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Kombucha from alternative raw materials – the review

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Abstract

Nowadays, people's awareness about the role of diet in maintaining well-being and good health has increased. Consumers expect that the products not only provide them with essential nutrients but will also be a source of biologically active substances, which are beneficial to their health. One of the "healthy trends," which has appeared among the consumers worldwide is kombucha, a tea drink with high antioxidant potential, obtained through the activity of a consortium of acetic acid bacteria and osmophilic yeast, which is also called "tea fungus." Kombucha obtained from tea is characterized by its health-promoting properties. Promising results in *in vitro* and *in vivo* studies have prompted research groups from around the world to search for alternative raw materials for tea fungus fermentation. Attempts are made to obtain functional beverages from leaves, herb infusions, vegetable pulp, fruit juices or milk. This review focuses on describing the progress in obtaining a fermented beverage and bacterial cellulose using tea fungus on alternative raw materials.

Keywords: tea fungus, kombucha, fermented beverages, fruit pomace, bacterial cellulose

1. Introduction

In recent years, consumer awareness about food quality and the role of diet in maintaining good health has increased. People expect that food products will not only be a convenient, ready to eat after minimal preparation, but will also be the source of essential nutrients as well as substances positively affecting health and well-being. Therefore, a continuous increase in demand for foods having desirable effects on the body has been observed, affecting the rapid development of a new food market. Such products are currently called "functional food." This term was introduced in the early 1990s and defined as food which provides not only basic nutrition but also exerts a positive effect on the human body by being a source of biologically active substances. These compounds can reduce the risk of certain diseases or slow down the ageing processes. The term "functional food" includes traditional foods with naturally occurring bioactive substances (e.g. dietary fiber, polyphenols), food with the addition of bioactive substances (e.g. peptides, antioxidants) and derived food ingredients introduced into ordinary foods (e.g. prebiotics). Health claims about the ability of functional food must be supported by significant scientific evidence (Martirosyan & Singh, 2015).

The concept of functional food is derived from the philosophical tradition of the East, in which there is no apparent difference between drugs and nutrition. A particular place in this topic is occupied by fermented products that have been obtained since ancient times. Fermentation is a method of food preservation, which allows extending the freshness of products, as well as causing favorable changes in the bioavailability of active compounds. Of particular interest are products, such as sauerkraut, kimchi, milk fermented beverages or kombucha whose functional properties have been thoroughly tested and described in the international literature (Hazra, Gandhi, & Das, 2018; Marco et al., 2017; Peñas, Martinez-Villaluenga, & Frias, 2016).

This work focuses on the review of the knowledge about the properties of kombucha beverages obtained from alternative raw materials, e.g. fruits, vegetables or herb infusions, and their comparison with the characteristics of fermented sweetened tea. The paper also describes the possibilities of using a waste product, bacterial cellulose created during the preparation of a drink.

2. Kombucha – fermented tea beverage

Kombucha is a fermented beverage with a specific refreshing, sweet and slightly sour flavor resembling carbonated cider. It is obtained from the sweetened medium, commonly black or green tea, by the action of a consortium of acetic acid bacteria and osmophilic yeast (so called "tea fungus"), which takes 7–21 days (De Roos & De Vuyst, 2018; Dickmann et al., 2017; Kapp & Sumner, 2019). Tea fungus, in the form of a cellulosic biofilm, transform the sugar and tea components into bioactive compounds with therapeutic effects.

2.1. Characteristics of microorganisms in tea fungus

Fermentation occurs rapidly after adding tea fungus to the sweetened tea. In this cellulosic biofilm yeasts are present, among others: *Candida stellimalicola*, *Candida tropicalis*, *Lachancea thermotolerans*, *Lachancea fermentati, Eremothecium cymbalariae, Kluyveromyces marxianus*, *Pichia mexicana*, *Dekkera bruxellensis*, *Dekkera anomala*, *Saccharomyces cerevisiae, Saccharomyces uvarum*, *Zygosaccharomyces bailii*, *Zygotorulaspora florentina* (Villarreal-Soto, Beaufort, Bouajila, Souchard, & Taillandier, 2018). In addition to yeast, bacteria are also present, including lactic acid bacteria (LAB) from the genus of *Lactobacillus* sp. – *Lactobacillus kefiranofaciens, Lactobacillus nagelli*, *Lactobacillus satsumensis* and *Lactococcus* sp. (Marsh, O'Sullivan, Hill, Ross, & Cotter, 2014). LAB delivered from kombucha can be considered as probiotics because they meet most of the criteria for these: they have a high tolerance for bile salts and they are able to survive in the human gut (Matei et al., 2018). However, the leading group of bacteria in tea

