrate (sucrose) concentration effect was also evaluated and concluded with a minimum, although positive, effect.

Keywords: Kombucha, fermented beverage, fermentation, industrial elaboration, microorganisms' metabolism.

RESUM

La kombutxa és una beguda fermentada efervescent reconeguda arreu del món, elaborada a partir de te, sucre i microorganismes (SCOBY, cultura simbiòtica de bacteris i llevats) que es coneix des de fa segles, però que actualment està guanyant cada vegada més popularitat pels seus beneficis per a la salut. Com a conseqüència d'això, la beguda fermentada coneguda que antigament es feia de forma casolana per uns pocs ara s'està produint a fàbriques a escala industrial. Malgrat la seva creixent popularitat, encara hi ha una manca de comprensió sobre el procés de fermentació de la kombutxa i com afecta la qualitat i les propietats del producte final.

A més, la producció industrial de la kombutxa encara està en les primeres etapes, amb poca estandardització del procés de producció i mesures de control de qualitat. Aquesta tesi té com a objectiu proporcionar una explicació exhaustiva de l'elaboració industrial de la kombutxa, combinant les bases teòriques amb les dades experimentals obtingudes al laboratori. Altrament, l'evolució de paràmetres com el pH (contingut àcid), densitat, sucres i contingut d'alcohol són indicadors del progrés de la fermentació i formen un sistema de seguiment per a aquest procés.

Els experiments realitzats, seguint l'evolució d'aquests paràmetres, han confirmat l'efecte de la temperatura en el metabolisme dels microorganismes, amb un rang de temperatura òptima de 21-28 ºC, sent aproximadament el doble de ràpid a 28 ºC que a 21 ºC. A més, s'ha avaluat l'efecte de les concentracions inicials del substrat (sucrosa) i s'ha conclòs que té un efecte mínim, tot i que positiu.

Paraules clau: Kombutxa, beguda fermentada, procés d'elaboració, microorganismes, metabolisme.

SUSTAINABLE DEVELOPMENT GOALS

El progrés industrial de la kombutxa pot incidir de manera efectiva en vàries metes dels 17 objectius en desenvolupament sostenible marcades per les Nacions Unides (ONU). És una indústria que necessita productes naturals d'arreu del món per crear el seu producte final i, per tant, treballant amb cooperatives de comerç just que lluiten per una compensació econòmica justa i decent pels treballadors i treballadores de la terra, pot extreure productes naturals i sostenibles de la millor qualitat. Aquest fet no només potencia les fites de l'ONU de *Reducció de les desigualtats* (ODS nº 10) i de *Fam zero* (ODS nº 2), de fer front a les situacions salarials dels països en desenvolupament així com millorar la regulació i el seguiment dels mercats i les institucions financeres mundials, sinó també crear un vincle entre països en desenvolupament i països desenvolupats (ODS nº 17, *Aliança pels objectius*).

A més a més, l'objectiu nº 9, *Indústria, innovació i infraestructures*, té com a meta actualitzar les indústries per fer-les més sostenibles i millorar l'adopció de tecnologies i processos industrials nets i ambientalment racionals. Tenint en compte que la kombutxa té una despesa energètica baixa pel que fa a termes de producció, pot ser una indústria que es sostingui només amb energies renovables, sense necessitat d'extreure energia de productes fòssils. De manera complementària, la generació de residus que comporta l'elaboració d'aquesta beguda, també concorda amb la meta de "reduir substancialment la generació de residus mitjançant la prevenció, la reducció, el reciclatge i la reutilització" (ODS nº 12, *Consum i producció responsables*), ja que l'envàs més utilitzat és el vidre (fàcilment reciclat) i el residu generat és matèria orgànica.

Finalment, promoure una beguda refrescant, ecològica, casolana i saludable com a substitutiu de les begudes ensucrades vetlla també per la salut i benestar de les persones i en conseqüència, complementa metes de *Fam zero* i *Salut i Benestar* (ODS nº 3).

1. INTRODUCTION

Kombucha is a fermented beverage that has been consumed for centuries and is currently gaining popularity worldwide due to its potential health benefits. Its fermentation process involves the use of a symbiotic culture of bacteria and yeast (SCOBY) to ferment tea, sugar, and other flavoring, so during the process, a variety of microorganisms transform the initial mixture into a complex beverage with unique flavor and physicochemical properties.

Therefore, exhaustive research on the theoretical aspects of the kombucha fermentation process, including the history and evolution of the beverage, its chemical composition, and its industrial process, will be carried out. Additionally, it will be complemented with a first-hand production of kombucha in order to experimentally analyze certain parameters such as density, pH, and alcohol content obtained during the fermentation process, as these qualities are known to evolve during the fermentation, thus providing reliable information on its progress. Finally, as a way of validating the obtained results, a complete comparation with known values will also be performed.

Overall, the present thesis contributes to the knowledge of the kombucha fermentation process and provides a basis for the development of a standardized production process and quality control measures. Moreover, it aims to highlight the importance of optimizing the fermentation conditions to achieve a high-quality kombucha product with consistent properties, which is of great interest to the industry and consumers alike.

1.1. HISTORY

The origins of kombucha are not known with certainty, and despite its documented existence for centuries, its true origin remains undefined. The fermentation of kombucha is a natural phenomenon that occurs when its three primary ingredients - tea, sugar, and microorganisms -

come into contact. This leads to the belief that the first fermented kombucha was obtained at an unknown time and place.

All documented research thus far points to ancient China as the likely origin of kombucha, with some discrepancies regarding the exact region, although many sources place it in Manchuria, northeastern China. The earliest records confirming the existence of this beverage date back to the Qin Dynasty in 220 B.C., where Emperor Qin Shi Huangdi was among the first to appreciate its energizing and detoxifying qualities. Additionally, it is known that in the year 414 A.C., the physician Dr. Kombu introduced this beverage to Japan to treat the digestive problems of Emperor Inkyo (Jayabalan et al., 2014).

Figure 1. Ancient Kombucha representation. (Petrzzello, 2023)

As trade routes expanded, this beverage became more and more popular around Europe, gaining a remarkable popularity in the 1930s (Stevens, 2003), first in Russia, later on Eastern Europe and Germany, and finally expanding throughout the continent in many European countries and Europe-dominated African countries. The habit of consuming this beverage became acceptable throughout Europe until World War II, when the shortage of tea and sugar supplies caused a dip in international consumption.

Later, in 1960, kombucha regained popularity after science researchers in Switzerland confirmed a study stating that its health benefits were similar to those of yogurt. Furthermore, this beverage had a meaningful market expansion around the 1990s when home-brewing kombucha predominated. Basic ingredients were sold in supermarkets to empower families to make their kombucha at home, but it wasn't until 1995 that the first industrial kombucha brewery opened up in Southern California (Troitino, 2017).

Since then, the industrial elaboration of kombucha has grown and is expected to keep on growing in significant numbers as statistics state it still hasn't reached its peak. More than 2 600

industrial breweries around the globe are nowadays producing kombucha, and more are yet to come.

In 2014, about 300 of these brewers associated and formed the KBI (Kombucha Brewers International, figure 2), an association with the goal to *"promote, protect, and enhance the overall well-being of the kombucha industry by creating an open line of communication between brewers, consumers, and regulators while advancing our industry through advocacy, education, research, and modern legislation."* (Pawar, n.d.)

Figure 2. KBI logo (Pawar, n.d.)

1.2. INUSTRIAL MARKET

As commented briefly before, the kombucha industry has witnessed noteworthy expansion in several parts of the globe and is still aiming to grow in the coming years. Presently, North America holds the dominant position as the largest market for kombucha, with the United States playing a pivotal role in driving growth in this region. This can be attributed to the escalating trend of health and wellness consciousness among consumers, a rise in consumer awareness of the various health advantages of kombucha, and the wide availability of an extensive range of flavors and variations. Following closely behind, Europe also stands as a significant market for kombucha, with countries like Germany, the United Kingdom, and France playing a significant role in propelling the market's growth in this region. Such facts are reflected in figure 3, referring to the market growth of this century. Based on 2021, *Polaris Market Research Analysis* affirms the market growth of that year was 2.59 billion USD and is predicted to scale up to 11.40 Billion USD by 2030 (Kombucha Market Size Global Report, 2022 - 2030, n.d.).

In addition, *Insight Ace Analytic* study on the Global Kombucha market states that as of 2022, the global kombucha market is valued at 2.52 billion and is forecasted to reach 9.01 billion by 2031, reaching an expected market growth of a 15.5 % CAGR during the estimated period 2023-2031 (Kombucha Market Share, Size, Growth and Forecast to 2031, n.d.). Alternatively, *Grand View Reaserch* states that 2022 kombucha market is valued in 3.53 billion and is predicted to

reach 9.70 billion by the year 2030 with a very similar Growth Rate (Kombucha Market Size, Share & Trends Analysis Report by Product (Conventional, Hard), by Distribution Channel (On-trade, Off-trade), by Region, and Segment Forecasts, 2022 - 2030, n.d.).

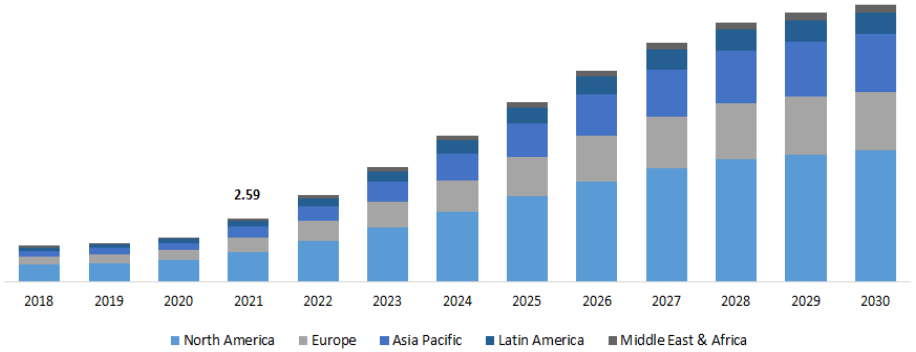


Figure 3. Kombucha market size, by region, 2018-2023 (USD Billion), (Kombucha Market Size Global Report, 2022 - 2030, n.d.).

Taking a closer look at market statistics in Spain, it can be said that the market has achieved a remarkable milestone in the industry. From generating a revenue of 3.1 million euros in 2020, it has sold 21.1 million euros worth of products in 2022. In terms of quantity, the sector has witnessed significant growth, going from selling 445,000 liters of kombucha to over 2.8 million liters. This translates to an increase from 1.16 million bottles to 8.19 million bottles sold annually.

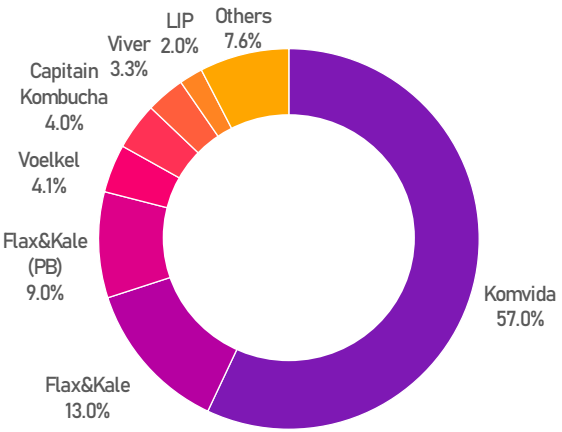


Figure 4: Leader producers of Kombucha in Spain. (Las Ventas De Kombucha Crecen Un 580% En Dos Años, n.d.).

According to the study done by *Inforetail*, the Extremaduran brand *Komvida* Kombucha is positioned as the current leader of kombucha in Spain, with a market share of nearly 57%. Followed by *Flax&Kale*, *Voelkel* and many more, the shares of the kombucha Spanish market are shown in figure 4, Catalonia being the major consumer in Spain, followed by Madrid and Andalusia (figure 5).

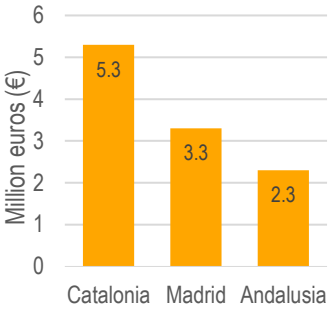


*Figure 5: Major consumers of kombucha in Spain (*Las Ventas De Kombucha Crecen Un 580% En Dos Años*, n.d.)*

1.3. CHEMICAL COMPOSITION

The chemical composition of kombucha encompasses a rich assortment of organic compounds that contribute to its unique properties. This fermented beverage is primarily composed of water, acetic acid, various types of organic acids (such as lactic acid and glucuronic acid), vitamins, minerals, enzymes, and active cultures of bacteria and yeast. These elements work together to create the tangy flavor, fizzy texture, and potential health benefits that kombucha is known for.

Meanwhile, bearing in mind the fact that the presence and quantities of specific chemical compounds are influenced by various factors (including the microorganisms present in the SCOBY, fermentation parameters such as time and temperature, concentration of sucrose, type of tea used, and the analytical method employed for measurement) the quantification of the chemical composition of kombucha varies, although it follows a certain trend.

Nevertheless, considering that the water content in kombucha (broth) is over 95%, the remaining 5% is distributed with a complex family of organic and non-organic compounds. Some of them are shown in table 1, a gathering of several studies made on the chemical composition of kombucha. Its results are exposed, referring to the initial sucrose content and the days fermented before the analysis (Villarreal-Soto et al., 2018).

Observing the table, note that Gluconic and Acetic acid are some significant organic compounds that not only have a certain relevance in the chemical composition but, also are responsible for the acidic taste in the beverage. Furthermore, it's worth noting that the alcohol content in kombucha is quite notable and relevant, bearing the fact that this compound must be regulated and can't exceed a certain concentration.

Table 1: General chemical composition of Kombucha. (Villarreal-Soto et al., 2018)

	Compound	***Average composition***	***Initial sucrose***	***Fermentation time** (days)*
Organic acids	Acetic acid	5.6 g/L	70 g/L	15 d
	Acetic acid	8.36 g/L	100 g/L	18 d
	Acetic acid	11 g/L	100 g/L	30 d
	Gluconic acid	39 g/L	100 g/L	60 d
	Glucuronic acid	0.0160 g/L	70 g/L	21 d
	Lactic acid	0.18 g/L	100 g/L	18 d
Vitamins	Vitamin B1	0.74 mg/mL	70 g/L	15 d
	Vitamin B2	8 mg/100 mL	70 g/L	10 d
	Vitamin B6	0.52 mg/mL	70 g/L	15 d
	Vitamin B12	0.84 mg/mL	70 g/L	15 d
	Vitamin C	25 mg/L	70 g/L	10 d
General composites	Ethanol	5.5 g/L	100 g/L	20 d
	Proteins	3 mg/mL	100 g/L	12 d
	Tea polyphenols	7.8 Mm GAE	100 g/L	15 d
Minerals	Cu, Fe, Mn, Ni, Zn	0.1 to 0.4 µg/mL	70 g/L	15 d
Anions	F−, Cl−, Br −, I−, NO3−, HPO4−, SO4−	0.04 to 3.20 mg/g	100 g/L	7 d

1.3.1. Organic acids

Kombucha is composed of several organic acids, such as acetic, gluconic, glucuronic, citric, L-lactic, malic, tartaric, malonic, oxalic, succinic, pyruvic, and usnic (Bishop et al., 2022a). The composition and metabolite concentration within the beverage can vary significantly depending on the factors stated in this section.

