Article **ECORFAN Journal-Ecuador**
June 2021, Vol.8 No.14 23-38

Production and effects of green tea Kombucha with blueberry and orange blossom honey without caffeine as probiotic inhibitor of pathogenic bacteria

Producción y efectos de Kombucha de té verde con arándano azul y miel de flor de azahar sin cafeína como probiótico inhibidor de bacterias patógenas

LAGUNA-MORALES, Leslie Asenat†, SANTIESTEBAN-LÓPEZ, Norma Angelica* and MALDONADO-RESÉNDIZ, Jorge Ángel

Benemérita Universidad Autónoma de Puebla. Facultad de Ciencias Biológicas, Puebla, Mexico.

ID 1st Author: *Leslie Asenat, Laguna-Morales* / **ORC ID:** 0000-0001-6103-1749

ID 1st Co-author: *Norma Angelica, Santiesteban-López* / **ORC ID:** 0000-0001-7700-4139, **CVU CONACYT ID:** 240825

ID 2nd Co-author: *Jorge Ángel, Maldonado-Reséndiz* / **ORC ID:** 0000-0003-1787-3660, **CVU CONACYT ID:** 1087863

DOI: 10.35429/EJE.2021.14.8.23.38 Received January 20, 2021; Accepted June 30, 2021

Abstract

Kombucha is a probiotic drink of Asian origin, whose symbiotic relationship between acetic bacteria and yeasts provides a wide range of compounds with antioxidant and antimicrobial power (against Gram-negative and Gram-positive bacteria); thus, improving digestion and preventing chronic diseases. Blueberry (*Vaccinium corymbosum*) of the *Ericaceae* family is one of the most studied and used fruits due to its high antioxidant benefits. In turn, orange blossom honey is one of several products rich in flavonoids obtained from the *Citrus sinensis* plant. Due to the high amount of phenolic compounds in these products, a homemade kombucha based on blueberry and orange blossom honey was developed to increase the beneficial properties of this probiotic, generating a value-added product. First, the tea fungus (SCOBY) was obtained in a caffeine-free medium of green tea and white sugar, through a process called "First fermentation". Then we performed a "Second fermentation" by adding the fruit and honey, giving it flavor and increasing its antioxidant properties. Finally, we tested it pleasant taste by means of a sensory evaluation of 30 people, which was statistically analyzed.

Kombucha, Caffeine-free, Green tea, Antioxidant

Resumen

La Kombucha, es una bebida probiótica de origen asiático, cuya relación simbiótica entre bacterias acéticas y levaduras aporta una amplia gama de compuestos con poder antioxidante y antimicrobiano (contra bacterias Gram negativas y Gram positivas); mejorando así la digestión y previniendo enfermedades crónicas. El arándano azul (*Vaccinium corymbosum*) de la familia *Ericaceae* es uno de los frutos más estudiados y utilizados debido a los beneficios de su alto poder antioxidante. A su vez, la miel de flor de Azahar es uno de los varios productos ricos en flavonoides obtenidos de la planta *Citrus sinensis*. Debido a la gran cantidad de compuestos fenólicos de estos productos, se desarrolló una kombucha casera a base de arándano azul y miel de flor de Azahar para aumentar las propiedades benéficas de este probiótico, generando un producto de valor agregado. Primero se obtuvo al hongo del té (SCOBY) en un medio sin cafeína de té verde y azúcar blanca, mediante el proceso denominado “Primera fermentación”. Después realizamos una “Segunda fermentación” agregado la fruta y miel, confiriéndole el sabor y aumentando sus propiedades antioxidantes. Finalmente, se probó la obtención de un sabor agradable, mediante una evaluación sensorial a 30 personas que se analizó estadísticamente.

Kombucha, Sin cafeína, Té verde, Antioxidante

Citation: LAGUNA-MORALES, Leslie Asenat, SANTIESTEBAN-LÓPEZ, Norma Angelica and MALDONADO-RESÉNDIZ, Jorge Ángel. Production and effects of green tea Kombucha with blueberry and orange blossom honey without caffeine as probiotic inhibitor of pathogenic bacteria. ECORFAN Journal-Ecuador. 2021. 8-14:23-38.

* Correspondence to Autor (E-mail: norma.santiesteban@correo.buap.mx)
† Researcher contributing as first author.

Introduction

One of the most important issues of our time is the strengthening of the immune system to resist or prevent diseases. Due to the current Covid-19 pandemic, several measures were taken to take care of the population and prevent the spread of the virus. According to the survey conducted by Sami et al. in 2021, out of 312 people surveyed in 2020, a total of 137 supported the consumption of functional foods to prevent an infection (Sami *et al.,* 2021).

Probiotics are a type of functional food based on microorganisms, whose consumption in adequate amounts, produces positive effects on human health. Their importance for the reinforcement of the immune system is thanks to the effects they exert through the brain and intestine, via the vagus nerve; these neuroendocrine pathways allow us to regulate not only the composition of the intestinal microbiota, which has immunomodulatory and anti-inflammatory functions, but also to demonstrate reductions in stress and depressive symptoms (Interián-Gómez *et al.,* 2021).

These foods also help in the regulation of homeostasis and the formation of regulatory T cells, which help in the production of metabolites that inhibit TNF-α (tumor necrosis factor alpha) and the cancer-related NF-kB protein complex (Darmawan *et al.,* 2020).

Due to these and other functions, the consumption of probiotics has become increasingly popular. Among these foods, we can highlight Kombucha, a probiotic beverage associated with protection against various pathologies produced by radical oxygen species (ROS), antimicrobial effects, detoxifying properties by accumulation of organic acids (such as acetic, gluconic, lactic and glucuronic acid), generation of bioactives such as vitamins, help against damage caused by antibiotics and contribution of tea polyphenols. It is worth mentioning that the natural flavors of this beverage are obtained from herbs and fruits, so the right choice of these elements could enhance its beneficial properties. (Uțoiu *et al.,* 2018).

On the other hand, caffeine is a popular compound consumed around the world, mainly in coffee, black tea and green tea. Currently, it is found in various products and foods; the compound is associated with many benefits and adverse effects around the amount of consumption, age, gender, lifestyle, and nutrition. Several studies, such as Doepker in 2018 have shown that caffeine in adequate doses avoids the negative effects mentioned above, however, in tea infusions it usually exceeds it, being counterproductive to the beneficial effects of this type of beverage (Doepker *et al.,* 2018).

Blueberry is one of the most popular fruits due to its high composition with polyphenols that confer antioxidant properties. It also contains flavonoids that represent up to 60% of the weight of the fruit, in addition to demonstrating that its anthocyanins reduce biomarkers and the risk to suffer diseases such as cardiovascular risks, diabetes Mellitus type 2, coronary heart disease, hypertension and neurological impairment. (Kalt *et al.,* 2020).

Orange blossom honey also contains flavonoids such as aringenin, hesperetin, kaempferol, chrysin, galangin and quercetin, as well as phenolic acids such as p-coumaric and caffeic acid at 3.64 mg/ 100 g (Escriche et al., 2011). Flavonoids possess anti-inflammatory, anti-atherogenic and anti-thrombotic properties (Parmenter *et al.,* 2021).

The compounds are present in different proportions in green tea, blueberries and orange blossom honey; for this reason, this article seeks to describe probiotics and their influence, highlighting the Kombucha, its origins, composition, antimicrobial properties and benefits, in addition to the description of the fruits and elements that, when added during fermentation, could enhance its benefits.

Showing thus, the production process of homemade Kombucha based on green tea without caffeine, which makes it different from other processes, avoiding the possible adverse effects of the consumption of this compound in the long run.

ISSN-On line: 1390-9959

Firstly, the bacterial consortium (SCOBY) was obtained, to later manufacture a homemade probiotic, in its two versions: in its natural form and a second flavored version that shares the properties of the three elements used, achieving the enhancemgent of its antioxidant properties, thus improving its antibacterial activity, taste, smell and pleasant texture, monitored through a sensory study and its statistical analysis.

