Scientific Bulletin. Series F. Biotechnologies, Vol. XXIX, No. 1, 2025
ISSN 2285-1364, CD-ROM ISSN 2285-5521, ISSN Online 2285-1372, ISSN-L 2285-1364

RESEARCH ON THE VALORISATION OF PLANT-BASED BY-PRODUCTS TO PRODUCE FOOD LINKED TO THE CIRCULAR ECONOMY CONCEPT

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Abstract

Circular economy is a sustainable approach that can help reduce waste, by valorising by-products and other materials for obtaining new products or for obtaining value added compounds. The aim of this study is to present a potential valorisation of various plant by-products through the brewing of Kombucha teas. This approach not only contributes to reducing food waste but also encourages the creation of more sustainable food systems by promoting resource efficiency. The plant-based Kombuchas were prepared separately using grape, lemon, broccoli, green tea and apple by-products. After the required aerobic fermentation process, the obtained samples showed regular pH values (3.3-3.5), Brix values (5.6-10.9), ethanol levels (<3 g/100 mL) and acidity levels (< 0.8% acetic acid). All of the samples, which were sterilised at 75 and 98°C respectively, showed no signs of any bacteria (Enterobacteriaceae and Listeria monocytogenes colonies) or fungi (yeasts and molds colonies). The results showed that discarded plant by-products have the potential to be used to obtain refreshing and natural Kombucha drinks.

Key words*: circular economy, valorisation, food residues, Kombucha tea, plant by-products.*

INTRODUCTION

Food waste is an increasingly serious problem leading to many future negative consequences such as severe environmental degradation, significant economic losses and social inequalities (Peng et al., 2024).
A 2011 report by the Food and Agriculture Organization (FAO) states that approximately one-third of the food produced globally is thrown away or wasted (Gustavsson et al., 2011).
In 2021, members of the United Nations Environment Programme (UNEP) estimate that 931 million tonnes of food, or 17% of the total food available to consumers in 2019, went into the waste bins of households, retailers, restaurants and other food services. According to new UN, research has been conducted to support global efforts to halve food waste by 2030 through the Food Waste Index report (UNEP, 2021).
In this context, the objective of this paper is to highlight the valorisation of agro-food by-products in the context of food preservation especially in the case of fruits and vegetables. This study strongly relates to the premises of circular economy by aiming to show beneficial and healthy alternatives to wasting precious resources.

MATERIALS AND METHODS

Materials

The materials utilized for the research were obtained from various suppliers in the Murcia region. The by-products that were intended to be discarded were: whole grapes, Verna lemon peels, broccoli stems, commercial green tea bags and Jonagold apple peels (a cross between Jonathan and Golden varieties).
To prepare the Kombucha tea, it has been used a liquid which was obtained by homogenizing the waste materials in an industrial mixer and removing the solids and all the polyphenols through an osmotic nanofiltration process. Afterwards, this liquid was boiled at 98°C for 2-3 minutes to sterilize it and let it cool down at a temperature of around 6°C for 2 hours. It is very important that it is left to cool off as to not kill the microorganisms which will be added later on.

Methods and equipment

In order to use the liquid in making tea, it must have a certain amount of sugar in its composition. By using a refractometer (Figure 1), the evolution of the Brix values of the beverages has been determined. These represent the number of solid compounds dissolved in a liquid and are commonly used to measure the dissolved **sugar content** of an aqueous solution.

Figure 1. The Brix Refractometer

The value of one Brix degree is the equivalent of one gram of sucrose in 100 grams of solution and represents the strength of the solution as part of a percentage by weight (% w/w). If the solution contains dissolved solids other than pure sucrose, then the °Bx only approximates the dissolved solid content. (Ryan, 2013)

By adding sugar to enhance the concentration level of the liquid, the Brix value was adjusted between 9 and 12°Bx (Table 1).

Table 1. The added sugar that was needed for each Kombucha tea

Sample	Added sugar (g)	Initial Brix value (°Bx)	Final Brix value (°Bx)
Grape	100	9	11
Lemon	0	12.2	12.2
Broccoli	640	3	10.6
Green Tea	140	0	10.9
Apple	250	3	10.8

During two weeks of incubation, 100 mL of each sample was collected every day under aseptic conditions using a micropipette and plastic sample containers. The analyses resumed two weeks after bottling and consisted in determinations such as: pH value, Brix degrees, total acidity, the amount of ethanol produced, the total *Enterobacteriaceae* colonies, the presence of *Listeria monocytogenes*, yeast and mold colonies, and the total number of lactic acid bacteria.