Yeast and bacteria hydrolyze sucrose into glucose and fructose using the enzyme invertase. Yeast within the matrix then produces ethanol using fructose as the primary substrate. Acetic acid bacteria use glucose to make gluconic acid and convert the ethanol produced by the yeast into acetic acid.

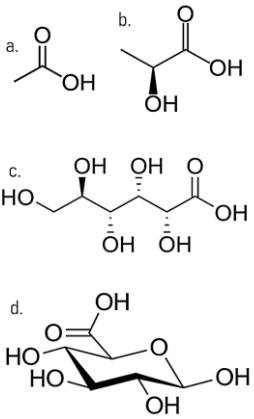


Figure 6. Organic acids found in Kombucha (a. Acetic acid, b. Lactic acid, c. Gluconic acid, d. Glucuronic acid).

Acetic acid is the organic compound responsible for the vinegary flavor and aroma commonly associated with kombucha. The concentration of this acid can vary; however, it tends to reach its peak at 11 g/L on day 30 of the fermentation process and will drop to 8 g/L by day 60 (Villarreal-Soto et al., 2018).The decrease in acetic acid is attributed to microorganisms in kombucha utilizing it as a carbon source after consuming all the sugar and ethanol in the fermentation matrix. Both ethanol and acetic acid in kombucha are known to exhibit antiseptic properties, inhibiting the growth of pathogenic microbes.

Moreover, other acids such as Lactic, gluconic and glucuronic are also found in fermented tea. On one hand, Lactic acid is found when fermenting green tea rather than other teas, for instance, black. On the other hand, Gluconic acid is formed in kombucha as a result of the metabolic activity of acetic acid bacteria present in the fermentation process. These bacteria utilize glucose to produce gluconic acid through an oxidation process. Gluconic acid contributes to the overall acidity and flavor profile of kombucha and serves as a precursor for the production of other beneficial compounds, such as glucuronic acid, which has detoxifying properties. This acid not only has a significant role in detoxifying the liver but also plays an important role in increasing the bioavailability of polyphenols and is a precursor for the biosynthesis of vitamin C.

1.3.2. Ethanol

Ethanol, a byproduct of yeast fermentation, is also an organic compound found in kombucha. The concentration of ethanol in kombucha will continue to increase as the fermentation progresses. A study carried out by Chen and Liu (2000) states that the ethanol concentration reached its maximum value of 5.5 g/L on day 20 of the fermentation, followed by a slow decrease (Chen & Liu, 2000). Although other studies conclude that the maximum of this compound is reached on the 6th day of fermentation (Jayabalan et al., 2014), followed also by a slow decrease.

The Food and Drug Administration (FDA) has conducted research and determined that the alcohol content of regular kombucha typically ranges between 0.7% and 1.3% by volume (ABV) (Bishop et al., 2022a). However, a new trend emerging in craft breweries is the production of "hard kombucha," which contains higher alcohol levels. Hard kombucha is known to have an alcohol content of approximately 3.5% to 5.5% ABV or even higher.

1.3.3. Vitamins and minerals

As seen in table 1, kombucha is enriched with a diversity of vitamins and minerals that make the composition more complex but have potential importance on the healthy properties of the beverage. To start, vitamins are known to help the metabolic and immune system of humans and therefore are considered to have a positive effect when absorbed. Kombucha is found to have high levels of vitamin C or ascorbic acid and small quantities of B vitamins. Figure 7 shows the concentrations of vitamins found in kombucha visualized next to the same vitamins found in milk.

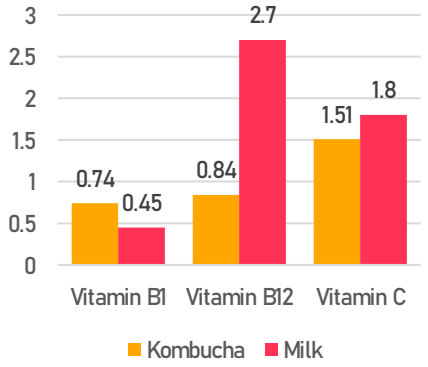


Figure 7. Vitamin values of kombucha and milk in mg/mL. (Alex & Alex, 2022; Vitamins &Amp; Minerals in Milk | MilkFacts.info, n.d.)

On the other hand, minerals are also found in kombucha due to the content of them in the tea itself. As well as vitamins, these compounds are consumed by the human body through several metabolic pathways and physiological functions. Essential minerals such as cobalt (Co^{2+}), potassium (K^{+}), magnesium (Mg^{2+}), copper (Cu^{2+}), iron (Fe^{2+}), and fluoride (F°) can be found in kombuchas with a green or black tea base. The portion of such minerals can fluctuate between 0.004 and 0.462 µg/mL depending on the mineral, following the study done by Bauer-Petrovska and Petrushevska-Tozi in 2000. This same study also analyzed the quantities of undesired, toxic, minerals and found small traces of Lead and Chromium (0.005 and 0.001 µg/mL respectively) (Bauer-Petrovska & Petrushevska-Tozi, 2000).

Other compounds such as Polyphenols, caffeine, and Amino acids are also found in the beverage at state. Polyphenols form up to 30% of tea leaves' weight and have a high antioxidant capacity. These, which endure through fermentation, are responsible for the health benefits associated with tea and kombucha, such as cancer prevention, improved immunity, reduced inflammation, and arthritis alleviation.

Regarding the caffeine content in kombucha, it constitutes approximately 3% to 6% of the tea leaves, with its concentration varying depending on cultivation conditions and subsequent processing (Sharma et al., 2007). In the context of kombucha, caffeine plays a crucial role during fermentation as it serves as a source of nitrogen for the yeast and bacteria, supporting their metabolic processes and cell growth. Additionally, it provides energy to the yeast and bacteria, enabling them to undergo the fermentation process effectively (Crum & LaGory, 2016).

1.3.4. Microorganisms

As aforementioned, kombucha is full of living microorganisms that not only attribute a probiotic activity to the beverage but also are an essential ingredient for preparation, being responsible for its fermentation. These microorganisms are often found in the so-called SCOBY, a *symbiotic culture of bacteria and yeast*, where both families coexist and contribute to the formation of fermented tea.

1.3.4.1. SCOBY

The SCOBY, also known as pellicle (Tran et al., 2020) or tea fungus (Jayabalan et al., 2014), is a cellulose biofilm formed during fermentation that is often found at the upper side of the brew. The biofilm formed in earlier fermentations is used as a yeast and bacteria source for the next batch and as the fermentation evolves, a new layer of SCOBY is formed on top of the old one.

The biofilm acts as a barrier to the diffusion of antibiotics due to the inherent viscosity of cellulose in its composition. This ultimately helps protect the kombucha from external bacteria (Stewart, 1996).

The SCOBY is the “mother” of the metabolic process and together with already fermented kombucha, it’s responsible for the fermentation process as well. It has a jelly-like texture and spreads a vinegary smell, an indicator that the fermentation is taking its course (Bishop et al., 2022b).

Several species of microorganisms that are consistently found in all SCOBYs are primarily made up of acetic acid bacteria and yeast. The SCOBY itself is formed by the presence of these microbes forming a zoogleal mat. Several studies confirm that the concentration of bacteria and yeast could reach 10^6 –10^8 CFU/mL in kombucha following a nine-day fermentation cycle (Dufresne & Farnworth, 200); Jarrell et al., 2000) and that yeast outnumbers the bacteria concentrations once the fermentation is complete (Chen & Liu, 2000; Goh et al., 2012).

1.3.4.2. Yeast

Yeast plays a crucial role in the SCOBY and is responsible for sugar metabolism and ethanol production in kombucha. Specifically, yeast from the *Saccharomyces* genus produces an enzyme called "Invertase" (also known as SInv or β-fructofuranosidase). This enzyme catalyzes the hydrolysis of sucrose, breaking down the disaccharide into its const,tuent parts; glucose and fructose. The resulting ethanol, a byproduct of yeast metabolism, serves as a defense mechanism to inhibit the growth of external microorganisms within the brewing solution (Stewart, 1996).

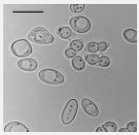
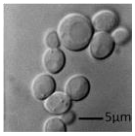
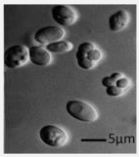
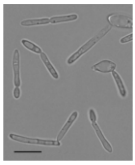
Given the complexity of kombucha's composition seen until this moment, yeast also plays a role in this matter. A broad spectrum of yeasts has been reported including species of *Saccharomyces, Saccharomycodes, Schizosaccharomyces, Zygosaccharomyces, Brettanomyces/Dekkera, Candida, Torulospora, Koleckera, Pichia, Mycotorula, and Mycoderma.* Several species have been identified through studies over the years. Therefore, the most common species are shown in table 2. Watawana et al. (2015) reported that *Zygosaccharomyces* is the predominant yeast found in kombucha at 84.1% of relative percentage of abundance, followed by Dekkera at 6% and Pichia at 5% (Watawana et al., 2015).

1.3.4.3. Bacteria

The primary bacteria found in kombucha tea culture are AAB (Acetic Acid Bacteria), which are aerobic bacteria capable of using alcohol as a substrate to produce acetic acid. They have a fundamental role in the fermentation of kombucha as they are responsible for the decomposition of alcohol formed by yeast. Unlike yeast, these bacteria rely heavily on oxygen for their growth and metabolic activity. The metabolic process involves the conversion of acetaldehyde into ethanol and acetaldehyde hydrate into acetic acid (Villarreal-Soto et al., 2018).

Kombucha contains a diverse range of acetic acid bacteria (AAB) belonging to more than seventeen genera, including *Acetobacter, Gluconobacter, Gluconacetobacter, and Komagataeibacter* (Wang et al., 2015). Among these, several studies have identified the dominant species of AAB as *Acetobacter xylinum, A. xylinoides, A. aceti, A. pausterianus, and Bacterium gluconicum* (Goh et al., 2012).

Table 2. Common yeast species isolated from Kombucha and their metabolic characteristics

Species	Morphology		Characteristics	Ref.
Zygosaccharomyces (Z.) bailii	White to cream colonies with brownish top, cylindrical or ellipsoidal shape, (3.5–6.0) (4.5–11.5) m in size		Tolerant to organic acids, Forms acetic acid, heat tolerance < 75 ºC Growth pH > 2 and < 7	(Redirect Notice, n.d.)
Zycosaccharomyces (Z.) rouxii	White to cream smooth colonies, round or oval shape		High osmotic stress and salt/sugar tolerant, grows under low oxygen and low water activity	(Villarreal-Soto et al., 2018).
Schizosaccharomyces (S.) pombe	Cream to tan, butyrous colonies, rod-shaped		Can convert malic acid to ethanol, high resistance to low water activity, low pH and wide range of temperature environments, highly sugar content tolerant	(Villarreal-Soto et al., 2018)
Saccharomycodes (S.) ludwigi	Cream, butyrous colonies, elongated shape, and swelling in the centre		Resistant to pressurized carbon dioxide, high sugar tolerant	(Redirect Notice, n.d.)
S. cerevisiae	White to cream, butyrous colonies, spherical or ovoid shape, 2.5–10.0 m (diameter)		Can convert glucose to ethanol, high ethanol tolerance, rapid fermentation rate	(Villarreal-Soto et al., 2018)
Brettanomyces (B.) bruxellensis	Distinctive elongated shape, 2.5–10.0 m (diameter)		Can produce high amounts of acetic acid and ethanol under aerobic conditions, high ethanol concentration (up to 15%), able to grow under low pH and oxygen environment, high efficiency to utilize nitrogen sources	(Redirect Notice, n.d.)

2. OBJECTIVES

The main objective of this work is to gather information regarding kombucha and its industrial process. To achieve this objective, specific sub-objectives have been defined from various perspectives, which are presented below.

From an industrial perspective:

- Explain as detailed as possible the industrial process of kombucha and create a thorough compilation of methods, materials, and equipment used during such process.

From an experimental perspective:

- Study the influence of temperature during the kombucha fermentation process.
- Analyse/check/study the influence of the initial concentration of sucrose on the final product.
- Create a guide for homebrewers to track their fermentations and have a clearer view of the process is going as expected.

3. INDUSTRIAL ELABORATION

There are several procedures to produce kombucha at an industrial scale. The different methods used by kombucha breweries normally diverge in the fermentation stage.

Traditionally, kombucha is understood to have two fermentations in its elaboration process. On one hand, the first one is characterized by the fermentation of plane sugar (the initial ingredient added at the beginning of the process) with the known culture of microorganisms called SCOBY (see section 1.3.4 for more information). On the other hand, the second fermentation is known for the fermentation of sugars contained in the flavoring ingredients added at the final stages of the elaboration, in addition to the residual sugars from the first fermentation.

For a clearer understanding of the industrial process of the commented beverage, figure 8 is a schematic block diagram of the global process where the different phases can be seen together with their sequential execution. Therefore, the following sections are explicitly focused on explaining the different phases seen in this figure.

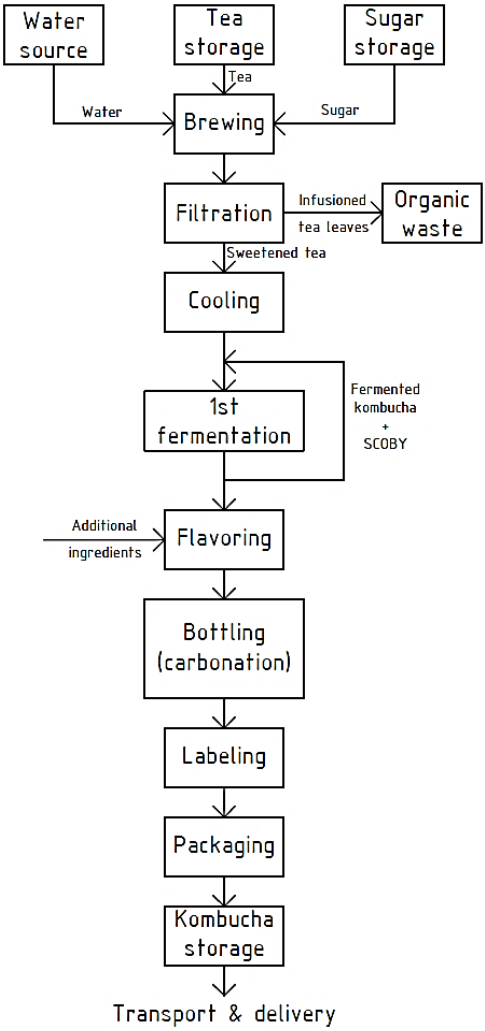


Figure 8: Block diagram on the elaboration of kombucha

3.1. RAW MATERIALS AND INGREDIENTS

3.1.1. Tea

Kombucha is made from sweetened black or green tea. Tea is known to be the second most consumed beverage in the world, behind water (Chang, 2015). It's derived from the perennial leafy plant known as *Camellia sinensis*, which belongs to the *Theaceae* family. It is cultivated in approximately thirty-five countries worldwide (Sharma et al., 2007), with China being the leading producer, accounting for nearly 46% of the global tea crop (Chang, 2015). India is the second-largest tea producer. However, a significant portion of the tea produced in these countries is consumed domestically rather than exported. The largest tea consumers globally include Turkey, Ireland, the United Kingdom, Russia, and Morocco.