Functional foods

The term currently known as "functional food" has its origin in Japan. Country where started its development in the 1980s, when the Japanese government launched and funded large-scale, systematic research programs on the development, analysis, regulation and molecular design of food functions. That project resulted in the introduction of a new concept that would describe those foods that were specifically developed to improve health and prevent disease, thus creating FOSHU (Valenzuela *et al.*, 2014).

FOSHU (Food with Specific Health Uses), is a category that since 1991 grouped foods such as prebiotics, probiotics, phytoestrogens, phytosterols, nutraceuticals, antioxidants, omega fatty acids, supplementary foods, among others. Foods that are intended to improve health and from which specific effects can be observed (Saarela, 2011).

This is how the concept of "functional foods" is described by several authors as those beverages and foods capable of providing health benefits through specific substances that can be consumed within a basic daily diet, regulating processes and preventing diseases (Saarela. 2011).

Probiotics and prebiotics

Probiotics are one of the best-known functional foods consumed by humans. The term "Probiotic" derived from Greek roots, means "for life". It was first used in 1965 by Lilly and Stillwell to describe "Substances secreted by one microorganism that stimulate the growth of another" (Lilly & Stillwell, 1965), mainly related to antibiotics.

Over the years, the definition gained a more general meaning, obtaining different hypotheses on the various beneficial effects it provides to health (Schrezenmeir & de Vrese, 2001).

The various definitions and studies on them allowed the consolidation of two clear ideas, the first is that probiotics are living organisms; and the second is that the positive effects they provide will depend on the adequate consumption of a dose of these microorganisms.By 2006, FAO/WHO gives a definition based on these two ideas, describing probiotics as those live microorganisms that administered in adequate amounts (> 6-7 log CFU/g) are capable of conferring health benefits to the host. Their functionality of course depends on the strain used; most of this type of commercialized products are made based on Gram-positive bacteria, as well as lactic acid bacteria, but Gram-negative bacteria such as Acetobacter and bifidobacteria have also been used (Nelson, 2017).

Among the benefits they have provided, we have reduction of Helicobacter pylori infections, reduction of allergy symptoms and constipation, inhibition of some harmful bacteria in the microbiome, effects on metabolism, cancer prevention and cholesterol reduction (Schrezenmeir & Vrese, 2001). In 2019, Plaza-Diaz et al. showed its role in the prevention of various problems such as digestive disorders, diarrhea caused by drugs (such as antibiotics), irritable bowel syndromes, diseases associated with Clostridium difficile, inflammatory diseases, gastric ulcers, atopic dermatitis and allergic rhinitis (Plaza-Diaz *et al.*, 2019). They also prevent and reduce the symptoms of respiratory diseases and their possible complications, a role mainly described in the Lactobacillus and Bifidobacterium genera (Carbonell, 2021).

The second most popular functional food that we will define, are prebiotics, which have been described as "a non-digestible food ingredient that beneficially affects the host by electively stimulating the growth and/or activity of one or a limited number of bacteria in the colon", this term was introduced by Gibson and Roberfroid in 1994, where they exchanged "pro" (from the definition of probiotic) meaning "before" for "pre" meaning "for" (Gibson & Roberfroid, 2004).

ISSN-On line: 1390-9959

The main difference between these two health beneficial foods is their main component, the probiotic has living organisms while the prebiotic has non-digestible substrates. This is the reason why many prebiotics are made from vegetables and fruits, since cellulose (the main component of these) is not digestible by humans, because we dispense with the enzyme cellulase. It is important to mention them since many probiotics are cultivated on prebiotic bases to stimulate their development and enhance their activity.

The origins of Kombucha tea

Kombucha is a fresh drink of Asian origin with a sweet and slightly sour taste obtained from the fermentation of an infusion of black or green tea leaves, through the cultivation of *Manchurian fungus*, a symbiotic association of bacteria (mainly *Acetobacter spp* and *Gluconobacter spp*) and heterofermentative yeasts commonly called "Tea Fungus", also named SCOBY (Heredia *et al.,* 2021).

This probiotic beverage originated in northwest China in Manchuria during the Tsin dynasty around 220 B.C., used for its benefits as a detoxifier and energizer. Thanks to the expansion of trade routes, Kombucha made its way to Eastern Europe and Russia appearing in different countries. In the early 20th century, researchers in Switzerland noted that Kombucha was equally beneficial as Yogurt consumption (Jayabalan *et al.,* 2014).

Kombucha preparation process

Its preparation is easy and not very elaborate, so it is possible to make it at home. Black tea is usually used, but green tea and oolong (also called blue tea) are also useful. According to Martinez and collaborators in 2018, it is usually used 5 g of tea leaves per liter of water.

Since this beverage is made from living microorganisms it requires a carbon source as substrate, the source in this case is sugar. The best sugar for this fermentation is refined white sugar, since it is of high purity (> 99.9%), obtained mainly from sugar beet or sugar cane, depending on the refining process we can say that it has a high quality and contains less minerals or inorganic elements that affect its purity and flavor.

The main compound of this type is sucrose, one of the carbohydrates most used by our acetobacter.

For Kombucha, 5 to 20% sugar of the total volume to be made is required (Pohl, & Stecka, 2011). Some authors such as Martinez et al. state that minimum 50 g of sugar per liter is sufficient to start the fermentation process (Martinez *et al.*, 2018). First, the water is heated to boiling point, then an infusion is prepared with the tea leaves, it is left to steep to obtain the concentrate, and finally sucrose is added until dissolved. The mixture is cooled and then the SCOBY is added, it is important that the preparation is at least 20° C before adding the "Tea Fungus" to avoid damaging or killing it. If there is no SCOBY, it can be generated from a mother ferment of another Kombucha, i.e., a minimum of 10% of the total volume that we intend to ferment is added with the volume of another previous Kombucha (Heredia *et al.,* 2021).

The process requires clean and sterile areas to avoid unwanted contamination. After adding to the SCOBY it should be covered with a cloth (Abel, & Andreson, 2020). The average fermentation time is from 7 to 14 days depending on the type of tea and conditions such as temperature, darkness, pH, type of carbon source. Authors such as Uțoiu have described for cases of obtaining a new SCOBY, that at room temperature it could take from 7 to 30 days (Uțoiu *et al.,* 2018).

If it is desired to obtain a Kombucha with added flavor and properties it is important to perform a second fermentation, repeating the previous steps by adding the fruits, herbs or seeds for the process This can be seen in more detail in the methodology section of the present research. The temperature can be from 21 °C to 25° C. The optimum pH described in literature is 3.2 in green tea and 3.5 in black tea, reaching a maximum of 4.2 without the overproduction of acetic acid changing the flavor and properties (Martínez *et al.,* 2018).

ISSN-On line: 1390-9959

The SCOBY

It is called SCOBY to the acronym of "Symbiotic Colony Of Bacteria and Yeast", this consortium known as tea fungus is a mixed culture of acid-acetic bacteria of which we find the genera *Bacterium, Gluconoacetobacter, Gluconobacter, Halomonas, Herbaspirillum, Komagataeibacter,* the presence of these can vary from one Kombucha to another, but thanks to its film we always find species such as *Acetobacter xylinum, Acet. Xylinoides or Bacterium gluconicum*, and yeasts such as *Schizosaccharomyces pombe, Saccharomyces ludwigii, Zygosaccharomyces rouxii, Candida spp.* or *Pichia membranaefaciens*

The base of SCOBY to carry out the fermentation process is a floating cellulosic film that is usually seen as a thin film on the medium with tea, ethanol and organic acids (mainly acetic acid and gluconic acid) (Chen & Liu, 2000). The longer the fermentation time, the thicker the film will be (González, 2021). The acids in this culture is what gives the beverage antibacterial activity and prevents its contamination by other pathogenic bacteria. This mixture may also contain traces of carbon dioxide (Martinez *et al.*, 2018).