fungus, the most numerous, are acetic acid bacteria (AAB), mainly species from the genera *Acetobacter* (*Acetobacter aceti*, *Acetobacter pasteurianus, Acetobacter nitrogenifigens*), *Gluconacetobacter* (*Gluconacetobacter* sp *A4*, *Gluconacetobacter sacchari, Gluconacetobacter oxydans*) and *Komagataeibacter* (*Komagataeibacter xylinus, Komagataeibacter kombuchae*) (Chakravorty et al., 2016). Sometimes bacteria from the *Propionibacterium* or *Enterococcus* genera are also isolated (Marsh et al., 2014). The microbial community in tea fungus may vary between fermentations, but some of the species remain unchanged (Chakravorty et al., 2016; Coton et al., 2017; Marsh, O'Sullivan, Hill, Ross, & Cotter, 2014). According to some authors, the biodiversity of the tea fungus ecosystems depends on the geographical and climatic conditions and on the types of wild yeasts and bacteria that occur locally. Also, fermentation conditions affect the bacterial ecosystem: a higher temperature promotes the growth of some bacteria genera e.g. *Propionibacterium*, *Corynebacterium* as well as *Lactobacillus*, *Lactococcus*, or *Streptococcus* (De Filippis, Troise, Vitaglione, & Ercolini, 2018).

The relationship between yeast and bacteria in tea fungus consortia is complex. At the same time, there may be a commensal and amensal association among them. Substances secreted extracellularly by microbes may stimulate or inhibit the growth of accompanying microflora. Their interactions should be subjected to comprehensive analysis to make it possible to understand this phenomenon of coexistence and close dependence of different microorganisms in one ecological system (Villarreal-Soto, Beaufort, Bouajila, Souchard, & Taillandier, 2018).

2.2. Sucrose metabolism by tea fungus consortia

Initially, sucrose, originating from the medium (sweetened tea), is hydrolyzed to glucose and fructose by invertase (β-fructofuranosidase, EC 3.2.1.26), produced mainly by *Saccharomyces cerevisiae* as well as other yeasts species (Kulshrestha, Tyagi, Sindhi, &

Yadavilli, 2013). This enzyme is active in an acidic pH (3.5–5.5); therefore, sucrose hydrolysis is not stopped by the organic acids formed at a later stage. From the resulting monosaccharides, yeasts synthesize ethanol. The maximum concentration of reducing sugars and ethanol occurs on day 7 of fermentation. Over the following days, the content of ethanol decreases as a result of oxidation to acetic acid by AAB. In addition, AAB enzymatically oxidizes D-glucose at the C-6 position and the aldehyde group of the β-D-glucose at the position of C1, resulting in the formation of significant quantities of glucuronic acid and D-glucano-δ-lactone, respectively. Microbial enzymes hydrolyze this latter metabolite into gluconic acid. At the same time AAB, mainly *K. xylinus* due to its specific metabolism produce cellulose from glucose (Amaniampong et al., 2017; Chakravorty et al., 2016; Jayabalan, Malbaša, & Sathishkumar, 2017; Ramachandran, Fontanille, Pandey, & Larroche, 2006; Villarreal-Soto et al., 2018). The roles of other microorganisms during fermentation are still not precisely described. Some of them excrete their metabolic products and affect each other. For example, Yang et al. (2010) showed that bacteria from the *Lactobacillus* genus have a positive effect on the growth of *Gluconacetobacter* sp. A4 and the production of D-saccharic acid-1,4-lactone, an essential bioactive compound.

2.3. Chemical composition and biological activity of kombucha

The primary substrates for the production of kombucha beverages are green or black sweetened tea. After fermentation, final products have a complex chemical composition and contain several compounds i.e. organic acids, vitamins, active enzymes, polyphenols and a variety of micronutrients (Kumar & Joshi, 2016). The composition of the beverages depends on many factors, e.g. the raw materials used and the carbon source, the tea's concentration, the microbial composition of the tea fungus, the time and temperature of fermentation and the pH of the process. Any changes in these parameters impact on the quality of the final product, its nutritional, biological and sensory properties. Even the hardness of the water used affects

148 the functional properties of the beverages. Kombucha obtained using water with a high
149 concentration of calcium ions had higher antibacterial activity against *Staphylococcus aureus*
150 than beverages achieved from water with a low content of calcium ions (Lawton & Kumar,
151 2016).

152 The dry weight of fresh tea contains about 0.5% of organic acids, mainly citric, malic,
153 tartaric, oxalic and succinic acids. During fermentation, microorganisms produce other
154 important acids: acetic, gluconic, glucuronic, L-lactic, malonic, pyruvic and usnic acids
155 (Villarreal-Soto et al., 2018). Acetic acid is produced in the highest concentration. It has been
156 shown that consumption of acetic acid in moderate amounts slows gastric emptying time,
157 blocks the action of the disaccharidases (enzymes hydrolyzing disaccharides into
158 monosaccharides) and increases glucose uptake by the liver and muscles which reduces its
159 level in the blood (Zubaidah, et al., 2018b). Additionally, it may also inhibit lipogenesis and
160 the cholesterologenesis pathway in the liver, so it is responsible for decreasing total
161 cholesterol, LDL cholesterol and triglycerides in serum (Zubaidah et al., 2018a; Zubaidah et
162 al., 2019).