This ingredient is essential for kombucha fermentation since it's a nutrient source for microorganisms. Tea, derived from the leaves of the *Camellia sinensis* plant, plays a crucial role in the nourishment and well-being of a SCOBY. The tea leaves contain essential nutrients such as nitrogen, caffeine, and theanine, which, when combined with sugar, feed the kombucha colony so it continues to thrive. Both black and green tea create an optimal environment for fermentation as they possess a high nutrient content, which facilitates the production of the beverage and promotes the well-being of the SCOBY (Kombucha 101 - Pick the Right Tea for Brewing, n.d.).

Furthermore, it is normally sold in bulk or paper sacks for large quantities used in this industry and therefore it's stored in large sacks or pellets depending on the production volume of the brewery. The properties of this ingredient do not require a climatized environment, but it is recommended to keep it in an airtight, cool space away from heat and sunlight.

3.1.2. Sugar

Sugar is one of the most important ingredients of kombucha elaboration and plays the integral role of feeding the yeast. To be more precise, the essential ingredient in this process is sucrose, a disaccharide formed by one glucose and one fructose linked by a glucosidic bond (figure 9).

Sugar, derived from sugarcane and sugar beets, is cultivated in over 100 countries worldwide. Among these, Brazil stands out as the largest producer, contributing to a quarter of the global sugar production. Additionally, other major sugar-producing nations include India, China, Thailand, and the United States (Walton, 2022).

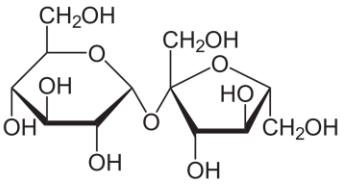


Figure 9. Chemical formula of sucrose.

Moreover, the used sugar in this industry is plain cane sugar. Either refined or simply organic, sucrose is broken down into its dysacards by yeast as the first chemcial reaction of kombucha's fermentation. Other sugar such as brown, honey, or agave, have a hard fermentation and can end up harming the microorganism's colony (Bond, 2023).

Like tea, sugar is sold in sacked pellets around the world. These pellets arrive at the brewery and are stored in similar conditions and often share space with tea pellets.

3.1.3. Water

Water is the medium of fermentation and plays a crucial role in the quality of the final product. Different water sources can be used for brewing kombucha although the most used one is filtered tap water. Well water has a high concentration of minerals beneficial to fermentation but is not treated in any way and therefore can't be used in this industry unless it's treated.

Tap water is treated with chlorine, fluoride, and other substances to ensure its safety and provide antimicrobial properties. Although this treatment is essential for safety purposes, it can have adverse effects on the SCOBY's fermentation process and the production of flavor compounds (Crum et al., 2016). In order to address the presence of chlorine or metabisulfite in the water, filtration systems are often employed, utilizing activated carbon. Additionally, UV lights are positioned prior to the carbon filtration system to disinfect the water and prevent any potential contamination of the carbon filters. This UV treatment helps break down organics, specifically chloramine, thereby minimizing absorption and contamination of the filter (Palmer, 2013).

These treatments can be carried out in the own brewery, or it is possible to have a direct supply of filtered water from a water treatment plant.

3.2. BREWING

Brewing is the act of creating a drink by mixing hot water with some species that can dissolve part of its content in it. Coffee, tea, beer, and kombucha, among others, are brewed beverages drunk every day around the world. Therefore, this stage of the process is characteristic for the formation of a balanced liquid mixture (formed by infused tea and dissolved sugar) creates the primary reactant of the fermentation, the raw kombucha, that will later become the vinegary and acidic beverage.

It's the first stage of the process and is concluded in a *brewery*, a big industrial pot made of stainless steel named after its clear duty to the process. The dimensions of such equipment depend on the volume of elaboration of the brewery usually moveing around 300 to 1 000 L but can scale up to 5 000 L.

These pots are often implemented with an internal heat exchanger that heats up the water inside to the optimal temperature. The exchangers used in this process have a large catalog, even though the most used ones are serpentine exchangers surrounding the interior walls of the machine. A common, but not essential accessory to this machinery is the agitation aspect. It is highly important to maintain the mixture as homogenized as possible to expect the best-resulting beverage and therefore agitation is added to cope with this matter. The most common agitation methods are the U-shaped anchor and paddle agitators.When the heat exchanger is external (found in the walls of the equipment) the

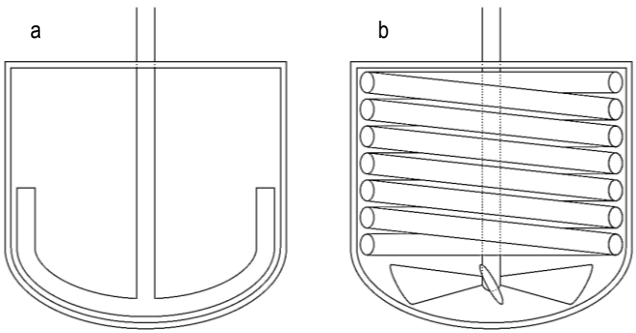


Figure 10. Agitation methods; a. U shaped anchor agitator, b. Paddle agitator with interior serpentine.

method used is the U-shaped anchor, while an internal serpentine is combined with a paddle agitator. Both models are shown in figure 10.

Moreover, referring to the elaboration process of this phase, the brewery is filled up with previously filtered water through plastic tubes and heated up to a value between 70 and 95 ºC (Tran et al., 2020). Once the water is up to heat, tea is added in a variable concentration from 1,5 to 10 g/L (Blanc, 1996; Chen & Liu, 2000; Chu & Chen, 2006; De Filippis, Troise, Vitaglione,& Ercolini, 2018; Goh et al., 2012a; Jayabalan et al., 2014).

There are several methods for adding tea in the brewery. With big industrial tea bags, with an internal stainless-steel filter, or with no container at all, and simply poured inside the pot. Tea is infused from 5 to 30 minutes (Antolak et al., 2021) to get the optimal dissolution of tea compounds in the mixture.

Furthermore, keeping the heat of the mixture elevated for the easier dissolution of sugar, this primary ingredient is added in a proportion of 50 to 100 g/L (Tran et al., 2020). This addition is often executed manually by pouring the sugar, previously weighed on a scale. Once all the sugar is dissolved the mixture passes along to the next phase of the elaboration process, filtration and cooling.

3.3. FILTRATION

This phase is not always necessary bearing the fact that frequently the tea is already added in a container and therefore major content of the tea leaves is already separated. When the practiced method is container-less this stage is necessary for the liquid mixture to be as clean as possible for proper fermentation later.

Furthermore, when needed, a simple stainless steel mesh filter between the brewery and the cooling system (figure 11) is enough to capture the tea leaves residue in the mixture.

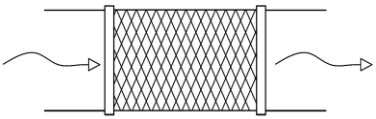


Figure 11. Stainless steel mesh filter disposal

Nevertheless, despite the method used to infuse tea, the organic waste residue generated in this phase is remarkable and therefore must be managed accordingly.

3.4. COOLING

In this stage, the mixture is cooled down to an optimal temperature so it is ready to start the fermentation process. It is an essential step due to the range of temperatures that the SCOBY tolerates. At high temperatures, yeast and bacteria are inhibited and even die. Therefore, the mixture must be at a sufficiently low temperature when it encounters the microorganisms for the fermentation stage.

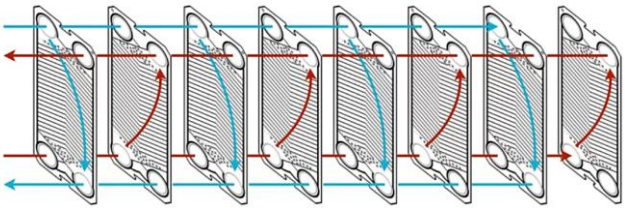


Figure 12. Plate heat exchanger flow diagram, (Hfmphe, 2023).

The refrigeration process is accomplished with a plate heat exchanger and water as the coolant, following the operation system in figure 12. The water can be reused as a primary ingredient in a future batch and accordingly, be filtered. If such action is not accomplished, water can be from a regular water supply network.

Starting at a temperature of 70 ºC or higher, the raw mix is cooled down to a minimum temperature of 39 ºC. Typically, yeasts exhibit optimal growth at temperatures ranging from 25 to 30 °C (Salvadó et al., 2011), whereas AAB (acetic acid bacteria) and LAB (lactic acid bacteria) thrive between 25 and 30 °C (Mamlouk & Gullo, 2013) and 20 to 40 °C (Matejčeková et al., 2016), respectively. Therefore, higher than 40 ºC the mixture reaches the fermenter at a temperature that is too high, resulting in the development of other undesirable chemical compounds that often contribute a yeasty taste to the beverage.

3.5. FIRST FERMENTATION

This phase is where the kombucha is actually made. The four main ingredients (tea, water, sugar, and microorganisms) come in contact and develop the beverage. This is a crucial stage of the process and the most important one.

During kombucha fermentation, a complex series of chemical reactions occur. The yeast in the SCOBY metabolizes the sugars in the tea, converting them into ethanol through the process of glycolysis. Simultaneously, the bacteria in the SCOBY, primarily acetic acid bacteria, utilize ethanol as a substrate and convert it into acetic acid through oxidation. This process, known as acetic acid fermentation, gives kombucha its characteristic tangy taste. In addition to acetic acid, various other organic acids are also produced during fermentation, contributing to the unique flavor profile and potential health benefits of kombucha.

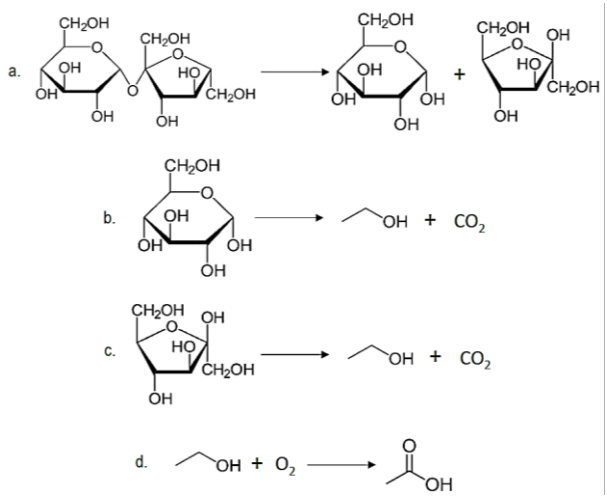


Figure 13. Main chemical reactions in kombucha. a. Sucrose hydrolysis, b. Glucose alcoholic fermentation, c. Fructose alcoholic fermentation, d. Ethanol oxidation.

This stage of the elaboration process is done at an optimal temperature range from around 20 to 30 ºC, depending on the SCOBY composition (Jayabalan et al., 2014). To carry out the fermentation at these temperatures, heat is supplied to the fermenters. This action can be done either by individual radiators for each fermenter or by a general climatized room with a thermostat.

The main reactions in this process are shown in figure 13. As commented, figure 13.a. is the hydrolysis of sucrose breaking down into glucose and fructose. Following, 13.b. and 13.c., glucose and fructose are metabolized by yeast to for ethanol and carbon dioxide. These reactions

are also the characteristic reactions of alcoholic fermentation in wine, beer, etc. Finally, 13.d. is the oxidation of ethanol made by AAB and acetic acid is formed as the final product. It can be observed that the ethanol oxidation process needs oxygen to go on and therefore this first fermentation of the elaboration is aerobic.

In addition, figure 14 is a schematic diagram of the chemical interactions in kombucha and the formation of other chemical compounds as well. As these reactions proceed, certain parameters such as density and pH suffer slight changes in their values. This fact provides information that is often used as a fermentation control system to ensure that the process is evolving as expected.

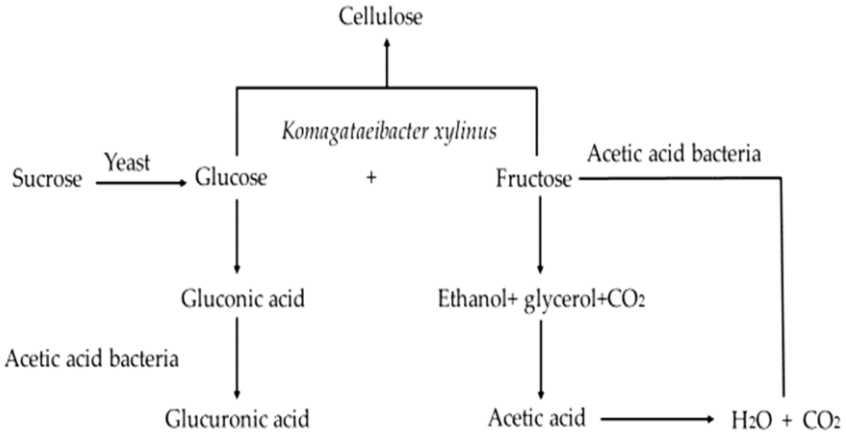


Figure 14. Main metabolic activity of Kombucha tea (Markov et al., 2003)

Breweries accomplish this stage in several different ways that will be explained next.

3.5.1. Small batch

This is the traditional method used for centuries in households around the world. It is a simple method characteristic for using glass jars as fermenters. From 10 to 30 liters capacity, the sweetened mix is added together with the SCOBY and a fraction of fermented kombucha from previous batches to act as starter liquid for the fermentation.

From an industrial scaling point of view, it could be said that this method does not modify its scale adapting it to an industrial production. The approach taken by some kombucha companies is to replicate the traditional method through a high number of fermenters, maintaining the volume of such containers rather than adopting bigger fermenters. Companies such as GT's or Health-Ade kombucha are pioneers in this technique of elaboration, Health-Ade ,for example, has a total of 300 000 glass jars for its daily production of 100 000 bottles (Insider, 2018).

Once the mixture is in place, the fermentation starts and is left for 7 to 14 days depending on the initial mixture and SCOBY composition (Jayabalan et al., 2014; Kitwetcharoen et al., 2023; Kumar & Joshi, 2016). The fermentation process is allowed to proceed for more than 14 days, although the acidity may increase to levels that could be potentially harmful to consume. To accomplish aerobic fermentation, this technique covers the upper opening of the jars with a cotton cloth to allow oxygen inside the jar but prevent external bacteria and contaminations away from the beverage. Moreover, figure 15 expresses the small batch disposition on shelves, together with the cotton cloth coverage.

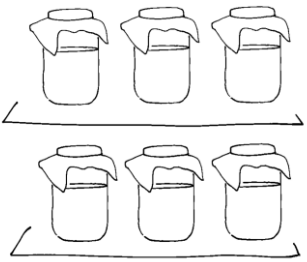


Figure 15: Small batch disposal in kombucha breweries

Additionally, as seen in previous sections (1.3.4), a daughter tea fungus is formed on the surface of the tea during fermentation. This new SCOBY is conserved with its mother and will form a new mother for other batches.

3.5.2. Large batch

This technique has the same methodology as the previous one, solving the industrial scaling factor. It follows the same fermentation time and conditions with a higher volume by batch. The equipment used in this method is stainless steel flat bottom 300 – 1 000 L tanks with a similar cotton or cotton-like cloth covering the upper opening of the tank to allow the aerobic fermentation to go on.