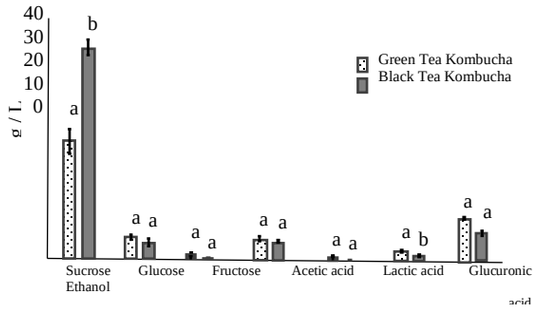
Composition and microbiome of Kombucha

According to Abel, & Andreson in 2020, the probiotic Kombucha beverage possesses compounds such as alcohols, aldehydes, ketones, esters, amino acids, tannins, terpenoids, saponins, flavonoids, phenols, alkaloids, CO2, enzymes, catechins, caffeine, among others (Based on Adzadogo, 2015). Biochemical analysis of Kombucha by Jayabalan and collaborators in 2014 indicated the presence of 14 amino acids, in addition to biogenic amines, purines, proteins, few hydrolytic enzymes, pigments, lipids, ethanol, antibiotically active matter, minerals, anions, DSL, yeast products and bacterial metabolites (Abel & Andreson, 2020).

Its metabolism is capable of producing glucose, fructose, vitamins C, B1 B2, B3, B6, B12, folic acid, organic acids such as acetic, gluconic, lactic, some enzymes, antibiotic active compounds, carbon dioxide, tea catechins and caffeine.

Of their therapeutic effects, they are known to come mainly from polyphenols and secondary metabolites obtained from fermentation, adding properties such as improving digestion, preventing cancer, eliminating toxic substances, relieving arthritis and preventing microbial infections (Kumar & Joshi, 2016).

As mentioned, the compounds vary according to the type of tea, so in Graph 1 (own elaboration of Figure 1 by Cardoso and collaborators in 2020), we can see that green tea has a greater amount of these compounds



Graphic 1 Own elaboration of "Figure 1. Chemical composition of the kombuchas. Results were expressed as mean of three repetitions. Error bars indicate ± standard deviation. Means followed by the same letter, for the same análisis, are not significantly different ($p < 0.05$)."
Source: (Cardoso et al., 2020)

According to Bardales and Pinto in 2021, the chemical composition of this probiotic beverage also varies around the time it takes to ferment, among these compounds, we have acids and alcohol usually increase proportional to the number of days. Thus, after 30 days, the amount of sucrose, glucose and fructose usually decreases, while the amount of gluconic acid, ethanol and acetic acid increases (Bardales & Pinto, 2021).

With respect to yeasts, these are responsible for the hydrolysis of sucrose, thus obtaining glucose and fructose. However, it is thanks to invertase that ethanol can be produced in the glycolysis process. The acetobacteria use this glucose to obtain gluconic acid and ethanol to obtain acetic acid. It is the set of organic acids produced during fermentation that provides a low pH value that confers a natural antimicrobial protection (Gonzalez, 2021).

ISSN-On line: 1390-9959

Some studies show that green tea and lemon balm tea have more stimulating effects than black tea, achieving fermentations in a shorter amount of time (Jayabalan *et al.,* 2014).

Regarding the amount of caffeine found in a beverage, Doepker, et al. in 2018 indicated that the daily intake of 400 mg per day of caffeine does not generate adverse health effects (equivalent to 10 g) in adults, similarly 300 mg in pregnant women and 2.5 mg for adolescents and children. However, when exceeding this dose we can find effects on bones, blood pressure, heart rate, cholesterol, mood, sleep, headache and withdrawal (Doepker *et al.,* 2018). In black tea-based kombucha, caffeine amounts of 16.64 mg/ g have been found, it has also been described that this proportion does not differ much from an infusion to the proportion in a fermentation, this is because, it does not influence the acidity of the beverage (Moya in 2020). In the literature, no standard has been reported for the amount of caffeine allowed or generated in green tea-based kombucha, but we did find in infusions, obtaining levels generally in the range of 141-338 mg/L depending on the brand (Ramiréz-Aristizabal *et al.,* 2016).

According to the FDA, although 400 mg is generally not related as a risk, there is a wide degree of effects on people due to their lifestyles. As we note the range of caffeine in green tea does not exceed the minimum level for consumption, noting that the adverse effects for a kombucha are minimal if the lifestyle is adequate, but in the long run the daily consumption and accumulation could generate effects just like the excessive consumption of a normal tea (FDA, 2018).

Antimicrobial capacity of Kombucha

This beverage, widely consumed in various parts of the world, has demonstrated a wide range of antimicrobial activity. The SCOBY symbiosis present in Kombucha is capable of inhibiting the growth of other highly contaminating bacteria. Activity against *Helicobacter pylori, E. coli, Staphylococcus aureus* and *Agrobacterium tumefaciens* has been reported due to the acetic acid produced during fermentation, in fact, it is considered the main antimicrobial compound.

Other authors such as Grenwalt et al. in 1998 cited by Battikhy collaborators in 2013, demonstrated activity in Kombucha made from green tea against bacteria *E. coli* serotype H10 (non-pathogenic), also for *E. coli* serotype H48 (pathogenic), the *S. typhimurium, Bacillus cereus* and *A. tumefaciens* (Battikh *et al.,* 2013). Table 1 based on Table 1 of Battikh et al. shows the comparison of the antimicrobial activity present in black tea and green tea based kombucha.

TABLE. ANTIBACTERIAL ACTIVITY OF KOMBUCHA[a]

Camellia sinensis type	Tested extracts	pH	Inhibition zone diameter (mm)[b] of target bacteria					
			Staphylococcus epidermidis CIP 106510	*Staphylococcus aureus* ATCC 25923	*Micrococcus luteus* NCIMB 8166	*Salmonella typhimurium* LT2	*Escherichia coli* ATCC 35218	*Listeria monocytogenes* ATCC 19115	*Pseudomonas aeruginosa* ATCC 27853
Black Tea	Fermented infusion (K_{25})[b]	2.59	18.5 ± 2.1	14.5 ± 2.1	16.5 ± 0.7	14.0 ± 1.4	10.5 ± 0.4	18.5 ± 2.1	19.0 ± 1.4
	Neutralized kombucha[c]	7.00	N.A.	9.5 ± 0.7	10.0 ± 0.0	N.A.	N.A.	N.A.	N.A.
	Unfermented infusion[d]	5.14	N. A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
	Acidified infusion[e]	2.59	N.A.	N.A.	N.A.	N.A.	N.A.	27.0 ± 1.4	15.5 ± 0.70
	Heat-denatured Kombucha[f]	2.59	16.5 ± 0.7	13.5 ± 2.1	13.5 ± 2.1	12.0 ± 0.0	13.0 ± 0.0	N.A.	11 ± 0.0
Green Tea	Fermented tea (Kombucha)[b]	2.54	22.0 ± 1.4	12.0 ± 0.0	22.0 ± 2.8	14.0 ± 1.4	14.5 ± 0.7	21.5 ± 2.1	18.0 ± 0.4
	Neutralized kombucha[c]	7.00	12.5 ± 0.7	N.A.	14.5 ± 0.7	N.A.	N.A.	N.A.	N.A.
	Unfermented tea[d]	5.08	10.0 ± 0.0	N.A.	16.0 ± 0.8	N.A.	N.A.	10.5 ± 0.7	N.A.
	Acidified tea[e]	2.54	27.0 ± 0.0	26.5 ± 0.7	20.5 ± 0.7	18.5 ± 0.7	13.0 ± 0.0	23.5 ± 2.1	13.0 ± 0.0
	Heat-denatured Kombucha[f]	2.54	19.0 ± 0.0	16.0 ± 1.4	19.5 ± 0.7	11.0 ± 1.4	12.0 ± 0.0	21.5 ± 2.1	9.0 ± 0.0

a Inhibition zone diameter (mean and standart deviation including wells diameter of 6 mm).
b Fermented infusion (Kombucha) at natural pH value without any adjustment.
c Neutralized kombucha: pH 7 fermented infusion adjusted with 1 M NaOH.
d Unfermented infusion prepared in the same way as that for making Kombucha, and 1 M HCL or 1 M NaOH was used to adjust their pH.
e Acidified infusion with acetic acid according to the acidity of Kombucha samples.
f Heat-denatured fermented infusions were treated at 120 C for 20 min.
N.A., no activity revealed.