163 Nonetheless, glucuronic acid is considered to be the principal therapeutic agent in
164 kombucha with the main role in liver detoxification by the process of glucuronidation (Coton
165 et al., 2017; Jayabalan et al., 2017; Martínez-Leal, Suárez, Jayabalan, Oros, & Escalante-
166 Aburto, 2018). Glucuronidation is based on the conjugation of glucuronic acid to the slightly
167 soluble or insoluble substrates, e.g. xenobiotics. This reaction is catalyzed by UDP-
168 gluconosyltransferases (EC 2.4.1.17). It is a detoxification process, which enables drugs to be
169 eliminated from the body through the excretory system. Glucuronidation occurs mainly in the
170 liver, but UDP-gluconosyltransferases are also found in other organs, e.g. kidneys, lungs and
171 ovaries and the prostate gland (Mróz & Mazerska, 2015). Fermentation at 30°C leads to
172 higher concentrations of gluconic and glucuronic acids than at 20°C. This is positively

correlated with the promotion of the growth of the leading producer of glucuronic acid, *Gluconacetobacter saccharivorans,* at the higher temperature, while at the lower temperature *K. xylinus* prevails in the fermentation (De Filippis et al., 2018).

DSL (D-saccharic acid-1,4-lactone) is created in kombucha beverages from D-glucaric acid as a result of the activity of bacteria belonging to *Gluconacetobacter* sp., especially by *Gluconacetobacter* sp. A4. DSL is not found in unfermented tea. Its concentration increases until the eighth day of fermentation, ranging between 58 and 133 mg/mL depending on the sample (Chakravorty et al., 2016; Martínez-Leal et al., 2018; Yang et al., 2010). DSL is considered to be the compound behind the hepatoprotective and hypocholesterolemic effects of kombucha (Bhattacharya, Gachhui, & Sil, 2013). Its hepatoprotective mechanism is based on inhibition of the activity of β-glucuronidase, an endogenic, human enzyme located in lysosomes, which hydrolyzes the complexes of glucuronic acid with toxins, formed in the process of glucuronidation, making it difficult to excrete them. DSL bonds with amino acids at the active site of the enzyme and blocks the binding of substrate (Iqbal et al., 2018; Jamil et al., 2018). Additionally, DSL can prevent hyperglycemia-induced hepatic dysfunctions by inhibiting liver apoptosis (Bhattacharya, Gachhui, & Sil, 2013).

Tea is a rich source of polyphenols, whose amount and composition varies depending on the type of tea. The polyphenols in the brewed green tea are mainly catechins, which account for 30–42% of the dry mass. Green tea polyphenols belong to four major classes: (-)-epicatechin, (-)-epicatechin gallate, (-)-epigallocatechin and (+)-epigallocatechin gallate (Sharma et al., 2018). In the case of black tea, during the production process, these compounds are oxidized and dimerized; therefore, the black tea polyphenols profile is different and contains thearubigins, theaflavins, flavonols as well as catechins. The concentration of the latter components is lower than in green tea (Ozdal et al., 2016; Sharma

et al., 2018; Warden, Smith, Beecher, Balentine, & Clevidence, 2001). It is well known that polyphenols can prevent chronic diseases due to their antioxidative properties. Some of these compounds were also shown to inhibit DNA methyltransferase 1 which may result in the demethylation of promotor regions of tumor suppressor genes, which are usually hypermethylated in tumor cells (Saldívar-González et al., 2018; Zhong, Xu, Reece, & Yang, 2016). Polyphenols are of particular interest to scientists because of their cytoprotective effect on healthy cells and simultaneously cytotoxic effect on cancer cells (Brglez Mojzer, Knez Hrnčič, Škerget, Knez, & Bren, 2016). During the tea fungus fermentation of sweetened tea, polyphenols are modified, and as a result, the new compounds are formed. With the extension of the fermentation time, the composition and concentration of polyphenolic compounds in kombucha changes. This may be due to the action of microbial enzymes that lead to the degradation of the complex tea polyphenols into simpler molecules, resulting in an increase of antioxidant activity of the beverage compared to unfermented tea. Hydrolysis of polyphenols during fermentation is probably caused by tannase, an enzyme extracellularly produced by yeast and bacteria. As a result of tannase activity, epigallocatechin, gallic acid and glucose are released from epigallocatechin gallate gallotannins, gallic acid esters and epicatechin gallate. The products of this enzymatic reaction possess higher antioxidant capacity than unhydrolyzed compounds (Baik et al., 2015; de las Rivas, Rodriguez, Anguita, & Munoz, 2019). Other extracellular enzymes produced by the microbes present in tea fungus, such as phytases and β-galactosidase, may also modify tea polyphenols. It was shown that addition of β-galactosidase to olive mill wastewater caused the release of simple phenolic compounds with high antioxidant activity from this raw material which is rich in polyphenols (Hamza, Khoufi, & Sayadi, 2012). Additionally, during tea fungus fermentation, part of the thearubigins from tea may be converted to theaflavin, which changes the color of the beverage from reddish brown to light brown (Chakravorty et al., 2016).