Additionally, to carry out the fermentation at its optimal temperature, this method can use either of the mentioned techniques, while the small batch method only relies on the climatized room thermostat.

The sweetened tea is added to the fermenter with the use of plastic tubes and pumps that connect both pieces of equipment, heat exchanger and fermenter. In some cases, the SCOBY and starter liquid are left inside the tank for upcoming batches. Under these circumstances, the tanks can contain two exit valves in the lower section to avoid extracting too much liquid and leave a minimum amount to certify the next fermentation.

3.5.3. Starter liquid SCOBY

A less commonly used method is the one done by *Jarr* kombucha brewery, creating a so-called liquid SCOBY with an in-situ oxygenized fermentation.

To achieve this goal, the starter liquid is brewed with slightly more sugar than the plain kombucha mixture and fermented in a large stainless-steel tank with continuous oxygenation for 14 days. This oxygenized technique promotes the reproduction of bacterial compounds over the yeast and gives a good start at the fermentation, using 25% of this mixture and 75% of sweetened tea and letting it rest for 10 - 12 more days (Mei Leaf, 2020). After this first fermentation, the cellulose layer formed on top is thrown away or given to possible takers.

3.6. FLAVORING

Continuing with the elaboration, the next phase of the process is flavoring. It is not an essential step bearing the fact that unflavored kombucha is also sold as a final product although all breweries produce flavored kombucha as well. Its where new ingredients are added to transform the beverage's flavors. To perform such a task, the fermented kombucha is mixed up with fruits and aromatic herbs.

These new ingredients are normally added as liquefied as possible and have been juiced out or infused before combining them with unflavored kombucha. These actions are done with an

industrial juicer and a brewery, respectively. On one hand, industrial juicers separate the juice from the pulp in a way that both parts of the ingredient can be used. The machine comes equipped with sharp blades that crush what is added through the machine's manual input. Once crushed, the pieces accumulate in large polymeric fiber bags, and once the bags are sufficiently full, they are compressed under pressure using two plastic plates with patterns that facilitate extracting the maximum amount of juice. This juice flows by gravity and is manually collected in bags, while the pulp is retained inside the mesh. Both parts are collected and often used in the production process as they contribute different flavors to the beverage. On the other hand, some ingredients are infused with water and then mixed up with unflavored kombucha. This task is done in a similar way to the initial sweetened tea brewing and therefore the equipment is often reused for both tasks.

In some cases, kombucha breweries also use oils and essences instead of natural products to give taste to their final beverage. By consuming these products, the resulting kombucha tends to be cleaner due to the natural unfiltered components in natural ingredients. Depending on the elaboration method of the brewery, the mixture is filtered before passing on to the next phase of the process. This second filtration not only captures the organic residue from added ingredients, but also can retain part of the yeast and, therefore, create a more stable product in terms of ethanol development.

3.7. BOTTLING AND LABELING

Bottling is not only the first packaging step of kombucha elaboration but also the beginning of the carbonation process to accomplish the final product. Until this stage, kombucha has been open to the atmosphere and has been generating carbon dioxide as a reaction product, which was not contained. Therefore, the beverage has not gained the fizzy texture that is normally attributed although small quantities of carbon dioxide are dissolved during the first fermentation and little carbonation is noted before bottling. To accomplish such fizzy texture the carbonation process can be fulfilled in two ways, with the natural carbonation generated by the beverage or forced carbonation with carbon dioxide.

3.7.1. Forced carbonation

After the flavoring process, the fermented kombucha is transferred from the fermenting vessel to the brite tank. The temperature is lowered to a range of 1 - 4 ºC, and carbon dioxide (CO_2) is introduced through a submerged carb stone in the brite tank. This process forces carbonation into the kombucha, giving it the desired effervescence (Booch Nesws, 2019).

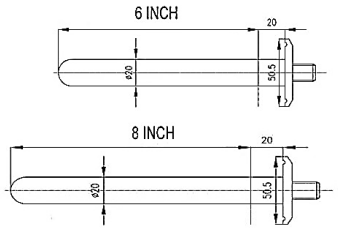


Figure 16. Stainless steal carbonation Stones desings (6 and 8 inch).

Carbonating kombucha using a carbonating stone, also known as carb stone, in a brite tank accelerates the carbonation process compared to natural carbonation methods. The carbonating stone (figure 16) enhances the surface area contact with the kombucha by creating small carbon dioxide bubbles that can be readily absorbed into the beverage. Prior to this process, it is essential to lower the temperature of the mixture to facilitate the dissolution of CO_2 into the kombucha. Ideally, the temperature of the kombucha should be reduced to a range of 1 to 2 ºC, although it should still be feasible even if the temperature is slightly higher, such as 3 or 4 ºC (*Brewing Equipment - Commercial Brewing & Home Brewing*, n.d.).

The duration of the forced carbonation depends on several parameters such as batch volume, carbonation volume, and carb stone pressure. On one hand, batch volume has a direct relation to the time needed, the bigger the batch, the more time it needs to accomplish the accurate carbonation. For example, an 800 000 L batch would need to be about one hour with forced CO_2. On the other hand, the working pressure is around 22 psi with

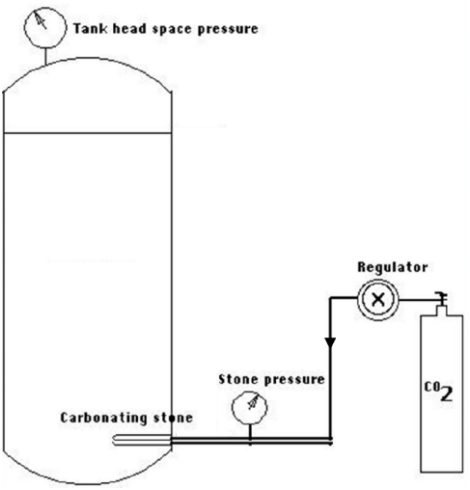


Figure 17. Forced carbonation diagram (Brewers Association Technical Committee)

slight changes depending on the carbonation volume desired. Carbonation volume refers to the measurement of carbon dioxide (CO_2) dissolved in a beverage, which determines its level of carbonation or fizziness. A common carbonation volume in kombucha is around 3, expressing that for one unit of liquid, 3 times that volume of CO_2 will be dissolved in it.

3.7.2. Natural carbonation

In contrast, this method carbonates the beverage from the natural fermentation of the remaining sugar from the initial brewing as well as the additional sugars from the new flavoring ingredients. Carbonatation occurs when the fermentation process is sealed inside a container and CO_2 formed during fermentation is absorbed by the kombucha since it has no exit. For this reason, the second fermentation is found when bottling kombucha in the final container and therefore, carbonating it.

The final containers of kombucha are either glass bottles or aluminum cans. Regardless of the type of container used, the bottling methodology is very similar. This operation is usually carried out in a semiautomatic bottling machine. The bottles are manually placed on an input tray where the machine picks them up, cleans them, fills them, and often also caps them. It is worth noting that just before being capped, the bottling machine has an air expulsion system that injects nitrogen or CO_2 into the bottle to remove any remaining air and oxygen that may be inside the bottle and prevent unwanted aerobic fermentation. This way, the oxygen is expelled from the bottle, and it is sealed until it is time to consume it. Figure 18 shows a schematic diagram of how industrial bottling machines work. After capping, a labeling

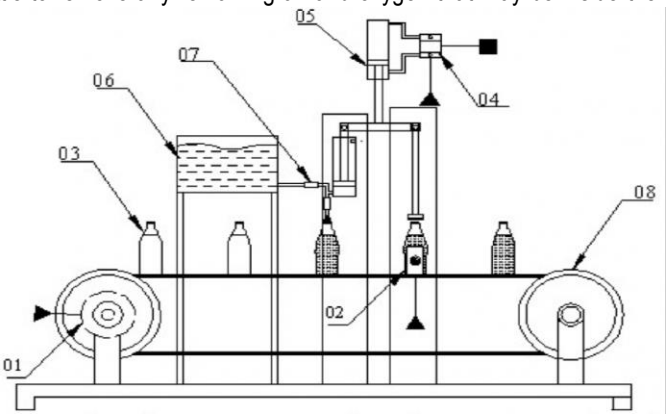


Figure 18. Automatic filling and cap closing machine. (1. Dc motor, 2. Proximity sensor, 3. Bottle, 4. Solenoid valve, 5. Pneumàtic cylinder, 6. Tank, 7. Non-return valve, 8. Conveyor) (Frigate Projects, 2022).

machine often follows and labels the bottles with the help of a sensor although this action can also be done manually. Finally, the bottling machine delivers the caped and labeled bottles on a tray where they are ready for packaging.

3.8. PACKAGING, STORAGE AND DELIVERY

Bottles are placed in boxes either manually or automatically, they are stacked and stored in two different ways. Storage can be either cold or at room temperature depending on the chosen carbonation method. If forced carbonation is used, as mentioned before, the beverage temperature should be below 4°C to ensure proper CO_2 dissolution. Therefore, when using this carbonation method, the beverage needs to be stored and kept cold after undergoing this process. On the other hand, if natural fermentation is employed, refrigeration is not necessary, and the product can be stored at room temperature. In fact, at higher temperatures, the secondary bottle fermentation is accelerated, and carbonation increases, which is necessary to achieve a carbonated final product.

In terms of delivery and consumption, forced carbonation kombucha can be delivered as soon as packed since the product is already finished. Natural carbonation kombucha needs an incubation time in the storage of one-week minimum for the second fermentation to occur. After this week it can also be sold and delivered.

4. PARAMETERS EVOLUTION IN FERMENTATION

Fermentation is a complex process influenced by various factors including temperature, pH, oxygen levels, dissolved CO_2, operating system, precursor availability, shear rate, and the composition of the medium. These factors can impact the fermentation rate, product properties, organoleptic characteristics, nutritional quality, and other physicochemical attributes. Variations

in sugar concentrations, fermentation duration, and the composition of the tea fungus can also contribute to differences in the final product's composition and biological activities.

4.1. PH

The pH level is a critical environmental factor that significantly impacts the fermentation process of kombucha. This is due to the formation of acids such as acetic and gluconic acids, which play a vital role in the biological activities and characteristics of the resulting beverages. The optimal pH ranges from 2,5 to 3,5 and has a direct correlation with microbial growth and the structural changes of phytochemical compounds, which can influence the antioxidant activity of the final product or the unwanted formation of harmful bacteria (Hur et al., 2014).

FDA guidelines state that when the pH of the food is 4.6 or below, it does not need further preservatives. Many microorganisms that typically cause food spoilage, such as *E. coli* and *Salmonella*, cannot survive in such a low-pH environment. This makes kombucha a safer beverage to consume. In contrast, if the pH is lower than 2, the beverage is too acidic to consume and will probably taste too sour. Nevertheless, while solutions with a high acetic acid content typically exhibit a lower pH level, it is important to note that the pH level is not solely determined by acetic acid. Hence, it is crucial to both test the pH and taste the kombucha to discern subtle flavor differences and determine if the fermentation process is complete. (Admin, 2023).

4.2. DENSITY

As the fermentation continues, sucrose is consumed and lighter compounds such as ethanol, acetic acid, or carbon dioxide are formed, leading to a slight reduction of density values.

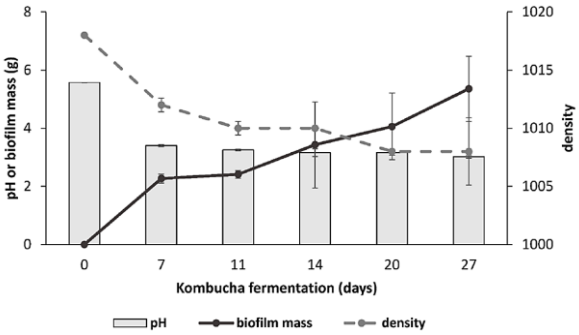


Figure 19. Physico-chemical parameters followed during 27 days Kombucha fermentations (Savary et al., 2021).

A study made by Savary et al. (2021) affirms that during the fermentation process, the density values show a rapid decrease from 1018 to 1010 within the first 11 days. Subsequently, the density stabilized between 1010 and 1008 until the completion of fermentation (27 days) (Savary et al., 2021).In figure 19, the pH and density values of this study can be observed together with biofilm mass.

4.3. ACID AND ALCOHOL CONTENT

As explained in sections 1.3.1 and 1.3.2, organic acids and ethanol are clear products of kombucha's fermentation. In figure 20, the concentrations of the most predominant acids (Lactic and acetic) and alcohol (ethanol) can be observed. Other organic acids are also formed during the process, but at lower concentrations, below 0.03 g/L (Savary et al., 2021).

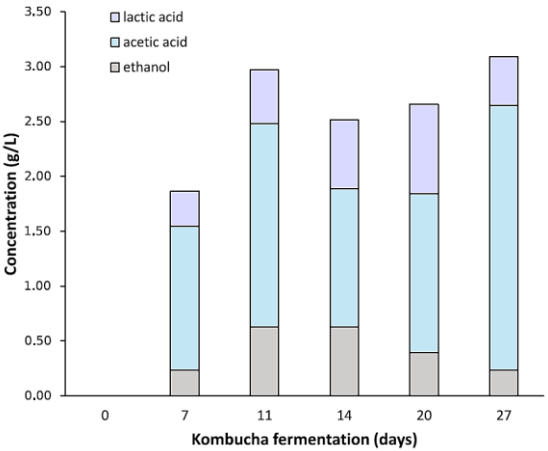


Figure 20. Changes in organic acids concentrations during Kombucha fermentations (Savary et al., 2021).

The formation of such organic compounds is reflected in the decrease in pH and density values (figure 20) as well as the taste of the final product. Moreover, other studies mention that acetic acid values can reach up to 11 g/L after 60 days of fermentation (Chen & Liu, 2000).

5. EXPERIMENTAL PART

5.1. METHODS

In order to achieve the mentioned objectives, 9 fermentations have been carried out in three different time periods, i.e., 3 simultaneous fermentations with a 15-day period. Two of these were conducted in the laboratory using a thermostatic bath, while the remaining one was performed at home without fixed temperature control. Such bath is shown in figure 21, where only one

Table 3. Experimental design of effectuated fermentations (at atmospheric pressure)

Date	***Temperature*** *[ºC]*	***Initial sucrose*** *[g/L]*	***Nomenclature[1]***	***Reference in graphs***
12/04-27/04	21 ± 3	50	Tamb - C50	
02/05-17/05	21 ± 3	65	Tamb - $C65_1$	
18/05-02/06	21 ± 3	65	Tamb - $C65_2$	
12/04-27/04	35	50	T35 - $C50_1$	
12/04-27/04	35	50	T35 - $C50_2$	
02/05-17/05	35	65	T35 - $C65_1$	
02/05-17/05	35	65	T35 - $C65_2$	
18/05-02/06	28	65	T28 - $C65_1$	
18/05-02/06	28	65	T28 - $C65_2$	

(1) The nomenclature used for this section is: "T" (temperature) with subindex referring to temperature value ("amb" stands for ambient, room temperature); dash (-); "C" (concentration) followed by initial sucrose value with subindexes indicating the replicates of each experiment.

fermenter is visualized, although two fermenters were simultaneously in. The volume capacity of both fermenters was 1 L, while the home fermenter had a capacity of 4 L. Table 3 displays the various experiments conducted, along with the respective set conditions for each of them. Concerning such table, experiments have been conducted at three different temperatures, while using two initial substrate (sucrose) concentrations.