Table 1 Own elaboration of "TABLE. ANTIBACTERIAL ACTIVITY OF KOMBUCHA."
Source: (Battikh *et al.,* 2013).

According to Cardoso et al. in 2020, Kombucha green tea inhibits the growth of pathogenic bacteria such as S. aureus, E. coli, Sallmonella and L. monocytogenes with a minimum concentration of 250 µL/mL while black tea only had activity against S. aureus and L. monocytogenes. This is due to the acidity of green tea being higher than that of other types of tea, with a pH of 3.2 and to the phenolic compound verbascoside which is unique to green tea.

Green tea

We denominate tea, to the use of any part of the *Camellia Sinesis* plant, to prepare infusions, by means of a preparation with boiling water. The leaves of this popular plant, native to Southeast Asia (South China, North India, Cambodia and Myanmar) and member of the *Theaceae* family, allow us to produce the famous green tea, whose catechins vary in quantity depending on the type of treatment used to dry them. Other variants of green tea known as black tea, Oolong tea, white tea and red tea are obtained from this same plant (Watson *et al.,* 2018).

ISSN-On line: 1390-9959

It possesses sugars, organic acids, amino acids, non-phenolic metabolites and bioactive phenolic compounds (Das *et al.,* 2019). Amino acids belonging to this tea variety comprise a total of 19, among these the most important are the non-proteinogenic ones such as L-Theanine, or 5-N-ethylglutamine which represents up to 50% of all free amino acids; it also possesses citrulline, ornithine and carnosine (Horanni and Engelhardt, 2013)

Its high antimicrobial and antioxidant activity is due to its xanthine compounds and polyphenols such as flavonoids, catechols, catechin tannins and acids. It has even been found to have a higher susceptibility against large positive than negative bacteria such as *Listeria monocytogenes*. In addition to conferring a pleasant odor and flavor to the taste (Mora *et al.,* 2013).

Kombucha in green tea has presented a total acidity of 0.36% (w / v acetic acid) higher than that of black tea (0.32 %). With respect to total phenolic properties, theaflavins, thearubigins and its antioxidant capacity, it has been found that black tea is 55.7% higher than green tea. Black tea is the beverage with the highest number of antioxidants (Cardoso *et al.,* 2020).

Blueberry or Blackberry

According to SAGARPA in 2017, one of the fruits with the highest antioxidant capacity are the berrys. *Vaccinium spp*. commonly called Blueberry is a berry native to the northeastern United States and eastern Canada currently cultivated in Mexico, it measures 1-2 cm in diameter and has a soft blue/purple coloration.
The literature describes that both the leaves and fruits of this plant possess high antioxidant activity, chlorogenic acid and quercetin glycosides are found in them (Chu *et al.,* 2017).

It possesses glucose, fructose and sucrose, in addition to phenolic compounds such as anthocyanins, quercetin, chlorogenic acids and proanthocyanidins.

Phenolic compounds are the main ones related to their antioxidant activity that allows them to neutralize substances such as free radicals that are related to chronic diseases, damage to the human body and cancer.

They have also demonstrated anti-inflammatory action, neuroprotection, cancer prevention and protection of organs such as the liver. The amount of these compounds in fruits varies according to the ripening stage and species, with anthocyanin being the compound that increases the most during ripening (Toyama *et al.,* 2021).

Orange Blossom Honey

The species *Citrus sinensis* known as Orange Blossom is a tree of Asian origin, from southwest China and the Malay Peninsula, this plant has a high commercial value due to the multiple products obtained from it, from orange to its honey. Currently, it is one of the species cultivated in Mexico, from which different products are exported in large quantities (SAGARPA, 2017).

The honey obtained from the citrus tree or "Orange blossom honey", is a light-colored honey and mild flavor with fresh and floral aroma distinctive of citrus (Kadar *et al.,* 2011). This honey possesses flavonoids such as chrysin, naringenin, kaempferol, luteolin, hesperetin, some galangin, pinocembrin and quercetin; the presence of phenolic acids was also found, specifically caffeic acid and p-coumaric acid (Escriche *et al.,* 2011). Orange tree flavonoids are important substances due to their ability to inhibit the oxidation of low-density lipoproteins in the blood, increase the amount of high-density lipoproteins and inhibit the activity of enzymes directly or indirectly involved in lipid peroxidation pathways such as cyclogenase, NO -synthase and NADH-oxidase (Atanelov *et al.,* 2018).

Studies by Escriche et al. in 2011 demonstrated the abundant presence of some lilac aldehydes and benzenacetaldehyde. Substances that, as seen, are part of the phenolic compounds that confer antioxidant power. Therefore, this honey is usually recommended for its antibacterial and healing properties, prevention of cancer and free radical damage, as well as reducing the risk of suffering chronic diseases (Escriche *et al.,* 2011).

ISSN-On line: 1390-9959

Methodology

Materials and reactives

Extra Special Orange Blossom Honey (370 g). Prometo PRODUCE brand Blueberry (156 g). Premium Green Tea brand STASH (40 g) herbal tea with 20 sachets without caffeine. EPURA brand jug water. Alquimia brand Kombucha lemon flavor black tea base (475 ml). Great Value brand refined white cane sugar (1 kg). Transparent glass jar of 42. 8 cm high with capacity of 4 liters. Electronic scale, Tefal brand, capacity up to 10 K. 100% cotton cloth of 47.5 cm by 34.6 cm. Elastic cloth band. Unireal nitrile gloves. A pewter spoon. Plastic spoon. One soup spoon. Two glass cups. One wooden stick. 1 measuring cup of 250 ml. 2-liter pewter pot. Hermetic refrigerator ACROS brand. 12 Estilo brand glass bottles with a capacity of 5600 ml each. Plastic funnel. AVEDISTANTE pH meter.

First fermentation

Boil 2 liters of Epura brand water for 5 minutes. Remove from heat and add 6 bags of Green Tea. Let stand covered for 10 minutes. Remove the tea bags and add 250 g of Great Value brand refined white sugar. Stir with a pewter spoon and let it stand for 10 minutes.

Subsequently, place the concentrate in the glass container. Add one liter of water at room temperature. Let stand 25 minutes then add 300 ml of commercial Kombucha Alquimia brand. Stir again with a plastic spoon and cover the mixture with the cloth, securing it with a rubber band. It is left to ferment for 7 days at room temperature in a dark and clean place (an airtight refrigerator without energy was used).

On the seventh day add a mixture of 300 g of refined sugar and 150 ml of commercial Kombucha. Stir with a plastic spoon. Let stand 14 days at a temperature of approximately 24 °C. Once the SCOBY has a suitable size, the Kombucha is tasted (it should have a sweet taste with a little sour). If the taste is as desired, the SCOBY is removed from the Kombucha with nitrile gloves to prepare the second fermentation. It is also consumable, as well as natural Kombucha.

NOTE: 2 sachets of Tea are placed per liter. Plastic spoons are used when mixing the concentrate with commercial Kombucha because it is an acidic beverage, if you use metal, the heat can oxidize.

Second fermentation

Once the SCOBY is carefully removed from the Kombucha. Place in a clean ceramic dish (with gloves). With the help of a funnel, pour the Kombucha into bottles with an airtight cap brand Estilo. Place 375 ml of the Kombucha in each bottle (3/4 of kombucha). Then add 30 g of orange blossom honey dissolved in 50 ml of Kombucha. Finally add 25 g of chopped blueberries (equivalent to two tablespoons).

Let it ferment for 4 to 5 days under the same conditions as the first fermentation. If it has the desired flavor, put it in the refrigerator at 4°C to stop fermentation.

To continue producing Kombucha, repeat the steps of the "First fermentation" procedure, but this time add 500 ml of the Kombucha concentrate obtained from the previous procedure. Add the SCOBY we generated and let it stand for 7 days or until the desired flavor is obtained.

Sensory Evaluation

Thirty people over 18 years of age were selected. They were given to taste ml of the two types of kombucha previously refrigerated. A sensory analysis test with a 9-point hedonic scale was applied. We plotted the results for the attributes of color, odor, taste and texture of the two types of Kombucha.