The **pH values** of all samples were measured every day using a pH meter, after which it was calibrated accordingly with sodium hydroxide (NaOH) representing an alkaline solution, and with hydrochloric acid (HCl) representing an acidic solution respectively. The electrode was kept immersed in the sample until the final result was displayed on the screen of the measuring device.

The **total acidity** values were determined by titrating the distillate sample with a 0.1 N sodium hydroxide (NaOH) solution in the presence of the indicator "phenolphthalein" under continuous stirring. The final values resulted from applying the mathematical formulas (Aveiga, 2024).

The **amount of ethanol** in the samples has been determined using a 785nm excitation laser with a Raman spectroscopy (Lucindo et al., 2023).

The method for the determination of the **total *Enterobacteriaceae* colonies** involves homogenization and serial decimal dilutions to ensure uniform distribution of microorganisms. In a Petri dish, 1 mL + 2% of the sample is inoculated into the culture media using the "lawn" technique with a Drigalski loop. 10-15 mL of pre-tempered Rapid *Enterobacteriaceae* agar is added and mixed thoroughly to allow to solidify. The mix is overlayed with an additional 5-10 mL of the same medium to create semi-anaerobic conditions. After solidification, the plates are inverted and incubated at 37 ± 1°C for 24 ± 2 hours. The colonies are subsequently counted using a magnifying glass on plates with 10 to 300 colonies. The number of total *Enterobacteriaceae* is calculated using the formula:

$$Total\ Enterobacteriaceae = \frac{(No.\ of\ colonies * Dilution\ factor)}{Volume\ of\ liquid\ on\ the\ plate}$$

The final result is measured in CFU ("colony forming units")/mL (Silbernagel, 2002).

The **presence of *Listeria monocytogenes*** determination requires prior homogenization to ensure uniform distribution of bacteria in the samples. 25 ml of each sample is introduced into a nutrient broth medium enriched with BLEB (“Buffed Listeria Enrichment Broth”) to promote the growth and development of *Listeria monocytogenes*. The samples are incubated for 24-48 hours at 30°C. After incubation, a portion of each sample is transferred to another Fraser-type nutrient broth medium. A second incubation is carried out for 24-48 hours at 35-37°C. At the end of the second incubation, the samples are inoculated onto Oxford media containing lithium chloride and antibiotics to inhibit other bacteria, except *Listeria*, whose colonies will appear black coloured. The third and final incubation is carried out at 35-37°C for 24-48 hours. Finally, they are observed under a microscope according to the colour acquired and counted. The measurements are made in numbers of colonies/25 ml (Singh, 2025).

The method for determining **colonies of yeast and molds** requires preliminary homogenization and decimal dilutions for a uniform distribution of microorganisms in the samples. In a Petri dish 1 mL + 2% of the sample is inoculated into the culture media using the “lawn” technique with a Drigalski loop. 10–15 mL of pre-tempered Symphony Agar is added and mixed thoroughly to allow to solidify. After solidification, the plates are inverted and incubated upright at 25°C for 54-72 hours. The colonies are subsequently counted using a magnifying glass on plates with 10 to 150 colonies. The total number of yeast and mold colonies is calculated using the formula:

$$Total\ yeasts\ and\ molds = \frac{(No.\ of\ colonies * Dilution\ factor)}{Volume\ of\ liquid\ on\ the\ plate}$$

The final result is measured in CFU (“colony forming units”)/ml (Silbernagel, 2002).

The determination of the **total number of lactic acid bacteria** involves homogenization and serial decimal dilutions to ensure uniform distribution of microorganisms. In a Petri dish, 1 mL + 2% of the sample is inoculated into the culture media using the “lawn” technique with a Drigalski loop. 10-15 mL of selective MRS Agar (de Man, Rogosa and Sharpe) medium. The samples are incubated for 24-72 hours at 30-37°C. Is added and mixed thoroughly to allow to solidify. The mix is overlayed with an additional 5-10 mL of the same medium to create semi-anaerobic conditions. After solidification, the plates are inverted and incubated at 30 ± 1°C for 72 ± 3 hours. The colonies are subsequently counted using a magnifying glass on plates with 10 to 300 colonies. The number of total lactic acid bacteria is calculated using the formula:

$$Total\ lactic\ acid\ bacteria = \frac{(No.\ of\ colonies * Dilution\ factor)}{Volume\ of\ liquid\ on\ the\ plate}$$

The final result is measured in CFU (“colony forming units”)/mL (Silbernagel, 2002).

