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Review Article

A novel technology for production of Kombucha extract by prolonged fermentation time and multiple enhanced concentration of beneficial ingredients

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Abstract

Nowadays, people's awareness of the role of diet in maintaining well-being and good health is increasing. Consumers expect that products will not only provide them with essential nutrients, but will also be a source of biologically active substances that are beneficial to their health. One of the "health trends" that has emerged among consumers around the world is kombucha with high antioxidant potential, obtained through the activity of a consortium of acetic acid bacteria and osmophilic yeast, also called "tea fungus". Kombucha is characterized by its health properties.

Consumption of kombucha has always been associated with various health benefits. There are many personal experiences and feedback from kombucha drinkers around the world about the kombucha's ability to protect against a huge number of metabolic and infectious diseases.

The promising results in in vitro and in vivo studies prompted us to create a new type of kombucha concentrat, which can be used directly for consumption, can be added to other drinks (juises, teas, herbal decoctions), might be used for preparation of carbonated and non-carbonated drinks, for food preservation, for vinegar preparation, as an additive in cosmetics and antiinflammatory products.

The aim of this article is to review research in search of experimental confirmation of the many health aspects of kombucha. The analysis of the literature data is performed in accordance with the latest concepts of health protection requirements. Attention is paid to the active compounds in kombucha, responsible for the specific effect and the mechanisms of their action. It has been shown that kombucha can be effective in health prevention and recovery due to four main properties: detoxification, antioxidant, energizing potentials and stimulation of suppressed immunity. In recent years, its consumption has increased due to its useful properties and ingredients, immunostimulatory, anti-inflammatory, anti-tumor, antioxidant and other properties. Recent experimental studies of kombucha consumption suggest that the drink is suitable for prophylaxis against broad-spectrum metabolic and infectious disorders. This makes kombucha attractive as a drink for health prevention.

In this article we describe the benefits of consuming the ancient traditional Chinese drink kombucha and describe the production of our new product using modern technology. Our combucha extract can be used for direct consumption and as a raw material for the production of various products containing kombucha.

KOMBUCHA EXTRACT of DALVITA is made by a special technology and is 100 times more concentrated than kombucha tea, which makes its action a hundred times stronger and reduces the required daily amounts a hundred times.

Keywords: kombucha; bioactivity; fermentation; active compounds; microbial symbiosis

Introduction

Kombucha is an alternative therapy that is gaining popularity as a means of treating various diseases such as rheumatism, intestinal disorders, aging, cancer, diabetes and others.

In the 1960s, Dr. R. Sklenar developed "biological therapy for cancer with Kombucha as the main agent" and his own system for diagnosing cancer. Sklenar's diagnosis of cancer is based on a diagnosis of the iris and a demonstration of the causative agent of the body with the help of "Blood picture according to Dr. Sklenar". He argues, on the one hand, that cancer is only one of many metabolic diseases, and on the other, that viruses,

according to him parasitic microorganisms in general, are responsible for the pathogenesis of cancer [1].

The antimicrobial activity of kombucha has been studied against a number of pathogenic microorganisms. It was found that Staphylococcus Aureus, Shigella sonnei, Escherichia coli, Aeromonas hydrophila, Yersinia enterolitica, Pseudomonas aeruginosa, Enterobacter cloacae, Staphylococcus epidermis, Campylobacter jejuni, Salmonella Enteritidis, Salmonella Typhimurium, Bacillus Cereus, Helicobacterpylori and Listeria monocytogenes are sensitive to kombucha. According to the literature, the acetic acid in kombucha is considered to be responsible for the inhibitory effect on the tested microbes and this is also valid in the study of Sreeramulu et al. [2]. However, in their study, kombucha proved that it exerts antimicrobial action against E. coli, Sh. sonnei, Sal. typhimurium, Sal. enteritidis, and Cm. jejuni, even at neutral pH and after thermal denaturation. This finding suggests the presence of antimicrobial compounds other than acetic acid and large proteins in kombucha. Velićansk et al. also report that the antibacterial activity of kombucha is due not only to acetic acid but also to some other components and metabolites in kombucha. [3]. Lactic acid increases its bioactivity [4].