Results and Discussion

Obtaining SCOBY in first fermentation

Recall that the popular beverage known as Kombucha, is generated through the symbiotic relationship of SCOBY (bacteria and yeasts), sustained within a cellulose film that can take 7 to 30 days to grow at room temperature (Uțoiu *et al.*, 2018). This film is synthesized mainly by *Acetobacter xylinum*, a bacterium from the most predominant group of microorganisms in fermentation, of the genus *Acetobacter* (Watawana *et al.*, 2015).

ISSN-On line: 1390-9959

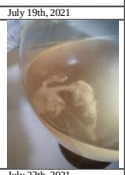
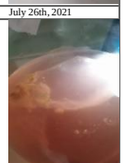
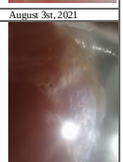
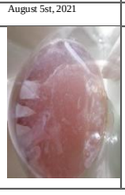
Date	July 7th, 2021	July 8th, 2021	July 9th, 2021	July 10th, 2021
Monitoring photo				
Date	July 11th, 2021	July 12th, 2021	July 13th, 2021	July 14th, 2021
Monitoring photo				
Date	July 15th, 2021	July 16th, 2021	July 17th, 2021	July 18th, 2021
Monitoring photo				
Date	July 19th, 2021	July 20th, 2021	July 21th, 2021	July 22th, 2021
Monitoring photo				
Date	July 23th, 2021	July 24th, 2021	July 25th, 2021	July 26th, 2021
Monitoring photo				
Date	July 27th, 2021	July 28th, 2021	July 29th, 2021	July 30th, 2021
Monitoring photo				
Date	July 31th, 2021	August 1st, 2021	August 2st, 2021	August 3st, 2021
Monitoring photo				
Date	August 4st, 2021	August 5st, 2021	August 6st, 2021	SCOBY- August 6st, 2021
Monitoring photo				

Table 2 Monitoring of SCOBY production from commercial Kombucha.
Source: (Own elaboration)

In Table 2 of the fermentation monitoring for obtaining a SCOBY, we noticed that, throughout the 30 days, the tea fungus took time to form a cellulose film, which on the 30th day reached the size of the surface of the liquid in the jar. It was very thin and with some lumps with a noticeable white-beige coloration.

The tea used for this process was caffeine-free, one of the compounds described as the main stimulator for cellulose synthesis (by bacteria) in green tea (Dufresne & Farnworth, 2000), whose composition is 3-6 % of the dry weight of the tea. Added to this, we have that the starter kombucha was caffeinated black tea. Accordingly, we can state the following, first, the bacteria and yeasts of our starter kombucha were accustomed to a medium with caffeine and xanthines, while the conditions of their new medium were different, since the consortium only possessed other xanthines in smaller amounts for their growth.

Secondly, we must remember that black tea has a higher pH and our starter kombucha was probably grown at an ideal temperature; Therefore, the change to a type of tea with a lower pH, the absence of caffeine and an initial ambient temperature of 21°C in the first 7 days, instead of the ideal 25°C (which was achieved in the remaining 23 days), caused an adaptation process, which is why the cellulose film obtained in Table 2 is thinner than the film obtained with a previous SCOBY used to the medium, as shown in Table 4.

Second fermentation kombucha

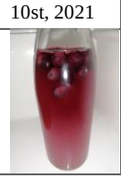
Date	Before fermentation	August 7st, 2021	August 8st, 2021	August 9st, 2021
Monitoring photo				
Date	August 10st, 2021	August 11st, 2021	August 12st, 2021	August 13st, 2021
Monitoring photo				

Table 3 Monitoring of the "Second Fermentation" process from the kombucha obtained in "First Fermentation" to add flavor and enhance properties with blueberry and orange blossom honey.
Source: (Own elaboration)

According to the literature, the optimum pH for fermentation in green tea is 3.2 with a maximum of 4.2. (Martinez *et al.*, 2018). The pH obtained in our Table 2 of natural Kombucha at 23 days was 2.42, having a final pH of 2.12 at 30 days.

ISSN-On line: 1390-9959

Its acidity confirms us the overproduction of acetic acid that can change the flavor and final properties, however, the final pH obtained from the second fermentation in Table 3, was 2.53. The increase in its final pH is due to the addition of blueberry and orange blossom honey, whose pH is 2.48 for the fruit (Stückrah *et al.*, 2007) and 4.14 for the honey (Periago *et al.*, 2019), thus resulting in a higher pH for the second fermentation but obtaining values outside the optimal range of growth for our consortium.

Since the base tea was more acidic due to the use of green tea, we can infer that it possesses antimicrobial activities against pathogenic bacteria such *as S. aureus, E. coli, Sallmonella and L. monocytogenes* (Cardosos *et al.*, 2020), part of the antimicrobial properties described in the literature giving an added economic value to our beverage as a probiotic.

First fermentation with SCOBY previously obtained

Date	August 8st, 2021	August 9st, 2021	August 10st, 2021	August 11st, 2021
Monitoring photo				
Date	August 12st, 2021	August 12st, 2021	August 12st, 2021	August 13st, 2021
Monitoring photo				
Date	SCOBY - August 13st, 2021			
Monitoring photo				

Table 4 Monitoring of the "first fermentation" of green tea based Kombucha with SCOBY previously obtained
Source: (Own elaboration)

For the first fermentation in table 4, with a previously obtained SCOBY, we measure an initial pH of 3.64 and a final pH after 7 days of 2.75 were achieved. The final pH of the natural Kombucha is higher than the pH of the cranberry-honey Kombucha because of the difference in time, ingredients used, and SCOBY obtained. Both are below the optimal growth pH range, however, if it was possible to obtain the desired tea fungus and pleasant flavor.

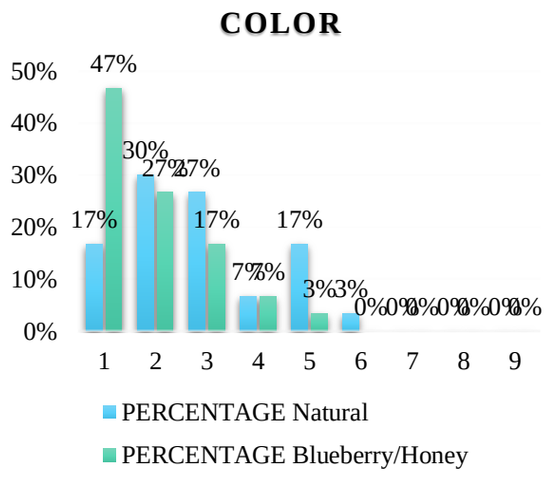
Respect to the SCOBY structure, we note that on the seventh day our cellulose film measures 0.4 millimeters, almost half a centimeter. This consortium is much thicker and stronger than the first cellulose film in Table 2, its coloration is white-beige similar to the first SCOBY, but unlike the previous one, the texture of this film is smooth.

The difference in growth time between the films is remarkable because the first SCOBY took 30 days to regulate itself to its environment in order to grow while the second film already had a tea fungus adapted to this environment, with a good control of the ideal temperature. We were able to obtain a strong SCOBY based on green tea and without caffeine. Also, we can confirm based on the literature, the presence of *Acetobacter xylinum* in both consortia, since in both cases a cellulose film was created.

To slow down fermentation, we must lower the temperature of our Kombucha. The ideal temperature is 4°C to avoid contamination by foodborne pathogens (Murphy *et al.*, 2018). The temperature change allows us to perform the sensory study of the beverage.

Sensory Evaluation

The application of a sensory evaluation using a nine-point hedonic scale allowed us to obtain 4 graphs, where we can compare the characteristics obtained through the production process of a natural Kombucha and the process of the blueberry Kombucha with honey.