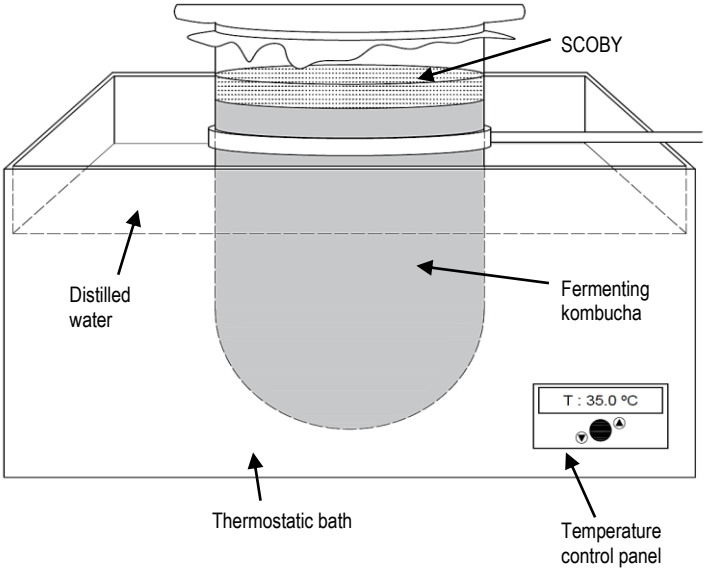


Figure 21. Experimental device diagram of laboratory fermentation at thermostatic bath.

Furthermore, each fermentation has been tracked by daily analysis of pH and density together with sugar, acid, and alcohol content. PH was determined with a *Basic 20 CRISON* pHmeter (0,01 precision), while density was measured by weighting 3 mL (3000 µL), pipetted with a *Proline® plus* 500-5000 µL pipette, on an *OHAUS® PIONEER™* precision balance (0.0001 g precision). Moreover, acid content was determined by an acid-base titration with 1000 µL of kombucha (analyte) and a sodium hydroxide solution of 0,02 M as titrant together with phenolphthalein solution 1% as pH indicator.

Sugar content was determined with a NOVEX Abbe laboratory refractometer, with refractive index (r.i.) (1,300 to 1,700; 0,0002 accuracy) and Brix scale (0-95 %). Such measurements require a thermostatic bath, that was fixed at 30 ºC and calibrated between 0 and 90 g/L of sucrose. Finally, alcohol content was measured with *HP/Agilent 6890 GC system* with a specific method of 5 minutes run time at 100 ºC oven temperature.

For all these analyses, a sample of 10-12 mL was sucked out with a 20 mL syringe from all the reactors. Additionally, an organoleptic test was also made at the 65 g/L ongoing room temperature experiments (Tamb - $C65_1$ and Tamb - $C65_2$).

5.2. RESULTS AND DISCUSSION

The explained methodology evaluating pH and density together with acid and sugar content, allowed the tracking of the fermentation status under three different temperatures and two initial substrate concentrations. In figures 22 and 23, the results regarding experiment T28-C65$_2$ are represented as an example of the results and trends obtained.

The acidity of the fermentation progressively increased from 1.32 ± 0.03 mL to 12.87 ± 0.21 mL as time passed while correspondingly, the pH of the mixture decreased towards a more acidic solution (3.74 to 2.77). This fact can be directly attributed to the formation of organic acids during fermentation, as it is known that these compounds are products of the reactions taking place.

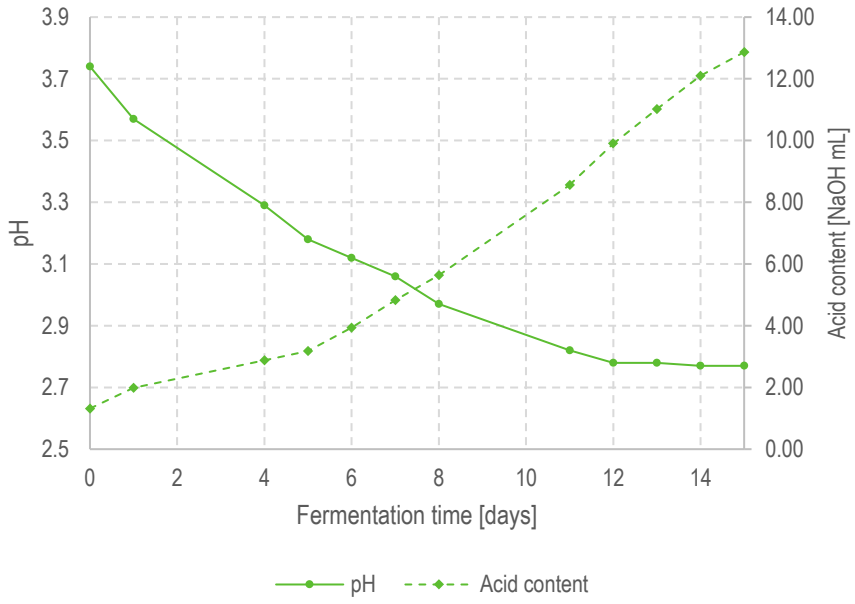


Figure 22. pH and acid content for the 15 days period time of T28-C65$_2$ experiment.

Regarding the density of the mixture, its value decreased after a pronounced peek between days 0 and 2 of fermentation, logical with other experiments mentioned earlier (section 4.2.). The formation of lighter compounds, such as carbon dioxide and ethanol, can be the consequence of this parameter's conversion. Moreover, the sugar content of the mixture also decreased to half its

content (55.43 %) with a consistent outcome of substrate consumption, also related to the density drop experienced.

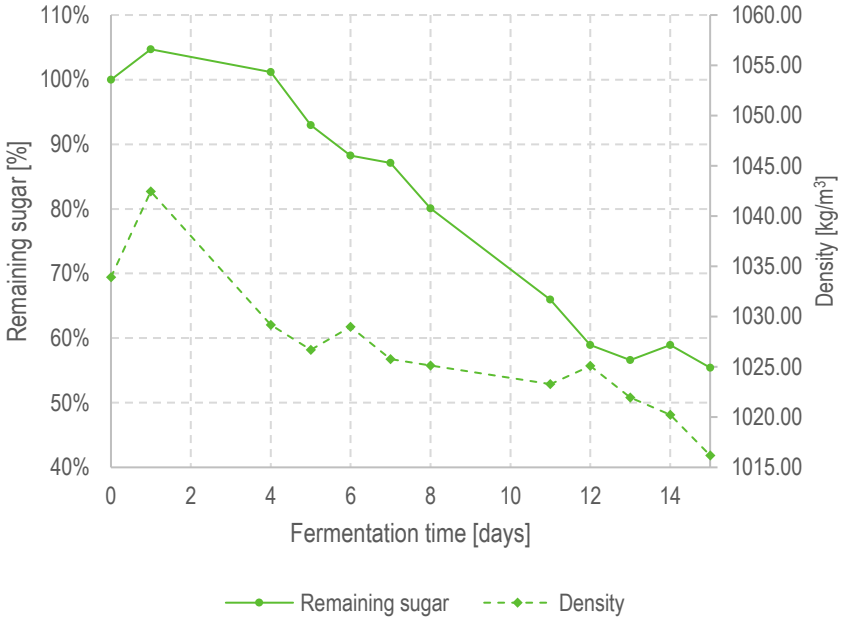


Figure 23. Density and sugar content for the 15 days period time of Tamb-C65$_2$ experiment.

The tendencies of the realized experiments are similar to the one just commented on, with some alterations that the following sections will address.

From a general point of view, pH before fermentation had a value of 3.37 ± 0.14 for experiments done with initial sucrose of 50 g/L, while 60 g/L values gave a pH of 3.59 ± 0.20. All experiments decreased progressively to values between 2.77 and 3.04 after 15 days of fermentation. Density tendencies had an increase during the first days of fermentation, followed by an irregular decrease at the final stages of fermentation and values varied from 1016.20 to 1056.46 Kg/m^3 with an error fluctuating between 0.009 and 2.470 %.

For the compound part of the analysis, acid content had a progressive increment, more pronounced in the second half of fermentation. Initial values differed from 1.13 to 4.55 mL of NaOH and escalate to final values between 4.43 and 12.87 mL (maximum error of 5%). Following

Savary's (2021) research, broadly speaking, 78 % of acid content during the first 14 days of fermentation is acetic acid, while the other 22 % is lactic acid. With such facts, undermining any conditioning effect of such research, it could be said that the acetic acid content differs between 6.2 and 11.8 g/L, while lactic acid varies between 1.8 and 5 g/L.

Additionally, sugar content suffered different effects depending on the working temperature which will be discussed in the next section (5.2.1.) Considering initial sucrose as 100 % sucrose content, after 15 days of fermentation the minimum sucrose content was 49.46 % of the initial sucrose added.

Finally, note that alcohol analysis with the effectuated method was inconclusive. There was no similar tendency in the various experiments and therefore will not be included in this section. Moreover, in the upcoming sections, only the most effective figures are shown for the understanding of the debated topic, although all figures can be found in appendices.

Due to the presence of multiple simultaneous reactions and the involvement of various microorganisms, it can be inferred that the mixture is a heterogeneous blend that does not react uniformly throughout the vessel. This justifies the irregular deviations that may be observed in some of the figures and suggests that the sampled data may not be 100 % representative. However, the obtained results do show trends in the evolution of the parameters, which allow for drawing certain conclusions about the effects, even though the values may differ in other parts of the reactor.

5.2.1. Temperature effect

In this matter, the temperature effect was studied at 28 ºC, 35 ºC, and room temperature (21 ± 3 ºC), and the results are shown in figure 24. As it can be observed, density for 35 ºC experiments, had an incremented evolution during the analyzed days. Such an increase could be attributed to the formation of heavier organic compounds, such as fructose, as there may be an initial decomposition of sucrose without the subsequent reaction to ethanol and carbon dioxide.

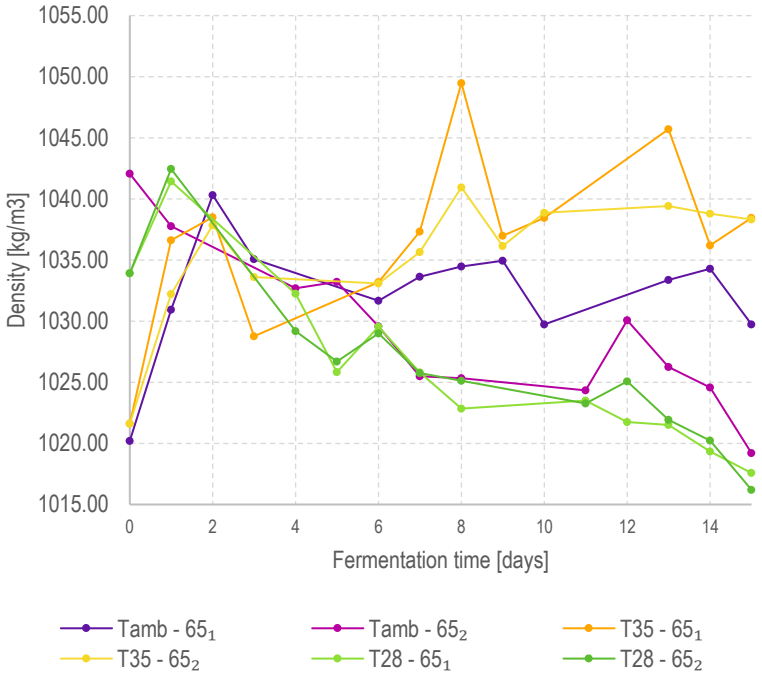


Figure 24. Density evolution during the first 15 days of fermentation with the same initial sucrose concentration (65 g/L).

On the other hand, 21 ± 3 and 28 ºC experiments decrease slowly after an initial pronounced peak on the first day of fermentation which could also affirm the irregular composition of the mixture commented before in this section. Within these two temperature values, note that 28 ºC experiments arrived at lower densities in the same amount of time, suggesting that it's the optimal temperature compared to the other two.

Moreover, in figure 25, the quantity of sugars in the different experiments is represented as the fermentation progressed. The two experiments conducted at 35°C did not show a decrease in the number of sugars, which suggests that the reaction was not proceeding in the expected manner. Alternatively, the experiments conducted at the other two temperatures did show a decrease in the quantity of sugars, with a greater slope observed in the experiments conducted at 28°C.

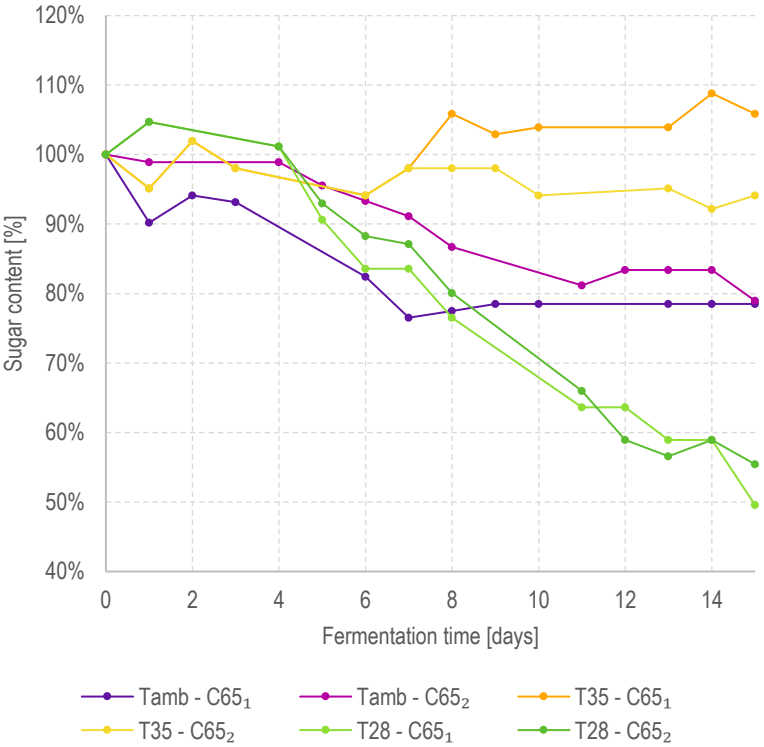


Figure 25. Sugar content evolution during the first 15 days of fermentation with the same initial sucrose concentration (65 g/L).

As commented in section 3.5., the optimal temperature range is 20 to 30 ºC, which is consistent with the obtained results. Experiments conducted at 35ºC have shown higher values of density and sugar content, while other parameters, although more inconclusive, have also been affected by this temperature exceeding 30 ºC. The experiments conducted at room temperature show slower reactions compared to those at 28 ºC, but they exhibit similar behavior to those conducted at 35 ºC. However, the sugar content in the experiments at room temperature and 35ºC show different effects, confirming that 35ºC is too high of a working temperature, while the reaction progresses correctly at room temperature, albeit at a slower rate than at 28 ºC.

5.2.2. Substrate effect

Regarding such effect, two substrate concentrations, 50 and 65 g/L, were evaluated in the commented experiments, while submitted to different temperatures. That being said, the pH and acid content titration results are shown in figures 26 and 27, with working temperatures being 35 ºC and room temperature, respectively.