Except for tea polyphenols in kombucha, isorhamnetin (O-methylated flavonol), a derivative of quercetin, was detected. This polyphenol is present, among others in cocoa or Ginkgo biloba, but not in tea. This suggested that tea fungus fermentation leads to the formation of this compound. Isorhamnetin and catechins have bacteriostatic and bactericidal activity (Bhattacharya et al., 2016; Li et al., 2016). The polyphenolic fraction of 14-day kombucha containing mainly catechin and isorhamnetin showed strong antibacterial activity against *Vibro cholerae*. These polyphenols may act as prooxidants by generating oxidative stress, which results in the degradation of bacterial cell membranes and leads to the inhibition of bacterial growth in a concentration-dependent manner. This phenolic fraction did not show a cytotoxic effect on human cells (Bhattacharya et al., 2018).

It has been shown that different carbon sources affect the total phenolic content in the product. Aspartame inhibited microbial growth and, as a consequence, the fermentation process did not proceed. Application of white or brown sugar as a carbon source during fermentation caused intensive growth of tea fungus and resulted in a high content of polyphenols in the final products. The use of honey as a carbon source results in a richer chemical composition in the final product, with a high content of e.g. organic acids, essential oils, alcohols, esters as well as polyphenols. Its original composition could lead to changes in the pH and modification of the fermentation process, and thus to changes in the polyphenol profile (Watawana, Jayawardena, Ranasinghe, & Waisundara, 2017).

Kombucha obtained from black or green tea is characterized by health-promoting properties. This beverage, rich in bioactive components, has a number of pro-health advantages: antimicrobial and antioxidant activity, as well as hepatoprotective and anticancer effect. Promising results in *in vitro* and *in vivo* studies have induced research groups from around the world to search for alternative raw materials for the tea fungus culture.

3. Alternative raw materials for kombucha production

Recently, in the world literature, there are more and more reports regarding using tea fungus to create new fermented functional products from raw materials other than tea, e.g. fruit or vegetable juices and cocktails, herbal or plant infusions, milk or food industry by-products. Some of them contain carbohydrates, which the tea fungus uses as a carbon source and in the fermentation process produce bioactive products with unique, pro-health properties (Aspiyanto et al., 2016; Gaggìa et al., 2018; Liamkaew, Chattrawanit, & Danvirutai, 2016; Moreno-Jiménez et al., 2018; Vázquez-Cabral et al., 2017; Vitas, Cvetanović, Mašković, Švarc-Gajić, & Malbaša, 2018; Yavari, Mazaheri-Assadi, Mazhari, Moghadam, & Larijani, 2017; Zubaidah et al., 2018a; Zubaidah et al., 2018b; Zubaidah et al., 2019). Depending on the composition of the raw material, the properties of the products vary on. It seems that the application of tea fungus to create new functional products based on various raw materials is still an open issue. Examples of the use of alternative raw materials for the tea fungus fermentation process found in the literature were collated and described below.

Tea with additives

Fermentation of sweetened green tea with the addition of cinnamon in the range 25–75% (w/v) resulted in increased amounts of organic acids amounts and high antioxidants and antimicrobial activity of the final products. These properties increased as the concentration of cinnamon in the tea was increased. The strong antibacterial activity of kombuchas with cinnamon is probably caused by the presence of cinnamaldehyde and eugenol derived from the cinnamon. These components disrupt the lipid bilayer of the bacterial cell membrane and cause higher permeability, which leads to extensive leakage of ions and important cell compounds (Nuryastuti et al., 2009; Shahbazi, Hashemi Gahruie, Golmakani, Eskandari, & Movahedi, 2018).

Kombucha made from black tea with the addition of 15% apple juice (v/v) after ten days of fermentation had a higher polyphenols content than kombucha made from tea alone

because apple juice contains a significant amount of polyphenols. The alcohol and acid content was also higher in apple-tea kombucha than tea kombucha. Further research should optimize the process to reduce the alcohol and acetic acid content (Liamkaew et al., 2016).

Pollen collected by bees has antimicrobial, antioxidant, antimutagenic and anti-inflammatory activity (Denisow & Denisow-Pietrzyk, 2016). It is also considered to exert also antitumoral, immunomodulatory, cardioprotective and anti-diabetic effects. The pollen grain wall has a complex structure resistant to degradation by digestive enzymes; therefore, the bioavailability of the phytonutrients from it is limited. Fermentation by tea fungus may be one of the methods of increasing the bioavailability of these valuable ingredients. After 30 days of fermentation of green tea with the addition of multi-floral pollen, the pollen grain wall was weakened and release of nutrients into the fermentation liquid took place. In the final result, fermented beverages containing pollen had higher polyphenol content than those without pollen. The addition of pollen also led to an increase in the LAB population, especially fructophilic LAB, which are the part of its microbiota. The final product was characterized by a high concentration of lactic acid and low content of gluconic acid in comparison to the product without pollen. This may suggest that LAB may inhibit the growth of AAB by way of competition. However, the addition of pollen indirectly induces the formation of short chain fatty acids (SCFA) in a beverage. SCFA are formed by the microbial fermentation of carbohydrates, such as dietary fiber. SCFA are bioactive molecules, called postbiotics, produced by bacteria, including LAB. Postbiotics refers to the metabolic products or by-products secreted by a bacteria cell. They may have anti-inflammatory, immunomodulatory, hypocholesterolemic and antioxidant activities (Aguilar-Toalá et al., 2018; Uțoiu et al., 2018).