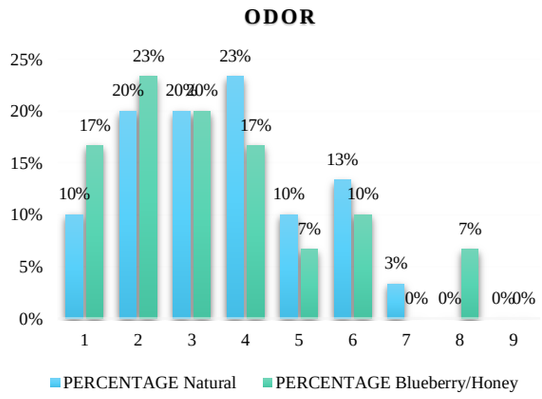


Graphic 2 Percentages obtained from the nine-point hedonic test on the color of natural Kombucha VS. Blueberry Kombucha with orange blossom honey
Source: (Own elaboration)

ISSN-On line: 1390-9959

Graphic 2 corresponds to color acceptance. For flavored kombucha, with a reddish tone, 47% of the population studied liked it very much, 27% liked it very much, 17% liked it moderately, 7% liked it slightly and 3% neither liked nor disliked it. With respect to natural kombucha with a greenish amber shade, we observe that 30% of the population likes it very much, 27% likes it moderately, 17% likes it very much, another 17% neither likes nor dislikes it, 7% likes it slightly and 3% dislikes it slightly.

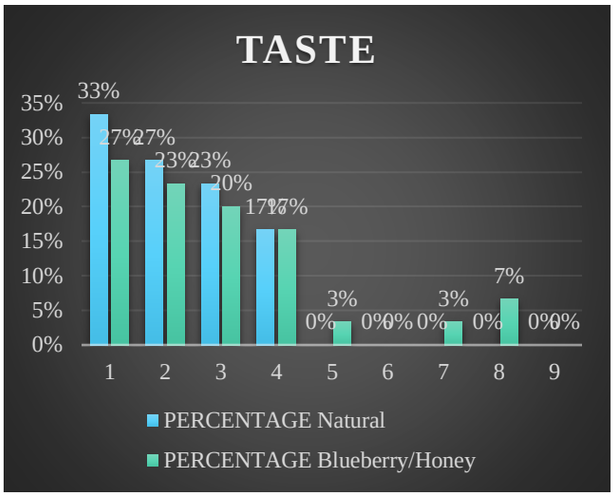
These data indicate that 97% of the population liked the color of the flavored drink, only 3% neither liked nor disliked it. On the other hand, 80% of the population liked the color of the natural drink, 17% neither liked nor disliked it and only 3% disliked it.



Graphic 3 Percentages obtained from the nine-point hedonic test for the odor of natural Kombucha VS. Blueberry Kombucha with orange blossom honey
Source: (Own elaboration)

Graphic 3 corresponds to the comparison of odor. With respect to blueberry kombucha with orange blossom honey, 23% of the population studied liked it very much, 20% liked it moderately, 17% liked it slightly, another 17% liked it very much, 10% indicated that they disliked it slightly, 7% neither liked nor disliked it, and another 7% disliked it very much. For natural kombucha, it was observed that 23% liked it slightly, 20% liked it very much and another 20% liked it moderately, 13% indicated that they disliked it slightly, 10% indicated that they liked it very much and another 10% indicated that they neither liked nor disliked the aroma, finally 3% indicated that they disliked it moderately.

The aroma obtained from the Kombucha resembled the aroma of acetic acid. The study found that 76% liked the aroma of the flavored beverage, 7% neither liked nor disliked it and 17% indicated that they disliked it. For the natural Kombucha, 74% liked it, 10% neither liked nor disliked it and 16% disliked it.



Graphic 4 Percentage of the nine-point hedonic test on the taste obtained from the production of a natural Kombucha VS. Blueberry Kombucha with orange blossom honey
Source: (Own elaboration)

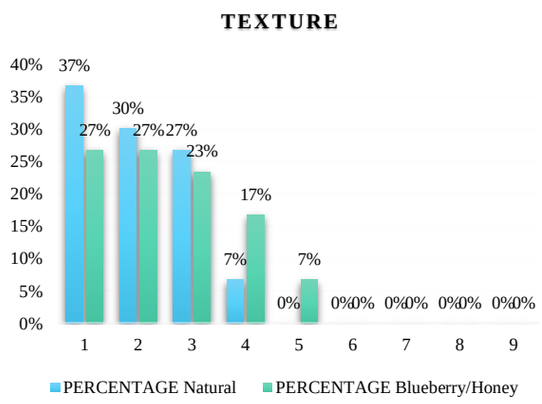
Graphic 4 shows the comparisons of the flavor obtained from the fermentation processes. For the taste of the blueberry kombucha with orange blossom honey fermented for 30 days, 27% of the population indicated that they liked the final taste obtained very much, 23% indicated that they liked it very much, 20% liked it moderately, 17% liked it slightly, 7% indicated that they disliked the taste very much, 3% indicated that they neither disliked it nor liked it, and finally another 3% indicated that they disliked it moderately. With respect to natural kombucha, 33% indicated that they liked it very much, 27% indicated that they liked it very much, 23% liked it moderately and 17% liked it slightly.

In terms of taste, it can be seen, that the cranberry drink with honey obtained 87% of acceptance as a pleasant taste, 3% indicated that it was neither unpleasant nor pleasant and only 10% indicated that it was unpleasant. For the natural drink, 100% indicated that they liked the taste.

ISSN-On line: 1390-9959

During fermentation, a slightly sour taste may predominate, due to the amount of acetic acid being produced. On some occasions, the flavor can be slightly alcoholic, similar to an apple cider (Marsh *et al.*, 2014). According to Ihsani et al., 2021, the ethanol increase in the first 8 days does not exceed 0.32% of its volume, nor does it affect the antioxidant quality, lactic acid levels or pH (Ihsani *et al.*, 2021). This probiotic beverage does not have an alcohol percentage higher than the 0.5% necessary to be considered an alcoholic beverage by the TTB (Alcohol and Tobacco Tax and Trade Bureau) of the United States or by the Mexican Ministry of Health (General Health Law, article 217), so this occasional taste does not affect its main function.

The sweet and fruity flavor it usually acquires depends on the type of sugar, fruit or herb used, as well as the time of the second fermentation. Its aroma is acidic, like vinegars, sometimes becoming fruity (Watawana *et al.*, 2015),



Graphic 5 Percentage of nine-point hedonic test applied for texture of a natural Kombucha VS. Blueberry Kombucha with orange blossom honey
Source: (Own elaboration)

Graphic 5 corresponds to the comparison of the texture of both kombuchas. Both beverages were refrigerated and served cold, obtaining an acceptance for the blueberry kombucha with orange blossom honey, 27% of the population liked it very much, another 27% liked it very much, 23% liked it moderately, 17% liked it slightly and 7% neither liked nor disliked it. The percentages on the texture of natural kombucha indicate that 37% liked it very much, 30% liked it very much, 27% liked it moderately and 7% liked it slightly.

The above data indicate that with respect to the cold texture of our beverage, 93% of the population considered the texture pleasant and only 7% as neither pleasant nor unpleasant, while our natural Kombucha obtained 100% acceptance as a pleasant beverage.

Based on the results obtained from the sensory evaluation, it can be seen that, the beverage with the highest acceptance in color and odor was the flavored Kombucha, but in the categories of flavor and texture, the highest acceptance was obtained by the natural beverage. However, for both probiotic beverages, their rejection rate was low, so both the natural and flavored probiotic have good texture and color. Its odor can be treated when refrigerated since after three days it was noticed that its initial sour aroma decreased drastically as the SCOBY stopped producing more organic acids. Regarding the main category in flavor, its acceptance rate is higher for the natural one, but the flavor was not very low, so the fermentation days could be decreased until the desired acid flavor is obtained. This indicates that its acceptance varies according to consumer tastes.

Acknowledgements

We thank the program of complementary support for the awarding of a scholarship to carry out the Summer of Research 2021, within the framework of the Interinstitutional Program for the Strengthening of Research and Postgraduate Studies of the Pacific "DELFÍN" in virtual mode. As well as to the organizers of the program for the opportunity to do research.

