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KOMBUCHA TRADICIONAL E SABORIZADAS COM PITANGA (*Eugenia uniflora L.*) E COM UMBU-CAJÁ (*Spondia tuberosa*): CARACTERIZAÇÃO QUÍMICA, COMPOSTOS VOLÁTEIS, BIOATIVOS E POTENCIAL ANTIOXIDANTE

JOÃO PESSOA – PB

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Existe inúmeros significados para "gratidão", busquei um e achei pertinente trazer, diz mais ou menos assim: "*Gratidão é o reconhecimento de uma pessoa por alguém que lhe prestou um benefício, um auxílio, um favor, em resumo é um agradecimento"*. Sinto-me exatamente assim, o coração recheado de gratidão por minha orientadora, Professora Angela Tribuzy, ela que me acolheu e até existe um vínculo muito afetuoso. Não tenho muita coisa para lhe dar em troca, trago apenas minhas palavras gentis e um coração grato por tudo que fez por mim, um eterno obrigado por tudo.

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"Então a nossa boca se encheu de riso e a nossa língua de cânticos. Então se dizia entre as nações: Grandes coisas fez o Senhor por eles. Sim, até aqui nos ajudou o Senhor, e por isso estamos alegres".

Salmo 126, 2-3

"Chore no começo para sorrir no fim".

Marta Vieira da Silva

Camisa 10 da Seleção Brasileira Feminina de Futebol

RESUMO

Kombucha é um chá fermentado de origem chinesa que se destaca devido as propriedades benéficas associadas ao seu consumo, como por exemplo a capacidade antioxidante, efeito anti-inflamatório e atividade anticâncer. Diante da ascensão da bebida, estudos vêm empregando matérias-primas alternativas para elaboração de kombuchas análogas, como as frutas. Nesse sentido, o principal objetivo do estudo foi elaborar kombucha tradicional de chá verde (KT) e avaliar os impactos da adição de polpas de pitanga (KSP) e de umbu-cajá (KSUC) nos diferentes tempos de armazenamento, quanto ao pH, acidez titulável total, teor de sólidos solúveis, perfis de ácidos orgânicos, açúcares, voláteis e fenólicos. Adicionalmente, avaliou-se a digestão gastrointestinal *in vitro* sobre a bioacessibilidade de compostos fenólicos e atividade antioxidante *in vitro* nas kombuchas produzidas. O estudo também viabilizou a elaboração de um artigo de revisão, contribuindo com informações relevantes sobre a bebida kombucha. Inicialmente a KT de chá verde foi elaborada a partir da infusão das folhas desidratadas a 1,25% (p/v), açúcar a 7,5% (p/v), 20% (v/v) do chá *starter* e 3% de SCOBY (p/v), mantida a 25°C ± 2°C em incubadora BOD. Após 7 dias de fermentação, a cultura foi removida, o chá foi filtrado e fracionado em três porções de mesmo volume e logo em seguida adicionado 15% (p/v) das polpas de frutas (pitanga e umbu-cajá) e na sequência as bebidas ficaram a 25°C (± 2°C) de temperatura por 48h em BOD. Finalizando essa etapa, as bebidas foram filtradas e envadas em garrafas estéreis (5°C ± 2°C). Todas as bebidas apresentaram, durante o período avaliado, pH reduzido, aumento na acidez, decaimento do teor de sólidos solúveis, e redução de açúcares (glicose e frutose). Maiores valores e menores perdas de açúcares foram registrados para as bebidas saborizadas evidenciando que os teores de glicose e frutose pré-existentes nas frutas contribuíram para bebidas mais doces. Os ácidos orgânicos acético, butírico, cítrico, succínico e málico foram identificados em todas as kombuchas, com exceção do ácido málico em KSP. Os terpenos (mais de 39% de área) foram os principais compostos voláteis relatados nas bebidas, pois contribuíram para o aumento e/ou surgimento de novos terpenoides, como o curzereno e o β-cariofileno. Foram identificados 15 polifenois em KT e 16 em KSP e KSUC, com destaque em todas as kombuchas o flavonoide galato de epigalocatequina, apresentando valores entre 63,69% a 76,84%. Contudo, os fenólicos mais bioacessíveis em todas as bebidas foram o ácido cafárico (22,38% a 29,98%), catequina (17,61% a 23,48%) e hesperidina (22,43% a 28,47%). Os resultados de atividade antioxidante das bebidas apresentaram variações entre si, e reduções consideráveis das frações bioacessíveis, entre os métodos DPPH e ORAC e tempos avaliados. No ensaio DPPH, a adição das polpas de pitanga e de umbu-cajá resultou no aumento na capacidade antioxidante em PF0 na KSP (2489,41 μM TEAC/100 mL) e na KSUC (2459,05 μM TEAC/100 mL), onde essas bebidas não apresentaram diferenças significativas ($p \leq 0,05$). Porém, KT (2690,98 μM TEAC/100 mL) foi a que apresentou a maior capacidade antioxidante ao final dos 7 dias de observação. Já pelo ensaio ORAC, todas as bebidas apresentaram aumento significativo ($p \leq 0,05$) ao final do armazenamento sob refrigeração. Nossos achados indicam que a utilização de pitanga e de umbu-cajá são interessantes alternativas na elaboração de kombucha, tanto para ampliar e diversificar sabores, mas também refletindo nas características químicas, bioativas e sensoriais de kombucha, revelando uma bebida mais doce, com tendência a aromas frutados e florais.

Palavras-chave: Bebida fermentada; Compostos bioativos; Kombucha análoga.

ABSTRACT

Kombucha is a fermented tea of Chinese origin that stands out due to the beneficial properties associated with its consumption, such as its antioxidant capacity, anti-inflammatory effect and anti-cancer activity. Given the rise of the drink, studies have been using alternative raw materials for the preparation of analogous kombuchas, such as fruit. In this sense, the main objective of the study was to prepare traditional green tea kombucha (KT) and to evaluate the impacts of the addition of pitanga (KSP) and umbu-cajá (KSUC) pulps in different storage times, in terms of pH, acidity total titratable, soluble solids content, organic acid, sugar, volatile and phenolic acid profiles. Additionally, the in vitro gastrointestinal digestion was evaluated on the bioaccessibility of phenolic compounds and in vitro antioxidant activity in the kombuchas produced. The study also enabled the elaboration of a review article, contributing with relevant information about the kombucha drink. Initially, the green tea KT was made from the infusion of dehydrated leaves at 1.25% (w/v), sugar at 7.5% (w/v), 20% (v/v) of the starter tea and 3 % SCOBY (w/v), maintained at 25°C ± 2°C in BOD incubator. After 7 days of fermentation, the culture was removed, the tea was filtered and fractionated into three portions of the same volume and then 15% (w/v) of the fruit pulps (pitanga and umbu-cajá) were added, followed by the drinks were kept at 25°C (± 2°C) for 48h in BOD. At the end of this step, the drinks were filtered and filled into sterile bottles (5°C ± 2°C). During the period evaluated, all beverages presented reduced pH, increased acidity, decreased soluble solids, and reduced sugars (glucose and fructose). Higher values and lower sugar losses were recorded for flavored beverages, evidencing that pre-existing glucose and fructose contents in fruits contributed to sweeter beverages. Organic acetic, butyric, citric, succinic and malic acids were identified in all kombuchas, with the exception of malic acid in KSP. Terpenes (more than 39% of area) were the main volatile compounds reported in beverages, as they contributed to the increase and/or emergence of new terpenoids, such as curzerene and β-caryophyllene. Fifteen polyphenols were identified in KT and 16 in KSP and KSUC, with the flavonoid gallate epigallocatechin standing out in all kombuchas, with values ranging from 63.69% to 76.84%. However, the most bioaccessible phenolics in all beverages were caffeine (22.38% to 29.98%), catechin (17.61% to 23.48%) and hesperidin (22.43% to 28.47%). The results of antioxidant activity of beverages showed variations among themselves, and considerable reductions in bioaccessible fractions, between the DPPH and ORAC methods and evaluated times. In the DPPH assay, the addition of pitanga and umbu-cajá pulps resulted in an increase in the antioxidant capacity in PF0 in KSP (2489.41 μM TEAC/100 mL) and in KSUC (2459.05 μM TEAC/100 mL), where these drinks did not show significant differences ($p \leq 0.05$). However, KT (2690.98 μM TEAC/100 mL) had the highest antioxidant capacity at the end of the 7 days of observation. As for the ORAC test, all beverages showed a significant increase ($p \leq 0.05$) at the end of refrigerated storage. Our findings indicate that the use of pitanga and umbu-cajá are interesting alternatives in the preparation of kombucha, both to expand and diversify flavors, but also reflecting on the chemical, bioactive and sensory characteristics of kombucha, revealing a sweeter drink, with a tendency to fruity and floral aromas.

Key words: Fermented beverage; Bioactive compounds; Kombucha analog.

SUMÁRIO

1 INTRODUÇÃO

O aumento no consumo de alimentos considerados saudáveis e que fornecem benefícios ao organismo está relacionado ao avanço das pesquisas científicas, elas correlacionam hábitos e estilos de vida saudáveis à qualidade de vida e saúde da população. E com isso, impulsionam a indústria pela elaboração de produtos que forneçam aporte de substâncias que reflitam diretamente na saúde (EMILJANOWICZ; MALINOWSKA-PAŃCZYK, 2019; ZUBAIDAH et al., 2019).

Nesta perspectiva, a bebida kombucha tem grande destaque e consumo no atual cenário mundial. De origem milenar chinesa, o kombucha transcende as barreiras do tempo, voltando à notoriedade pelas reivindicações funcionais associadas ao seu consumo. Originária do nordeste da China, esta bebida é elaborada com as folhas de *Camellia sinensis*, possui características sensoriais refrescante, levemente gaseificada e ácida, sendo obtida através da fermentação por uma cultura de bactérias acéticas e leveduras que interagem entre si que estão presentes em uma rede celulósica chamada de SCOBY (*Symbiotic Culture of Bacteria and Yeast*) (COTON et al., 2017; JAYABALAN et al., 2014; JAYABALAN; MALBAŠA; SATHISHKUMAR, 2016; MORALES, 2020).

Diversas propriedades benéficas são associadas ao consumo de kombucha, como atividade antioxidante (CARDOSO et al., 2020), atividade anti-inflamatória (BANERJEE et al., 2010), ação antimicrobiana (BATTIKH, BAKHROUF, AMMAR; 2012) ação hipoglicemiante (ZUBAIDAH et al., 2019) e anticâncer (VITAS et al., 2018). As substâncias bioativas existentes na matriz vegetal utilizada na fermentação, bem como os produtos gerados do metabolismo microbiano são responsáveis por essas propriedades (CHAKRAVORTY et al., 2019). Novas perspectivas científicas estão inovando o preparo da kombucha com a utilização de outros substratos ou agregando outras matrizes vegetais à bebida, como ervas e frutas, apresentando novos sabores para uma aceitação sensorial e diversificando o conteúdo químico (EMILJANOWICZ; MALINOWSKA-PAŃCZYK, 2019; KAPP; SUMNER, 2019; ULUSOY; TAMER, 2019; VITAS et al., 2018).

Espécies frutíferas podem ser utilizadas na elaboração de kombucha, pois irão contribuir na composição de químicas destas bebidas agregando principalmente com ácidos fenólicos e flavonoides (EMILJANOWICZ; MALINOWSKA-PAŃCZYK, 2019). Deste modo, a biodiversidade brasileira apresenta uma infinidade de destas espécies que ganharam popularidade devido a sua composição, principalmente os

compostos fenólicos (DUTRA et al., 2017; MACEDO et al., 2019). Embora essas frutas possuam uma infinidade de fenólicos, poucos relatos são documentados sobre os efeitos da digestão *in vitro* de kombucha e suas análogas (ABUDUAIBIFU; TAMER, 2019; DEĞIRMENCIOĞLU et al., 2021).

Propostas interessantes como a pitanga (*Eugenia uniflora L.*) e o umbu-cajá (*Spondia tuberosa*) são exemplos espécies da biodiversidade brasileira que apresentam sabor e aroma exóticos e agradáveis, com significativa presença de vitaminas, minerais e uma variedade de ácidos fenólicos e flavonoides. Ambos os frutos possuem versatilidade e potencialidade de utilização para obtenção de novas formulações de produtos alimentícios (FRAZON et al., 2018; GALVÃO et al., 2011; LIMA; SILVA; OLIVEIRA, 2018; RAMALHO et al., 2019), podendo ser utilizadas na elaboração de kombucha impactando principalmente nas características físico-químicas e no conteúdo de substâncias bioativas.

Considerando os aspectos apresentados, o presente estudo possui dois produtos científicos, primeiro traz inicialmente informações relevantes sobre a bebida milenar por meio de um compilado de estudo em formato de artigo de revisão narrativo, abordando o contexto histórico, formulação, substratos alternativos, principais características e efeitos benéficos associados ao consumo da kombucha.

O segundo produto científico se trata de um artigo experimental que objetivou avaliar o impacto da adição das polpas de pitanga e de umbu-cajá na elaboração de kombuchas a partir de uma segunda fermentação da kombucha tradicional de chá verde, uma vez que não há estudos evidenciando a utilização dessas matrizes vegetais na elaboração de análogas. Este estudo propôs avaliar parâmetros físico-químicas, perfil de açúcares, ácidos orgânicos e voláteis, bem como avaliar os compostos fenólicos e atividade antioxidante pré e pós digestão *in vitro*.

2 REFERENCIAL TEÓRICO

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2.1 ARTIGO DE REVISÃO

KOMBUCHA: FORMULATION, CHEMICAL COMPOSITION, AND THERAPEUTIC OTENTIALITIES

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ABSTRACT

Kombucha is a millennial beverage of great prominence in the world due to its functional claims. From the infusion of black or green tea leaves *(Camellia sinensis)* sugared and fermented by a symbiotic culture of bacteria and yeasts (SCOBY), an acidic and lightly carbonated beverage is produced. It presents in its composition phytoconstituents with relevant nutritional properties, among these, flavonoids that stand out for their antioxidant, anti-inflammatory characteristics and because they are associated with decreased risk of development of various diseases. Thus, promising results of *in vivo* and *in vitro* tests using the beverage were obtained in several studies. These studies led to/awakened the search for alternative raw materials for tea fermentation, in addition to factors that interfere in the production, constitution and nutritional potentialities of the beverage, as well as its functionality in the face of diseases. Thus, this review compiles relevant scientific data on kombucha involving its origin, production, nutritional potential, and possible health benefits associated with its consumption.

Key words: Fermented Tea; Bioactive Compounds; Fermented beverage.

Introduction

Kombucha is a refreshing, lightly carbonated, acidic beverage, fermented by a culture of bacteria and yeast, called SCOBY (Symbiotic Culture of Bacteria and Yeast). These microorganisms interact symbiotically, from the use of sucrose previously added in green or black tea *(Camellia sinensis).* Despite being a beverage of ancient tradition, it presents wide and emerging consumption worldwide, mainly due to its functional claims reported in several studies.[1–4]

Plant sources are increasingly associated with a healthy diet, since they present, in addition to macronutrients and micronutrients, bioactive substances, among them, phenolic compounds. [5] Scientific evidence proves that the intake of these substances is directly associated with several benefits to the body and in the prevention of diseases. [6,7] In this context, *Camellia sinensis* (CS), used for kombucha production, is a plant widely reported because it is a natural source of these compounds, especially flavonoids, a broad class of phytochemicals belonging to polyphenols and presenting several biological potentialities. [8]

The fermentation process used for the formulation of kombucha promotes the release and/or extraction of bioactive compounds, contributing to alter the composition, bioactivity and digestibility of the fermented beverage, in addition to improving sensory characteristics and prolonging the shelf life of the final product. [9,10] Other compounds also present in the beverage are organic acids, water-soluble vitamins such as C and B complex, and probiotic microorganisms, capable of contributing to the modulation of the intestinal microbiota and thus protecting against diseases such as diabetes and cancer. [11,12]

Additionally, kombucha has been the target of several current studies with the aim of evidencing the beverage as promising food, capable of contributing to the maintenance of health and disease prevention. [13] However, studies still need to be expanded in humans, to take into account all bioavailability complexity and mechanisms of action within the body, since most tests are *in vitro* or in animals.

Considering these aspects, this study aimed to present research, involving kombuchas, that address kombucha's production characteristics, as well as its nutritional properties and the potential health benefits associated with its consumption.

Origin and expansion of Kombucha

Records show that kombucha has been a product appreciated for thousands of years, and its consumption began around 220 BC., in Manchuria, located in northeastern China. During the Chinese dynasty "*Tsin*", the beverage became popular in that country, as it was already believed in its energy and detoxifying properties. [1,13–16]

The consumption of kombucha expanded in Japan around AD 414 through the Korean physician "*Kombu*", whose name was later used to name the current beverage, plus the suffix "*Cha"* (which translates to Tea). [1,13,15,17] It is also reported that this doctor used kombucha in the treatment of emperors of the time, for the relief of digestive symptoms. [1,15,17]

Given these nutritional and functional potentialities, kombucha spread throughout the world and became also known in Europe. Studies report that it first arrived in Russia via commercial sea routes and expanded to Germany and Italy in the 20th century, shortly after World War II. In the 1950s, kombucha also became popular in France and North Africa. [1,15,18]

However, the designation for kombucha was not always the same. During this process of dissemination throughout the world, it presented distinct names, being known

as "*Mo-gu*" and "*Kambucha*" in Russia; "*Kombuchaschwamm*" in Germany; and "*Funkochinese*" in Italy. [1,13,15,17]

In this scenario, with the wide acceptance and possible health claims, at the time, some questions about the possible toxicity of the beverage were pointed out. However, in the 1960s, a study developed by Swiss researchers demonstrated that kombucha was a safe beverage and its consumption could provide positive health effects, thus being even more included in the menu of the world population. [1,19] The Figure 1 shows briefly initial expansion of kombucha.

Currently, kombucha has gained prominence again and has been widely disseminated in the world market for beverages and products with functional claims. This has reflected in investments and research on the development of new formulations and flavors by the food industry through the production of traditional beverages and its analogous. Furthermore, it has been gaining evidence in studies related to its antioxidant action, influence on microbiota and biological activities. [3,13,19–21]

>>> FIGURE 1 <<<

Production of kombuchas

The process of obtaining kombucha is considered simple, since it does not require large equipment and hard-to-reach ingredients. Beverageing water, leaves of CS, sucrose and SCOBY (Symbiosis of bacteria and yeasts) are required for the production of the beverage. [4,13] According to the region and the studies developed, there are variations in the specificities and proportions of the materials used, as described in Table 1, [3,16,22–29] but the methodology described by Jayabalan et al. [1] is referred to as the standard process [19] as shown in Figure 2.

The collection of kombucha starts from the preparation of the tea, by infusion of CS leaves, then the sugar is added and, at room temperature, the inoculation of SCOBY to promote the natural fermentation characteristic of the beverage. The literature also reports the use of previously fermented tea to be used in place of SCOBY, transforming it into a starter species, which can be used to start the fermentation process. [1,13,15]

>>> TABLE 1 <<<

>>> FIGURE 2 <<<

Substrates for kombucha formulation

The dehydrated leaves of CS are indispensable ingredients for the production of traditional kombucha. They are used in their fresh *(green* tea) or fermented (black tea) form. This herb is widely reported in the literature as a source of important phytochemicals with functional potentialities such as catechin, epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC) and epigallocatechin gallate (EGCG), belonging to the flavonoid class. [8,22–24]

The obtention of tea happens by aqueous extraction, under high temperature conditions, and green tea is referred to as the most consumed beverage in the world. [13,24,25] This broad consumption is mainly related to its health claims, as reported by Zhu et al. [24] who, in an *in vivo* study, associated the consumption of green tea with changes in the intestinal microbiota of mice and protection against the development of obesity in these animals, even under the stimulus with the supply of high-fat diets.