Infusions

297 Coffee contains over a thousand bioactive compounds, some of which have potential
298 therapeutic effects. It is an important source of antioxidants, mainly caffeine, caffeic acid and
299 its derivative, chlorogenic acid, diterpenes, cafestol and kahweol. It is well known that coffee
300 shows pro-health properties such as antioxidant, anti-inflammatory, antifibrotic, or anticancer
301 activity. Fermentation of black tea enriched with CoffeeBerry® extract resulted in final
302 beverages with a higher polyphenol content and higher antioxidant activity than black tea
303 kombucha (Essawet et al., 2015). Tea fungus fermentation also takes place in sweetened
304 coffee extract without tea. Seven-day fermentation of coffee infusions improves their
305 therapeutic properties. It was observed that coffee kombucha has higher antioxidant activity
306 than a coffee infusion as well as a higher chlorogenic and caffeic acid content. The fermented
307 coffee infusion inhibited the activity of starch hydrolase to a greater extent than an
308 unfermented beverage. Therefore, it is stated that coffee kombucha can delay starch digestion
309 and reduce the amount of glucose in the blood. In this way, the fermented beverage is useful
310 in maintaining health and wellness (Poole et al., 2017; Watawana, Jayawardena, &
311 Waisundara, 2015; Yamagata, 2018).

312 Herbal infusions have been used for many years in the home treatment of various
313 ailments. Their health-promoting activity can be increased after the fermentation process
314 carried out by the tea fungus. Velićaniski et al. (2014) showed that kombucha from
315 sweetened lemon balm (*Melissa officinalis* L.) had greater antioxidant activity than a non-
316 fermented infusion. The same relationship has been demonstrated for kombucha from winter
317 savory (*Satureja montana* L.) (Cetojevic-Simin, et al., 2008). Both types of fermented
318 beverages also showed antibacterial activity against many gram-positive and gram-negative
319 species of pathogenic bacteria (Velićaniski et al., 2014; Cetojevic-Simin, et al., 2008). In
320 addition, kombucha from winter savory inhibited the growth of HeLa cells (cervix epithelioid
321 carcinoma) by 20% (Cetojevic-Simin, et al. 2008).

Yarrow (*Achillea millefolium*) is a widely used medicinal plant. It has astringent, antiseptic and anti-inflammatory properties and is used for the treatment of wounds, burns, hemorrhages, digestive disorders, menstrual cramps or flatulence. It contains over a hundred bioactive compounds, e.g. achilleine, apigenin, azulene, camphor, coumarin, menthol, quercetin, rutin, succinic and salicylic acid (Tadić et al., 2017). Yarrow extract, obtained as a result of supercritical extraction, fermented by tea fungus showed higher antioxidant activity and a higher content of organic acids (acetic, succinic, malic and oxalic) in comparison to the yarrow infusion fermented by tea fungus. Both types of yarrow kombucha showed good antimicrobial and antioxidant activity. Yarrow infusion kombucha showed antiproliferative activity against cells of human rhabdomyosarcoma and human cervix carcinoma Hep2c (HeLa) (Vitas et al., 2018).

The beverage obtained from ten-day tea fungus fermentation of a ginger infusion possessed ginger bioactive components e.g. 6-gingerol and 6-shogaol, which have anti-inflammatory and antitumor activity leading to the inhibition of tumour proliferation and stimulation of its apoptosis. The fermented ginger infusion decreased catalase, glutathione and malondialdehyde activity in tumour homogenate (Salafzoon, Mahmoodzadeh Hosseini, & Halabian, 2018).

Leaves

Rooibos tea does not contain catechins, so kombucha made from rooibos has a lower antioxidant activity than kombucha made from green or black tea. However, rooibos kombucha has a glucuronic acid amount comparable to kombucha made from black tea and contains other valuable compounds, e.g. rutin, aspalathin, orientin and isoorientin, all with antioxidant activity. Rooibos kombucha showed a significant positive effect on the recovery of H_2O_2 induced oxidative damage of fibroblast cell lines (Gaggìa et al., 2018).

346 Guava (*Psidium guajava*) is an evergreen shrub native to South and Central America
347 and the Caribbean. Its leaves, after drying, are used in the traditional medicine: as an anti-
348 inflammatory, hypoglycemic, antidiarrheal, antioxidant and antibacterial agent. During tea
349 fungus fermentation of guava leave extracts, new products with a completely different
350 composition and potential health-promoting effect than tea kombucha are created (Moreno-
351 Jiménez et al., 2018). It was shown that the primary polyphenol in guava leaves is quercetin
352 with lower concentrations of other flavonoids, e.g. kaempferol (Alnaqeeb et al., 2019;
353 Metwally, Omar, Ghazy, Harraz, & El Sohafy, 2011). The content of flavan-3-ols (catechin,
354 gallocatechin and epicatechin) in guava kombucha was lower than in tea kombucha, but
355 unlike the tea beverage, the concentration of these compounds increased with the time of
356 fermentation. Tea polyphenols are pH-sensitive: they are more stable in an acidic pH. The
357 maximum amount of organic acids in tea kombucha was observed on the fifth day of
358 fermentation, while in guava kombucha the maximum concentration of organic acids is
359 reached after nine days of fermentation. The different time for formation of organic acids
360 results in differences in pH and, as a result, influences the profile of polyphenols (Zeng, Ma,
361 Li, & Luo, 2017).