Cetojevic-Simin et al. also reported that traditional kombucha showed higher activity against Staphylococcus aureus and Escherichia coli, as well as bacteriostatic activity on Salmonella enteritidis [5]. A study by high affinity liquid chromatography investigating the efficacy of kombucha against enterotoxigenic Escherichia coli, Vibrio cholerae, Shigella flexneri and Salmonella Typhimurium found cabucin, catechin and isoramhamnetin as the main antibacterial compounds present in this polyphenolic fraction. To the best of our knowledge, this is the first report of the presence of isoramhamnetin in kombucha. The overall study suggests that kombucha can be used as a potent antibacterial agent against enteropathogenic bacterial infections, which is mainly attributed to its polyphenolic content. [6]. Kombucha can be considered as a potential alternative source of antivirulent polyphenols against V. cholera [7].

When studying the effect of kombucha tea on the changes caused by oxidative stress in rats treated with chromate - a strong oxidant, the results show that kombucha tea has powerful antioxidant and immunopotentiating effects [8].

In a three-year study of 64 mice until they die of natural death - old age, half of which systematically drank kombucha, mice that drank kombucha lived longer than controls [9].

The changes in the main components and microbes in Kombucha broth, prepared from nine different sources during continuous fermentation up to 60 days, were studied. Acetic acid increases slowly up to 30 days then decreases gradually. Gluconic acid contributes to the titratable acidity and the taste of the broth during the last stage of fermentation [10].

Kombucha tea was found to prevent lipid peroxidation and significantly reduce paracetamol-induced hepatotoxicity in experimental rats [11].

To evaluate the effect of oral administration of kombucha tea on lead oxidative stress, rats were given orally 1ml of 3.8% lead acetate solution daily alone or in combination with kombucha tea for 45 days and was antioxidant status and lipid peroxidation were assessed. The use of lead lowers the levels of a number of antioxidant enzymes and destroys DNA in the liver. Oral administration of kombucha tea to rats exposed to lead reduced lipid peroxidation and DNA damage. The addition of kombucha tea has alleviated lead-induced immunosuppression to significant levels. The results of this study suggest that kombucha tea has powerful antioxidant and immunomodulatory properties [12].

According to other studies in rats, intraperitoneal administration of kombucha may be useful in preventing peritoneal adhesions [13].

Exposure to high concentrations of carbon tetrachloride (including vapors), used in the past as a cleaning agent, for firefighting, in refrigerators, etc., affects the central nervous system, liver and kidneys. Kombucha tea has shown the strongest hepatoprotective and healing properties compared to other types of tea against experimentally induced carbon tetrachloride toxicity in male rats [14].

Another study in rats showed that kombucha can repair damage caused by environmental pollutants such as trichlorethylene and may be beneficial for patients suffering from kidney damage [15].

Addition of kombucha tea reduces phenol-induced cytotoxicity in experimental mice [16].

Studies show that kombucha tea prevents paracetamol and carbon tetrachloride-induced hepatotoxicity in mouse hepatocytes and could be beneficial against liver disease [17]. The antioxidant effect of kombucha has been proven for other oxidants in mouse hepatocytes [18]. Kombucha tea has also shown a healing effect on ulcers [19].

The use of kombucha tea causes attractive healing effects on diabetic rats, especially in terms of liver and kidney function. Therefore, kombucha tea can be considered as a potential strong candidate for future use as a functional supplement for the treatment and prevention of diabetes [20]. Also, diabetic complications associated with increased oxidative stress can be suppressed by antioxidant molecules formed during the fermentation period of the beverage [21].

A model of pulmonary silicosis in rats was induced by injecting silica powder into the lungs of rats, which were then treated by spraying with kombucha and Chinese herbal kombucha for a month. The rats were then killed and the effects of the treatments were assessed by examining the extent and severity of histopathological lesions in the lungs of the animals, measuring their organic coefficients and lung collagen content, determining the dry and wet weight of the lungs, and measuring the contents of the lungs, free silica in dried lungs. The levels of free silica in the lungs of animals treated with Chinese herbal kombucha were found to be significantly lower than for any other group exposed to silica. These preliminary results show that spraying with Chinese herbal preparations from kombucha can effectively promote the expulsion of silicon dust from lung tissues. Thus, inhalation of Chinese herbal kombucha may be a useful new treatment for silicosis and other pneumoconiotic diseases [22].