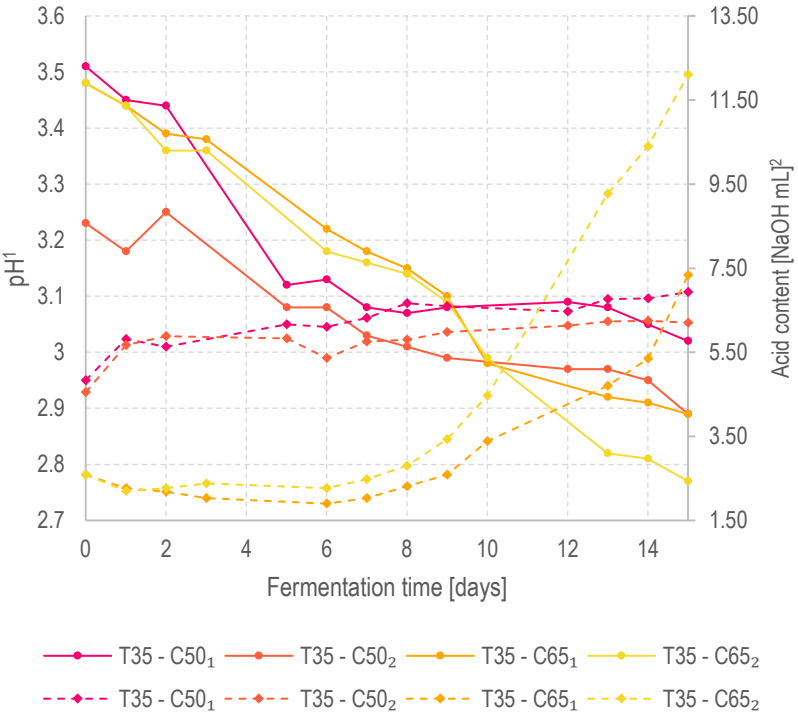


Figure 26. pH and acid content by titration evolution during the first 15 days of fermentation with same work temperature (35 ºC).
(1) pH representations stand for the continuous lines with doted markers
(2) Acid content representations stand for dashed lines with rhombus markers

At 35 ºC, the 65 g/L experiments resulted more acidic than the ones containing 50 g/L.Both pH and titration, coincide in that the higher substrate concentration experiments have a lower initial acidic value (higher pH, but lower NaOH consumption) than those with a lower concentration, while at the end of the analyzed period, the experiments with a concentration of 65 g/L show higher acidic values compared to the other concentration.

On the other hand, at room temperature, the three represented experiments in figure 25 showed a similar progression regardless of the initial substrate concentration. The two 65 g/L replicates differ drastically from the initial pH value, while 50 g/L remained between the two other experiments for most of the executed time. From the titration point of view, lower substrate concentration showed higher values of acidic content during the entire period, not consistent with results from 35 ºC experiments.

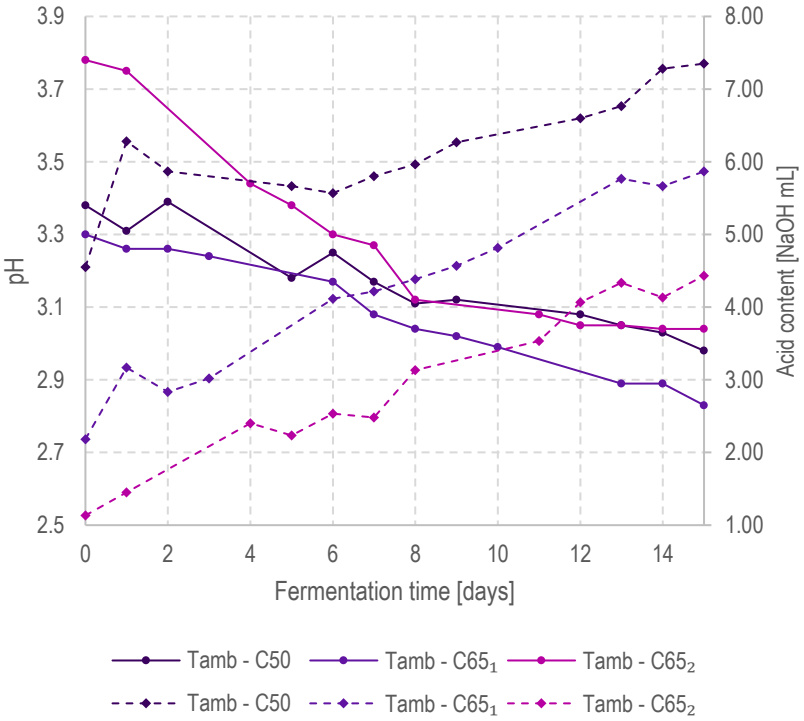


Figure 27. pH and acid content by titration evolution during the first 15 days of fermentation with same work temperature (room temperature, 21±3).
(1) pH representations stand for the continuous lines with doted markers
(2) Acid content representations stand for dashed lines with rhombus markers

In conclusion, for experiments at 35 ºC a remarkable effect of substrate concentration is noted while for room temperature, that effect is not visualized with the obtained results.

5.2.3. Time effect

By tasting the homemade experiments, an interval of the evaluated parameters was determined with the optimal taste range, which can be observed in table 4. Lower values of the indicated minimum suggested a sweetened, not yet fermented, taste, while higher values indicated a sour mixture not pleasant for consumption.

Table 4: Appropriate taste intervals for pH, density, acid and sugar content from Tamb-C65$_1$ and Tamb-C65$_2$ organoleptic test.

	pH	***Density*** *[kg/m3]*	***Acid content*** *[mL NaOH]*	***Sugar content*** *[i.r.; Brix%]*	
MIN.	*2.89*	1019.196	3.13	1.3391	4.25
MAX.	*3.12*	1030.085	5.67	1.340	4.8

With these ranges in mind, a comparison with the 28 ºC fermentations was conducted to observe the time needed for such fermentations to go on to accomplish appropriate taste and therefore end the process at the optimal moment. The in-range values came between days 4 and 14 of fermentation, while all parameters in-range values share days 6 and 7 of fermentation.

Table 5.T28-C65$_1$ and T28-C65$_2$ results with colored zone referring to values inside the appropriate taste range from table 4.

T28 - C65$_1$						***T28 - C65$_2$***					
Ferm, days	**pH [-]**	**Density** [g]	**Acid content** [mL NaOH]	**Sugars** [i.r - % Brix]		Ferm. Days	**pH [-]**	**Density** [g]	**Acid content** [mL NaOH]	**Sugars** [i.r - % Brix]	
0	3.74	1033.93	1.32	1.3405	5.2	*0*	3.74	1033.93	1.32	1.3405	5.2
1	3.59	1041.44	2.07	1.3409	5.4	*1*	3.57	1042.45	1.98	1.3409	5.4
4	3.23	1032.24	3.55	1.3406	5.25	*4*	3.29	1029.19	2.88	1.3406	5.25
5	3.16	1025.83	4.03	1.3397	4.6	*5*	3.18	1026.71	3.18	1.3399	4.75
6	3.1	1029.55	4.62	1.3391	4.25	*6*	3.12	1029.00	3.93	1.3395	4.5
7	3.06	1025.82	5.07	1.3391	4.25	*7*	3.06	1025.76	4.83	1.3394	4.4
8	2.98	1022.85	5.83	1.3385	3.8	*8*	2.97	1025.13	5.63	1.3388	4
11	2.87	1023.51	8.40	1.3374	3	*11*	2.82	1023.27	8.57	1.3376	3.25
12	2.8	1021.74	9.14	1.3374	3	*12*	2.78	1025.09	9.90	1.337	2.75
13	2.8	1021.52	10.90	1.337	2.75	*13*	2.78	1021.94	11.02	1.3368	2.7
14	2.81	1019.33	11.13	1.337	2.75	*14*	2.77	1020.24	12.10	1.337	2.75
15	2.8	1017.57	12.60	1.3362	2.25	*15*	2.77	1016.20	12.87	1.3367	2.5

5.2.4. Replicates

As shown in table 3, all experiments have one replicate except Tamb-C50. Moreover, the results obtained with each replicate have similarities in some aspects and differ in others. In general terms, the replicates exhibited similar trends in all cases, despite having significant discrepancies in numeric results. Since kombucha fermentation involves a wide variety of reactions that result in irregularities in the composition of the fermenters, these discrepancies could be justified by the complexity of the analyzed fermentation.

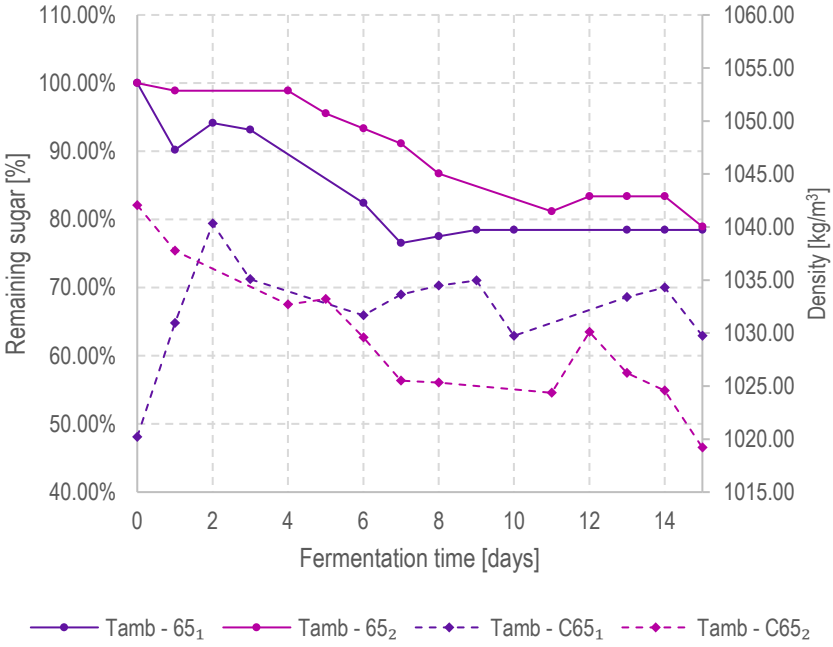


Figure 28: Density and sugar content for the 15 days period time of experiments Tamb-C65₁ and Tamb-C65₂.
(1) Sugar content representations stand for the continuous lines with doted markers
(2) Density representations stand for dashed lines with rhombus markers

The most irregular replicate was the one conducted at room temperature with initial sucrose of 65 g/L. As seen in figure 28, where density and sugar content from such experiments are represented, adopting an irregular evolution in time. In contrast, figure 29 presents the two replicates conducted at 28 ºC where the obtained results were like one another, although some irregularities are also observed.

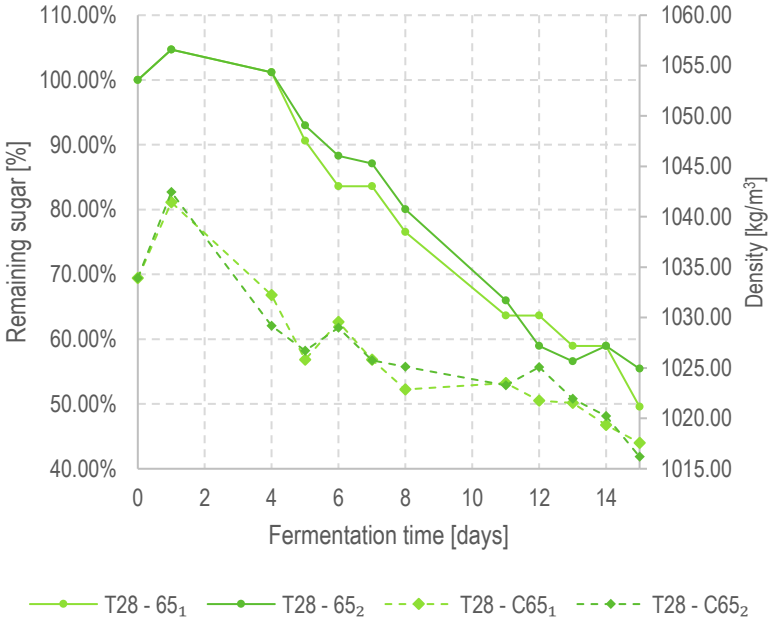


Figure 29. Density and sugar content for the 15 days period time of experiments Tamb-C651 and Tamb-C652.

(1) Sugar content representations stand for the continuous lines with doted markers

(2) Density representations stand for dashed lines with rhombus markers

6. CONCLUSIONS

After the effectuated experimental part, it can be said that temperature does have a significative effect on the fermentation of kombucha. Jayabalan (2014) stated that the optimal range was between 20 and 30 ºC, while the experiments conducted align with this data. At temperatures of 35°C, the reaction was less favorable and inconsistent, with odd fluctuations in sugar and density levels. However, at room temperature and 28°C, a more notable evolution was observed. The latter temperature, 28°C, has shown the best compatibility with the experiment and yielded more consistent results.

As for the initial substrate quantity, the results were not entirely conclusive. In the experiments conducted at 35°C, a change in substrate increase was observed, while those performed at room temperature did not show any observable changes. Since it was observed that the reaction is not favored at 35°C, it can be concluded that the effect of substrate concentration is relatively minor, despite having a positive effect in some experiments.

Moreover, after the organoleptic test, some values of the evaluated parameters are now attributed to a favorable taste in kombucha. Although it is highly recommended to taste the kombucha batch to know how its fermentations are evolving, these values can be of help for following fermentations at home. pH ranges from 3.12 to 2.89, while density moved between 1030 and 1019 kg/m^3. Acid content varied between 3.13 and 5.67 NaOH mL, while sugar content varied from 1.340 to 1.3391.

Finally, the replicates of the fermentation together with the results indicate a high complexity in the fermentation, combining several organic compounds reacting and interacting between them, generating some discrepancies difficult to justify.

REFERENCES AND NOTES

Admin. (n.d.). *Global Sugar Markets*. Gro Intelligence. https://www.gro-intelligence.com/insights/global-sugar-markets

Admin. (2023). What Is Your Kombucha pH Telling You? *Precision Laboratories*. https://www.preclaboratories.com/kombucha-ph-telling/

Alex, H. A., & Alex, H. A. (2022). Kombucha, Vitamins & the Rise of Nutritionism. *Kombucha Kamp*. https://www.kombuchakamp.com/kombucha-benefits-vitamin-content-and-bioavailability-in-nutrition

Antolak, H., Piechota, D., & Kucharska, A. (2021). Kombucha Tea—A Double Power of Bioactive Compounds from Tea and Symbiotic Culture of Bacteria and Yeasts (SCOBY). *Antioxidants*, *10*(10), 1541. https://doi.org/10.3390/antiox10101541

Bauer-Petrovska, B., & Petrushevska-Tozi, L. (2000). Mineral and water soluble vitamin content in the Kombucha drink. *International Journal of Food Science and Technology*, *35*(2), 201–205. https://doi.org/10.1046/j.1365-2621.2000.00342.x

Bishop, P., Pitts, E., Budner, D. M., & Thompson-Witrick, K. A. (2022a). Chemical Composition of Kombucha. *Beverages*, *8*(3), 45. https://doi.org/10.3390/beverages8030045

Bishop, P., Pitts, E., Budner, D. M., & Thompson-Witrick, K. A. (2022b). Kombucha: Biochemical and microbiological impacts on the chemical and flavor profile. *Food Chemistry Advances*, *1*, 100025. https://doi.org/10.1016/j.focha.2022.100025

Bond, S. (2023). The Best Sugar For Kombucha. *Brew Buch*. https://brewbuch.com/best-sugar-for-kombucha/

Booch Nesws. (2019). Guest Posting: Scaling up your Homebrew Kombucha Operation, by Stout Tanks and Kettles, LLC. *'Booch News*. https://www.boochnews.com/2019/04/13/guest-posting-scaling-up-your-homebrew-kombucha-operation-by-stout-tanks-and-kettles-llc/#:~:text=The%20fermented%20kombucha%20is%20transferred,%2C%20bottles%2C%20cans%2C%20etc.