362 Fermentation of sweetened infusion of oak leaves (*Quercus* spp.) by tea fungus
363 changes its sensory properties. This is due to the microbiological degradation of compounds
364 present in unfermented beverages, which cause its tartness and bitter taste (e.g. flavan-3-ols,
365 hydroxybenzoic acid derivatives and hydroxycinnamic acids). The microbial modification of
366 these compounds leads to an increase in beverage sensory acceptability. The content of other
367 polyphenols in oak leaf kombucha e.g.: benzoic acid, vanillic acid, gallic acid, caffeic acid, 4-
368 hydroxybenzaldehyde, 2-hydroxybenzoic acid, 4-hydroxy-phenylethanol, and coumaric acid
369 was higher than in the infusion of unfermented oak leaves. In the case of gallocatechin, its
370 concentration in the fermented oak leaf beverage was similar to the amount in black tea

kombucha. The presence of quercetin glucuronide in oak leaf kombucha is also responsible for its antioxidant properties and anti-inflammatory activity. Fermented oak leaf beverages reduce the nitric oxide production (NO) in macrophages stimulated with lipopolysaccharide (LPS) – a major element of the outer membrane of gram-negative bacteria. Macrophages stimulated with LPS produce proinflammatory cytokines, prostaglandins and high levels of free radicals, such as NO (Fujihara et al., 2003). NO destroys phagocytosed cells and is involved in the host immune response. Its production is associated with the induction of inflammation. This compound is unstable and in the presence of superoxide anions may form toxic peroxynitrite, causing oxidative damage. Oak leaf kombucha treatment reduced the production of NO to a similar level to that obtained by macrophages without LPS stimulation (Vázquez-Cabral et al., 2017, 2014).

Fruits

Salak is a fruit growing in a palm from the *Arecaceae* family in Indonesia. It is commonly called "snake fruit" due to its brown, scaly skin. Beverages obtained as a result of a 14-days of tea fungus fermentation of the salak juice displayed anti-hyperglycaemic activity. It was shown that 28-day oral administration of salak kombucha for diabetic rats (doses 5-15 mL/kg body weight/day) caused a significant glucose reduction in blood plasma (31-59%) (Zubaidah et al., 2018b). According to the authors, this is due to the high content of antioxidants, such as tannins, polyphenols and organic acids, such as acetic, citric and lactic, which can decrease the fasting plasma glucose level by increasing the glucose uptake of cells. Additionally, salak kombucha enhances superoxide dismutase (SOD) activity and decreases malondialdehyde (MDA) level in blood serum. Probably, the flavonoid compounds present in kombucha are responsible for increasing the SOD activity by indirectly influencing on the synthesis of SOD in cells (Zubaidahet al., 2018b; Zubaidah et al., 2019). Zubaidah et al., (2018a) also showed that consumption of kombucha from salak juice by rats resulted in the

regeneration of their pancreatic β-cells. The salak kombucha was more effective in treating streptozotocin-induced diabetes than kombucha from black tea, due to the differences in total phenolics and acids content. Salak kombucha's activity in lowering fasting plasma glucose levels, reducing oxidative stress and lipid profiles was comparable to the activity of metformin, what indicates that salak kombucha could potentially replace this drug in diabetes therapy (Zubaidah et al., 2018a).

Vegetables

Vegetables fermented by tea fungus can be used to produce products with bioactive components. It has been shown that after 14 days of tea fungus fermentation of blanched spinach pulp, the total polyphenol content increases about 93% (Aspiyanto et al., 2016). Fermented spinach pulp had a significantly higher content of folic acid that the raw vegetable. The freeze-dried fermented spinach pulp of spinach could be a good source of folates. Such dry products could be used as functional food additives (Nugraha, Susilowati, Aspiyanto, Lotulung, & Maryati, 2017).

Juices

Tea fungus fermentation of pasteurized juices from pomegranate, red grape, sour cherry and apple allowed kombucha vinegar (4% of acetic acid) to be obtained. During fermentation process of all juices, there were similar physicochemical changes: significantly increasing of acids and fructose concentration, lowering the pH and content of alcohol and sucrose. The lowest concentration of acetic acid was noted in fermented apple juice, while the highest in fermented pomegranate juice. The raw material for fermentation determines the flavor of the product (Akbarirad, Assadi, Pourahmad, & Khaneghah, 2017). Fermentation of the juices from pomegranate and sour cherry also leads to the tea fungus producing considerable amounts of glucuronic acid, 17.07 and 132.5 g/l, respectively. Kombucha from

fruit juices may be a component of a diet supplementing the intake of this important compound (Yavari, Assadi, Moghadam, & Larijani, 2010; Yavari et al., 2017).