A study of the effects of Camellia sinensis (GT) Linn and kombucha, two natural drinks, for cholesterol and antioxidant status, using a model of rats with hypercholesterolemia, compared the free radical scavenging abilities and polyphenol levels of GT and kombucha. Cholesterol-rich rats received kombucha or GT for 16 weeks. The free radical scavenging activity of kombucha turned out to be higher than that of GT. Compared to GT, kombucha significantly reduces cholesterol, triglycerides, LDL and VLDL, while increasing HDL levels. Kombucha induced a 55% reduction in TBARS levels in the liver and 44% in the kidneys compared to rats fed only a high-cholesterol diet. These results reveal that the use of kombucha leads to attractive therapeutic effects on hypercholesterolemia, especially with respect to liver and kidney function in rats [23]. Kombucha contributes to the fight against fatty liver diseases by changing the intestinal microbiota [24]. Kombucha protects hepatocytes from lipid toxicity by affecting lipid metabolism and suppressing inflammation and fibrosis, which contributes to liver repair in mice with steatohepatitis [25].

Studies in mice have shown that kombucha reduces lipid accumulation and protects the liver from damage by promoting liver repair [26].

The foot-and-mouth disease virus is sensitive to acids and can be inactivated by exposure to low pH conditions. Spraying animals at risk of infection with suspensions of acid-forming microorganisms has been identified as a potential strategy to prevent the disease. Kombucha is one

of the most acid-forming symbiotic probiotics and thus can be an effective agent to implement this strategy. Previous studies have shown that Chinese herbal kombucha effectively inhibits foot-and-mouth disease replication in vitro. To evaluate the inhibitory effects of Chinese herbal kombucha against foot-and-mouth disease, pigs were infected by intramuscular injection of foot-and-mouth disease. After treatment with Chinese herbal kombucha, they are partially protected against infection. In a large-scale field study spraying cattle in an outbreak area of foot-and-mouth disease with kombucha proved to be protected against infection. Chinese herbal kombucha can be a useful probiotic agent to control foot-and-mouth disease outbreaks [27].

The antioxidant activity of kombucha against oxidative damage caused by H2O2 in activated monocytes (macrophages) was studied. In addition, anti-inflammatory activity was determined using lipopolysaccharide (LPS) - stimulated macrophages; in particular, the production of nitric oxide (NO), TNF-alpha and IL-6 is evaluated. Levels of pro-inflammatory cytokines IL-6 and TNF-alpha were significantly reduced by kombucha treatment. Similarly, NO production is lower with kombucha treatment compared to LPS-stimulated macrophages. Fermented oak beverages effectively reduce NO production, while pro-inflammatory cytokines (TNF-alpha and IL-6) in macrophages are stimulated by LPS. In addition, the phytochemicals present in kombucha reduce oxidative stress [28].

Seborrheic dermatitis is a chronic and recurrent superficial dermatitis in which Malassezia species play an important role. There are various species of Malassezia that have recently been reported to be resistant to common antifungals. Kombucha is believed to have potential antimicrobial properties. In this regard, a study was performed to investigate the antifungal activity of the kombucha tea ethyl acetate fraction against Malassezia species obtained from patients with seborrheic dermatitis. The results of the study show the antifungal properties of kombucha. Therefore, this extract can be advertised as an adjunct to the treatment of infections caused by Malassezia [29].