Product preparation

In a glass container it was added the liquid, a starter which is composed of nutrients and over 1 billion live microorganisms, promoting the development of the culture medium but also a SCOBY disc (“symbiotic culture of bacteria and yeast”) (Figure 2), where the SCOBY community creates a thick cellulose biofilm and a sparkling acidic broth with the infusion (El-Shall et al., 2023).

Figure 2. The SCOBY disc

The mixture is covered with a filter which allows both maintaining the sterility of the medium and its access to oxygen which is needed for the aerobic fermentation. The containers were stored in a dry and dark place at an ambient temperature.

After the 2 designated weeks for fermentation, the teas were divided into 3 distinct categories, each with the same amount of liquid. The first category was the blank sample to which no modification was applied. The second category was subjected to sterilisation by boiling for 1 minute at a temperature of 75°C, and the third at a temperature of 98°C (Figure 3).

Figure 3. Grape Kombucha tea bottles
at the three boiling temperatures

Comparison of the samples with commercial Kombucha tea

To make a proper comparison with the commercial products, a bottle of lemon Kombucha tea from a supermarket has been subjected to the same set of analyses as the samples prepared in the laboratory. Being lemon tea, the results were compared to those of the one sterilised at 98°C in the laboratory.

After the release of the microbiological analysis results, a sensorial examination was carried out on the samples sterilized at the 98°C temperature, and compared with the commercial lemon sample. The examination followed 3 sensory parameters, namely the taste, colour, and smell of the samples, as well as the global value represented by the average of the 3 parameters. The analysis was carried out on a group of approximately 50 untrained consumers, part of Murcia's Technological Center staff. The subjects appreciated each category with grades on a 5-point hedonic scale.

RESULTS AND DISCUSSIONS

After the first two weeks, due to the added microorganisms through the starter solution but also with the help of the optimal growth and development conditions created, through the aerobic fermentation process, the SCOBY disc grew on the surface of the liquids forming a gelatinous film around it with the role of ensuring a much more stable connection with the oxygen they need to survive (Figures 4 and 5).

Figure 4. The Grape Kombucha tea freshly
brewed on day 0

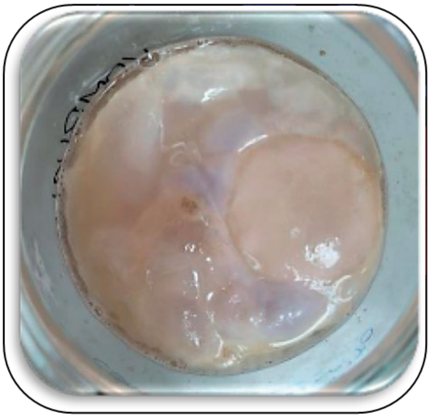

Figure 5. The Grape Kombucha tea on the 15th day
of fermentation

During the fermentation process, the acidity level of the samples increased, achieving a lower pH, which can also be observed by the formation of gas bubbles in the sample jars, with the lids slightly bulging from the accumulated pressure (Figure 6), due to the increasing level of carbon dioxide (CO_2) which also holds the SCOBY disc at the surface of the liquid throughout the entire process.

Figure 6. Lemon Kombucha sample on the 10th day of fermentation

After the initial fermentation timeframe of 15 days, both the SCOBY disc and other solid particles were removed and the liquid was poured into the bottles.

The following data has been obtained from the course of various determination methods during a total period of 5 weeks starting at the very beginning of the fermentation process (day 0) and towards the end of the 3 weeks of bottling (day 35).

Over time, the pH values of most samples had a favourable evolution. Due to the fermentation process, the acidity level in the media increased, resulting in a significant decrease in pH (Figure 7).

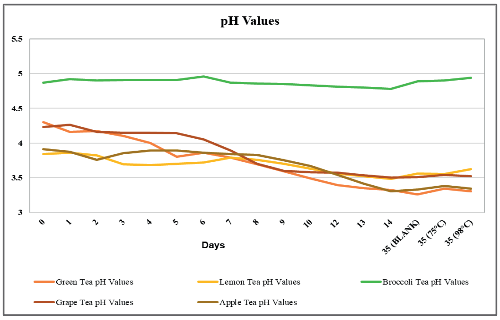

Figure 7. Evolution of pH levels throughout the fermentation period

Green, grape and apple teas showed the largest variations in 2 weeks, with values from 4.3 to 3.32, 4.23 to 3.5 and 3.91 to 3.3, respectively. These values are similar to other studies (Barbosa et al., 2021; Morales et al., 2023; Zubaidah et al., 2018). In their research, after 2 weeks of fermentation the pH values were: 3.8 for green tea (Barbosa et al., 2021), 2.8 for grape tea (Morales et al., 2023) and 3.00 for "Red Delicious" apple tea (Zubaidah et al., 2018). However, the evolution of the Broccoli Kombucha Tea proved to be problematic, because the fermentation process didn't occur. The explanation could be that, compared to the other samples, broccoli being a vegetable instead of a fruit, meant that its sugar content was too low to start a fermentation, requiring additional amounts to kick-start the process. As such, for future experiments, sugar must be added in order to have a proper fermentation.