By-products and wastes

Soybean whey is a by-product of soy processing, which contains a lot of valuable substances, such as proteins, oligosaccharides, isoflavones, organic acids and minerals. Beverages obtained from soybean whey during six days of fermentation had fruity and floral flavors from nonanal and undecanal aldehydes formed by microorganisms. It was shown that these products had higher antioxidant activity than unfermented soy whey and antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli* (Tu, Tang, Azi, Hu, & Dong, 2019).

Another example of an interesting fermented product is obtained from banana peel extract. It is characterized by a new taste, smell and color. In comparison with traditional tea kombucha, beverages from banana peels extracts have a lower pH and higher phenolic content than unfermented extracts. Final products showed significant antioxidant activity, which may result from the microbial fermentation of the protein in banana peels (Pure & Pure, 2016).

Milk

Tea fungus can be used for the preparation of fermented milk products without or with additives, such as transglutaminase, whey concentrates or extracts from other plants. During fermentation, substances with health-promoting effects are formed from milk compounds, e.g. peptides with the ability to inhibit an angiotensin-converting enzyme (ACE), which causes elevated blood pressure and congestive heart failure. Synthetic drugs for hypertension, such as captopril have many side effects, so bioactive peptides delivered naturally during food fermentation arouse much interest. Moreover, rats fed with fermented milk products had low harmful LDL cholesterol, glucose and aminotransferases in the blood (Al-Dulaimi, Abd-

Alwahab, & Hasan, 2018; Elkhtab, El-Alfy, Shenana, Mohamed, & Yousef, 2017; Iličić et al., 2017; Iličić, Milanović, Kanurić, Vukić, & Vukić, 2016; Kanurić et al., 2018).

4. Cellulose synthesis by Kombucha culture

Bacterial cellulose (BC) is an extracellular metabolite of many bacterial species (Picheth et al., 2017). This is a biopolymer with exceptional material properties, e.g. lack of impurities like pectin, lignin or hemicellulose, high tensile strength and great water-uptake capacity (Ullah, Santos, & Khan, 2016). The pathway of cellulose biosynthesis in bacteria is complex and consists of several stages. The substrates in biosynthesis may be glucose, fructose, ethanol, acetic acid, citric acid, or glycerol. Enzymatic transformations lead to the formation of cellulose fibrils that combine with each other to form chains, then macrofibrils and finally a 3-D structure of about 1,000 separate glucan chains. BC has an excellent water capacity – it can hold up to 200 times more water than its dry mass (Semjonovs et al., 2017; Villarreal-Soto et al., 2018). BC thermal stability arises from its high crystallinity. This property allows for sterilization of BC at 121°C and that causes it to have superiority over other polymers that typically change their properties above 100°C [Cacicedo et al. 2016].

BC can be produced by both gram-negative and gram-positive bacteria, such as *Aerobacter* sp., *Agrobacterium* sp., *Achromobacter* sp., *Aerobacter* sp., *Azotobacter* sp., *Rhizobium* sp., *Sarcina* sp., *Salmonella* sp., *Pseudomonas* sp. and *Gluconacetobacter* sp.. Bacteria from the genus *Komagataeibacter* (family *Acetobacteraceae*) are usually used for the industrial production of BC, especially *K. xylinus* (formerly *Gluconacetobacter xylinus*) (Mohammadkazemi, Azin, & Ashori, 2015; Villarreal-Soto et al., 2018). The enzymatic activity of *Komagataeibacter* leads to the synthesis of uridine diphosphoglucose, which is a precursor of cellulose, then the single cell can polymerize up to 200,000 glucose residues per second with β-1,4-glycosidic bonds, so the yield of cellulose is highly effective. This may be due to the presence of a CcpA protein called “cellulose complementing factor.” It is encoded

only in the *Komagataeibacter* genus and may be responsible for its high activity in the synthesis of cellulose (Römling & Galperin, 2015).

BC is produced extracellularly in the form of fibrils attached to the cells. When the culture is static at the air-liquid interface, one floating biofilm is formed. However, when the culture is agitated (e.g. by continuous mixing) irregular masses of fibrillar structures are distributed in the medium (Neera, Ramana, & Batra, 2015). BC is a mechanical protection for the cells and by retaining them on the surface of the fermented liquid ensures oxygen for the bacteria. Additionally, it is assumed that BC can form a reticulation in which nutrients move by diffusion, so bacteria located deep inside the structure have access to them (Iguchi, Yamanaka, & Budhiono, 2000).