To obtain black tea, the leaves of CS, in the post-harvest period, are submitted to fermentation. [25] This process promotes intentional changes in the chemical constitution of leaves and sensory aspects of tea, which may also vary according to the degree of processing of the raw material. [13,26] Fermentation stimulates enzymatic activity acting on the cleavage of the flavonoid ring structure or at phenolic conjugation points, resulting in structural rearrangements that can act in increasing the bioavailability of these compounds. Among these arrangements is the formation of theaflavins and thearubigins, compounds also considered bioactive due to their antioxidant, antimicrobial and anti-inflammatory properties. [25–27]

Sucrose is also a necessary ingredient for the formulation of kombucha, as it provides favorable conditions for microbial metabolism, functioning as an energetic substrate, essential for the symbiotic culture to ferment the tea. [16,20] As a result of this metabolism there is the production of ethanol, acetic acid and other compounds, such as glucuronic acid, which has detoxifying properties. Such compounds are obtained through microbial enzymes and specific metabolic routes. [16,28,29]

SCOBY (Figure 3) [30] is responsible for the fermentation process which results in kombucha. Its acquisition is usually carried out through donation or can still be acquired from companies specialized in the commercialization of this biological material. Although commonly reported as SCOBY, the symbiotic culture may have other designations, such as, mother of kombucha, tea fungus and tea mushroom.[1,12,15]

>>> FIGURE 3 <<<

Composed of a cellulosic polymer matrix, SCOBY houses a multitude of beneficial microorganisms that vary from species and/or genera, but interact beneficially for both. Thus, it presents predominantly acetic bacteria, of the genera *Acetobacter* and *Gluconobacter*, which produce acetic acid and other secondary metabolites resulting from biochemical processes. They also produce a cellulosic network called "tea fungus" that floats as a biofilm on the surface of the liquid medium and that arises around 3 to 4 days after the beginning of the fermentation process. [1,12,15,17,31]

This process involves yeasts and bacteria in different metabolic activities, and yeasts are responsible for hydrolyzing sucrose in glucose and fructose by the action of the enzyme invertase with ethanol formation. Acetic bacteria oxidize glucose in gluconic and glucuronic acid and use ethanol to produce acetic acid and synthesize and polymerize cellulose chains forming the "tea fungus". It is estimated that a single cell of the genus *Acetobacter*, can polymerize up to 200,000 glucose residues in chains β-1,4-glucan. [1,12,20]

Among the yeasts present in SCOBY, the highest predominance of species is the *genus Saccharomyces*, which uses sucrose of the medium for ethanol production. However other genera have also been identified as *Brettanomyces, Candida, Hanseniaspora, Kloeckera, Kluyveromyces, Lachancea, Torulaspora, Pichia, Schizosaccharomyces* and *Zygosaccharomyces*. [13,32,33]

Furthermore, research indicates the existence of probiotic strains in SCOBY that may contribute to the modulation of the intestinal microbiota, especially species belonging to the genus *Lactobacillus*. [2,31] However, further studies are needed to emphasize this action on the human microbiota, as well as its resistance and adherence to the intestinal wall. [34]

Analog Kombuchas beverages

Because of the popularization and visibility of kombucha, researchers have been investigating the variation not only of the concentrations of the ingredients of its original formulation, but of new raw materials and processes. This perspective of innovation increases the possibility of flavors and functionalities of the beverage, which further contributes to the acceptance of these products, called kombucha analogs. [20,21]

Given this reality, current research has been replacing CS or associating with other herbs, fruits and vegetables for the production of the beverage. [35–37] These tests have occurred in a varied way, either using these raw materials to prepare the direct infusion and/or its addition to induce a second fermentation, which favors the flavoring and acceptance of the final product, besides potentiating the profile of bioactive compounds of the beverages. [3,20] Figure 4 [24,46–48] briefly shows some research using alternative substrates for the elaboration of kombucha analogs.

>>> FIGURE 4 <<<

In the study developed by Vitas et al. [3] , the researchers developed fermented beverage using their previously fermented kombucha itself in the proportion of 10% (v/v), added to infusions or supercritical aqueous extracts *of Achillea millefolium* L. The authors observed that according to the parameters of the fermentation process (pH, total acidity and biomass yield), chemical composition (organic, phenolic acids and vitamin C) and sensory analysis, the analogous kombucha produced with the addition of herb extracts stood out in relation to the infusion. Similarly, Abuduaibifu and Tamer [35] developed analogous kombuchas, but using the infusion of red goji berry (Lycium*barbarum* L.) and black goji berry *(Lycium ruthenicium.)* for fermentation, and both were compared with traditional kombucha obtained with black tea. All kombucha samples showed higher antioxidant activity than their infusions. However, the analogous of black goji berry was the most appreciated beverage regarding the sensory parameters analyzed.

Zubaidah et al. [38] used sugary juices from five varieties of salak fruit, also known as snake fruit (*Salacca zalacca* (Gaerth.) Voss), for fermentation for 14 days in a kombucha (SCOBY). The beverages were evaluated for the physical-chemical and sensory properties and observed that one cultivar stood out among the others (Salak Suwaru), and also observed that the fermentative process contributed to increase the antioxidant activity of snake juices, since the fermented juices presented more significant levels of phenolics, tannins and flavonoids. To Xia et al.[39], fermentation with kombucha further increased the beneficial properties of soy milk, since they used SCOBY to produce the fermented beverage. The authors observed the multiplication of lactic and acetic acid bacteria, with the formation of new bioactive compounds associated with the fermentation process, in addition to considerable levels of ferulic and chlorogenic acids in the samples.

Another alternative substrate already utilized was bee pollen, which presents high levels of bioactive compounds, but with compromised bioavailability related to the complex external structure of the cell wall. Pollen was used to assist in the fermentation of SCOBY, with the production of a differentiated beverage, with an increase in prebiotic contents, in addition to higher antioxidant activity and bioactive compound content, presenting greater probiotic action *in vitro*.[40]

Given the broad approach in the use of alternative substrates for the elaboration of analogous kombuchas, research has revealed favorable changes in chemical, functional and sensory variety of beverages. This has awakened the demand for new plant sources for the production of kombucha analogs, which add flavor and functionality in order to meet the demands of consumers.

Chemical Composition

Kombucha has very interesting nutritional qualities, mainly due to the benefits of CS that are already well described in the literature. As for the characteristic acidity of the beverage, it may vary according to the time and speed of fermentation and occurs due to the production of organic acids, especially acetic acid. [3,26,41] Acetic bacteria, the majority in the culture, synthesize acetic acid from the ethanol produced by yeasts. However, there are other acids that also constitute kombucha, such as lactic, gluconic, glucuronic, malic and tartaric acids, among others. [3,13,26,42]

They are also found in kombucha, ethanol, sugars, mainly glucose and fructose, but also sucrose fractions that are not degraded by yeasts, in addition to amino acids, B complex and C vitamins, minerals such as iron, zinc and manganese and polyphenols that may vary according to the raw material used and the conditions of production applied. [1,12,15,22,43]

Thus, studies show that the chemical constitution of the beverage suffers direct interference of the ingredients and their proportions, as well as the variation of the established fermentation parameters. Thus, these variations can potentiate the production of specific nutritional compounds. Examples include phenolic compounds, known to have several health benefits and are associated with disease prevention. [44,45] Among the compounds found in kombucha are flavonoids, especially catechins and their derivatives, considered functional substances. [4,13,15–17]

Still, kombucha is reported to present probiotic microorganisms in its constitution, but its concentration and effect on the microbiota will depend on the microorganisms of

SCOBY used in the fermentation process. Therefore, research involving this possible probiotic effect of kombucha has invested in researching the microbial profile of symbiotic culture in order to evidence the existence of strains that can contribute to this effect. [34,46]

Biological effects associated with kombucha

The biological activities of kombucha and their respective functional and therapeutic potentialities have been associated with the chemical constituents of the beverage and are commonly reported in studies involving *in vitro* and/or *in vivo* tests.

Antioxidant activity

One of the most mentioned and studied biological activities in the scientific literature on kombucha and its analogous is antioxidant action using *in vitro* methods. These techniques are widely disseminated, such as the snare activity of the DPPH radical and ABTS, reduction of the ferric ion (FRAP), among others. This activity has relevance due to the effects they can cause to the body, as they are able to reduce oxidative stress when we ingest these chemical components. [13,47]

Thus, kombucha has been reported throughout the researches as a beverage of high antioxidant potential, due to polyphenols present in plant matrices, which are used for the production of the beverage. [48,49] In addition, antioxidant compounds can also be potentiated or come from the fermentative process performed for the preparation of the beverage. [33]

In this sense, studies indicate that microbial metabolism can promote changes in the concentration of antioxidants due to the complex chemical interaction that happens between microorganisms and phenolic molecules, which can also influence their ability to be absorbed by the body. [22,50]

In vitro trials conducted by Jayabalan et al.[22] in India and the Republic of Korea and by Cardoso et al.[26] in Brazil compared the total antioxidant activity (TAA) of kombucha produced with black tea and green tea, but using different analytical methods (DPPH and ABTS, respectively). According to the study conducted in the Asian continent, green tea kombucha showed higher responses than those produced with black tea, obtaining lower DPPH radical reduction values by 88% when compared to the sample

produced with black tea. In contrast, Cardoso et al.[26] identified more expressive TAA in black tea kombucha with values of 65.32% inhibition of the ABTS radical.

The differences found may be related to the form of production of the beverage, fermentation period, methods and even to the raw materials used. In a study in Serbia, Vitas et al.[3] observed that when formulating analogous kombuchas from supercritical extracts of *Achillea millefolium* L. antioxidant activity varied with increased concentration of the herb, showing that the amount of raw material used can directly influence the response of antioxidant capacity of beverages.

In the study promoted by Zubaidah et al, [38] in Indonesia in cooperation with France, it was observed that the formulation of the analogous kombucha using the exotic snake fruit (*Salacca zalacca* (Gaerth.) Voss) also produced a beverage of high antioxidant and antimicrobial activity, even when compared to the fresh fruit. However, research developed in Turkey by Abuduaibifu and Tamer, [35] when comparing traditional black tea kombucha with analogous from two distinct species of the genus *Lycium*, showed that antioxidant activity, either in the pre or post-digestion phase *in vitro*, during bio accessibility study, was higher for black tea kombucha, followed by the one made from black goji *(Lycium ruthenicium* Murr.) and the red goji *(Lycium barbarum* L.). They also observed that the longer fermentation period also contributed to higher levels of total phenolic compounds and antioxidant activity.

The use of cereals has also been reported as raw materials for the production of kombucha analogues. In trials produced in Egypt, using *in vitro* techniques, Ahmed et al. [41] studied the antioxidant activity of analogous kombuchas prepared with rice and barley compared to traditional black tea kombucha and identified that the beverage formulated from barley infusion was the one that presented the highest prominence in the results reported in their manuscript. Vászquez-Cabral et al. [36] in Mexico, identified a reduction in oxidative stress in macrophages stimulated by lipopolysaccharides, when they were submitted to kombucha produced with oak leaves.

As already reported in this review, the main characteristic of kombucha is its antioxidant activity in *in vitro* trials (in which they are most found in the scientific literature), but there is still little information on *in vivo* processes to demonstrate its antioxidant potential against the body. [47] Another topic that can be taken into consideration to increase this antioxidant characteristic is the use of plant materials that can contribute positively from the chemical point of view. That is, transforming kombucha into a beverage with this appeal, because in this point of view, the antioxidant

potential of traditional and analogous kombuchas can contribute to the supply of these bioactive substances to the body.

Anti-inflammatory activity

The presence of flavonoids provides functional claims to kombucha and anti-inflammatory action. [24] Inflammatory processes are proven to be directly related to the appearance and worsening of diseases, since they are complex responses to infections and organic imbalances. [6] In this sense, *in vitro* and in vivo research have associated the effect of kombucha on these processes. [13,42]

Banerjee et al,[51] in a research conducted in India, evaluated the positive effect of kombucha on gastric ulcers in mice. It was found that the traditional beverage was more effective when compared to unfermented black tea. In this *in vivo* study, traditional black tea kombucha or an analogous kombucha fermented by a strain of *Candida parapsilosis* were administered. The results showed that kombucha showed antioxidant activity *in vitro* and a more significant phenolic content when compared to the analogous beverage. Similarly, the healing process of gastric ulcers was more effective in animals that received traditional kombucha.

A Mexican study investigated the anti-inflammatory effect of traditional black tea kombucha, prepared for 7 days, at 28 °C and its fermented analog, with oak leaves, under the same conditions. Macrophages stimulated by lipopolysaccharides were used and the production of pro-inflammatory cytokines were evaluated. They were nitric oxide (NO), TNF-alpha and interleukin-6 (IL-6). The researchers observed that the analogous kombucha of oak leaves promoted a significant reduction in IL-6 and TNF-alpha levels and negatively regulated the production of NO. [36]

The research by Villarreal-Soto et al, [43] in France, evaluated the fermentation kinetics, in addition to the compounds and microorganisms produced during the process, as well as potential health promotion properties of three consortia of kombuchas. Thus, they identified that *in vitro* assays showed remarkable results including antioxidant capacity against DPPH radical and 15-lipoxygenase enzyme, indicating a potential anti-inflammatory activity.

Antimicrobial activity

Kombucha has its own physicochemical characteristics that favor microbial control, such as: high acidity and presence of phytoconstituents and bioproducts with

antimicrobial action from the raw material and the fermentation process, respectively. The pH of a food matrix is considered an intrinsic antimicrobial factor, which can hinder the growth of pathogens, especially when it is in a pH range between 2.8 to 3.8.[26] The organic and phenolic acids found in the beverage also have antibacterial action in food. [3,52–54]

In this context, a few studies have shown the antimicrobial potential of kombucha against pathogens such as: *Enterobacter cloacae, Pseudomonas aeruginosa, Aeromonas hydrophila, Escherichia. coli, Salmonella enteritidis, Salmonella typhimurium, Yersinia enterocolitica, Shigella sonnei, Campylobacter jejuni, Helicobacter pylori, Bacillus cereus, Staphylococcus epidermis e Staphylococcus aureus*. [42]

These antimicrobial effects also extend to fungi considered pathogenic, as identified in the study developed in Tunisia by Battikh et al. [53] The researchers identified effects of metabolites generated in the kombucha beverage on fungi: *Candida albicans, Candida tropicalis, Candida parapsilosis, Candida glabrata, Candida dubliniensis*. Furthermore, in a current research developed in Brazil, the microbial effect of green tea kombucha was evaluated. The kombucha showed inhibition capacity against *Alicyclobacillus* spp., which is a deteriorating agent in food capable of causing economic losses in processed vegetables. Its detection only happens when the product presents undesirable sensory changes. [55]

However, this antimicrobial capacity may also vary or even have no effects on certain microbial species. The plant material and the quantities used are a determining factor for the efficiency of the antimicrobial potential of the kombucha beverage. [42]

Analyzing the antimicrobial characteristic of kombucha and its analogous, further studies are needed to prove the effect on pathogenic microorganisms. Mainly, these studies focus on microorganisms linked to food-transmitted diseases in order to mitigate risks and obtain strict control in the supply of products.

Anti-hyperglycemic activity

Diabetes is a pathology characterized by increased glucose in the blood plasma due to insufficiency or lack of control in insulin secretion or difficulty in the action of this hormone. Diabetes *mellitus* type 2 is the most common type of diabetes and is associated with inadequate eating habits and lifestyle of the individual. [42,45]

The effects of kombucha on hyperglycemia has been reported by Zubaidah et al, [45] in an *in vivo* study. In this research, different groups of Wistar rats with type 2 diabetes induced by streptozotocin were used. These animals, by groups, received traditional kombucha or their analogous produced with snake fruit juice and metformin medication. The authors showed that the group of animals that received the analogous kombucha presented better responses to hypoglycemic action, being the superior effect even when compared to the group that received metformin, but affirm the need for further studies to evaluate this efficiency in humans.

Similarly, Srihari et al [56] also evaluated antihyperglycemic effect in type 2 diabetic rats, also induced by streptozotocin. The authors administered the lyophilized kombucha extract for 45 days and evidenced that kombucha supplementation in the group of animals decreased glycosylated hemoglobin, increased plasma insulin levels, and found an increase in hemoglobin and tissue glycogen, important factors in the process of blood glucose uptake, as well as tissue recovery.

Similarly, kombucha action on pathophysiological changes resulting from diabetes has already been identified. In a study using black tea kombucha and unfermented tea in diabetic rats supplemented for 14 days, the results showed that both groups presented positive responses regarding the recovery from the pathophysiological changes induced by alloxan, but kombucha stood out in this aspect. According to the authors, this effect can be attributed to the presence of molecules with antioxidant function of beverages and potentiated with the fermentative process. [57]

The incidence of chronic non-communicable diseases such as diabetes has been reported. In this case, kombucha has been shown to be promising as to its possible hypoglycemic action, but also with potential action on the effects associated with this pathology. However, further studies in humans are needed to evaluate the mechanisms of action and bioavailability of their compounds.

Anticancer activity

Cancer is identified as a global public health problem, responsible for millions of deaths every year.[58] Considered to be a multicausal disease, it may be associated with external factors (eating habits and lifestyle) and/or internal factors (genetics).[59] Oxidative stress is one of the factors that can induce carcinogenesis and, in this sense, food components may present interesting therapeutic effects. [60–62] Phytochemicals are

examples of these compounds, as they present high antioxidant potential and important roles in cellular metabolism. [58,63]

For this reason, kombucha has been studied for its possible effect on cancer cells. An *in vitro* study identified antiangiogenic effect of black tea kombucha in human prostate cancer cell lines, inhibiting growth and reducing the possibility of metastasis. [56] Similarly, green tea kombucha has also been identified with antiproliferative activity against cancer cell lines, and this effect is attributed to the presence of catechins, an abundant compound in the beverage. [26]

More specifically, Kaewkod, Bovonsombut & Tragoolpua [58] in Thailand, detected kombucha as a potential source of toxicity in colorectal cancer cells, while Uțoiu et al, [34] in Romania, found moderate antitumor activity of kombucha fermented with pollen, in two human cell lines of laryngeal carcinoma (20 mg/mL) and colon adenocarcinoma (15 mg/mL).

Despite the promising effects described in this section, it is necessary to deepen *in vivo* studies on the interaction of kombucha and neoplasms in a broad context, taking into account bioavailability of compounds with antitumor actions, as well as their effective action and the mechanisms that involve these effects.

Final considerations

Kombucha has been being increasingly spread because of its properties: it is a soft, refreshing, slightly carbonated beverage with potential functional properties. Originally elaborated from the leaves of *Camellia sinensis*, research has identified in kombucha a beverage that can vary in its formulation, as well as its nutritional and sensory characteristics, through the elaboration of similar beverages. The literature reports changes in the production process, diversity of plant matrices used, presence of second fermentation, as well as variations in time, concentration of ingredients and temperature of the fermentation process, which may interfere in the composition of the beverage. Antioxidant, antimicrobial, anti-inflammatory, hypoglycemic and anticancer biological properties are usually attributed to kombucha by *in vivo* and in *vitro* tests already confirmed in studies conducted on almost all continents. However, further studies are needed, including *in vivo* clinical trials and bio accessibility assessment, to investigate the

various biological effects associated with kombucha consumption and all the complexity of the body's systems.