Conclusions

It was possible to develop the production of homemade Kombucha based on caffeine-free green tea with antioxidant properties added by means of a fruit and honey from orange blossom. Thus, obtaining a probiotic beverage with added properties, thanks to the wide variety of microbial species of the consortium and the added fruits.

Despite having different environmental conditions than the initial commercial Kombucha, a SCOBY was obtained and together with it, a Kombucha with enhanced antioxidant properties was produced (shown in Table 2 and 3), a process carried out by means of a second fermentation.

ISSN-On line: 1390-9959

Due to the current situation of COVID-19, it was not possible to perform antimicrobial experiments to test the inhibitory activity of Gram-positive and Gram-negative bacteria of the probiotic beverage, through bacterial growth tests with strains, which could be achieved in other studies; but it did perform a sensory evaluation of natural Kombucha and flavored Kombucha to approximate the added value of the product. Along with this and based on the literature, it was confirmed that the growth of the tea fungus was possible due to the existing broad consortium, which in other studies has demonstrated high effectiveness against pathogens such as Gram-negative and Gram-positive bacteria. Thus, obtaining a probiotic beverage with beneficial properties for the human microbiota, with a very pleasant color and texture, with a characteristic acetic acid odor that is controllable and 87% accepted in taste.

Possible improvements to achieve greater acceptance in flavor would be to reduce the number of days for the first fermentation to obtain SCOBY, since the excess of acetic acid changed the expected organoleptic properties. Also, the temperature control could be improved from the first day, verifying that it is a constant 25°C in the dark, along with this, the field of study for sensory analysis could be expanded, thus yielding even more accurate values.

References

Abel, M., & Andreson, H. (2020). Effect of simultaneous inoculation of commercial yeast starter cultures on Kombucha fermentation. *Agronomy Research, 18((S3))*, 1603–1615. https://doi-org.proxydgb.buap.mx/10.15159/AR.20.140

Atanelov, N., Arutinashvili, N., Burjanadze, G., Kuprava, G., & Koshoridze, N. (2018). Isolation of Flavonoid Extract from Georgian Citrus Sinensis Peel and Its Antioxidative Properties. *Georgian Medical News, 283*, 162–165.

Bardales Espinoza, F. M., & Pinto Perez, C. P. (2021). Estudio de prefactibilidad para la instalación de una planta procesadora de una bebida en base a hojas de té verde y un cultivo probiótico (*Medusomyces gisevi*).

Battikh, H., Chaieb, K., Bakhrouf, A., & Ammar, E. (2013). Antibacterial and Antifungal Activities of Black and Green Kombucha Teas. *Journal of Food Biochemistry, 37(2),* 231–236. https://doi-org.proxydgb.buap.mx/10.1111/j.1745-4514.2011.00629.x

Carbonell Gonzálvez, Á. (2021). Análisis sobre las recomendaciones dietético-nutricionales con relación a la COVID-19.

Chen, C., & Liu, B. Y. (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

Chu, W., Gao, H., Cao, S., Fang, X., Chen, H., & Xiao, S. (2017). Composition and morphology of cuticular wax in blueberry (Vaccinium spp.) fruits. Food chemistry, 219, 436–442. https://doi.org/10.1016/j.foodchem.2016.09.186

Darmawan, G., Tendean, M., Dharmawan, A., William, Tan, H. T., Atmogo, T., & Gunardi, W. D. (2020). The Possible Role of Probiotics in Combating COVID-19. Indonesian *Journal of Gastroenterology, Hepatology & Digestive Endoscopy, 21(3)*, 226–230.

Das, P. R., Kim, Y., Hong, S., & Eun, J.-B. (2019). Profiling of volatile and non-phenolic metabolites—amino acids, organic acids, and sugars of green tea extracts obtained by different extraction techniques. *Food Chemistry*. doi:10.1016/j.foodchem.2019.05.194

Doepker, C., Franke, K., Myers, E., Goldberger, J. J., Lieberman, H. R., O'Brien, C., Peck, J., Tenenbein, M., Weaver, C., & Wikoff, D. (2018). Key Findings and Implications of a Recent Systematic Review of the Potential Adverse Effects of Caffeine Consumption in Healthy Adults, Pregnant Women, Adolescents, and Children. *Nutrients, 10(10),* 1536. https://doi.org/10.3390/nu10101536

Dufresne, C., & Farnworth, E. (2000). Tea, Kombucha, and health: a review. *Food Research International, 33(6),* 409–421.

ISSN-On line: 1390-9959

Escriche, I., Kadar, M., Juan-Borrás, M., & Domenech, E. (2011). Using flavonoids, phenolic compounds and headspace volatile profile for botanical authentication of lemon and orange honeys. *Food Research International, 44(5*), 1504–1513. https://doi-org.proxydgb.buap.mx/10.1016/j.foodres.2011.03.049

Gibson, G. R., Probert, H. M., Loo, J. V., Rastall, R. A., & Roberfroid, M. B. (2004). Dietary modulation of the human colonic microbiota: updating the concept of prebiotics. *Nutrition research reviews, 17(2),* 259–275. https://doi.org/10.1079/NRR200479

González Arranz, S. (2021). Diseño de un nuevo material sostenible a partir de celulosa bacteriana de residuos orgánicos de producción local en un marco de economía circular.

Graph 1: Cardoso, R. R., Neto, R. O., dos Santos D'Almeida, C. T., do Nascimento, T. P., Pressete, C. G., Azevedo, L., Martino, H. S. D., Cameron, L. C., Ferreira, M. S. L., & Barros, F. A. R. de. (2020). Kombuchas from green and black teas have different phenolic profile, which impacts their antioxidant capacities, antibacterial and antiproliferative activities. *Food Research International, 128*. https://doi-org.proxydgb.buap.mx/10.1016/j.foodres.2019.108782

Heredia, A. S. R., Tello, I. P. S., Mancheno, C. I. F., & Andrade, G. I. M. (2021). Utilización de tres sustratos para la determinación de la viabilidad del acetobacter aceti y saccharomyces cerevisiae presentes en el medusomyces gisevi, y su posible aplicación agroindustrial. *ConcienciaDigital, 4(3.1),* 122-141.

Horanni, R., & Engelhardt, U. H. (2013). Determination of amino acids in white, green, black, oolong, pu-erh teas and tea products. *Journal of Food Composition and Analysis, 31(1),* 94–100. doi:10.1016/j.jfca.2013.03.005

Ihsani, N., Hernahadini, N., & Muhammad, F. (2021, febrero). The variation of ethanol concentration and kombucha characterization on several incubation periods. *Journal of Physics: Conference Series, 1764(1)*. DOI:10.1088/1742-6596/1764/1/012008

Interián-Gómez, L., Aguila-Gutiérrez, S. E., Esquivias-López, K. M., Pulido-De la Cruz, V. A., Silva-Arzola, N. J., & Gonzalez-Becerra, K. (2021). Componentes alimenticios, estado de ánimo y su relación con el sistema inmune en COVID-19. RESPYN *Revista Salud Pública y Nutrición, 20(4), 73-86.*

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

Kalt, W., Cassidy, A., Howard, L. R., Krikorian, R., Stull, A. J., Tremblay, F., & Zamora-Ros, R. (2020). Recent Research on the Health Benefits of Blueberries and Their Anthocyanins. *Advances in Nutrition (Bethesda, Md.), 11(2),* 224–236. https://doi-org.proxydgb.buap.mx/10.1093/advances/nmz065

Kombucha desde cero | la guía completa. (2020, 25 abril). [Vídeo]. *YouTube*. https://www.youtube.com/watch?v=N3SCuxaIpsQ

Kumar, V. & Joshi, V. 2016. Kombucha: technology, microbiology, production, composition and therapeutic value. *Intl. J. Food. Ferment. Technol. 6,* 13–24.