BC can be successfully used as an emulsion stabilizer, thickener and source of dietary fiber in the diet. It has higher activity in lowering serum triglycerides, LDL and total cholesterol as well as liver total lipids and liver total cholesterol than plant cellulose. BC could be used as a promising low-calorie food ingredient for different applications e.g. as dietetic snacks (Chau, Yang, Yu, & Yen, 2008). Additionally, it can be used in the cosmetic industry as a facial mask, scrubs, cleansing formulation and, due to its biocompatibility, permeability to liquid and gases and transparency, as a material for contact lenses. In addition, BC is applied in medicine. It is successfully used as a material for wounds dressings, burn treatments and as a drug delivery systems. Tests on animals have shown the possibility of using BC as cardiovascular implants (Kołaczkowska et al., 2019). Additionally, it can be used as artificial blood vessels, cartilage, bones, skin and as a wound healing scaffold for the tympanic membrane (Cacicedo et al., 2016; de Oliveira Barud et al., 2016; Ullah et al., 2016).

Despite its advantages, unique properties and comprehensiveness of applications, BC is rarely produced because of its price. Bacteria are grown on complex media, with the

addition of ethanol, which improves cellulose yield up to four times (Islam, Ullah, Khan, Shah, & Park, 2017). Methods leading to cheaper and more efficient BC production are being sought and alternative media such as fruit juices or waste are being tested. The addition of apple juice into the static culture of a Hestrin–Schramm medium inoculated with *K. rhaeticus* P 1463 isolated from kombucha allowed the production of cellulose with a high yield. Prolonged fermentation for 14 days and gradual supplementation of the carbon substrate led to a cellulose yield of about 9.5 g/L. The obtained biomaterial has good physical and mechanical properties (Semjonovs et al., 2017). A similar yield of cellulose (about 9.1 g/L) was obtained in diluted pineapple juice using the *K. xylinus* strain DFBT (Neera et al., 2015).

The carbon source also is a crucial factor for affecting the properties of bacterial cellulose. The addition of mannitol to the Hestrin–Schramm medium leads to the most efficient BC production by *K. xylinus*, whereas food-grade sucrose and date syrup the yield of cellulose is not satisfacory (Mohammadkazemi et al., 2015). However, another study proves that sucrose as a carbon source for a strain of *K. xylinus* DFBT – results in a high yield of BC (Neera et al., 2015).

Fermentation of sweetened black tea using the tea fungus also leads to the production of BC with high efficiency (Al-Kalifawi, 2014). Our preliminary studies showed that BC obtained during the fermentation of chokeberry pomace extracts by tea fungus exerts high antioxidant and antimicrobial activity (unpublished data). Such cellulose can be used in many ways, for example as a functional food additive, as dietary fiber with antioxidant and anitimicrobial properties, ora as an active packaging material. The possibility of using BC obtained on media with a high antioxidant potential must be confirmed in studies. In the available literature (to the best of our knowledge), there is very little data on the properties od BC obtained during the production of kombucha beverages Zhu, Li, Zhou, Lin, & Zhang (2014) showed that BC obtained by the tea fungus fermentation of black tea had

biocompatibility with Schwann cells and did not exert hematological and histological toxic effects on nerve tissues.

5. Conclusion and perspectives

The health benefits of drinking fermented tea, such as its antioxidant and anti-inflammatory activity, the ability to reduce LDL cholesterol and blood glucose, and its hepatoprotective properties cause the drink to be very popular. However, more research should be performed to fill in an existing gap in the direct evidence about the functionality of kombucha products. It is a necessary to determine the various factors affecting the functional features of kombucha and its safety.

The idea to use tea fungus for the fermentation of alternative raw materials arises from the unique properties of tea kombucha and the desire to obtain an edible product with unusual functional properties e.g. with the content of uncommon pro-health substances or with an increased amount of biologically active compounds i.e. polyphenols. Additionally, tea fungus fermentation may release bioactive components from raw materials into the fermentation liquid, which allows a product with a high biological value and an enriched composition to be obtained. There is still a large number of potential raw materials that have not been tested in terms of suitability for the production of a functional, fermented product, such as fruit pomace. Pomaces are a result of processing fruits and vegetables and are usually treated as waste. They constitute 10–35% of the mass of raw material and contain many valuable substances, such as vitamins, polyphenols, minerals or fiber, so they should be treated as an intermediate product for further processing e.g. by fermentation by tea fungus. Such processes may lead to new products with health-promoting properties. Our preliminary research indicates that tea fungus effectively ferments extracts from fruit pomaces, leading to the creation of beverages with interesting sensory and functional properties (data unpublished). Properties of new fermented beverages should also be tested depending on the

starter cultures used or the fermentation time (Amarasinghe, Weerakkody, & Waisundara, 2018; Gaggìa et al., 2018; Ii & Kumar, 2016; Vázquez-Cabral et al., 2014).

The large microbial diversity of kombucha and the complex interactions between microorganisms make it challenging to investigate and understand the functioning of this unique ecosystem. However, understanding the interactions between microorganisms, determining the relationships between them and gaining knowledge about how they create specific niches closely associated with each other, would allow for the selection of appropriate media, optimal fermentation conditions, and directing the fermentation process. This would favour increasing the biosynthesis efficiency of the desired bioactive compounds in kombucha.

Declaration of interest

The authors declare no corporate/business, funding or founder sponsor conflict of interest.

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