Brewing Equipment - Commercial Brewing & Home Brewing. (n.d.). https://conical-fermenter.com/

Chen, C., & Liu, B. X. (2000). Changes in major components of tea fungus metabolites during prolonged fermentation. *Journal of Applied Microbiology*, *89*(5), 834–839. https://doi.org/10.1046/j.1365-2672.2000.01188.x

Crum, H., & LaGory, A. (2016). *The Big Book of Kombucha: Brewing, Flavoring, and Enjoying the Health Benefits of Fermented Tea*. Storey Publishing.

Dufresne, C., & Farnworth, E. R. (2000). Tea, Kombucha, and health: a review. *Food Research International*, *33*(6), 409–421. https://doi.org/10.1016/s0963-9969(00)00067-3

Frigate Projects. (2022, September 20). *Automatic Bottle Filling and Cap Closing Machine - Frigate*. Frigate. https://frigateprojects.com/shop/projects/pneumatic/automatic-bottle-filling-and-cap-closing-machine/

Goh, W. N., Rosma, A., Kaur, B., Fazilah, A., Karim, A., & Bhat, R. (2012). Fermentation of black tea broth (Kombucha): I. Effects of sucrose concentration and fermentation time on the yield of microbial cellulose. *International Food Research Journal*, *19*(1), 109–117. http://www.ifrj.upm.edu.my/19%20(01)%202011/(15)IFRJ-2011-105%20Rajeev.pdf

Hfmphe. (2023). Plate Heat Exchanger Design - How to Design a Heat Exchanger. *Hofmann Industry, Plate Heat Exchanger & Fluid Handling Supply Platform*. https://www.hofmann-heatexchanger.com/phe-service/plate-heat-exchangers-design

Insider. (2018, July 27). *How Kombucha Is Made* [Video]. YouTube. https://www.youtube.com/watch?v=l078iZpF9wg

Jarrell, J. A., Cal, T., & Bennett, J. V. (2000). The Kombucha consortia of yeasts and bacteria. *Mycologist*, *14*(4), 166–170. https://doi.org/10.1016/s0269-915x(00)80034-8

Jayabalan, R., Malbaša, R. V., Lončar, E. S., Vitas, J., & Sathishkumar, M. (2014). A Review on Kombucha Tea-Microbiology, Composition, Fermentation, Beneficial Effects, Toxicity, and Tea Fungus. *Comprehensive Reviews in Food Science and Food Safety*, *13*(4), 538–550. https://doi.org/10.1111/1541-4337.12073

Kitwetcharoen, H., Phung, L. T., Klanrit, P., Thanonkeo, S., Tippayawat, P., Yamada, M., & Thanonkeo, P. (2023). Kombucha Healthy Drink—Recent Advances in Production, Chemical Composition and Health Benefits. *Fermentation*, *9*(1), 48. https://doi.org/10.3390/fermentation9010048

Kombucha 101 - Pick the right tea for brewing. (n.d.). https://www.teatulia.com/brewing/kombucha-101-pick-the-right-tea-for-brewing#:~:text=The%20most%20important%20ingredient%3F,colony%20so%20it%20continues%20thrive.

Kombucha Market Share, Size, Growth and Forecast to 2031. (n.d.). https://www.insightaceanalytic.com/report/global-kombucha-market/1052

Kombucha Market Size Global Report, 2022 - 2030. (n.d.). Polaris. https://www.polarismarketresearch.com/industry-analysis/kombucha-market

Kombucha Market Size, Share & Trends Analysis Report By Product (Conventional, Hard), By Distribution Channel (On-trade, Off-trade), By Region, And Segment Forecasts, 2022 - 2030. (n.d.). https://www.grandviewresearch.com/industry-analysis/kombucha-market

Kumar, V., & Joshi, V. (2016). *Kombucha*: Technology, Microbiology, Production, Composition and Therapeutic Value. *International Journal of Food and Fermentation Technology*, *6*(1), 13. https://doi.org/10.5958/2277-9396.2016.00022.2

Las ventas de kombucha crecen un 580% en dos años. (n.d.). https://www.revistainforetail.com/noticiadet/las-ventas-de-kombucha-crecen-un-580-en-dos-anos/4624c46d90561d0232e691b2a1aa7923

Mamlouk, D., & Gullo, M. (2013). Acetic Acid Bacteria: Physiology and Carbon Sources Oxidation. *Indian Journal of Microbiology*, *53*(4), 377–384. https://doi.org/10.1007/s12088-013-0414-z

Matejčeková, Z., Liptáková, D., Spodniaková, S., & Valík, Ľ. (2016). Characterization of the growth of *Lactobacillus plantarum* in milk in dependence on temperature. *Acta Chimica Slovaca*, *9*(2), 104–108. https://doi.org/10.1515/acs-2016-0018

Mei Leaf. (2020, February 1). *TOUR A COMMERCIAL KOMBUCHA BREWERY - See how JARR make their booch!* [Video]. YouTube. https://www.youtube.com/watch?v=jLyVONKQcfA

Palmer, J., & Kaminski, C. (2013). *Water: A Comprehensive Guide for Brewers*. Brewers Publications.

Pawar, A. (n.d.). *Kombucha Brewers International*. Kombucha Brewers International. https://kombuchabrewers.org/

Redirect Notice. (n.d.-a). https://www.google.com/url?sa=i&url=https%3A%2F%2Fwww.sciencedirect.com%2Ftopics%2Fimmunology-and-microbiology%2Fzygosaccharomyces-bailii&psig=AOvVaw1Xyo-_yKRZV-hPXyNjqR0K&ust=1684604124219000&source=images&cd=vfe&ved=0CBMQjhxqFwoTCND2-Lr4gf8CFQAAAAAdAAAAABAI

Redirect Notice. (n.d.-b). https://www.google.com/url?sa=i&url=https%3A%2F%2Fscoiattolorampante.wordpress.com%2F2016%2F01%2F30%2Fcorso-microbiologia-alimentare-muffe-e-lieviti%2Ffig-1-saccharomycodes-ludwigii-lievito-isolato-dal-vino%2F&psig=AOvVaw1OdD0Csz6o-AfcCyegQh_f&ust=1684604791716000&source=images&cd=vfe&ved=0CBMQjhxqFwoTCJCxz4P4gf8CFQAAAAAdAAAAABAY

Redirect Notice. (n.d.-c). https://www.google.com/url?sa=i&url=https%3A%2F%2Fwww.sciencedirect.com%2Ftopics%2Fagricultural-and-biological-sciences%2Fbrettanomyces-bruxellensis&psig=AOvVaw0bl4G7IKRcDYEy4azg5Ecl&ust=1684604862332000&source=images&cd=vfe&ved=0CBMQjhxqFwoTCIiGlKX4gf8CFQAAAAAdAAAAABAE

Redirect Notice. (n.d.-d). https://www.google.com/url?sa=i&url=https%3A%2F%2Fwww.indiamart.com%2Fproddetail%2Factivated-carbon-filters-22559468597.html&psig=AOvVaw2HQfxecf-EA5v__hbCqqlK&ust=1685467907322000&source=images&cd=vfe&ved=0CBEQjRxqFwoTCLjktrGHm_8CFQAAAAAdAAAAABB3

Salvadó, Z., Arroyo-López, F. N., Guillamón, J. M., Salazar, G., Querol, A., & Barrio, E. (2011). Temperature Adaptation Markedly Determines Evolution within the Genus *Saccharomyces*. *Applied and Environmental Microbiology*, *77*(7), 2292–2302. https://doi.org/10.1128/aem.01861-10

Savary, O., Mounier, J., Thierry, A., Poirier, E., Jourdren, J., Maillard, M., Penland, M., Decamps, C., Coton, E., & Coton, M. (2021). Tailor-made microbial consortium for Kombucha fermentation: Microbiota-induced biochemical changes and biofilm formation. *Food Research International*, *147*, 110549. https://doi.org/10.1016/j.foodres.2021.110549

Sharma, V., Bhattacharya, A., Kumar, A., & Hl, S. (2007). Health Benefits of Tea Consumption. *Tropical Journal of Pharmaceutical Research*, *6*(3). https://doi.org/10.4314/tjpr.v6i3.14660

Stevens, N. (2003). *Kombucha: el té extraordinario*. Sirio Editorial.

Stewart, P. S. (1996). Theoretical aspects of antibiotic diffusion into microbial biofilms. *Antimicrobial Agents and Chemotherapy*, *40*(11), 2517–2522. https://doi.org/10.1128/aac.40.11.2517

Tran, T., Grandvalet, C., Verdier, F., Martin, A., Alexandre, H., & Tourdot-Maréchal, R. (2020). Microbiological and technological parameters impacting the chemical composition and sensory quality of kombucha. *Comprehensive Reviews in Food Science and Food Safety*, *19*(4), 2050–2070. https://doi.org/10.1111/1541-4337.12574

Troitino, C. (2017, February 1). Kombucha 101: Demystifying The Past, Present And Future Of The Fermented Tea Drink. *Forbes*. https://www.forbes.com/sites/christinatroitino/2017/02/01/kombucha-101-demystifying-the-past-present-and-future-of-the-fermented-tea-drink/?sh=7df156a04ae2

Villarreal-Soto, S. A., Beaufort, S., Bouajila, J., Souchard, J., & Taillandier, P. (2018). Understanding Kombucha Tea Fermentation: A Review. *Journal of Food Science*, *83*(3), 580–588. https://doi.org/10.1111/1750-3841.14068

Vitamins & Minerals in Milk | MilkFacts.info. (n.d.). http://www.milkfacts.info/Milk%20Composition/VitaminsMinerals.htm

Walton, J. (2022). The 5 Countries That Produce the Most Sugar. *Investopedia*. https://www.investopedia.com/articles/investing/101615/5-countries-produce-most-sugar.asp

Wang, B., Shao, Y., Chen, T., Chen, W., & Chen, F. (2015). Global insights into acetic acid resistance mechanisms and genetic stability of Acetobacter pasteurianus strains by comparative genomics. *Scientific Reports*, *5*(1). https://doi.org/10.1038/srep18330

Watawana, M. I., Jayawardena, N., Gunawardhana, C. B., & Waisundara, V. Y. (2015). Health, Wellness, and Safety Aspects of the Consumption of Kombucha. *Journal of Chemistry*, *2015*, 1–11. https://doi.org/10.1155/2015/591869