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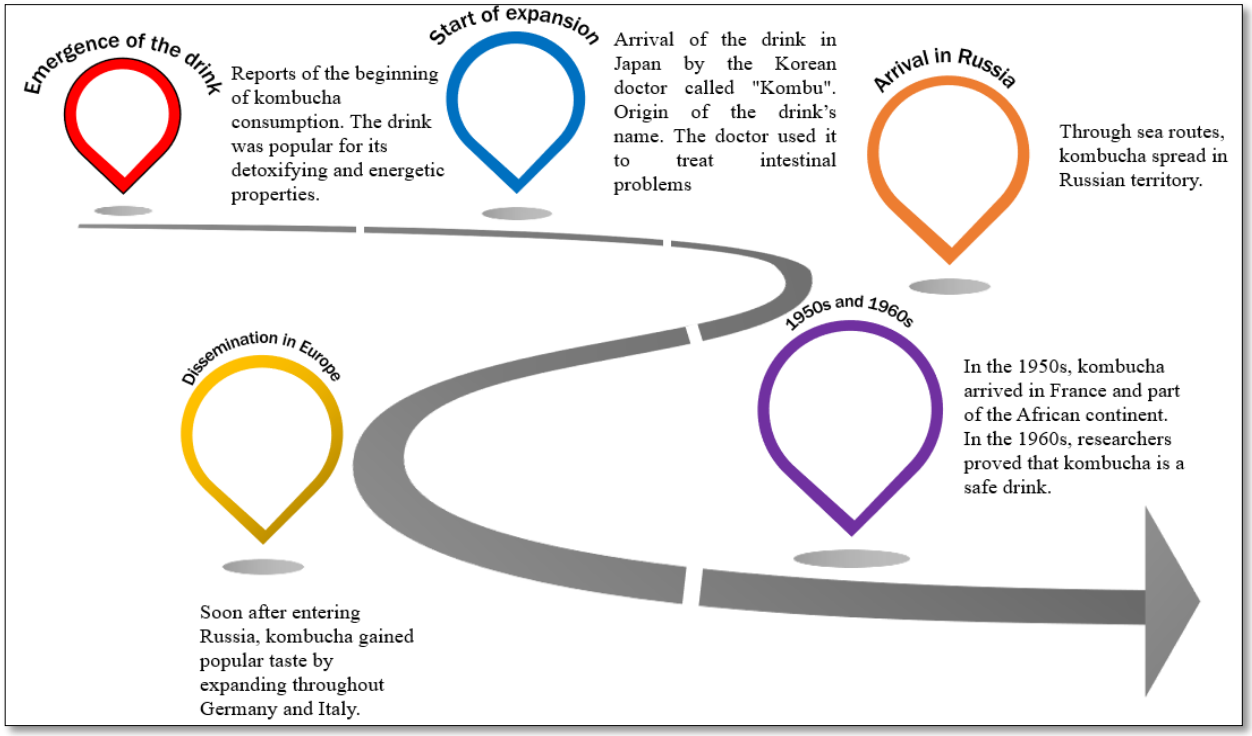
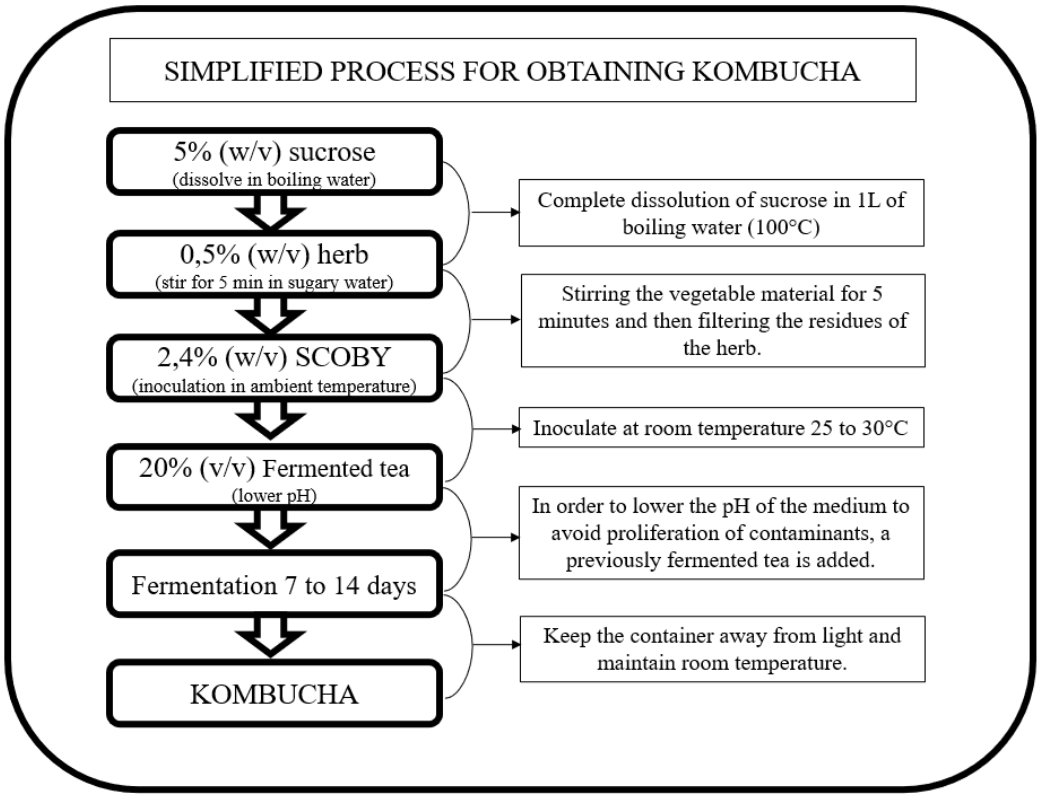
Figure 1. Brief timeline of the origin and expansion of kombucha.



Source: Illustration Developed by authors (2020).

Figure 2. Flowchart for obtaining the kombucha beverage.

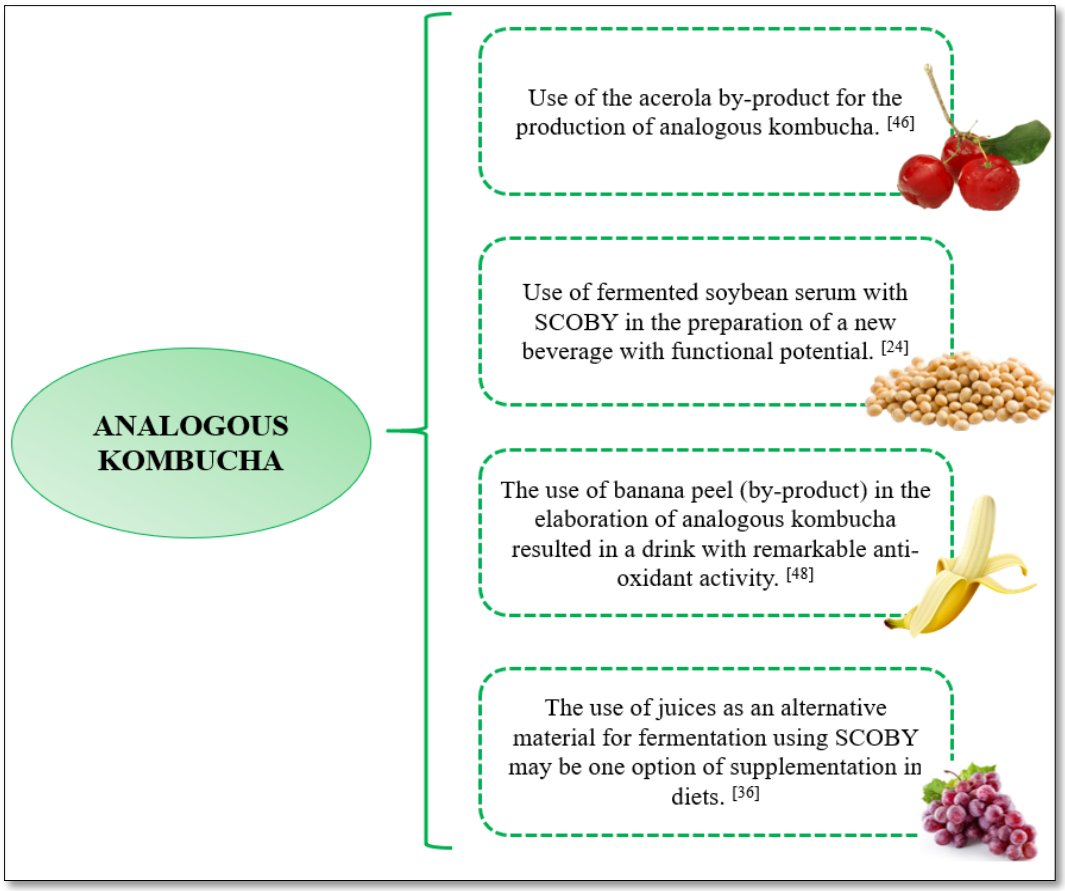


Source : Translated and adapted from Jayabalan et al. [1] (2014).

Figure 3 – SCOBY – Symbiotic Culture of Bacteria and Yeast.

Source: Mousavi et al.,[38] (2018)

Figure 4. Utilization of unusual substrates in the elaboration of analogous kombucha.



Source: Illustration Developed by the authors (2020).

Table 1 - Proportions of the main raw material used in the formulation of kombucha according to several studies.

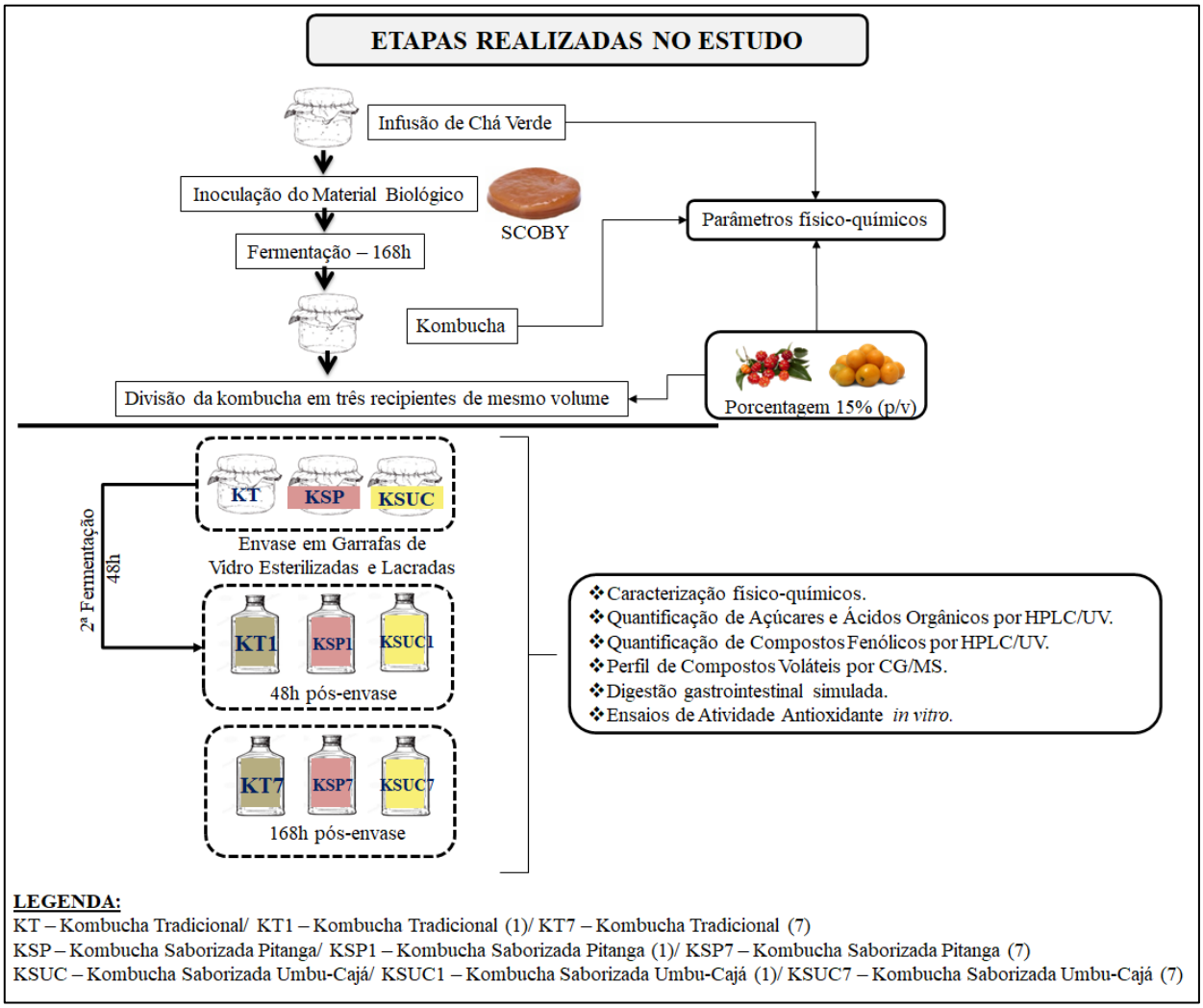
TRADITIONAL KOMBUCHA				
	Traditional Raw Materials			
Authors	**Water (mL)**	**Herb (g)**[*1] (w/v)	**Sucrose (g)**[*1] (w/v)	**SCOBY or Kombucha**[*1,2]
Martinez *et al* [16]	1000	0.50%	5%	10.00%(w/v)
Rahmani *et al* [22]	1100	0.95%	7%	0.63% (w/v)
Ashrafi *et al* [23]	1000	0.40%	10%	3.00%(w/v)
Tu *et al* [24]	1000	1.00%	10%	10.00%(w/v)
Malbaša *et al* [25]	1000	0.15%	7%	10.00%(v/v)
Zubaidah *et al* [26]	500	2.00%	10%	10.00%(v/v)
De Filippis *et al* [27]	1000	1.00%	10%	2.00%(w/v)
Villarreal-Soto *et al* [28]	1000	1.00%	7%	4.00%(w/v)
Kallel *et al* [29]	1000	1.20%	10%	3.00%(w/v)
Vitas *et al* [3]	500	0.45%	7%	10.00%(v/v)

[1]Percentages based on the volume of water used in the studies. [*2]Utilization of SCOBY or Fermented tea as starter of the fermentation process.

3 MATERIAS E MÉTODOS

Todos os ensaios e procedimentos analíticos foram desenvolvidos e realizados na Universidade Federal da Paraíba (UFPB) e nas suas dependências. No Núcleo de Pesquisa e Extensão – Laboratório de Combustíveis e Materiais (NPE – LACOM/UFPB), nos laboratórios do Centro de Tecnologia (CT): Laboratório de Análise de *Flavour* (LAF/CT) e Laboratório de Processos Microbianos em Alimentos (LPMA/CT), e no Laboratório Analítico CVT-SAN, localizado no Centro de Tecnologia e Desenvolvimento Regional (CTDR). Na **Figura 1** tem-se o detalhamento das etapas envolvidas e realizadas no presente estudo, bem como as análises propostas.

Figura 1 – Desenho experimental do estudo.



Fonte: Desenho desenvolvido pelo Autor (2020).

3.1 AQUISIÇÃO DO MATERIAL BIOLÓGICO

A cultura simbiótica de bactérias e leveduras foi adquirida por meio de compra online do laboratório certificado “White Labs Pure Yeast And Fermentation” (San Diego, EUA). A empresa atesta que o material biológico está isento de microrganismos patogênicos, além de informar as bactérias e leveduras benéficas identificadas geneticamente da cultura mãe. Assim, a SCOBY adquirida possui uma proporção dos seguintes microrganismos: **BACTÉRIAS –***Bacillus sp. liqueniformis* (99%); *Bacillus sp. cereus** (99%); *Bacillus sp. pumillus/ aerophilus/ safensis/ altitudinis* (99%); *Acetobacter tropicalis* (99%); *Bacillus sp. aerophilus* (96%); *Bacillus sp. arvabhattai* (98%); *Gluconacetobacter saccharivorans* (99%); *Micrococcus sp.* (98%); *Gluconacetobacter rhaeticus* (98%); *Paenibacillus taichungensis* (97%); *Bacillus sp. subtilis* (99%). **LEVEDURAS –***Brettanomyces bruxellensis* (99%); *Saccharomyces cerevisiae* (92%); *Zygosaccharomyces sp.* (97%); *Candida sp.* (97%). *Segundo o revendedor, esta espécie não é patogênica, ela é comumente encontrada em probióticos (ANEXO a e b).

3.2 AQUISIÇÃO DOS INSUMOS

As folhas de chá verde (*Camellia sinensis*) desidratadas, o açúcar cristal orgânico e os frutos *in natura* de umbu-cajá (*Spondia tuberosa*) foram adquiridos no comércio local de João Pessoa – PB, porém a origem do umbu-cajá é da cidade de Santa Rita, Paraíba, Brasil, que fica a 12 km da capital da Paraíba. As pitangas (*Eugenia uniflora L.*) foram adquiridas por meio de doação e coletadas no mês de agosto de 2020 na área urbana da cidade de Cabedelo (7 ° 00'07,8 "S 129 34 ° 49'50,3" W), Paraíba, Brasil no mesmo município.

3.3 OBTENÇÃO DAS POLPAS DOS FRUTOS

Inicialmente, os frutos foram adquiridos em estádio de maturação comercial, em relação a sua integridade, as espécies vegetais estavam isentas lesões mecânicas ou quaisquer tipos de deterioração de ordem microbiana. Os frutos de pitanga e umbu-cajá foram submetidos a uma lavagem inicial com água corrente para remoção de grandes sujidades, na sequência foram sanitizados por meio de imersão em solução de hipoclorito

de sódio a 100 ppm por 10 minutos, sendo esse procedimento realizado por duas vezes. Logo após o processo de sanitização, os frutos passaram pelo processo enxágue utilizando água destilada e secos por 30 minutos em cabine de biossegurança em temperatura ambiente (26°C ± 2°C). O procedimento de despolpamento foi realizado manualmente, logo após, a polpa foi peneirada em peneira doméstica estéril para separação das sementes e películas protetoras. Antes do armazenamento sob congelamento (-18°C ± 2°C), as polpas foram submetidas análises de pH, acidez titulável total e sólidos solúveis.