Compounds that have the ability to inhibit angiotensin-converting enzyme (ACE) are used to treat hypertension. The presence of such compounds naturally in food is potentially useful for the treatment of a disease state. Lactic acid bacteria have been studied for their ability to produce new potent ACE-inhibiting peptides during milk fermentation. Strains of Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus delbrueckii ssp. bulgaricus, Lactobacillus helveticus, Lactobacillus paracasei, Lactococcus lactis, Leuconostoc mesenteroides and Pediococcus acidilactici. A symbiotic consortium of yeast and bacteria used in the commercial network for the production of kombucha tea was also tested. Several new peptides with potent ACE-inhibitory activity have been isolated. Some of these peptides have been synthesized and their ACE inhibitory activity has been confirmed. The use of organisms producing these unique peptides in food fermentation can make a positive contribution to human health [30].

Abnormal metabolism is a common occurrence in cancer cells. For example, increased production of reactive oxygen species (ROS), especially due to aerobic respiration during the invasive stage, leads to cancer progression. The effect of kombucha tea made from ginger on the change of antioxidant agents has been evaluated in an animal model of breast cancer. Two types of kombucha tea with or without ginger were administered to mice before and after tumor induction. Superoxide dismutase (SOD), catalase, glutathione (GSH) and malodialdehyde (MDA) were measured in tumor, liver and kidney. The application of ginger kombucha tea significantly reduces catalase activity, as well as the level of GSH and MDA in tumor homogenates. A significant decrease in SOD activity and an increase in the amount of MDA was found in the kidneys of mice consuming ginger kombucha tea. The results show that the consumption of kombucha prepared from ginger can have an antioxidant effect by balancing many antioxidant factors in different tissues in breast cancer models [31].

Natural ingredients have always been an interesting approach to prolonging the youthful appearance of the skin. One of the natural compounds is kombucha tea, which has been used mainly as an energy drink in Asian countries for a long time. Previous reports indicate that it has pharmaceutical and beneficial wound healing effects. The beneficial properties of kombucha are believed to be mainly due to the presence of fermentation products such as flavonoids and other polyphenols with inhibition of hydrolytic and oxidative enzymes and anti-inflammatory effects. These properties provide an opportunity to study the anti-aging potential of kombucha in aging mice. Young and old mice are used as controls. The ethyl acetate fraction of kombucha, which has the highest flavonoid content, was administered intradermally to old mice. Its application significantly increases the collagen content, the level of NAD + / NADH and at the same time improves the connective tissue disorders in aged skin. This finding suggests that the ethyl acetate fraction of kombucha may be a suitable candidate as a cosmetic product for improving aging-related skin disorders and regeneration of aged skin [32].

In a study to determine the mechanism of antibacterial activity of a polyphenolic fraction composed mainly of catechin and isorhamhamnetin isolated from kombucha against the enteropathogen Vibrio cholerae N16961, bacterial growth was found to be severely impaired by the polyphenolic fraction up to. Scanning electron microscopy demonstrates morphological changes in bacterial cells when exposed to the polyphenolic fraction in a concentration-dependent manner. Permeabilization assays confirm that the fraction disrupts the integrity of the bacterial membrane in terms of both time and dose, which are proportional to the production of intracellular reactive oxygen species (ROS). In addition, each of the polyphenols catechin and isoramhamnetin shows the ability to penetrate bacterial cell membranes by generating oxidative stress, thus suggesting their role in the antibacterial potential of kombucha. Thus, the main mechanism of antibacterial activity of the polybutane fraction of kombucha against V. cholerae includes bacterial membrane permeabilization and morphological changes that may be due to the generation of intracellular ROS. This study demonstrates the membrane-mediated antibacterial mechanism of kombucha, which is mainly due to its polyphenolic content. This study also suggests the exploitation of kombucha as a potential new source of bioactive polyphenols against V. cholerae [33].

Kombucha maintains the quality of minced beef, significantly slowing down the growth of microbes, extending the shelf life of minced beef to 3 days. This makes it a potential food storage material [34].

The health benefits of pollen are increased by fermentation with kombucha. The content of bioactive compounds (polyphenols, soluble silicon species and SCFA) is higher in fermented pollen and the product shows a moderate antitumor effect on human colorectal cancer cells [35].