ACRONYMS

ONU – Organització de les Nacions Unides

ODS – Objectius de desenvolupament sostenible

SCOBY – Symbiotic culture of bacteria and yeast

B.C. – Before Christ

A.C. – After Christ

USD – United States dollar

CAGR – Compound Annual Growth Rate

ABV – Alcohol by volume

CFU – Colony forming units

AAB – Acetic Acid Bacteria

CO_2 – Carbon dioxide

r.i.– Refractive index

NaOH – Sodium hydroxide

Min. – Minimum

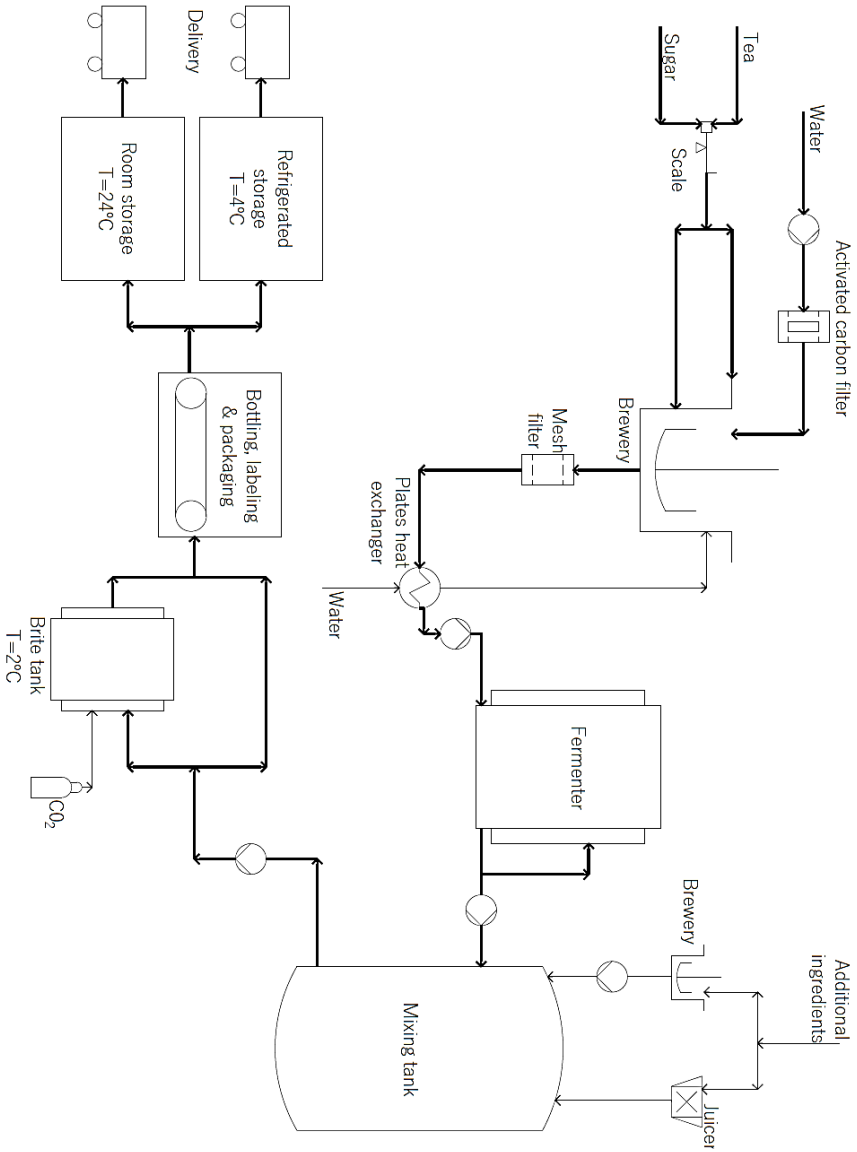
Max. – Maximum

Ferm. days – Fermented days

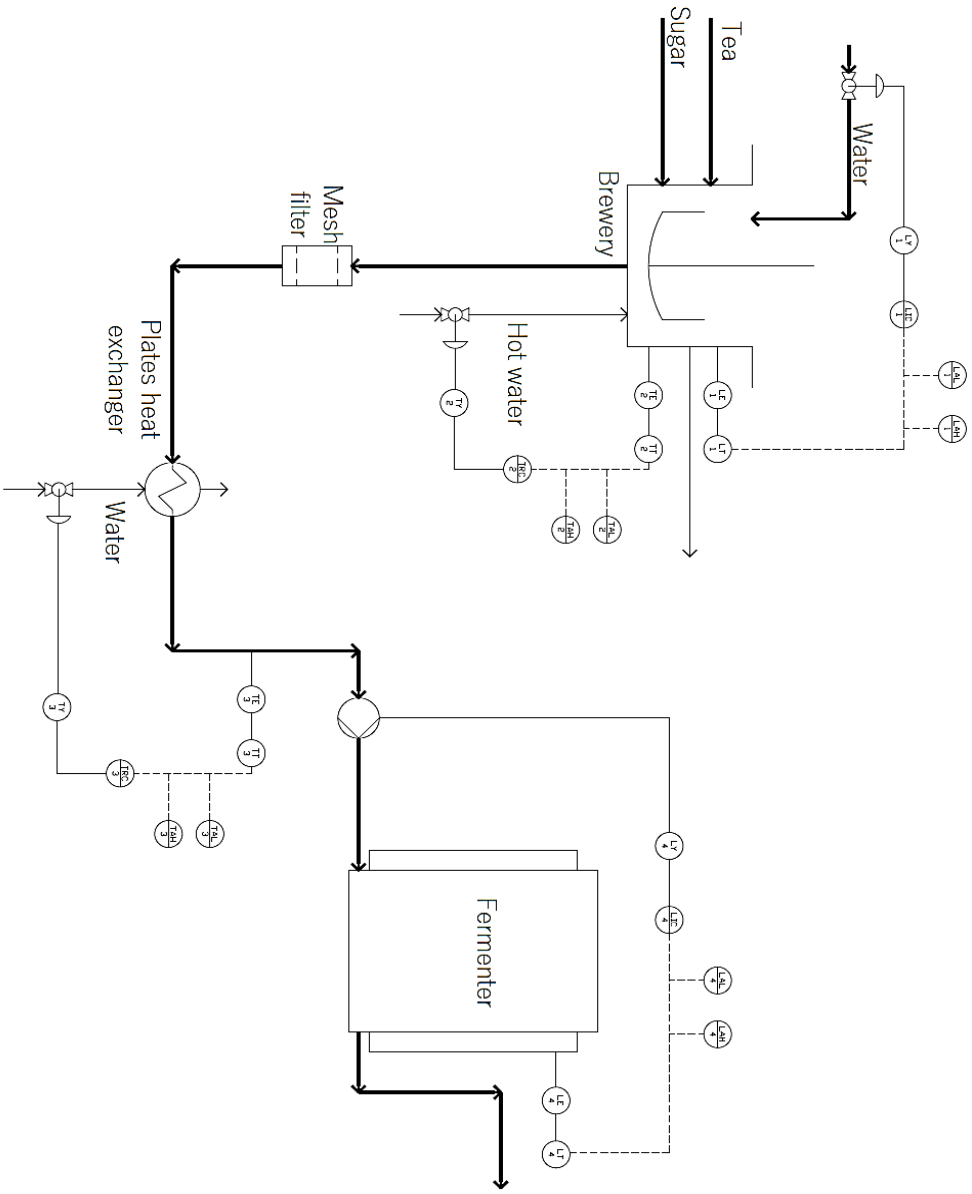
Ref. – Reference

APPENDICES

APPENDIX 1: PFD



APPENDIX 2: PID PROPOSAL FOR KOMBUCHA BREWERY AUTOMATIZATION



APPENDIX 3: DENSITY AND SUGAR CONTENT FOR REPLICATE EXPERIMENT

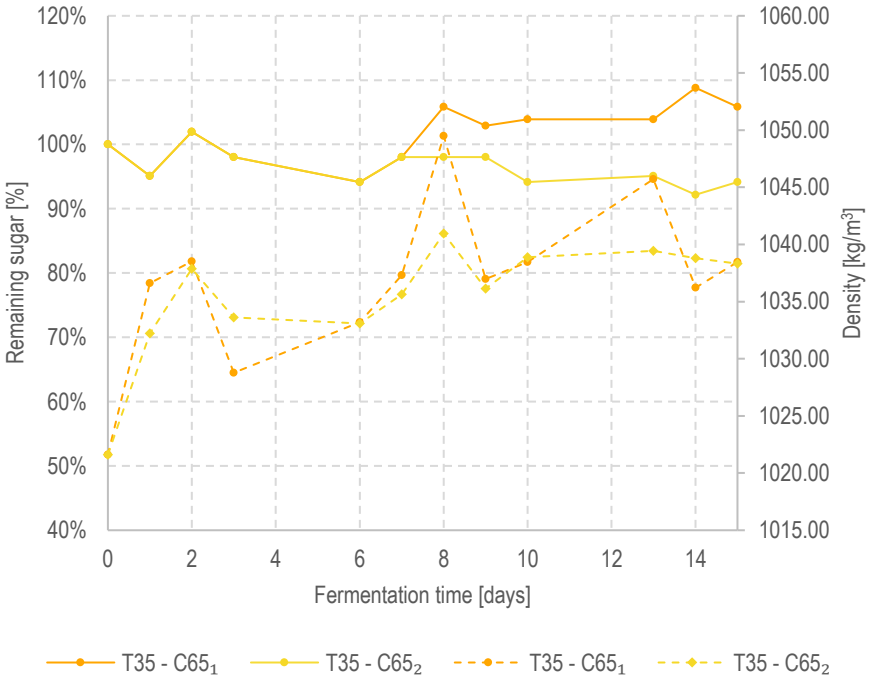


Figure 30. Density and sugar content for the 15 days period time of experiments T35-C65₁ and T35-C65₂.
(1) Sugar content representations stand for the continuous lines with doted markers
(2) Density representations stand for dashed lines with rhombus markers

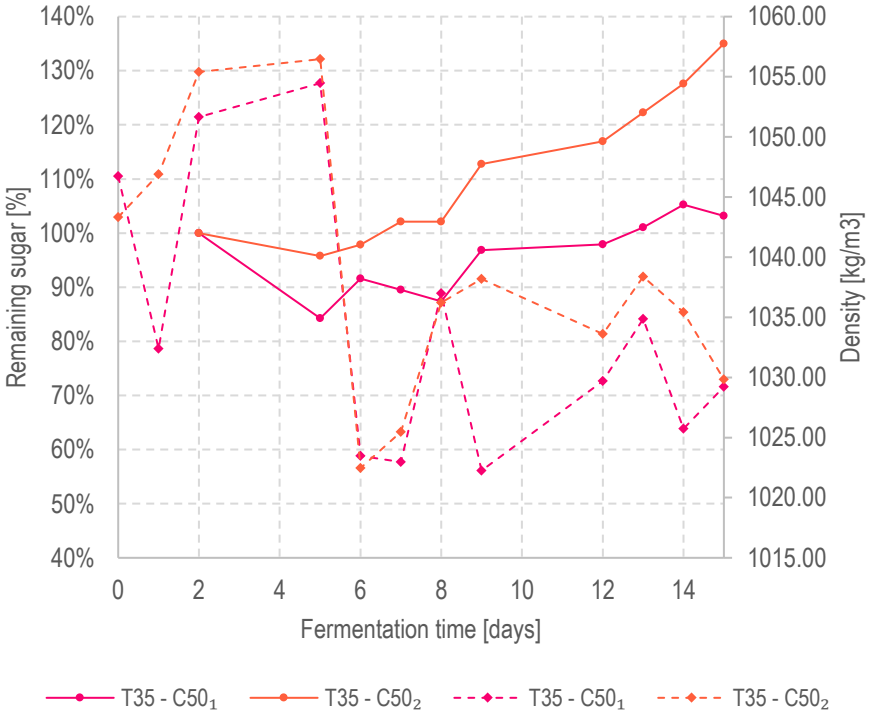


Figure 31. Density and sugar content for the 15 days period time of experiments T35-C50₁ and T35-C50₂.
(1) Sugar content representations stand for the continuous lines with doted markers
(2) Density representations stand for dashed lines with rhombus markers

APPENDIX 4: ACID CONTENT TITRATION AND PH VALUES FOR REPLICATE EXPERIMENTS

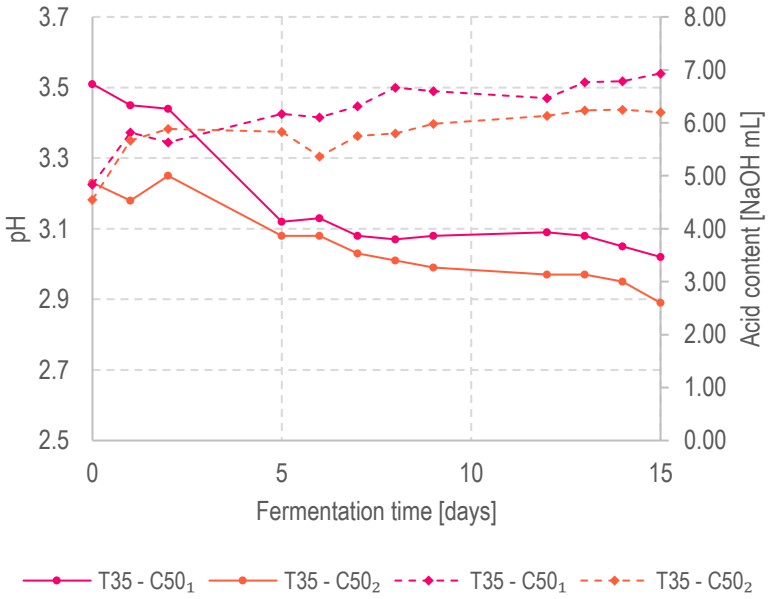


Figure 32. pH and acid content by titration evolution during the first 15 days of fermentation with same work temperature (35 ºC), T35-C50₁ and T35-C50₂
(1) pH representations stand for the continuous lines with doted markers
(2) Acid content representations stand for dashed lines with rhombus markers

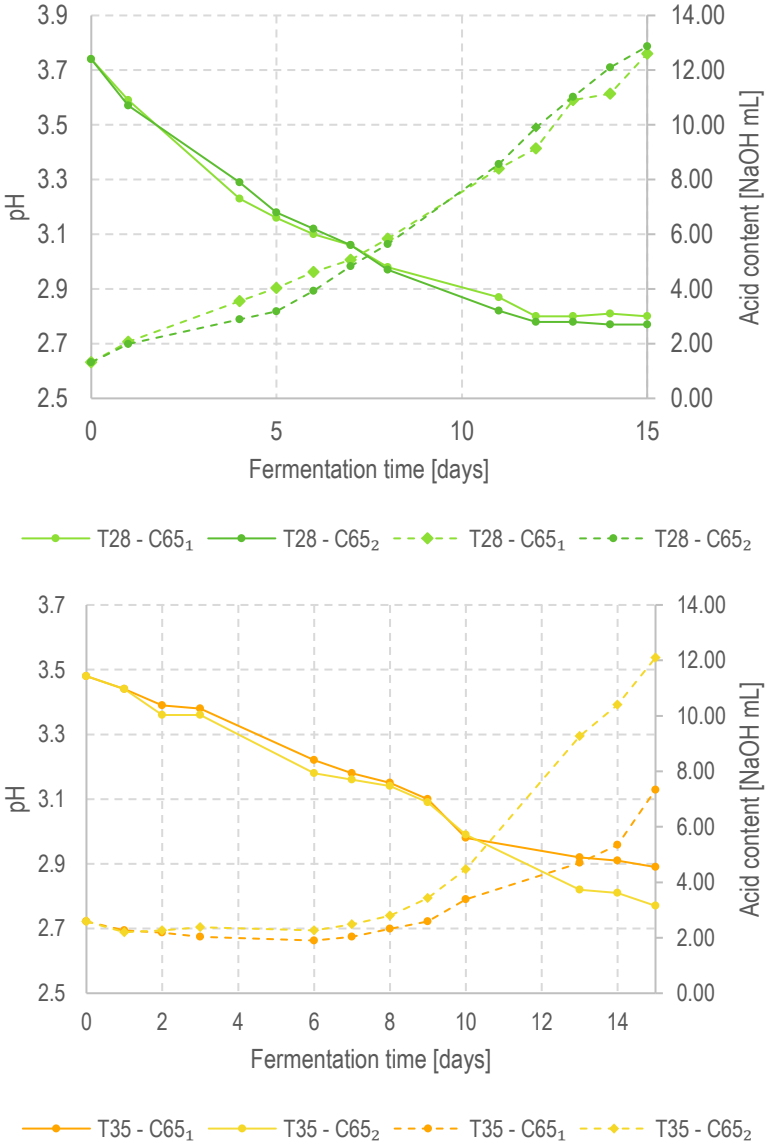


Figure 33. pH and acid content by titration evolution during the first 15 days of fermentation with same work temperature (35 ºC), T28-C65$_1$, T28-C65$_2$, T35-C65$_1$ and T35-C65$_2$
(1) pH representations stand for the continuous lines with doted markers
(2) Acid content representations stand for dashed lines with rhombus markers

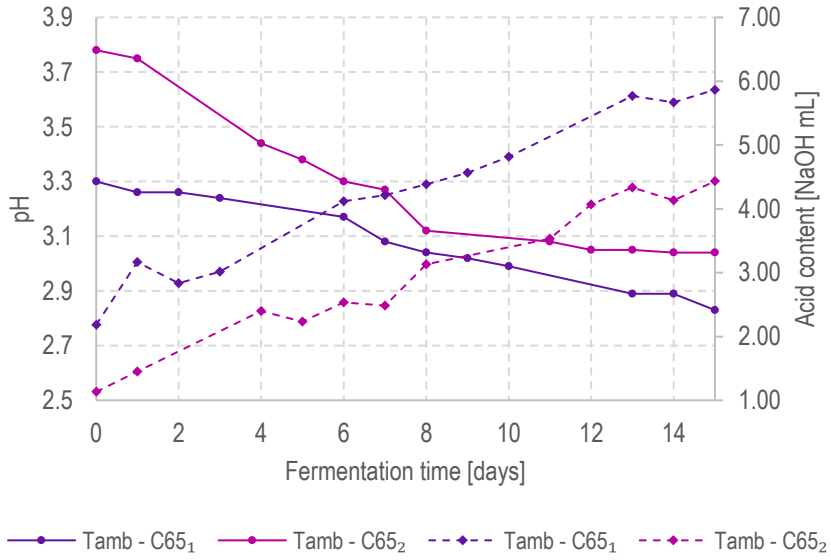
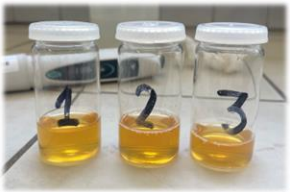


Figure 34. pH and acid content by titration evolution during the first 15 days of fermentation with same work temperature (35 ºC), Tamb-C65$_1$ and Tamb-C65$_2$
(1) pH representations stand for the continuous lines with doted markers
(2) Acid content representations stand for dashed lines with rhombus markers

APPENDIX 5: PHOTOGRAPHIC COMPILATION OF THE EXPERIMENTAL PART

EXPERIMENTAL DESIGN



SCOBY

HOMEMADE FLAVORING AND BOTTLING