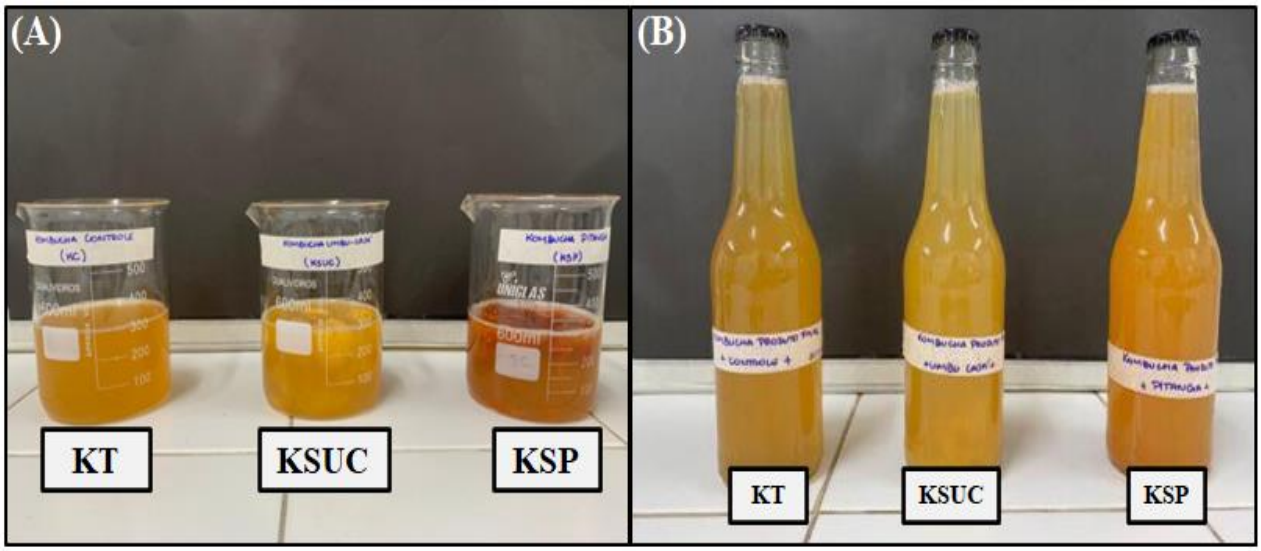
3.4 ELABORAÇÃO DA KOMBUCHA TRADICIONAL E SABORIZAÇÃO

A Kombucha Tradicional (KT) foi obtida por meio da infusão das folhas desidratadas de chá verde. Água potável foi aquecida a 100 °C e cessado o aquecimento foi adicionado 1,25% (p/v) das folhas que ficaram submersas, em recipiente de vidro estéril tampado, por 15 minutos. O chá então foi filtrado e ainda quente adicionado 7,5% (p/v) de açúcar cristal orgânico, sendo homogeneizado até sua completa dissolução. A mistura foi resfriada naturalmente até alcançar a temperatura ambiente (26 °C ± 2 °C). Em seguida, foi realizada a inoculação da SCOBY a 3% (p/v) juntamente com 20% (v/v) de chá *starter*. Um tecido permeável estéril foi utilizado para cobrir e amarrar o gargalo do frasco de vidro onde estava a mistura para formulação da kombucha. Posteriormente, o recipiente foi direcionado a estufa incubadora BOD (CALTECH – MODELO EI – 08F1, Pernambuco, Brasil) com temperatura controlada de 25 °C ± 2 °C por todo período de fermentação, que inicialmente foi de 7 dias. Todos os parâmetros para obtenção da KT foram estabelecidos previamente por meio de estudo piloto.

A segunda etapa deste trabalho iniciou com o preparo de 3 (três) amostras de kombuchas, uma foi referente a tradicional (KT), e as demais foram saborizadas com as polpas e submetidas a segunda fermentação. Para tanto, foram adicionados 15% (p/v) da polpa de pitanga (KSP) e 15% (p/v) da polpa de umbu-cajá (KSUC) (**Figura 2A**). Os três frascos foram cobertos com tecido permeável e acondicionados novamente em estufa incubadora BOD (CALTECH – MODELO EI – 08F1, Pernambuco, Brasil) a 25 °C ± 2 °C, por 48 horas. As bebidas foram filtradas em peneiras estéreis, envasadas em garrafas de vidros de 330 mL esterilizadas e lacradas com auxílio de um arrolhador de garrafas (ALL BREW, Rio Grande do Sul, Brasil) (**Figura 2B**), e posteriormente armazenadas em BOD, com ajuste da temperatura para 3 °C ± 2 °C. As bebidas obtidas após segunda

fermentação (PF0), 24h após o envase (PF1) e 168h depois do envase (PF7) foram submetidas a análises que serão descritas ao longo desta seção. A quantidade de polpa adicionada na saborização foi estabelecida por meio de um estudo piloto.

Figura 2 – Kombucha tradicional e saborizadas com polpa de pitanga e umbu-cajá.



Fonte: Acervo do Autor (2020).

3.5 pH, ACIDEZ TITULÁVEL TOTAL E SÓLIDOS SOLÚVEIS TOTAIS

O valor de pH foi obtido utilizando um pHmetro digital (Quimis, São Paulo – Brasil) previamente calibrado com soluções de pH 4,0 e 7,0. Já a acidez titulável total foi obtida por titulação das amostras com hidróxido de sódio a 0,1 N e os resultados foram expressos em porcentagem de ácido acético. O conteúdo de sólidos solúveis totais foi medido com o auxílio de um refratômetro digital (modelo HI 96801, São Paulo, Brasil) a 25 ± 1 ° C, e os resultados foram expressos em °Brix (AOAC, 2019).

3.6 PERFIL DE ÁCIDOS ORGÂNICOS E AÇÚCARES

A identificação e quantificação dos ácidos orgânicos e açúcares foi realizada por Cromatografia Líquida de Alta Eficiência (CLAE) (COELHO et al., 2018). Para o preparo das amostras, inicialmente foram centrifugadas a 4000 *g* por 15 minutos. Após a separação das fases o sobrenadante foi coletado com auxílio de uma seringa e posteriormente filtrado através de um filtro Millex-HÁ de 0,45 μm (Millipore Co.,

Bedford, MA) e armazenados em vial. Uma alíquota de 10 µL foi injetada em cromatógrafo Agilent 26 (modelo 1260 Infinity LC, Agilent Technologies, EUA) acoplado a um detector de arranjo de diodo (DAD) e um detector de índice de refração (DIR). As condições foram as seguintes: uma coluna Agilent Hi-Plex H (7,7 × 300 mm, 8 µm), onde a temperatura do compartimento da coluna foi mantida a 70°C ± 2°C; H_2SO_4 4 mmol /L em água ultrapura como fase móvel (vazão 0,5 mL / min). A identificação dos ácidos orgânicos foi dada através de um detector DAD a 210 nm, e a dos açúcares através do RID. Os picos obtidos das análises dos ácidos orgânicos e dos açúcares foram identificados comparando seus tempos de retenção com padrões dos ácidos orgânicos (acético, butírico, cítrico, fórmico, láctico, málico, propiônico, succínico e tartárico) e dos açúcares (frutose, glicose, maltose e ramnose) da Sigma Aldrich (St. Louis, EUA). Os resultados foram expressos em gramas por litro (em ambas as análises) de bebida.

3.7 PERFIL DE FENÓLICOS

Para identificação do perfil de fenólicos, as amostras foram também submetidas ao preparo antes da injeção no equipamento. Primeiramente, as amostras das bebidas foram centrifugadas 4000 *g* por 15 minutos, o sobrenadante foi filtrado utilizando filtro Millex-HA de 0,45 µm (Millipore Co., Bedford, MA). Por conseguinte, os compostos fenólicos presentes nas bebidas foram identificados e quantificados utilizando uma coluna Zorbax Eclipse Plus RP-C18 (100 × 4,6 mm, 3,5 µm) e a pré-coluna Zorbax C18 (12,6 × 4,6 mm, 5 µm). A temperatura da coluna foi ajustada para 35 °C, A "fase A" era composta por água acidificada a pH 2,0 com ácido fosfórico 0,1 mM/L e a "a fase B" por metanol acidificado com ácido fosfórico a 0,5%. A vazão foi mantida a 0,8 mL/min. As aquisições de dados do DAD foram processadas utilizando o software Open LAB CDS Chem Station EditionTM (Agilent Technologies) (PADILHA, 2017). Os picos obtidos da análise de compostos fenólicos foram identificados comparando seus tempos de retenção com padrões de Ácido cafárico, Ácido caféico, Ácido clorogênico, Ácido gálico, Ácido p-cumárico, Ácido siríngico, Catequina, Cianidina 3-glucosídeo, Cianidina 3,5-diglucosídeo, Cis-resveratrol, Delfinidina 3-glucosídeo, Epicatequina, Galato de epicatequina, Galato de epigalocatequina, Hesperidina, Kaempferol 3-glucosídeo, Malvidina 3-glucosídeo, Malvidina 3,5-diglucosídeo, Miricetina, Naginarina, Pelargonidina 3-glucosídeo, Pelargonidina 3,5-diglucosídeo, Petunidina 3-glucosídeo, Procianidina A2, B1 e B2, Quercitina 3-glucosídeo, Rutina e Trans-resveratrol foram da

Sigma Aldrich St. Louis, EUA. Os resultados foram expressos em porcentagem relativa referente ao conteúdo total dos compostos fenólicos encontrados nas bebidas.

3.8 PERFIL DE VOLÁTEIS

Os compostos voláteis foram extraídos por microextração em fase sólida por *headspace*. A fibra utilizada foi Divinilbenzeno/Carboxen/Polidimetilsiloxano (DVB / CAR / PDMS) (Supelco, Bellafonte, PA, EUA) e foi acondicionada de acordo com as instruções do fabricante antes da extração. Primeiro, 15 mL de kombucha (de cada amostra) foram transferidos para um frasco de *headspace* de fundo plano de 20 mL com um selo magnético de PTFE/septos de silicone e tampa. Os compostos voláteis foram extraídos colocando o frasco em banho-maria a 40 ºC com agitação magnética interna. A amostra atingiu o equilíbrio em 10 minutos e foi então exposta à fibra por 50 minutos (ZAPATA et al., 2019). Um cromatógrafo de gás (GC) 7890B acoplado a um espectrômetro de massa (MS) Agilent® Technologies 5977B (Little Falls, DE, EUA) e uma coluna capilar de sílica fundida de baixo sangramento / MS Varian® VF-5 MS (5% fenil / 95 % PDMS, 60 mx 0,25 mm ID x 0,25 µm de espessura de filme) foram usados para separar e identificar os voláteis coletados por SPME. A temperatura do injetor GC foi ajustada em 240 °C e a taxa de fluxo do gás portador de hélio foi de 1,2 mL / minuto. A temperatura inicial do forno foi de 40 °C, que foi mantida por 10 minutos, seguida por uma elevação de temperatura de 5 °C / minuto até 240 °C por 11 minutos. O espectrômetro de massa foi operado no modo de impacto eletrônico a 70 eV. As temperaturas da linha de transferência do GC, da fonte de íons e do analisador quadrupolo foram definidas em 250, 230 e 150 °C, respectivamente. Uma faixa de massa de m / z 35–350 foi registrada no modo de varredura completa. Os dados foram adquiridos e analisados usando o programa de software Mass Hunter (Agilent). Os componentes voláteis foram provisoriamente identificados por comparação de seus espectros de massa com espectros do NIST / EPA / NIH Mass Spectral Database (Versão 2.2 2014), mostrando os coeficientes Match> 600 e RMatch> 700. Além disso, o índice de retenção linear publicado (LRI) foi usado para auxiliar na confirmação estrutural dos compostos identificados. A quantidade relativa de componentes individuais foi expressa como área e área percentual do pico em relação à área total.

3.9 DIGESTÃO GASTROINTESTINAL SIMULADA

Este procedimento metodológico simula as condições gastrointestinais humanas, realizado em três fases: a fase oral, gástrica e por último a fase intestinal. Em cada recipiente foram adicionados 5 mL das bebidas, em seguida 3,5 mL de solução salivar e 975 μL de água destilada, 25 μL de $CaCl_2$ 0.,3 M, 0,5 mL de enzima α-amilase (1500 U/mL α-amilase) foram incorporados com a amostra, a mistura foi submetida a agitação mecânica a 90 rpm por 2 minutos a 37 °C. Após o processo para obtenção da fração oral, foi adicionada a mistura a solução gástrica com 0,695 mL de água destilada, 5 mL de $CaCl_2$ 0,3 M, 1,6 mL de solução de pepsina diluída na solução gástrica (2000 U/mL), para ajustar o pH a 2, foi utilizado HCl 0,1 M utilizando um pHmetro digital (Quimis, São Paulo – Brasil). Após o processo anterior, a mistura obtida foi incubada e submetida à agitação mecânica a 90 rpm por 2h sob temperatura de 37 °C. Logo em seguida, para finalização do processo gastrointestinal, foi incorporada a 20 mL fração gástrica, 11 mL solução intestinal com 1,3 mL de água destilada, 40 μL de $CaCl_2$ 3M e 5 mL de pancreatina diluída na solução intestinal (800 U/mL), sendo o pH novamente ajustado para 3,0 utilizando HCl 0,1 M ou NaOH 0,1 M. Este procedimento foi submetido novamente a agitação mecânica sob as mesmas condições anteriormente descrita. Por fim, após a finalização da última etapa para a obtenção da fase intestinal, as amostras foram submetidas ao banho de gelo até atingir a temperatura ambiente e foram armazenadas adequadamente em tubos do tipo Falcon de 50 mL e congeladas até o momento das análises (MINEKUS et al., 2014). Por fim, a fração bioacessível foi analisada através dos métodos de atividade antioxidante DPPH e ORAC que serão descritas a seguir.

3.10 DETERMINAÇÃO DA ATIVIDADE ANTIOXIDANTE

3.10.1 Atividade Antioxidante pelo Sequestro do Radical Livre DPPH•

A solução do radical livre DPPH• foi elaborada a 80 μM, em uma microplaca, foi adicionada 180 μL da solução anterior, em seguida 20 μL das amostras, a mistura obtida tem volume final de 200 μL. Em seguida, a microplaca contendo a mistura foi submetida a repouso por 30 minutos para que acontecesse a reação. Por fim, foi realizada a leitura em um leitor de microplaca modelo Fluorstar Omega, BMG LABTEC, Alemanha, com absorção a 515 nm. O método utilizado foi descrito por Brand-Williams, Cuvelier e Berset

(1995) com modificações propostas por Rufino, Fernandes, Alves e Brito (2009) e adaptações de Oliveira et al., (2020). Os resultados obtidos foram expressos μmol TEAC/100 mL.

3.10.2 Atividade Antioxidante pelo Método ORAC

O método consiste em sequestrar os radicais peroxil gerados pelo radical AAPH (2,20-azobis (2-metilpropionamidina), com metodologia proposta por Zulueta et al., (2009) com adaptações. Para o ensaio, foi utilizado um leitor espectrofotométrico de microplacas (Fluostar Omega, BMG, Alemanha). Foram adicionadas alíquotas de 20 μL das amostras, com 120 μL de fluoresceína (116,66 nM). Em seguida, a microplaca foi submetida a incubação durante 30 minutos a 37 °C. Após essa etapa, foi adicionada em cada poço uma alíquota de 60 μL de AAPH (178 nM) para iniciar a reação, sendo assim, o volume final da mistura em cada poço foi de 200 μL e todos os reagentes foram preparados com tampão fosfato 75 mM com pH 7,4. A intensidade de fluorescência foi medida cineticamente a cada 1 minuto (excitação: 485 nm, emissão: 520 nm). Uma curva de calibração foi obtida previamente e os resultados foram expressos em μmol TEAC / 100 mL.

3.11 ANÁLISES ESTATÍSTICAS

Todos os procedimentos analíticos foram realizados em triplicata (três repetições distintas) e os resultados expressos em médias seguidas de seu desvio padrão. Os dados foram submetidos à análise de variância (ANOVA), seguida do teste de Tukey com o software Statistica 10.0 (Stat Soft Inc., Tulsa, OK, EUA) para determinar diferenças significativas ($p \leq 0,05$) entre as médias.

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4 RESULTADOS E DISCUSSÃO

Os resultados da dissertação estão apresentados em formato de artigo experimental, desta forma, atendendo a Norma Complementar **Nº 03/2011** do Programa de Pós-Graduação em Ciência e Tecnologia de Alimentos (PPGCTA), da Universidade Federal da Paraíba (UFPB).

O artigo intitulado "***Traditional and flavored kombuchas with pitanga and umbu-cajá pulps: chemical properties, antioxidants, and bioactive compounds***" está formatado de acordo com as normas da revista. O referido manuscrito encontra-se redigido na língua inglesa e foi submetido ao periódico internacional "*Food Bioscience*" (**Qualis A2 – CAPES**).

Traditional and flavored kombuchas with pitanga and umbu-cajá pulps: chemical properties, antioxidants, and bioactive compounds.

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Abstract

This study aimed to evaluate the impact of the addition of pitanga (*Eugenia uniflora*) and umbu-cajá (*Spondia tuberosa*) pulps, during storage, in the physicochemical characteristics, volatile, phenolic and antioxidant profile of traditional kombuchas (TK). All beverages TK, PFK (kombucha added with pitanga) and UCFK (kombucha added with umbu-cajá) showed, over the 7 days evaluated, a reduced pH, an increase in acidity, a reduction in the content of soluble solids and sugars (glucose and fructose) characteristic of the fermentation process. Higher values and lower losses of sugars were recorded for flavored beverages, showing that the levels of glucose and fructose pre-existing in fruits contributed to sweeter beverages. Acetic, butyric, citric, succinic, and malic acids were identified in all kombuchas, except for malic acid in PFK. Terpenes were the main volatile compounds reported in beverages, favoring the sensory profile of flavored kombuchas, as they contributed to the increase and/or appearance of new terpenoids, such as Curzerene and β-caryophyllene. In contrast, TK presented acetic acid as the major component. High antioxidant activity was observed for flavored kombuchas and among the identified phenolics, epigallocatechin gallate was the predominant component in all beverages (above 63%). The bioaccessibility of phenolics reduced after simulated gastrointestinal digestion and reflected in the significant drop in the antioxidant capacity of kombuchas. Present study demonstrated that the fruits of pitanga and umbu-cajá contributed to diversify and improve the chemical, bioactive and sensory characteristics of the kombucha, revealing a sweeter beverage, with a tendency to fruity and floral aromas.

Keywords: kombucha analogues; fermented beverage; *Eugenia uniflora*; *Spondia tuberosa*.

1. Introduction

The incentive to consume food with health claims is growing, since the appearance of several chronic non-communicable diseases are often associated with inappropriate eating habits throughout life (Marsola et al., 2021; Rusin et al., 2013). In this sense, kombucha stands out on the world stage as a fermented beverage with interesting nutritional potential due to the presence of bioactive substances and with antioxidant characteristics. Traditionally prepared by infusing the leaves of Camellia sinensis, sweetened and fermented by SCOBY (Symbiotic Culture of Bacteria and Yeasts), it has an acidic, carbonated and slightly sweet characteristic (Chakravorty et al., 2019).

SCOBY consists of a polymeric cellulose film, in addition to acetic bacteria and yeasts, responsible for the fermentation process and the main characteristics of the beverage (Chakravorty et al., 2019; Jayabala et al., 2014; Morales, 2020). Among the potential beneficial effects of kombucha are the high antioxidant, antimicrobial, anti-inflammatory and antiproliferative activity (Chakravorty et al., 2019; Jayabalan et al., 2014; Vitas et al., 2018; Zubaidah et al., 2018). Organic acids, B vitamins, minerals (magnesium, iron, cobalt, among others) and phenolic compounds, mainly catechins and their derivations are compounds commonly identified in kombuchas (Jayabalan et al., 2016; Kapp and Sumner, 2019; Morales, 2020).