The Brix values of the samples decreased during the fermentation, subsequently increasing back to their initial values for some of them (Figure 8).

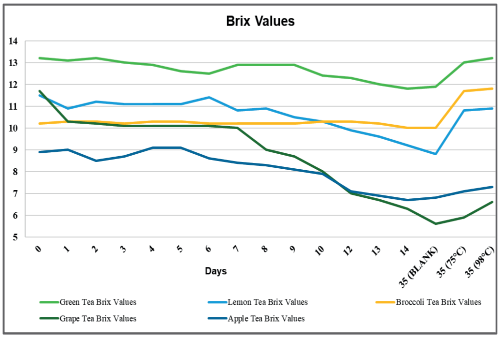

Figure 8. Evolution of Brix values throughout the fermentation period

For the lemon tea with values from 11.5° on day 0 to 10.9° on day 35, and for the green tea from 13.2° on day 0 to 13.2° on day 35. The largest decrease was observed in the grape tea, which from the initial value of 11.7° reached 5.6° for the blank sample after bottling. In regards to the apple teas evolution from 8.9° initially to 6.7° after 2 weeks, the results correlate to Zubaidah et al. (2018) once more, having achieved 6.00° for "Red Delicious" apple tea during the 14 days of fermentation.

The total acidity levels had a relatively slow initial increase during the first week of preparation (Figure 9).

Green tea, apple tea, and lemon tea had significantly different evolutions from each other during the fermentation period but reached similar values after bottling. The green tea had a relatively fast peace, increasing from 0.07% acetic acid to 0.16% acetic acid in seven fermentation days. The result is similar to that of Tu (2008) where the green tea had 0.12% acetic acid in a single week.

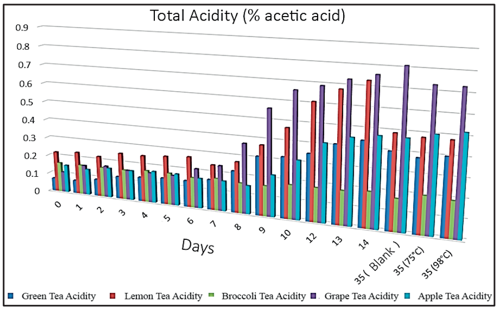

Figure 9. Total Acidity of the samples

Ethanol production was measured after a preliminary fermentation period of approximately 4-5 days. These values increased significantly up to 3 g/100 mL initially but decreased slightly with a maximum level of 1.5 g/mL lost after bottling (Figure 10).

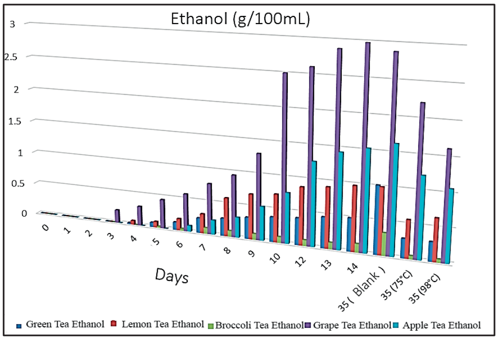

Figure 10. Ethanol production chart

The quick increase of the green tea from 0.02 g/100 mL on day 4 to 0.5 g/100 mL on day 14 is similar to the green tea of Barbosa et al. (2021) which had a total of 0.7 g/100 mL during the two weeks period.

As for the microbiological determinations, both for the presence of *Listeria monocytogenes* and the formation of *Enterobacteriaceae* colonies, the results indicated no signs of either of them in any of the analysed samples.

The formation of yeast and mold colonies was analysed after bottling the 5 types of teas. For the thermally treated samples, insignificant values of viable yeast and mold were detected, namely < 1 cfu/mL. As for the blank samples, the highest quantity was detected in the lemon tea, reaching a value of 780,000 cfu/mL (Figure 11).