Lilly, D. M., & Stillwell, R. H. (1965). Probiotics: growth-promoting factors produced by microorganisms. *Science (New York, N.Y.), 147(3659*), 747–748. https://doi.org/10.1126/science.147.3659.747

Maria Saarela. (2011). Functional Foods : Concept to Product: Vol. 2nd ed. *Woodhead Publishing.*

Marsh, A. J., O'Sullivan, O., Hill, C., Ross, R. P., & Cotter, P. D. (2014). Sequence-based analysis of the bacterial and fungal compositions of multiple kombucha (tea fungus) samples. *Food microbiology, 38*, 171–178. https://doi.org/10.1016/j.fm.2013.09.003

ISSN-On line: 1390-9959

Martínez Leal, J., Valenzuela Suárez, L., Jayabalan, R., Huerta Oros, J., & Escalante-Aburto, A. (2018). A review on health benefits of kombucha nutritional compounds and metabolites. CyTA: *journal of food*, *16*, 390-399. doi: 10.1080/19476337.2017.1410499

Mora, Andreína, Parra, Jonathan, Chaverri, José M, & Arias, María Laura. (2013). Determination of the antimicrobial capacity of green tea (Camellia sinensis) against the potentially pathogenic microorganisms Escherichia coli, Salmonella enterica, Staphylococcus aureus, Listeria monocytogenes, Candida albicans and Aspergillus niger. *Archivos Latinoamericanos de Nutrición, 63(3*), 247-253. Recuperado en 16 de julio de 2021, de http://ve.scielo.org/scielo.php?script=sci_arttext&pid=S0004-06222013000300008&lng=es&tlng=en.

Moya Patiño, E. E. (2020). Concentración de cafeína a partir de té negro, hoja de guayusa y café con ganoderma, durante la fermentación del Hongo Kombucha (Medusomyces Gisevi) (Bachelor's thesis, Universidad Técnica de Ambato. *Facultad de Ciencia e Ingeniería en Alimentos y Biotecnología.* Carrera de Ingeniería en Alimentos).

Murphy, T. E., Walia, K., & Farber, J. M. (2018). Safety Aspects and Guidance for Consumers on the Safe Preparation, Handling and Storage of Kombucha -- A Fermented Tea Beverage. *Food Protection Trends, 38(5),* 329–337

Nelson, D. L. (2017). Functional Foods: Sources, Health Effects and Future Perspectives. *Nova Science Publishers, Inc*.

Nutrition Division. (2006). Propiedades saludables y nutricionales y directrices para la evaluación - Informe de la Consulta de Expertos FAO/OMS sobre Evaluación de las Propiedades Saludables y Nutricionales de los Probióticos en los Alimentos (*FAO Food and Nutrition Paper ed., Vols. 1014–2916).* FAO/OMS. http://www.fao.org/3/a0512s/a0512s.pdf

Office of the Commissioner. (2018, 12 diciembre). Spilling the Beans: How Much Caffeine is Too Much? *U.S. Food and Drug Administration*. https://www.fda.gov/consumers/consumer-updates/spilling-beans-how-much-caffeine-too-much

Parmenter, B. H., Dalgaard, F., Murray, K., Cassidy, A., Bondonno, C. P., Lewis, J. R., Croft, K. D., Kyrø, C., Gislason, G., Scalbert, A., Tjønneland, A., Hodgson, J. M., & Bondonno, N. P. (2021). Habitual flavonoid intake and ischemic stroke incidence in the Danish Diet, Cancer, and Health Cohort. *American Journal of Clinical Nutrition, 114(1),* 348–357. https://doi-org.proxydgb.buap.mx/10.1093/ajcn/nqab138

Periago Castón, M. J., Navarro-González, I., Alaminos, A. B., Elvira-Torales, L. I., & García-Alonso, F. J. (2019). Parámetros de calidad en mieles de diferentes orígenes botánicos producidas en La Alpujarra granadina. *Anales de Veterinaria de Murcia, 32,* 59–71. Recuperado a partir de https://revistas.um.es/analesvet/article/view/369371

Plaza-Diaz, J., Ruiz-Ojeda, F. J., Gil-Campos, M., & Gil, A. (2019). Mechanisms of Action of Probiotics. *Advances in nutrition (Bethesda, Md.), 10*(suppl_1), S49–S66. https://doi.org/10.1093/advances/nmy063

Pohl, P., & Stecka, H. (2011). Elemental Composition of White Refined Sugar by Instrumental Methods of Analysis. *Critical Reviews in Analytical Chemistry, 41(2),* 100–113. https://doi-org.proxydgb.buap.mx/10.1080/10408347.2011.538585

Ramiréz-Aristizabal, L. S., Ortiz, A., & Ospina-Ocampo, L. F. (2016). Determinación de compuestos fenólicos (catequinas, cafeína, ácidos orgánicos) en té verde (camellia sinensis) usando cromatografía líquida de alta resolución/ Determination of phenolic compounds (catechins, caffeine, organic acid) of green tea (camellia sinensis) using high performance liquid chromatography resolution. *Vitae, 23,* S245.

ISSN-On line: 1390-9959

SAGARPA, 2017. Frutas del Bosque arándano, frambuesa zarzamora mexicanas. Ciudad de México: *Secretaría de Agricultura y Desarrollo Rural,* pp.1-3.

SAGARPA, 2017. Planeación agrícola nacional 2016-2030. Cítricos, Limón, naranja y toronja mexicanos. Ciudad de México: *Secretaría de Agricultura y Desarrollo Rural,* pp.12-15.

Sami, R., Alshehry, G., Elgarni, E., & Helal, M. (2021). Saudi Community Care Awareness Food Facts, Nutrients, Immune System and Covid-19 Prevention in Taif City among Different Age Categories. *African Journal of Food, Agriculture, Nutrition & Development, 21(1),* 17213–17233. https://doi-org.proxydgb.buap.mx/10.18697/ajfand.96.20440

Schrezenmeir, J., & de Vrese, M. (2001). Probiotics, prebiotics, and synbiotics--approaching a definition. *The American journal of clinical nutrition, 73(*2 Suppl), 361S–364S. https://doi.org/10.1093/ajcn/73.2.361s

Secretaría de Salud. (2021, 1 junio). Reglamentos de la Ley General de Salud. *Ley General de Salud*. https://www.gob.mx/insabi/prensa/reglamentos-de-la-ley-general-de-salud-275018?idiom=es

Stückrath, Rosemarie, & Petzold, Guillermo R. (2007). Formulación de una Pasta Gelificada a Partir del Descarte de Arándanos (Vaccinium corymbosum). *Información tecnológica, 18(2),* 53-60. https://dx.doi.org/10.4067/S0718-07642007000200008

Toyama, Y., Toshima, S., Hirano, T., Yamasaki, M., & Kunitake, H. (2021). Polyphenol contents, antioxidant activities, and anti-cancer cell proliferation properties at each stage of fruit development in intersectional hybrids between highbush blueberry and shashanbo (<italic>Vaccinium bracteatum</italic> Thunb.). *Journal of Berry Research, 1–16.* https://doi-org.proxydgb.buap.mx/10.3233/jbr-210713

Uțoiu, E., Matei, F., Toma, A., Diguță, C. F., Ștefan, L. M., Mănoiu, S., Vrăjmașu, V. V., Moraru, I., Oancea, A., Israel-Roming, F., Cornea, C. P., Constantinescu-Aruxandei, D., Moraru, A., & Oancea, F. (2018). Bee Collected Pollen with Enhanced Health Benefits, Produced by Fermentation with a Kombucha Consortium. *Nutrients, 10(10*). https://doi-org.proxydgb.buap.mx/10.3390/nu10101365

Valenzuela B, Alfonso, Valenzuela, Rodrigo, Sanhueza, Julio, & Morales I, Gladys. (2014). Alimentos funcionales, nutraceúticos y foshu: ¿vamos hacia un nuevo concepto de alimentación?. *Revista chilena de nutrición, 41(2), 198-204.* https://dx.doi.org/10.4067/S0717-75182014000200011

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, 1–11.* https://doi-org.proxydgb.buap.mx/10.1155/2015/591869

Watson, R. R., Preedy, V. R., & Zibadi, S. (2018). Polyphenols: Mechanisms of Action in Human Health and Disease (English Edition) (2.a ed.). *Academic Press. 55*, 715-721, https://doi.org/10.1016/B978-0-12-398456-2.00055-4.

ISSN-On line: 1390-9959