However, the search for the use of new plant matrices in the formulation of the beverage has diversified beyond the sensory aspects, the very chemical content of kombuchas (Aung and Eun, 2021; Emiljanowicz and Malinowska-Pańczyk, 2019; Zubaidah et al., 2018). So, for the development of kombucha analogues, the use of different vegetable raw materials has already been reported, such as A*chillea millefolium* L. (Vitas et al., 2018), *Salacca zalacca* (Gaerth) Voss (Zubaidah et al., 2018), *Brassica*

tournefortii (Rahmani et al., 2019), coffee (Bueno et al., 2021), *Porphyra dentata* (Aung and Eun, 2021) and wastes from the fruit pulp industry (Leonarski et al., 2021). In this sense, fruits are recognized as important substrates for formulating these beverages, as they present varied flavors and colors, in addition to high nutritional potential and sources of bioactive compounds (Emiljanowicz and Malinowska-Pańczyk, 2019; Macedo et al., 2019).

Exotic fruits such as pitanga (*Eugenia uniflora* L.) and umbu-cajá (*Spondia tuberosa*) are widely accepted in northeastern Brazil and arouse interest in its sensory and phytochemical characteristics, in addition to presenting high potential for application in the development of new food products (Emiljanowicz and Malinowska-Pańczyk, 2019; Macedo et al., 2019).

In this perspective, this study aimed to i) elaborate traditional kombucha (TK) and add pitanga (PFK) and umbu-cajá (UCFK) pulps and characterize them, at different storage times, in terms of pH, total titratable acidity, soluble solids content, profiles of organic acids, sugars, volatiles, phenolics and antioxidant activity, and ii) to evaluate the impact of the addition of fruit pulps on the antioxidant activity and bioaccessibility of phenolic compounds present in the traditional beverage, during storage.

2. Materials and Methods

2.1 Chemical and reagents

Ethyl alcohol, hydrochloric acid (37% w / w) and sulfuric acid were purchased from Neon (São Paulo, Brazil). α-amylase, pepsin, pancreatin and bile salts were purchased from Sigma Aldrich St. Louis, USA. The chemicals DPPH (2,2′-diphenyl-2-picrilhidrazil), trolox (hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid),

fluorescein and AAPH - 2,20-azobis (2- methylpropionamidine) were obtained by Sigma -Aldrich Chemical, SA (Hamburg, Germany).

The analytical standards used in the analysis of sugars (fructose, glucose, maltose and rhamnose) and organic acids (acetic acid, butyric acid, citric acid, formic acid, lactic acid, malic acid, propionic acid, succinic acid and tartaric acid), as well as the patterns of phenolic compounds (caffeic acid, caffeic acid, chlorogenic acid, gallic acid, p-cumaric acid, siringic acid, catechin, cyanidin 3-glucoside, cyanidin 3,5-diglucoside, cis-resveratrol, delfinidine 3-glucoside , epicatechin, epicatechin gallate, epigallocatechin gallate, hesperidin, kaempferol 3-glucoside, malvidin 3-glucoside, malvidin 3,5-diglucoside, myricetin, naginarine, pelargonidine 3-glucoside, pelargonidine 3,5-diglucoside, petun 3-glucoside, petun 3 , procyanidin A2, B1 and B2, quercitin 3-glucoside, rutin and trans-resveratrol were purchased from Sigma Aldrich St. Louis, USA. All chemicals used in the experiments were of analytical reagent grade.

2.2 Inputs for the production of kombuchas

Dehydrated green tea leaves, organic crystal sugar and beverageing water were purchased in the city of João Pessoa, Paraíba, Brazil. SCOBY with safety certification and absence of pathogenic microorganisms was acquired from the White Labs Pure Yeast and Fermentation laboratory (San Diego, USA) through online commerce.

2.3 Acquisition, processing and obtaining of the pulps

Pitanga fruits were collected in August 2020 in the urban area (7 ° 00'07.8 "S 34 ° 49'50.3" W), in the city of Cabedelo, Paraíba, Brazil. While the umbu-cajá fruits were

acquired, in November 2020, at an open market in the market of João Pessoa, Paraíba, Brazil. According to the trader's information, the umbu-cajá fruits come from the city of Santa Rita, 12 km away from the capital João Pessoa, PB. The selection of fruits was based on commercial maturity and integrity, and those with mechanical damage or deterioration aspects were discarded. Initially, they were washed to remove dirt and sanitized with sodium hypochlorite (100 ppm) for 10 minutes followed by rinsing with repetition of the entire process, for subsequent drying in a biosafety cabin (25 °C ± 2 °C) for 30 minutes. The fruits were manually pulped, and their pulp was stored in a domestic freezer (-18 °C ± 2 °C) until the moment of use.

2.4 Preparation of traditional kombucha and kombuchas with fruits

Previously, a pilot study was carried out to define the quantity of inputs to be used. To obtain the infusion, 1.25% (w/v) dehydrated leaves of green tea were submerged in boiling water for 15 minutes, followed by filtration through a sterile sieve and homogenization with 7.5% (w/v) sugar. The mixture was cooled to room temperature (26 °C ± 2 °C), when 3% (w/v) SCOBY was inoculated along with 20% (v / v) of green tea kombucha. The preparation was incubated in a BOD oven (CALTECH - MODEL EI - 08F1) with the controlled temperature of 25 °C ± 2 °C for 7 days.

After that period, the traditional kombucha (TK) was obtained, the SCOBY was then removed, and the filtered liquid divided into three portions of the same volume and stored in a glass container directed to the traditional kombucha and those flavored with 15% (w/v) of pitanga pulp (PFK) and umbu-cajá (UCFK). The beverages were incubated again in BOD for another 2 days, obtaining the final products at time 0 (FP0). The fruit pulps were removed by filtration through a sterile sieve, the beverages were then filled

and sealed with the aid of a manual bottle opener (ALL BREW, Erechin, Brazil). Again, they were stored in BOD under refrigeration temperature (3 °C ± 2 °C) and analyzed after 1 day of production (FP1) and with 7 days (FP7) of storage.

2.5 pH, Total Titratable Acidity and Total Soluble Solids

The pH, Total Titratable Acidity (ATT) and the Total Soluble Solids (SST) content of fermented beverages were obtained following the standard AOAC (2019) procedure. The pH was measured with the aid of a digital pH meter from Quimis (São Paulo, Brazil) and the titratable acidity expressed as a percentage of acetic acid. Soluble solids were expressed in ° Brix, using a refractometer, model HI 96801, São Paulo, Brazil.

2.6 Profile of organic acids and sugars

To obtain the profile of organic acids and sugars, High Performance Liquid Chromatography (HPLC) was used (Coelho et al., 2018). 10 µL aliquots of each sample were filtered with the aid of a 0.45µm Mille-HA filter and injected into an Agilent chromatograph (model 1260 Infinity LC, Agilent Technologies, USA) coupled to a diode array detector (DAD) and a detector of refractive index (RID). The detection of organic acids and sugars was performed at 210nm under the following analytical conditions: an Agilent Hi-Plex H column (7.7 × 300mm, 8µm); H_2SO_4 mmol / L in ultrapure water as a mobile phase (flow rate 0.5mL/min). The peaks obtained were identified by comparing their retention times with those of the Sigma Aldrich standards (St. Louis, USA) and the quantification was performed through the average area of the injections, with calibration curves of R2 >0.998. The results obtained after the analysis (organic acids and sugars) were expressed in g/L.

2.7 Profile of volatile compounds

The volatile compounds from the kombucha samples were extracted by headspace - solid phase microextraction and divinylbenzene/carboxen /polydimethylsiloxane fiber (DVB/CAR/PDMS) (Supelco, Bellafonte, PA, USA) (Zapata et al., 2019). The extractions took place in a gas chromatograph (Shimadzu Corporation, Kyoto, Japan) (GC2010), coupled to a mass spectrometer (QP2010) with Rtx-5 column (60 m×0.25 mm×0.25 μm). The conditions employed are gas flow rate of 1.2 mL/min, initial extraction temperature of 40 °C, subsequently increased at the heating rate of 5 °C min^{-1} to 60 °C, after 3 °C min^{-1} rate up to 90 °C and later 4 °C min^{-1} rate up to 240 °C, with a plateau at that temperature of 10 min. The temperatures of the GC transfer line, the ion source and the quadrupole analyzer were set at 250, 230 and 150 °C, respectively. A mass range of 35–350 m/z was recorded in full scan mode. The data were analyzed using the Mass Hunter software program (Agilent). The volatile components were identified by comparing their mass spectra with NIST/EPA/NIH Mass Spectral Database (Version 2.2 2014) spectra, showing the Match >600 and RMatch coefficients >700. The results were expressed as a percentage of volatile compound area.

2.8 Bioaccessibility of kombuchas, determination of phenolic composition and Antioxidant activity in vitro predigestion and postdigestion

The in vitro digestion procedure (oral, gastric, and intestinal) followed the methodology described by Dutra et al. (2017) with modifications. Initially, to simulate the oral phase, 5 mL of each beverage was mixed with 3.5 mL of salivary solution (975 μL of distilled water, 25 μL of 0.3 M CaCl2, 0.5 mL of α-amylase enzyme (1500 U/mL α-amylase)). The mixture was subjected to mechanical stirring at 90 rpm for 2 minutes at 37 °C. For simulation of the gastric phase, the sample was added 5 mL of 0.3 M CaCl2,

1.6 mL of pepsin solution diluted in the gastric solution (2000 U/mL), adjusting pH to 2, with 0.1 M HCl. The mixture was then incubated and subjected to mechanical stirring at 90 rpm, at 37 °C for 2h. To finish the digestion process, the gastric phase was added 11 mL of intestinal solution (1.3 mL of distilled water, 40 μL of 3M CaCl2 and 5 mL of pancreatin diluted in the intestinal solution (800 U/mL)). The pH was adjusted to 3 using 0.1 M HCl solutions and finally, the mixture was taken to mechanical stirring, under the same conditions as previously described. After completion of the simulated gastrointestinal process, the bioaccessible fractions were obtained and submitted to analysis of antioxidant activity in vitro and to the phenolic profile.

The analysis of the phenolic profile followed the methodology of Padilha et al., (2018) using an Agilent 1260 Infinity LC System liquid chromatograph (Agilent Technologies, Santa Clara - USA) coupled to a diode array detector (DAD) (model G1315D). The column used was Zorbax Eclipse Plus RP-C18 (100 × 4.6 mm, 3.5 μm) and the Zorbax C18 pre-column (12.6 × 4.6 mm, 5 μm) (Zorbax, USA). The data were processed using the OpenLAB CDS ChemStation Edition software (Agilent Technologies, Santa Clara - USA) and the identification and quantification were made by comparison with external standard Sigma Aldrich standards (St. Louis, USA). The results were expressed in relative percentage from the total value of the phenolic compounds found in each beverage and in its bioaccessible phases.

Antioxidant activity was measured using the DPPH method, described by Brand-Williams, Cuvelier and Berset (1995) with modifications proposed by Rufino, Fernandes, Alves, and Brito (2009) and ORAC method with methodology proposed by Zulueta, Esteve and Frígola (2009). The results were expressed in μM Trolox/100mL of beverage.

2.9 Statistical analysis

The analytical procedures were performed in triplicate and the results were expressed as means followed by their standard deviation. Statistics was applied to determine significant differences ($p \leq 0.05$) between the means, applying the analysis of variance (ANOVA), followed by the Tukey test using the Statistica 10.0 software (StatSoft Inc., Tulsa, OK, USA).

3. Results and discussion

3.1 Physico-chemical parameters, sugar profile and organic acids

The values for pH, titratable acidity, and total soluble solids of the kombuchas are shown in Table 1. Initially, pH of 2.77 (TK), 2.80 (PFK) and 2.19 (UCFK) were found with reduction over time for all beverages. This decrease is mainly attributed to the production of organic acids from fermentation, but the addition of fruits can also contribute to a reduction in the pH of the medium (Chakravorty et al., 2016; Leonarski et al., 2021). Note that the use of pitanga and umbu-cajá pulps influenced the pH of the kombuchas, showing the same trend for this parameter (Supplementary Table 1).

Organic acids are also primarily responsible for increasing the total titratable acidity of kombuchas (Jayabalan et al., 2016; Vitas et al., 2018). In the present study, acidity showed significant differences ($p \leq 0.05$) in all treatments and was shown to increase over time. The same behavior has been observed in other studies using snake fruit (*Salacca zalacca* (Gaerth.) Voss) (Zubaidah et al., 2018), grape juice (*Vitis vinifera*) (Ayed et al., 2017) and red and black goji berry (Abuduaibifu and Tamer, 2019) as alternative matrices in the production of kombuchas.

The content of soluble solids decreased during storage, with less expressive values in traditional kombucha. Similar behavior was observed by Abuduaibifu and Tamer, (2019) in kombucha produced with red and black goji berry and stored also under refrigeration. Soluble solids are related to the content of simple carbohydrates in the medium that tend to reduce over time, since microorganisms use these compounds as energetic substrates (Chakravorty et al., 2016). This reduction in sugars (glucose and fructose) was also identified in all evaluated beverages (Figure 1).

Traditional kombucha showed greater glucose reduction during storage (14.19 g / L> 13.80 g / L> 11.43 g / L) when compared to flavored beverages (PFK: 18.02 g / L> 17, 44 g / L> 16.75 g / L and UCFK: 18.55 g / L> 18.16 g / L> 17.80 g / L). This is because fruits are natural sources of sugars (Lima et al., 2018; Vizzotto et al., 2011b). The same trend was observed for the fructose content with a more expressive decrease in TK (11.23 g / L> 10.81 g / L> 8.81 g / L), while the flavored kombuchas varied differently between them. The fermented pitanga beverage (PFK) showed a reduction in fructose in FP1 (17.28 g / L) with a small increase in FP7 (17.53 g / L). Different behavior was observed for UCFK where there was a significant increase ($p \leq 0.05$) of fructose levels in FP1 (18.19 g / L), and on the 7th day of storage under refrigeration (FP7) a value like FP0 was recorded. This variation in flavored beverages may be associated with the difference in fructose concentrations in pitanga and umbu-cajá fruits (Lima et al., 2018; Vizzotto et al., 2011b). Still, the smaller fluctuations in the fructose levels when compared to the glucose levels, in the different times of flavored beverages, are associated with the preferential pathway of the microorganisms by glucose, mainly yeasts (Chakravorty et al., 2016, 2019; Jayabalan et al., 2014; Leonarski et al., 2021).

In this perspective, Zubaidah et al., (2018) identified a 40-50% reduction in the total sugar used in the production of snake fruit kombucha, after 14 days of fermentation.

Leonarski et al. (2021) used acerola pulp residue (5%, 3% and 1%) to produce kombucha with 15 days of fermentation and evaluated that the reduction in total sugars was 56.6%, 43, 4% and 31.6%, respectively. They also reported that the reductions in glucose levels were always more expressive than those of fructose. In addition, several parameters can influence the sugar levels of a kombucha, such as the type and amount of sucrose used, the chosen plant matrix, the microbial population, and its preferred metabolic pathway, as well as the fermentation time and temperature employed, which shows that the beverage may present profiles of very particular chemical components (Chakravorty et al., 2016; Villarreal-Soto et al., 2018).

The identified and quantified organic acids are shown in Table 2. Acids, butyric, citric, succinic and malic acids were identified in all kombuchas. Except for malic acid that was not detected in the beverage flavored with pitanga. The major organic acids identified in TK were butyric and malic acids. Acetic acid, commonly reported in kombuchas (Ahmed et al., 2020; Cardoso et al., 2020; Vitas et al., 2018) was also identified in TK and as well as butyric, malic, and succinic, presented reduction of the contents on the seventh day of storage under refrigeration.

The addition of the fruits in the traditional kombucha modified the concentrations of these compounds. It is observed in FP0 that acetic acid was higher in flavored kombuchas, prevailing at other times in UCFK. Butyric acid was also more evident in UCFK, during storage. Other studies have also identified butyric acid in kombuchas (Ansari et al., 2019; Chandrakala et al., 2019), however, not as a majority (Jayabalan et al., 2014; Leal et al., 2018). Butyric acid is a short-chain fatty acid obtained as a product of the fermentation of polysaccharides by bacteria. When produced by the microbiota it is directly associated with the health of the intestine. Thus, several studies have correlated

this organic acid with health improvement and disease prevention (Chen et al., 2019; Liu et al., 2021).

Citric acid was the most expressive in the beverage flavored with pitanga, and despite the reduction in PFK on the seventh day of observation, it still represented more than 35% of the total organic acids. This acid is a natural constituent of fruits, including cherry. It has an antioxidant characteristic, used as a preservative in food and cells, it has a protective effect on liver tissue, in addition to an important role in intermediate metabolism, since it is one of the components of the Krebs cycle (Abdel-Salam et al., 2018; Vinholes et al., 2018).

Thus, the use of the pulps showed changes in the physical-chemical characteristics of the beverages and allowed an increase in the concentration of interesting organic acids, with high functional potential. The results corroborate with other studies that attribute variations in the production of Kombucha to changes in the final composition of the product (Abuduaibifu and Tamer, 2019; Jayabalan et al., 2016; Morales, 2020).

3.2 Profile of beverage volatiles

The class of terpenes were the main compounds reported in the samples (Supplementary Table 2) and probably play an important role in the sensory profile of kombuchas. TK exhibited 23 compounds, distributed among the classes of terpenes (9), alcohols (4), esters (4), acids (3), hydrocarbons (2) and alkane (1). The major compound was acetic acid, in all periods analyzed, prevailing in PF0 with 43% of the other volatiles and at the end of storage with 47%. However, most of the compounds present in TK belong to the terpene class, representing in FP0 more than 39% of the total content. Terpenes are a large class of plant substances, responsible for the formation of the aroma

of plants (Huang et al., 2018), especially of the active aromatic compounds of green tea, which can contribute to floral or fruity odors (Lee et al., 2013; Lin et al., 2012), with attention to the volatile linalool also identified in this study. It is recorded that storage under refrigeration caused partial or total losses of some compounds in all analyzed beverages, suggesting spontaneous loss. These findings corroborate those found by Zhao et al., (2018) who found loss of volatiles in traditional kombucha over 14 days of fermentation.

The addition of pitanga pulp in the traditional kombucha contributed to the emergence of new compounds, with 55 volatile components being detected in PFK, with the predominance of terpenes (36), but with identification of esters (9), alcohols (4), acids (3), ketone (1), ether (1) and alkane (1). The terpene class represented 84.53% of the initial total content of the compounds analyzed in PFK, remaining with 63.74% in PF7. Among the terpenes, Curzerene was the most expressive compound in FP0 and FP1, but with considerable losses over time. The highlight at the end of the 7 days of storage was the concentration of β-caryophyllene (33%) in relation to the other substances. Curzerene is a volatile compound already identified in the constitution of pitanga fruits (Rodrigues et al., 2019), as well as β-caryophyllene, but not expressive as trans-b-ocimene, the main volatile component identified in pitanga fruit and an important volatile constituent of other Brazilian tropical fruit species, such as *Spondias citherea* (Oliveira et al., 2006). According to Lee et al. (2013) terpenes, among other substances, can be considered essential aromatic components, contributing to the sensory quality of green tea infusions, the main raw material of this beverage.