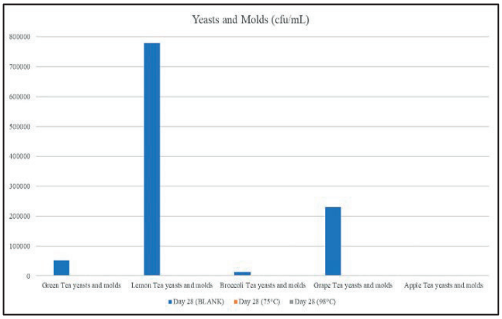

Figure 11. Total yeast and molds CFU before and after sterilization

Similar to the analyses performed to detect the formation of yeast and molds, for determining the total lactic acid bacteria, the samples subjected to boiling had insignificant values of < 1 cfu/mL. The most significant value was found in the grape tea, producing quantities of up to 2,400,000 cfu/mL of total lactic acid bacteria (Figure 12).

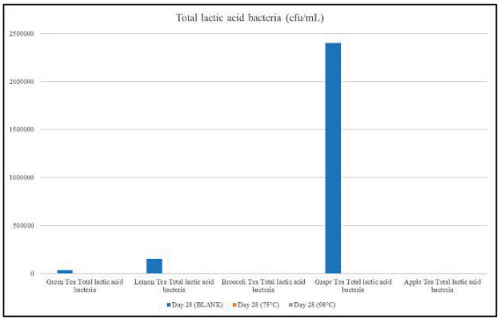

Figure 12. Total Lactic Acid Bacteria CFU before and after sterilization

The commercial lemon Kombucha tea was analysed similar to the sample teas obtained in the laboratory and the results were compared with the laboratory lemon tea sterilised at 98°C (Table 2).

The pH and Brix values were relatively similar, but the total acidity and ethanol values differ significantly indicating the presence of a possible acidity enhancer or different sweeteners that give the drink a more refreshing taste and a longer shelf life than in the case of the laboratory teas subjected to traditional and more natural preparation methods.

Table 2. Comparison between commercial lemon Kombucha tea and laboratory lemon Kombucha tea sterilised at 98°C

	Commercial lemon Kombucha tea	Laboratory lemon Kombucha tea
pH	3.8	3.62
Brix Values (°)	9.7	10.9
Total Acidity (% acetic acid)	0.7	0.48
Ethanol (g/100 mL)	1.82	0.64
Total *Enterobacteriaceae* (cfu/mL)	<1	<1
Listeria monocytogenes (/25 mL)	0	0
Yeast and Molds (cfu/mL)	<1	<1
Total Lactic Acid Bacteria (cfu/mL)	<1	<1

In addition, all the sample teas sterilized at 98°C and the commercial lemon Kombucha tea were subjected to a sensory analysis carried out with a panel of 50 untrained consumers. The examination followed 3 sensory parameters, namely taste, colour, and smell of samples, as well as the global value represented by the average of the 3 parameters. The subjects appreciated each category with grades on a 5-point hedonic scale. The results indicated that apple tea was the most overall appreciated having all 3 of the parameters with the most points compared to the others with a score of 4.23 points and the grape tea obtaining the second position with a score of 3.47 points (Table 3).

Table 3. Organoleptic examination results

Sample	Parameter	Result
Grape Kombucha	Smell	2.23
	Appearance	3.98
	Taste	4.22
	Global value	**3.47**
Lemon Kombucha	Smell	3.23
	Appearance	3.77
	Taste	2.92
	Global value	**3.30**
Broccoli Kombucha	Smell	1.88
	Appearance	3.13
	Taste	2.09
	Global value	**2.36**
Green Tea Kombucha	Smell	2.29
	Appearance	3.46
	Taste	4.08
	Global value	**3.27**
Apple Kombucha	Smell	4.01
	Appearance	4.12
	Taste	4.56
	Global value	**4.23**
Lemon Kombucha (commercial)	Smell	3.31
	Appearance	3.69
	Taste	2.92
	Global value	**3.30**

Regarding the other teas, except for Broccoli tea, they all achieved similar results with the commercial lemon Kombucha tea.

CONCLUSIONS

The results obtained indicate that the Kombucha samples represent an ideal example for highlighting the potential of a circular economy approach in the food and beverages sector. By providing them with the optimal growth and development conditions specific to an aerobic fermentation process, refreshing and natural drinks from food waste can be successfully obtained. The best results were registered for grape and apple teas, which were highlighted by their chemical and microbiological descriptors, as well as the overall organoleptic parameters (from the slight acidity to the sweet aroma specific for the fruit sample).

ACKNOWLEDGEMENTS

The materials, equipment, methods and results utilised belong and were carried out within the Power Plant Laboratory of the CTNC (Centro Tecnológico Nacional de la Conserva) of Molina de Segura, Spain during an Erasmus+ mobility program in the year of 2023. All rights reserved and my gratitude to the institute and the employees that have made these analyses possible.

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