In UCFK the variation of volatile substances was even greater, with 63 compounds detected among terpenes (36), esters (11), alcohols (6), hydrocarbons (6), acids (3) and ketone (1). More than 75% of the total volatile compounds identified in FP0

are represented by terpenes. β-Caryophyllene also appears as a major compound in UCFK, however in times FP0 (34.02%) and FP1 (40.05%), with a marked reduction in FP7, not reaching 1%. According to Narain et al., (2007) β-Caryophyllene is characteristic of fruits, and its contents may vary during ripening. Ribeiro et al., (2021) also identified the presence of β-Caryophyllene as one of the compounds present in the largest percentage of area in umbu-cajá jellies. Thus, flavored beverages showed a greater diversity of volatile compounds characteristic of the fruits used, which may make them sensorially more attractive.

3.3 Phenolic profile and antioxidant activity of beverages and bioaccessible fractions

The phenolic profile of kombuchas (TK, PFK and UCFK) and the bioaccessibility of phenolic compounds identified after simulated gastrointestinal digestion, over time, are shown in table 3 and figure 2, respectively. Initially, 15 polyphenols were identified in TK and 16 in kombuchas flavored with pitanga and umbu-cajá pulp. The major compound in all kombuchas was of the flavonoid class, epigallocatechin gallate, presenting values between 63.69% to 76.84%. The expressiveness of epigallocatechin gallate is attributed to the green tea leaves used to prepare kombuchas, since they are identified as the source of these phytochemicals (Cardoso et al., 2020).

However, the bioaccessible fractions of the studied beverages showed qualitative and quantitative reductions in phenolic compounds (Figures 2C, 2D and 2E). Nine compounds were identified in the fractions of traditional beverages (TK) and flavored with umbu-cajá (UCFK), while the digested fraction of PFK showed the release of only eight phenolics. Despite the expressiveness of epigallocatechin gallate in beverages, this

compound was not identified in the digested fraction of PFK and showed low representativeness in the bioaccessible fractions of TK and UCFK.

Among the most bioaccessible phenolics identified after in vitro digestion, of all kombuchas, at different times, are caftaric acid, and flavonoids catechin and hesperidin. These three compounds that most resisted the simulated exposures of gastrointestinal digestion, reached bioaccessibility varying from 17 to 32%. Polyphenols have been associated with the high antioxidant capacity of kombuchas, the profiles of these phytochemicals being modified from the raw materials used in the production of beverages and due to the fermentation process (Chakravorty et al., 2016, 2019; Morales, 2020). However, evidence demonstrates that phenolics, especially flavonoids, are unstable at alkaline pH, characteristic of the final stage of simulated digestion to obtain the bioaccessible fraction. This change in pH and the chemical structure of phenolics themselves can contribute to instability of these compounds when subjected to in vitro digestion (Dutra et al., 2017; Kamiloglu and Capanoglu, 2013; Mosele et al., 2015; Thakur et al., 2020). Inada et al. (2020), emphasized a significant reduction of anthocyanins in jaboticaba (*Plinia jaboticaba*) fruits when submitted to intestinal pH in a simulation of in vitro digestion, but with increased bioaccessibility specifically for ellagic acid. They also reported that phenolic bioaccessibility decreased to 19% after fermentation due to the metabolism of the compounds by the intestinal microbiota.

The results of the antioxidant capacity of the kombuchas and their digested fractions, determined by the DPPH and ORAC assays, are shown in Figure 2 and Table 4. It is observed in the DPPH assay, that the addition of the pitanga and umbu-cajá pulps resulted in an increase in the antioxidant activity in FP0. However, for this same method, TK was the one that showed the highest antioxidant activity at the end of the 7 days of observation. As for the ORAC assay, the same beverages did not differ significantly in

time between FP0 and FP1 and, showed a significant increase ($p \leq 0.05$) at the end of storage under refrigeration. In flavored beverages, PFK showed a reduction in antioxidant activity in FP7 by the DPPH method, while UCFK did not indicate changes during storage. While using the ORAC assay, there was a significant increase in antioxidant activity in FP7 in both flavored kombuchas.

Fruits can contribute with different phytochemicals with antioxidant characteristics, such as carotenoids, anthocyanins, and vitamins, so these compounds may have contributed to obtaining these results (Abuduaibifu and Tamer, 2019; Franzon et al., 2018; Leal et al., 2018; Leonarski et al., 2021). Thus, the difference between the values obtained may be associated with the fruits, since they have a different chemical composition, but also with the methods used to quantify the antioxidant potential by different mechanisms (Casagrande et al., 2018; Zeraik et al., 2016). However, the high antioxidant activity of the analyzed samples corroborates the profile of identified phenolics, with epigallocatechin gallate, as the major compound, which presents hydroxyl groups that give them the characteristic of a compound with high antioxidant activity (Hidalgo et al., 2010).

After the digestion process, it was found that the amounts of phenolic compounds released from the kombuchas reflected negatively on the results of antioxidant activity, in both methods (Figure 2). Possible chemical and structural changes in the compounds present in the samples may justify the differences, since the digestive process involves changes in the pH of the medium, in addition to the action of digestive enzymes that can directly interfere in the final content of the bioaccessible samples (Değirmencioğlu et al., 2020; Leonarski et al., 2021). However, the addition of fruits to taste kombuchas influenced different times and methods. For the DPPH assay, the digested fractions of TK and PFK showed significantly reduced antioxidant activity at the end of 7 days. While

the UCFK results showed to be increasing during the evaluation period (Figures 2A and 2B). The highest antioxidant activity of the bioaccessible fractions recorded was for PFK in FP0 (21.53 ± 1.37 µM TEAC / 100mL). In the ORAC method assay, the highest antioxidant capacity was observed for UCFK fraction in FP1, representing 19.42 ± 2.00 µM TEAC / 100mL. While the PFK fraction showed the highest antioxidant activity in FP7 (14.70 ± 3.94 µM TEAC / 100mL). Compounds such as phenolics, anthocyanins are unstable at alkaline pH and their stability is easily influenced by the concentration of oxygen and ions and can be partially degraded under the simulated conditions of the gastrointestinal tract. Furthermore, these compounds can interact with digestive enzymes and cause losses during digestion (Ananingsih et al., 2013; Inada et al., 2020), which could justify this reduction in antioxidant activity after digestion of beverages.

4. Conclusion

This was the first study to use pitanga and umbu-cajá fruits to taste traditional green tea kombucha. The beverages TK, PFK and UCFK were, over the time evaluated, within the physical-chemical parameters. Fruits contributed to the increase in citric acid and butyric acid, respectively in PFK and UCFK. Terpenes were the main volatile compounds reported in beverages, favoring the sensory profile of kombuchas with floral and fruity aromas. The flavored kombuchas showed greater antioxidant activity both by the DPPH method (FP0) and in the ORAC test (FP7). Epigallocatechin gallate stood out as a major component in the evaluated kombuchas. However, after simulated gastrointestinal digestion, the bioaccessibility of kombucha phenolics reduced, reflecting the significant drop in antioxidant capacity. The complex chemical interaction between microorganisms and phenolics during the fermentation and in vitro digestion process may have supposedly altered the bioaccessibility of antioxidants. Our findings indicate that

the use of pitanga and umbu-cajá are interesting alternatives to expand and diversify the chemical, bioactive and sensory characteristics of kombucha. Studies involving sensory evaluation may be part of future research.

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Conflict of interest

The authors have declared no conflicts of interest for this article.

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Table 1. Physico-chemical parameters of traditional kombucha and flavored with pitanga and umbu-cajá pulps.

Drink	pH			Total Titratable Acidity (% acetic acid w/v)			Total Soluble Solids (°Brix)		
	FP0	FP1	FP7	FP0	FP1	FP7	FP0	FP1	FP7
TK	$2.77^{aA} \pm 0.04$	$2.58^{bB} \pm 0.03$	$2.53^{bC} \pm 0.00$	$0.34^{cB} \pm 0.01$	$0.32^{cC} \pm 0.01$	$0.35^{cA} \pm 0.00$	$7.13^{cA} \pm 0.06$	$6.97^{bB} \pm 0.06$	$6.40^{cC} \pm 0.00$
PFK	$2.80^{aB} \pm 0.01$	$2.85^{aA} \pm 0.01$	$2.67^{aC} \pm 0.01$	$0.48^{bC} \pm 0.00$	$0.51^{bB} \pm 0.00$	$0.59^{bA} \pm 0.00$	$8.10^{aA} \pm 0.00$	$8.00^{aB} \pm 0.00$	$7.10^{aC} \pm 0.00$
UCFK	$2.19^{bA} \pm 0.03$	$2.22^{cA} \pm 0.01$	$2.09^{cB} \pm 0.01$	$0.63^{aC} \pm 0.00$	$0.65^{aB} \pm 0.01$	$0.70^{aA} \pm 0.01$	$8.00^{bA} \pm 0.00$	$8.00^{aA} \pm 0.00$	$7.00^{bB} \pm 0.00$

The results are expressed mean (n=3) ± standard deviation. The averages followed by the same lower case letter vertically evaluate the beverages at the same time, and averages followed by the same upper case letter horizontally evaluate the same beverage flavor at different times, thus not differing statistically from each other. Tukey's test $p \leq 0.05$ was applied. FP0 - Finished Product Time 0; FP1 - Finished Product Time 1; FP7 - Finished Product Time 7; TK - Traditional Kombucha; PFK - Pitanga Flavored Kombucha; UCFK - Umbu-Cajá Flavored Kombucha.

Table 2. Organic acid profile of traditional kombucha and flavored with pitanga and umbu-cajá pulps.

Organic Acids (g / L)	TK			PFK			UCFK		
	FP0	**FP1**	**FP7**	**FP0**	**FP1**	**FP7**	**FP0**	**FP1**	**FP7**
Acetic	$1.20^{bA} \pm 0.01$	$1.21^{bA} \pm 0.00$	$0.93^{cB} \pm 0.01$	$1.24^{aA} \pm 0.01$	$1.24^{abA} \pm 0.05$	$1.20^{bA} \pm 0.01$	$1.24^{aB} \pm 0.00$	$1.30^{aA} \pm 0.01$	$1.29^{aA} \pm 0.01$
Butyric	$2.53^{bB} \pm 0.05$	$2.64^{bA} \pm 0.02$	$1.02^{cC} \pm 0.01$	$1.18^{cB} \pm 0.00$	$1.18^{cB} \pm 0.01$	$1.89^{bA} \pm 0.01$	$2.92^{aA} \pm 0.02$	$2.72^{aB} \pm 0.06$	$2.71^{aB} \pm 0.01$
Citric	$0.64^{bA} \pm 0.01$	$0.38^{bC} \pm 0.04$	$0.47^{bB} \pm 0.01$	$2.25^{aB} \pm 0.01$	$2.31^{aA} \pm 0.02$	$2.20^{aC} \pm 0.01$	$0.17^{cB} \pm 0.00$	$0.22^{cA} \pm 0.00$	$0.17^{cB} \pm 0.00$
Malic	$2.32^{aA} \pm 0.01$	$1.86^{aB} \pm 0.01$	$1.70^{aC} \pm 0.01$	N/D	N/D	N/D	$0.03^{bC} \pm 0.00$	$0.08^{bA} \pm 0.00$	$0.07^{bB} \pm 0.00$
Succinic	$0.71^{bA} \pm 0.01$	$0.51^{aB} \pm 0.01$	$0.39^{cC} \pm 0.01$	$0.89^{aA} \pm 0.11$	$0.24^{cB} \pm 0.01$	$0.85^{aA} \pm 0.01$	$0.44^{cB} \pm 0.01$	$0.47^{bA} \pm 0.00$	$0.46^{bA} \pm 0.01$

The results are expressed mean (n=3) ± standard deviation. The averages followed by the same lower case letter horizontally evaluate the same acid between beverages at the same time and averages followed by the same upper case letter horizontally evaluate the same beverage flavor at different times. thus not differing statistically from each other. Tukey's test $p \leq 0.05$ was applied. N/D - Not detected; FP0 - Finished Product Time 0; FP1 - Finished Product Time 1; FP7 - Finished Product Time 7; TK - Traditional Kombucha; FPK - Pitanga Flavored Kombucha; UCFK - Umbu-Cajá Flavored Kombucha.

Table 3. Profile of phenolic compounds from traditional kombucha and flavored with pitanga and umbu-cajá.

Compounds	TK			PFK			UCFK		
	FP0	**FP1**	**FP7**	**FP0**	**FP1**	**FP7**	**FP0**	**FP1**	**FP7**
Syringic acid	1.67	3.60	1.57	3.42	3.73	1.66	4.17	4.48	3.52
Caftaric acid	10.77	10.79	9.56	7.19	11.72	10.57	8.87	8.96	10.65
Chlorogenic acid	1.20	1.11	1.07	1.05	1.35	1.14	0.98	0.91	1.12
Caffeic acid	0.96	0.92	1.00	0.44	0.83	0.87	0.62	0.64	0.86
p_Coumaric acid	0.16	0.09	0.14	0.09	0.10	0.09	0.09	0.09	0.09
Procyanidin B1	0.56	0.46	0.50	0.35	0.52	1.14	0.80	0.46	1.03
Catechin	2.31	2.40	2.14	1.84	2.80	2.45	1.95	2.01	2.23
Procyanidin B2	4.70	4.80	4.35	3.16	5.19	4.72	3.90	3.93	4.64
Epigallocatechin gallate	70.97	69.00	73.16	76.84	63.69	68.38	72.14	72.21	68.04
Myricetin	2.79	2.77	2.64	2.81	4.77	4.37	2.13	2.19	2.66
Quercitin 3-glucoside	N/D	N/D	N/D	0.26	0.31	0.26	0.18	0.18	0.17
Rutin	0.24	0.28	0.21	0.35	0.83	0.79	0.18	0.18	0.26
Kaempferol 3-glucoside	0.08	0.09	0.29	0.09	0.10	0.09	0.27	0.18	0.26
Hessperidin	1.52	1.48	1.36	0.96	1.76	1.48	1.33	1.37	1.63
cis-Resveratrol	1.36	1.38	1.21	0.88	1.45	1.22	1.15	1.10	1.37
trans-Resveratrol	0.72	0.83	0.79	0.53	0.83	0.79	1.24	1.28	1.46
Total (%)	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**

N/D - Not detected; FP0 - Finished Product Time 0; FP1 - Finished Product Time 1; FP7 - Finished Product Time 7; TK - Traditional Kombucha; PFK - Pitanga Flavored Kombucha; UCFK - Umbu-Cajá Flavored Kombucha

Table 4. Antioxidant activity of kombuchas by DPPH and ORAC assays.

	DPPH (µM TEAC/100mL)		
	FP0	**FP1**	**FP7**
TK	$2142.12^{bC} \pm 42.06$	$2485.76^{aB} \pm 10.52$	$2690.98^{aA} \pm 69.41$
PFK	$2489.41^{aA} \pm 33.49$	$2133.62^{cC} \pm 37.86$	$2350.98^{bB} \pm 11.71$
UCFK	$2459.05^{aA} \pm 23.13$	$2350.76^{bA} \pm 74.49$	$2361.91^{bA} \pm 52.58$
	ORAC (µM TEAC/100mL)		
	FP0	**FP1**	**FP7**
TK	$182.49^{aC} \pm 7.53$	$203.19^{aB} \pm 4.09$	$370.83^{bA} \pm 3.44$
PFK	$190.44^{aB} \pm 14.58$	$200.21^{aB} \pm 3.64$	$474.46^{aA} \pm 15.23$
UCFK	$194.31^{aC} \pm 3.34$	$207.87^{aB} \pm 2.47$	$296.60^{cA} \pm 3.02$

The results are expressed mean (n=3) ± standard deviation. The averages followed by the same lower case letter vertically evaluate the beverages at the same time, and averages followed by the same upper case letter horizontally evaluate the same beverage flavor at different times, thus not differing statistically from each other. Tukey's test $p \leq 0.05$ was applied. FP0 - Finished Product Time 0; FP1 - Finished Product Time 1; FP7 - Finished Product Time 7; TK - Traditional Kombucha; PFK - Pitanga Flavored Kombucha; UCFK - Umbu-Cajá Flavored Kombucha.

Figure 1. Variations in the sugar content (glucose and fructose) of kombuchas, during storage.

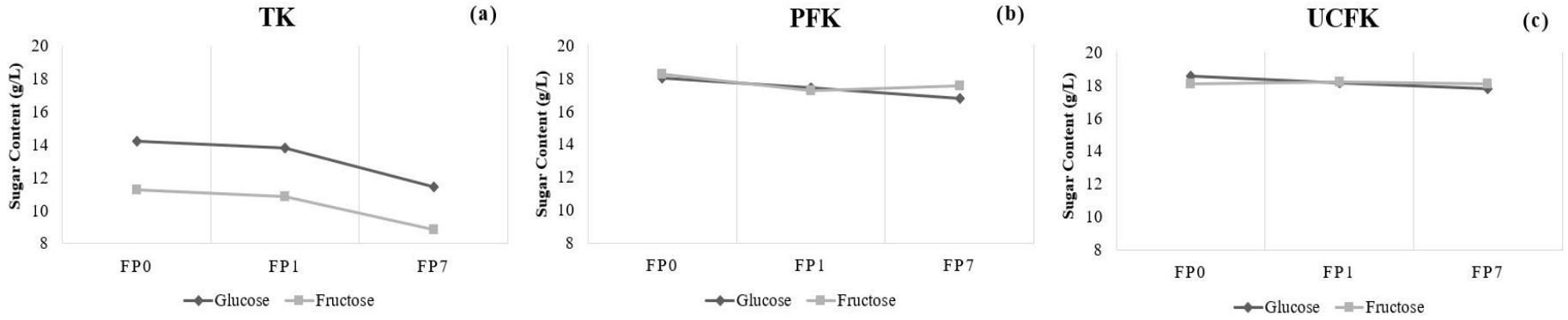
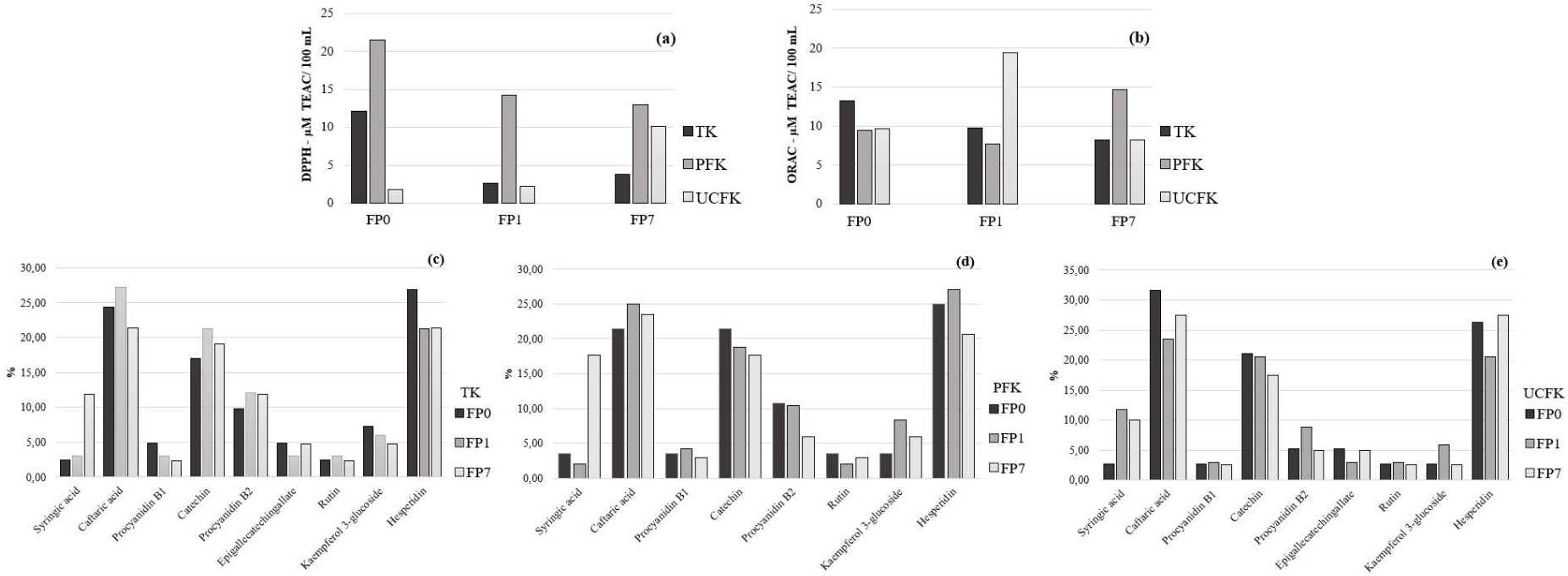


Figure 2. Antioxidant activity and phenolic profile of bioaccessible fractions in kombuchas, during storage**:** (A) Antioxidant Activity - DPPH, (B) Antioxidant Activity - ORAC, (C) TK phenolic profile, (D) PFK phenolic profile and (E) UCFK phenolic profile.



Supplementary Table 1. Physico-chemical characteristics of the infusion, kombucha (7th day) and the pulps used in the study.

PHYSICAL-CHEMICAL PARAMETERS				
PARAMETERS	Green Tea Infusion	Kombucha (7 days)	Pitanga pulp	Umbu-Cajá pulp
pH	5.52 ± 0.03	2.91 ± 0.05	3.13 ± 0,01	2.14 ± 0.01
ATT	0.01[*1] ± 0.00	0.28[*1] ± 0.01	1.63[*2] ± 0,02	1.85[*2] ± 0.02
SST	8.23 ± 0.06	7.10 ± 0.00	10.30 ± 0,00	11.37 ± 0.06

The results are expressed mean (n=3) ± standard deviation. [*1]Values expressed as% of acetic acid (w / v); [*2] Values expressed as% of citric acid (w / v).

Supplementary Table 2. Profile of volatile compounds from TK, PFK and UCFK kombucha by HS-SPME-GC-MS

Code	CAS Number	Compound	Class	RI (lit)	RI (cal)	FP0*		FP1*		FP7*	
						Area x 10^5	%Area	Area x 10^5	%Area	Area x 10^5	%Area
TK											
T1	64-19-7	Acetic acid	Acid	698	734	304.19±59.25[a]	36.39±3.10	131.37±34.34[b]	46.00±5.12	191.96±18.48[b]	47.09±6.32
T2	535-77-3	β-Cymene	Terpene	1023	1023	10.78±5.39	1.33±0.73	nd	nd	nd	nd
T3	5989-27-5	(+)-(R)-Limonene	Terpene	1030	1027	69.21±15.42	8.49±2.60	2.64±1.21	0.90±0.29	nd	nd
T4	470-82-6	Eucalyptol	Terpene	1032	1030	40.01±11.88	4.88±1.68	2.67±1.13	0.92±0.29	nd	nd
T5	104-76-7	2-Ethylhexanol	Alcohol	1033	1033	42.33±6.09[a]	5.13±0.92	11.47±0.13[b]	4.11±0.56	14.33±5.44[b]	3.51±1.42
T6	3779-61-1	trans-β-Ocimene	Terpene	1043	1041	8.85±9.84	1.10±1.26	nd	nd	nd	nd
T7	78-70-6	β-Linalool	Terpene	1100	1100	58.19±3.26[a]	7.05±0.74	24.84±11.10[b]	8.70±3.22	13.58±2.27[b]	3.34±0.70
T8	60-12-8	Phenylethyl Alcohol	Alcohol	1112	1112	10.64±5.25	1.25±0.53	nd	nd	nd	nd
T9	123-07-9	4-Ethylphenol	Alcohol	1163	1163	6.66±2.46	0.79±0.21	nd	nd	nd	nd
T10	98-55-5	α-Terpineol	Terpene	1182	1181	11.96±6.81	1.39±0.63	3.75±1.21	1.34±0.44	nd	nd
T11	106-32-1	Ethyl octanoate	Ester	1190	1189	110.50±25.85[a]	13.31±3.01	19.14±3.14[b]	6.76±0.19	54.05±12.94[b]	13.59±4.67
T12	106-24-1	trans-Geraniol	Terpene	1250	1250	3.16±0.62	0.38±0.04	nd	nd	nd	nd
T13	2785-89-9	4-Ethylguaiacol	Alcohol	1277	1276	7.28±1.19	0.87±0.05	2.80±0.52	1.02±0.33	nd	nd
T14	334-48-5	n-Decanoic acid	Acid	1381	1381	51.44±15.38[a]	6.17±1.70	25.11±6.36[b]	9.24±3.63	30.21±3.32[ab]	7.40±0.88
T15	110-38-3	Ethyl decanoate	Ester	1399	1397	50.78±21.93	5.99±2.06	34.62±13.15	12.46±5.04	70.86±53.46	15.79±8.27
T16	629-59-4	Tetradecane	Alcane	1400	1400	6.51±1.70	0.78±0.15	nd	nd	nd	nd
T17	87-44-5	β-Caryophyllene	Terpene	1424	1422	13.18±6.21[a]	1.64±0.86	2.79±0.66[b]	0.98±0.11	3.83±3.07[ab]	0.85±0.49
T18	6753-98-6	α-Humulene	Terpene	1458	1457	4.33±1.60	0.53±0.23	nd	nd	nd	nd
T19	4537-11-5	(5-Decyl)benzene	Hydrocarbon	1535	1539	3.39±2.55	0.40±0.29	1.91±0.27	0.68±0.13	nd	nd
T20	143-07-7	Dodecanoic acid	Acid	1568	1565	7.79±1.38	0.95±0.22	6.39±1.08	2.25±0.12	5.88±1.85	1.40±0.13
T21	106-33-2	Ethyl dodecanoate	Ester	1597	1595	4.42±1.05	0.53±0.12	9.85±3.91	3.41±1.01	31.36±22.64	7.03±3.43
T22	4537-15-9	(1-Butylheptyl)benzene	Hydrocarbon	1633	1637	3.11±2.39	0.36±0.26	2.43±0.46	0.89±0.29	nd	nd
T23	628-97-7	Ethyl hexadecanoate	Ester	1994	1994	2.30±1.36	0.28±0.18	0.93±0.12	0.33±0.03	nd	nd
PFK											
P1	64-19-7	Acetic acid	Acid	610	<800	119.20±28.43	3.98±1.61	130.05±59.85	2.61±0.73	138.67±79.73	5.84±2.72
P2	110-93-0	6-Methyl-5-heptene-2-one	Cetone	986	990	7.18±2.41	0.23±0.07	6.78±0.94	0.14±0.01	nd	nd
P3	123-35-3	β-Myrcene	Terpene	991	993	4.99±5.74	0.12±0.09			6.92±4.24	0.28±0.11
P4	1569-60-4	Methylheptenol	Alcohol	994	996	20.47±9.40	0.62±0.12	6.78±0.94	0.14±0.01	nd	nd
P5	123-66-0	Ethyl hexanoate	Ester	1000	1002	nd	nd	nd	nd	3.67±2.03	0.15±0.06

P6	5989-27-5	D-Limonene	Terpene	1030	1027	37.06±4.26	1.29±0.60	2.36±1.04	0.05±0.01	17.83±14.75	0.53±0.17
P7	470-82-6	Eucalyptol	Terpene	1032	1030	17.26±1.82	0.60±0.27	nd	nd	nd	nd
P8	104-76-7	2-Ethylhexanol	Alcohol	1033	1033	23.92±9.80^{a}	0.74±0.26	8.97±3.15^{b}	0.18±0.03	6.33±1.79^{b}	0.35±0.29
P9	3779-61-1	trans-β-Ocimene	Terpene	1043	1042	nd	nd	nd	nd	180.71±127.02	6.37±1.06
P10	78-70-6	β-Linalool	Terpene	1099	1100	54.47±19.53	1.72±0.59	23.68±7.11	0.48±0.05	nd	nd
P11	7216-56-0	(4E,6Z)-allo-Ocimene	Terpene	1131	1127	nd	nd	nd	nd	26.97±20.18	0.90±0.12
P12	93-89-0	Ethyl benzoate	Ester	1165	1164	6.07±4.50	0.20±0.18	nd		14.52±5.51	0.73±0.53
P13	98-55-5	α-Terpineol	Terpene	1182	1182	15.21±5.37^{b}	0.53±0.29	5.03±2.48^{b}	0.10±0.03	34.14±9.69^{a}	1.87±1.58
P14	119-36-8	Methyl salicylate	Ester	1185	1184	25.09±18.59	0.74±0.59	15.92±0.99	0.33±0.04	14.00±6.15	0.84±0.84
P15	106-32-1	Ethyl octanoate	Ester	1190	1189	73.29±38.05	2.26±1.19	124.07±23.14	2.66±0.99	141.23±78.57	5.80±2.31
P16	101-97-3	Ethyl benzeneacetate	Ester	1243	1239	2.82±1.14	0.09±0.04	nd	nd	nd	nd
P17	106-24-1	trans-Geraniol	Terpene	1250	1250	5.86±3.29	0.17±0.05	nd	nd	13.33±4.87	0.71±0.59
P18	2785-89-9	4-Ethylguaiacol	Alcohol	1277	1276	5.23±2.87	0.15±0.04	nd	nd	nd	nd
P19	20307-84-0	δ-EIemene	Terpene	1338	1339	12.41±9.15^{a}	0.34±0.09	7.87±1.98^{b}	0.16±0.01	nd	nd
P20	334-48-5	n-Decanoic acid	Acid	1373	1380	39.33±12.70^{ab}	1.23±0.19	12.50±4.64^{b}	0.25±0.05	50.48±17.97^{a}	2.59±1.98
P21	515-13-9	(-)-β-Elemene	Terpene	1391	1394	48.12±33.90^{a}	1.35±0.32	27.74±12.71^{b}	0.56±0.15	nd	nd
P22	110-38-3	Ethyl decanoate	Ester	1396	1397	99.12±49.88	2.97±0.98	285.90±29.03	5.95±0.56	296.46±189.64	10.65±1.45
P23	629-59-4	Tetradecane	Alcane	1400	1400	6.75±3.19	0.20±0.03	nd	nd	nd	nd
P24	87-44-5	β-Caryophyllene	Terpene	1419	1423	49.87±39.19	1.37±0.45	123.54±19.81	2.56±0.09	1089.32±821.57	33.04±8.08
P25	29873-99-2	(-)-γ-Elemene	Terpene	1433	1438	237.60±202.27^{b}	6.42±2.58	391.37±70.32^{a}	8.09±0.12	nd	nd
P26	489-39-4	(+)-Aromadendrene	Terpene	1440	1443	7.52±5.29	0.21±0.06	7.57±3.87	0.15±0.05	nd	nd
P27	6753-98-6	α-Humulene	Terpene	1454	1458	nd	nd	6.30±2.30^{b}	0.13±0.02	381.36±299.81^{a}	11.05±3.93
P28	25246-27-9	(-)-Alloaromadendrene	Terpene	1461	1465	nd	nd	5.86±3.60	0.12±0.05	nd	nd
P29	112-53-8	1-Dodecanol	Alcohol	1473	1475	nd	nd	nd	nd	3.91±2.30	0.19±0.13
P30		2-Isopropenyl-4a,8-dimethyl-1,2,3,4,4a,5,6,7-octahydronaphthalene	Terpene	1478	1479	29.58±23.43	0.81±0.27	54.00±17.71	1.10±0.15	23.37±17.49	0.75±0.10
P31	6980-46-7	γ-Amorphene	Terpene	1488	1485	nd	nd	24.78±5.76	0.51±0.02	nd	nd

P32	17066-67-0	(+)-β-Selinene	Terpene	1486	1490	51.92±38.43	1.44±0.40	76.17±21.77	1.56±0.15	98.24±78.13	2.92±0.86
P33	28624-23-9	δ-Selinene	Terpene	1493	1495	14.62±12.95	0.39±0.17	28.47±8.49	0.58±0.06	nd	nd
P34	3691-11-0	δ-Guaiene	Terpene	1505	1501	nd	nd	246.98±66.98	5.06±0.40	nd	nd
P35	17910-09-7	Curzerene	Terpene	1498	1505	1111.00±452.00[a]	34.31±7.19	863.09±205.12[ab]	17.74±0.79	160.61±127.34[b]	4.75±1.44
P36	18319-31-8	8,14-Cedranoxide	Eter	1539	1517			4.24±1.48	0.09±0.01		
P37	483-76-1	(+)-δ-Cadinene	Terpene	1524	1528	15.78±12.20	0.43±0.14	31.18±7.20	0.64±0.02	17.84±12.66	0.61±0.05
P38	58893-88-2	Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethylidene)-, (4aR,8aS)-	Terpene	1544	1541	70.15±55.33	1.92±0.63	155.34±39.33	3.19±0.19	nd	nd
P39	5951-61-1	(±)-Cadinene	Terpene	-	1544	nd	nd	81.83±23.79	1.67±0.17	nd	nd
P40	6813-21-4	3,7(11)-Selinadiene	Terpene	1542	1548	102.17±82.42	2.78±0.96	241.94±53.70	4.98±0.14	nd	nd
P41	15423-57-1	Germacrene B	Terpene	1564	1564	88.34±55.62[b]	2.53±0.40	198.05±28.48[a]	4.11±0.21	nd	nd
P42	143-07-7	Dodecanoic acid	Acid	1568	1572	nd	nd	nd	nd	19.56±11.87	0.81±0.35
P43	106-33-2	Ethyl dodecanoate	Ester	1595	1597	51.02±14.63	1.70±0.81	161.67±92.80	3.59±2.61	222.13±144.90	7.96±1.49
P44	20303-60-0	(4S)--trans-β-Elemenone	Terpene	1605	1613	nd	nd	540.22±135.17	11.09±0.64	nd	nd
P45	5945-72-2	Neointermedeol	Terpene	1631	1620	7.96±4.73	0.23±0.07	7.40±2.99	0.15±0.03	nd	nd
P46	472-07-1	(+)-Junenol	Terpene	1625	1626	13.50±6.95	0.40±0.11	14.54±5.41	0.29±0.05	nd	nd
P47	1474790	α-epi-Cadinol	Terpene	1640	1648	nd	nd	7.24±1.31	0.15±0.00	nd	nd
P48	6989-21-5	Atractylone	Terpene	1673	1669	225.52±139.42	6.49±0.95	271.95±98.80	5.52±0.97	nd	nd
P49	117066-77-0	epi-γ-Eudesmol	Terpene	1675	1673	7.77±2.12	0.25±0.04	6.78±0.42	0.14±0.04	nd	nd
P50	6902-91-6	Germacrone	Terpene	1705	1705	465.19±201.60	14.12±2.60	495.63±85.33	10.25±0.23	nd	nd
P51	6831-17-0	Aristolone	Terpene	1745	1740	8.47±4.46	0.25±0.05	10.25±1.90	0.21±0.00	nd	nd
P52	62332-96-1	Germazone	Terpene	1746	1752	132.76±57.25	4.03±0.76	121.65±30.05	2.50±0.14	nd	nd
P53	489-84-9	Guaiazulene	Terpene	1778	1782	1.29±0.89	0.04±0.01	4.92±2.16	0.11±0.07	nd	nd
P54	124-06-1	Ethyl tetradecanoate	Ester	1794	1794	4.19±1.28	0.15±0.09	6.37±4.11	0.14±0.11	4.99±2.78	0.20±0.07
P55	628-97-7	Ethyl hexadecanoate	Ester	1993	1994	6.05±2.37	0.21±0.12	5.22±1.30	0.11±0.05	3.16±1.56	0.15±0.10
UCFK											

U1	64-19-7	Acetic acid	Acid	698	<800	124.67±8.78[ba]	5.83±1.94	78.93±51.64[b]	2.64±0.29	192.45±21.80[a]	9.90±2.75
U2	105-54-4	Ethyl butanoate	Ester	802	803	11.21±1.07	0.52±0.16	3.43±1.77	0.13±0.04	nd	nd
U3	123-35-3	β-Myrcene	Terpene	991	992	7.07±64.42	0.28±0.09	8.42±4.57	0.32±0.10	nd	nd
U4	123-66-0	Ethyl hexanoate	Ester	1000	1002	4.59±0.90	0.21±0.04	5.94±4.01	0.19±0.02	nd	nd
U5	142-92-7	Hexyl acetate	Ester	1020	1023	5.76±3.59	0.23±0.07	nd	nd	nd	nd
U6	5989-27-5	D-Limonene	Terpene	1030	1028	52.16±33.20	2.07±0.69	23.94±16.20	0.79±0.11	nd	nd
U7	470-82-6	Eucalyptol	Terpene	1032	1030	25.43±12.86	1.05±0.17	nd	nd	nd	nd
U8	104-76-7	2-Ethylhexanol	Alcohol	1033	1033	14.85±1.89	0.68±0.19	6.32±2.59	0.26±0.13	nd	nd
U9	3779-61-1	trans-β-Ocimene	Terpene	1043	1041	55.09±29.67	2.25±0.46	226.95±165.79	6.95±1.35	nd	nd
U10	3338-55-4	cis-β-Ocimene	Terpene	1048	1051	15.00±7.04	0.63±0.07	43.42±34.63	1.24±0.47	nd	nd
U11	111-87-5	1-Octanol	Alcohol	1071	1075	4.52±0.56	0.21±0.06	3.23±0.19	0.18±0.17	nd	nd
U12	78-70-6	β-Linalool	Terpene	1100	1101	129.95±41.85[a]	6.19±3.34	90.58±56.49[ab]	3.04±0.13	30.24±20.04[b]	1.46±0.81
U13	60-12-8	Phenylethyl Alcohol	Alcohol	1116	1111	23.19±12.68	1.03±0.53	nd	nd	nd	nd
U14	3016-19-1	(4E,6E)-Allocimene	Terpene	1128	1127	18.88±5.58	0.83±0.08	42.44±31.85	1.32±0.28	nd	nd
U15	93-89-0	Ethyl benzoate	Ester	1165	1164	18.77±2.12	0.87±0.25	11.22±7.31	0.38±0.05	nd	nd
U16	562-74-3	4-Terpineol	Terpene	1170	1170	4.48±0.07[a]	0.21±0.08	2.18±1.36[b]	0.09±0.05	nd	nd
U17	98-55-5	α-Terpineol	Terpene	1182	1182	50.07±10.00	2.26±0.45	17.20±14.33	0.53±0.17	nd	nd
U18	119-36-8	Methyl salicylate	Ester	1184	1184	30.68±3.09[a]	1.42±0.43	7.50±6.79[b]	0.24±0.10	7.77±2.73[b]	0.38±0.03
U19	106-32-1	Ethyl octanoate	Ester	1190	1189	48.47±14.12	2.13±0.21	74.21±40.96	2.68±0.57	54.68±28.77	2.52±0.32
U20	112-14-1	Octyl acetate	Ester	1200	1201	3.54±1.45	0.15±0.01	3.29±2.19	0.11±0.01	nd	nd
U21	106-24-1	trans-Geraniol	Terpene	1250	1250	21.50±6.22[a]	0.95±0.15	11.42±6.99[ab]	0.39±0.03	2.09±0.42[b]	0.10±0.02
U22	2785-89-9	4-Ethylguaiacol	Alcohol	1277	1276	4.89±0.09[a]	0.23±0.09	1.50±0.94[b]	0.05±0.01	2.08±0.39[b]	0.10±0.02
U23	3856-25-5	Copaene	Terpene	1376	1378	25.18±7.99	1.10±0.08	19.14±7.81	0.87±0.58	nd	nd
U24	334-48-5	n-Decanoic acid	Acid	1381	1382	nd	nd	24.84±14.27	1.15±0.81	40.81±6.80	2.07±0.45
U25	105-87-3	Geranyl acetate	Terpene	1387	1386	21.76±8.76	0.93±0.03	17.21±8.24	0.68±0.28	nd	nd
U26	110-38-3	Ethyl decanoate	Ester	1399	1398	79.85±22.25	3.53±0.40	156.33±107.98	6.13±2.97	176.92±125.76	7.84±2.44
U27	87-44-5	β-Caryophyllene	Terpene	1424	1427	810.27±365.73	34.02±3.12	1265.86±837.90	40.65±2.27	23.56±26.72	0.93±0.75

U28	357414-37-0	10,10-Dimethyl-2,6-dimethylenebicyclo[7.2.0]undecane	Terpene	1440	1433	nd	nd	13.68±9.73	0.43±0.07	nd	nd
U29	29873-99-2	γ-Elemene	Terpene	1433	1437	nd	nd	nd	nd	38.70±16.04	1.83±0.18
U30	489-39-4	(+)-Aromadendrene	Terpene	1443	1443	nd	nd	3.74±2.43	0.13±0.03	nd	nd
U31	889360-49-0	4,11,11-trimethyl-8-methylenebicyclo[7.2.0]undec-3-ene	Terpene	1460	1454	6.95±2.43	0.30±0.01	428.16±297.98	13.28±1.88	nd	nd
U32	689-67-8	6,10-Dimethyl-5,9-undecadien-2-one	Cetone	1456	1456	nd	nd	nd	nd	24.65±14.69	1.11±0.24
U33	6753-98-6	α-Humulene	Terpene	1458	1459	256.25±138.47	10.47±2.14	10.08±5.21	0.38±0.12	nd	nd
U34	112-53-8	1-Dodecanol	Alcohol	1473	1475	22.02±18.89	0.81±0.54	6.02±4.63	0.28±0.23	nd	nd
U35		2-Isopropenyl-4a,8-dimethyl-1,2,3,4,4a,5,6,7-octahydronaphthalene	Terpene	1478	1479	18.00±8.87	0.75±0.11	24.90±16.75	0.82±0.09	13.98±10.43	0.61±0.23
U36	17066-67-0	(+)-β-Selinene	Terpene	1491	1491	75.14±38.20	3.10±0.51	106.30±75.38	3.32±0.48	34.50±26.26	1.53±0.58
U37	28624-28-4	(+)-δ-Selinene	Terpene	1497	1495	nd	nd	nd	nd	12.15±14.61	0.47±0.43
U38	473-13-2	α-Selinene	Terpene	1501	1500	6.38±4.13	0.25±0.09	7.26±5.76	0.21±0.08	nd	nd
U39	17910-09-7	Isogermafurene	Terpene	1506	1503	nd	nd	nd	nd	351.02±116.72	17.09±3.20
U40	3691-11-0	δ-Guaiene	Terpene	1501	1510	6.38±5.85	0.24±0.12	7.26±5.76	0.21±0.08	nd	nd
U41	56633-28-4	α-Panasinsene	Terpene	1519	1522	1.91±1.19	0.08±0.02	2.50±1.67	0.08±0.01	nd	nd
U42	483-76-1	(+)-δ-Cadinene	Terpene	1528	1527	17.01±8.22	0.71±0.09	17.84±10.56	0.63±0.12	nd	nd
U43	4537-11-5	(5-Decyl)benzene	Hydrocarbon	1535	1539	5.06±1.39	0.22±0.03	nd	nd	nd	nd
U44	58893-88-2	Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethylidene)-, (4aR,8aS)-	Terpene	1544	1540	nd	nd	nd	nd	48.65±46.17	2.02±1.17
U45	4537-12-6	(1-Propylheptyl)benzene	Hydrocarbon	1546	1547	4.91±1.24	0.22±0.03	nd	nd	nd	nd
U46	6813-21-4	3,7(11)-Selinadiene	Terpene	1547	1547	nd	nd	nd	nd	60.87±50.08	2.61±1.14
U47	15423-57-1	Germacrene B	Terpene	1557	1563	nd	nd	nd	nd	27.22±22.40	1.17±0.51
U48	143-07-7	Dodecanoic acid	Acid	1568	1565	11.96±2.01	0.54±0.13	11.89±4.93	0.58±0.47	93.68±47.21	4.56±1.76
U49	913176-41-7	Caryophyllenyl alcohol	Alcohol	-	1575	12.31±2.79	0.55±0.09	10.56±2.58	0.51±0.38	nd	nd
U50	106-33-2	Ethyl dodecanoate	Ester	1597	1595	60.03±12.18	2.99±1.79	60.99±45.61	2.29±1.05	nd	nd

U51	20303-60-0	β-Elemenone	Terpene	1605	1612	nd	nd	nd	nd	320.51±63.40	16.14±3.32
U52	472-07-1	(+)-Junenol	Terpene	1625	1626	nd	nd	nd	nd	4.88±0.58	0.27±0.13
U53	4537-15-9	(1-Butylheptyl)benzene	Hydrocarbon	1633	1637	7.00±0.82	0.32±0.09	2.96±1.67	0.13±0.09	nd	nd
U54	5945-72-2	(-)-Neointermedeol	Terpene	1660	1661	62.38±21.46	2.70±0.27	5.08±3.54	0.16±0.0.02	nd	nd
U55	4536-86-1	(1-Propyloctyl)benzene	Hydrocarbon	1643	1646	nd	nd	2.96±1.67	0.14±0.09	nd	nd
U56	6989-21-5	Atractylone	Terpene	1673	1669	nd	nd	nd	nd	147.89±97.35	6.57±1.85
U57	6902-91-6	Germacrone	Terpene	1705	1705	nd	nd	nd	nd	297.32±107.68	14.30±0.93
U58	2719-63-3	5-Phenyldodecane	Hydrocarbon	1730	1735	nd	nd	1.54±1.26	0.05±0.02	nd	nd
U59	6831-17-0	Aristolone	Terpene	1745	1740	nd	nd	nd	nd	7.11±2.17	0.35±0.04
U60	62332-96-1	Germazone	Terpene	1746	1752	nd	nd	nd	nd	75.16±20.64	3.70±0.52
U61	2400-00-2	3-Phenyldodecane	Hydrocarbon	1766	1769	nd	nd	1.14±1.00	0.04±0.02	nd	nd
U62	124-06-1	Ethyl tetradecanoate	Ester	1795	1794	nd	nd	5.27±3.91	0.17±0.06	3.27±1.52	0.18±0.14
U63	628-97-7	Ethyl hexadecanoate	Ester	1994	1995	33.24±24.97	1.27±0.63	3.37±1.29	0.17±0.13	3.46±1.00	0.19±0.12

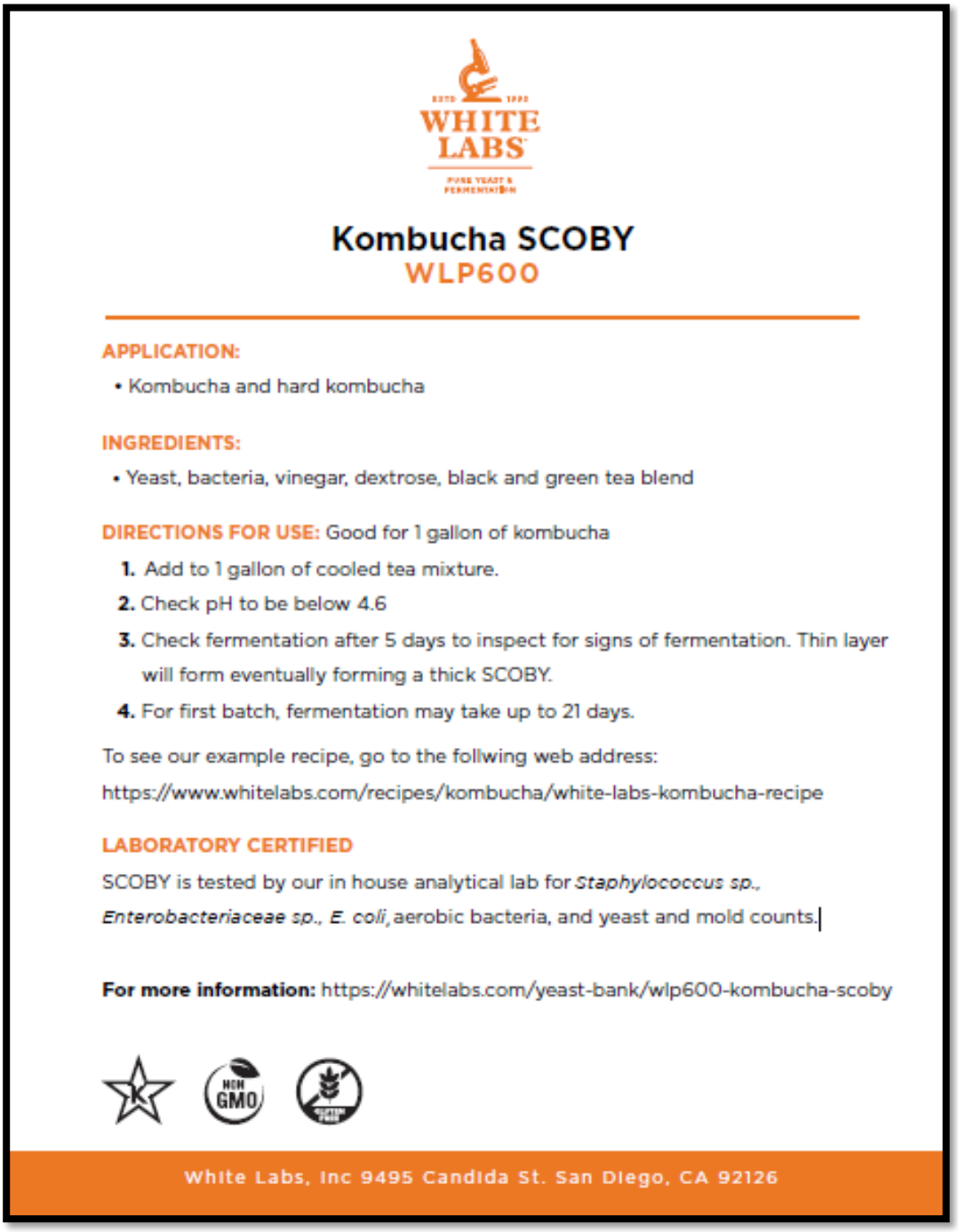
Footnote: LRI (lit.): linear retention index in the literature; LRI (cal.): Linear retention index calculated; * Data expressed as mean ± standard deviation; na = not detected; a, b, c Area means within a line with different envelopes differ significantly ($P \leq 0.05$), n = 3

5 CONSIDERAÇÕES FINAIS

A utilização de materiais vegetais alternativos, como as frutas, mostrou-se viável para elaboração de kombucha análoga. Em nosso estudo pioneiro utilizando polpas de pitanga e umbu-cajá, possibilitou diversificar o sabor, aroma e compostos químicos da bebida kombucha. As kombuchas elaboradas apresentaram, ao longo do período de avaliação, dentro dos parâmetros físico-químicos. A saborização de kombucha tradicional de chá verde com as polpas de pitanga e umbu-cajá contribuiu para o aumento e/ou surgimento de novos compostos voláteis, com destaque os terpenoides: curzereno e β-cariofileno. Remetendo ao conteúdo de ácidos orgânicos, o processo de adição com as polpas mostrou interessante, pois houve uma diversificação no conteúdo, onde o ácido cítrico e butírico foram mais evidentes. Com relação a capacidade antioxidante das bebidas desenvolvidas, o conteúdo químico das polpas contribuiu para o aumento dos valores nos métodos utilizados, isso por conta da presença de flavonoides como o galato de epigalocatequina. A bioacessibilidade dos fenólicos das kombuchas reduziu após digestão gastrointestinal simulada refletindo na queda significativa da capacidade antioxidante. Mesmo assim, nossos achados indicam que o uso de pitanga e umbu-cajá são alternativas interessantes para expandir e diversificar as características químicas, bioativas e sensoriais do kombucha, revelando uma bebida mais doce, com tendência a aromas frutados e florais. Estudos envolvendo avaliação sensorial podem fazer parte de pesquisas futuras.

ANEXOS

a) Folha Técnica WL600 Kombucha SCOBY emitida pelo laboratório White Labs



WHITE LABS
PURE YEAST & FERMENTATION

Kombucha SCOBY
WLP600

APPLICATION:

- Kombucha and hard kombucha

INGREDIENTS:

- Yeast, bacteria, vinegar, dextrose, black and green tea blend

DIRECTIONS FOR USE: Good for 1 gallon of kombucha

1. Add to 1 gallon of cooled tea mixture.
2. Check pH to be below 4.6
3. Check fermentation after 5 days to inspect for signs of fermentation. Thin layer will form eventually forming a thick SCOBY.
4. For first batch, fermentation may take up to 21 days.

To see our example recipe, go to the follwing web address:
https://www.whitelabs.com/recipes/kombucha/white-labs-kombucha-recipe

LABORATORY CERTIFIED

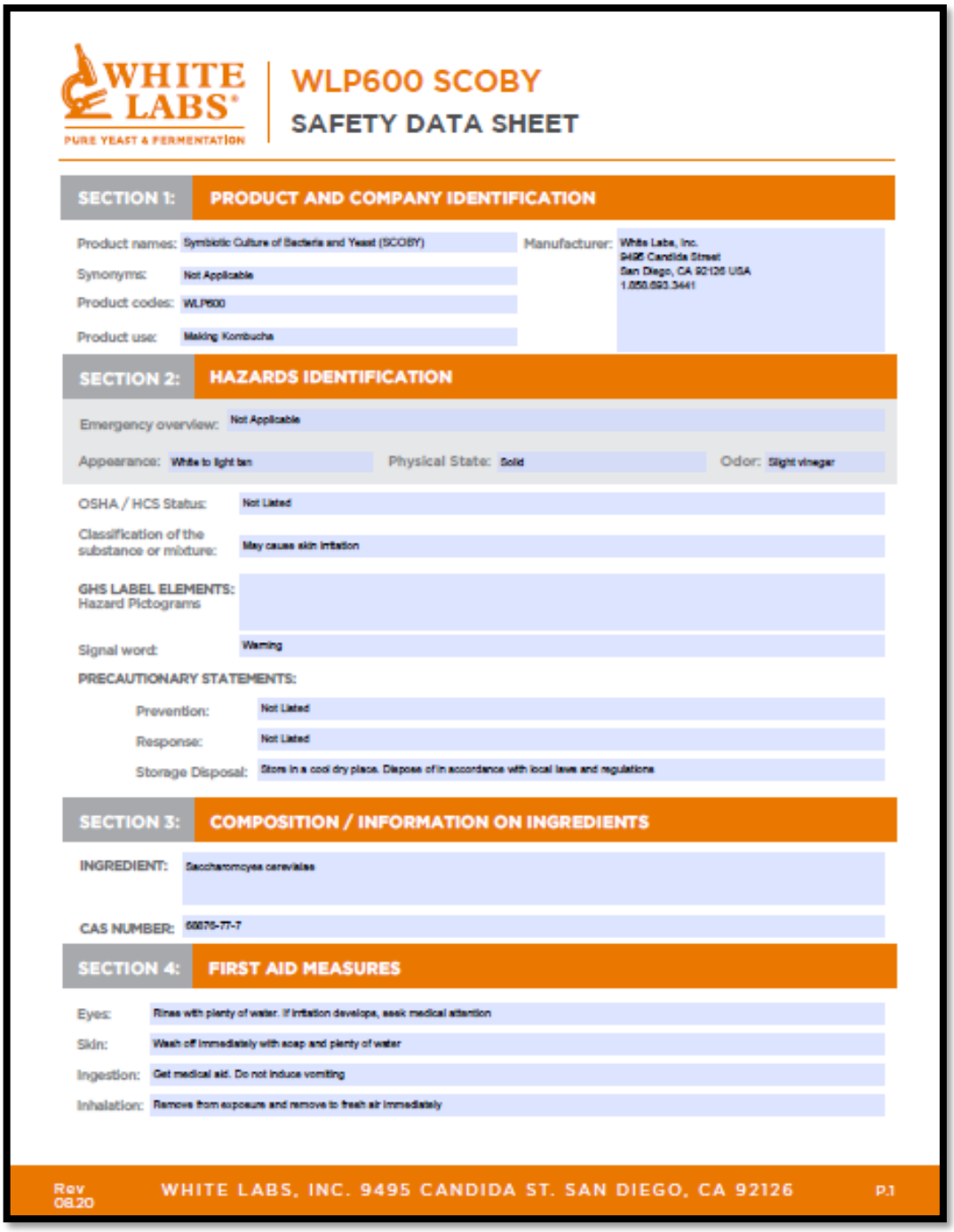
SCOBY is tested by our in house analytical lab for *Staphylococcus sp.*, *Enterobacteriaceae sp.*, *E. coli*, aerobic bacteria, and yeast and mold counts.

For more information: https://whitelabs.com/yeast-bank/wlp600-kombucha-scoby

NON GMO

White Labs, Inc 9495 Candida St. San Diego, CA 92126

b) Ficha de Dados de Segurança da SCOBY emitida pelo laboratório White Labs



WHITE LABS®
PURE YEAST & FERMENTATION

WLP600 SCOBY
SAFETY DATA SHEET

SECTION 1: PRODUCT AND COMPANY IDENTIFICATION

Product names: Symbiotic Culture of Bacteria and Yeast (SCOBY)
Synonyms: Not Applicable
Product codes: WLP600
Product use: Making Kombucha

Manufacturer: White Labs, Inc.
9495 Candida Street
San Diego, CA 92126 USA
1.858.693.3441

SECTION 2: HAZARDS IDENTIFICATION

Emergency overview: Not Applicable

Appearance: White to light tan
Physical State: Solid
Odor: Slight vinegar

OSHA / HCS Status: Not Listed
Classification of the substance or mixture: May cause skin irritation

GHS LABEL ELEMENTS:
Hazard Pictograms

Signal word: Warning

PRECAUTIONARY STATEMENTS:

Prevention: Not Listed
Response: Not Listed
Storage Disposal: Store in a cool dry place. Dispose of in accordance with local laws and regulations

SECTION 3: COMPOSITION / INFORMATION ON INGREDIENTS

INGREDIENT: Saccharomyces cerevisiae
CAS NUMBER: 68876-77-7

SECTION 4: FIRST AID MEASURES

Eyes: Rinse with plenty of water. If irritation develops, seek medical attention
Skin: Wash off immediately with soap and plenty of water
Ingestion: Get medical aid. Do not induce vomiting
Inhalation: Remove from exposure and remove to fresh air immediately