The effect of adding various medicinal plants to kombucha has been studied: cinnamon, cardamom or thyme. The results show that cinnamon-containing kombucha samples show higher antioxidant and antimicrobial activities, more organic acids and better sensory results. As the concentration of cinnamon increases, certain increases in the amounts of organic acids and in the values of antioxidants and antimicrobial activities are observed. This indicates that the antioxidant and antimicrobial activities of kombucha can be increased by adding medicinal plants, especially at higher concentrations [36].

Rooibos has shown an effect on the repair of oxidative damage to fibroblast cell lines against oxidative stress. These results make rooibos leaves interesting for making a drink kombucha with rooibos with health benefits [37].

The amount of glucuronic acid at temperatures of 20 ° C and 30 ° C for 21 days and the antibacterial properties of kombucha black tea on the growth of Salmonella typhimurium, Staphylococcus aureus and Lactobacillus rhamnosus were studied. The formation of glucuronic acid changes at 20 ° C from 17.0 mg/L on day 1 to approximately 27.2 mg/L on day 21. The amount of this acid at 30 ° C increases from 42.2 mg/L on day 1 to 48.0 mg/L on day 21. The amount of glucuronic acid produced at 30°C is significantly higher than at 20°C. Kombucha black tea has antibacterial action against Salmonella typhimurium and Staphylococcus aureus, but not against Lactobacillus rhamnosus. There were no statistical differences in the antibacterial activity of kombucha between incubation at 20°C and 30°C. This study offers a perspective on glucuronic acid production (especially at 30°C, not 20°C) and the antibacterial activity of black tea kombucha [38].

In addition, it inhibits the development of pathogenic enteric bacteria: Escherichia coli. E. coli O157: H7. Shigella dysenteriae, Salmonella Typhi and Vibrio cholera, a kombucha made from green tea and black tea, have demonstrated toxicity to colon cancer cells. Therefore, kombucha tea can be considered as a potential source of antioxidant, inhibition of pathogenic enteric bacteria and toxicity to colon cancer cells [39].

Leaky gut syndrome is a disease in which the structure of the gut, due to the breakdown of intracellular and intercellular binding proteins, is so loose and worn that nutrients pass undigested through the intestinal mucosa. Once in this form in the bloodstream, they activate the immune system, which recognizes them as foreign particles and attacks them, which in turn triggers other immune processes that are usually undesirable. Leaky gut syndrome is seen in some of the gastrointestinal and inflammatory bowel diseases. Therapeutic approaches aim to reduce inflammation, restore the production of binding proteins and reduce intestinal permeability. The therapeutic effect of kombucha has been studied in mice with this syndrome. The results show kombucha as a promising therapeutic candidate for reducing intestinal permeability. Young animals with colitis showed more severe clinical signs and a lower survival rate than older mice with colitis, but they responded better to kombucha treatment than older mice [40].

Aspergillus Fumigatus is one of the most common fungi that causes infection in immunocompromised and immunosuppressive patients. Laboratory studies show that kombucha may exert inhibitory effects on the growth and expression of certain genes in Aspergillus Fumigatus strains [41].

Conclusion

Nowadays, people's awareness of the role of diet in maintaining well-being and good health is increasing. Consumers expect that products will not only provide them with essential nutrients, but will also be a source of biologically active substances that are beneficial to their health. One of the "health trends" that has emerged among consumers around the world is kombucha with high antioxidant potential, obtained through the activity of a consortium of acetic acid bacteria and osmophilic yeast, also called "tea fungus". Kombucha is characterized by its health properties. The promising results in in vitro and in vivo studies prompted us to create a new type of kombucha extract, which can be used directly for consumption, can be added to other drinks (juises, teas, herbal decoctions), might be used for preparation of carbonated and non-carbonated drinks, for food preservation, for vinegar preparation, as an additive in cosmetics and antiinflammatory products.

Kombucha is the most popular non-alcoholic fermented beverage. The microbiology of kombucha is remarkable because it includes all the enzymes described in known fermented foods: lactic acid bacteria, acetic acid bacteria and yeast. The mode of action that has been studied and demonstrated the most is probiotic. However, it can be expected that the fermentation metabolites may be prebiotic or directly affect the health of the host.

Kombucha is composed of a multi-species microbial ecosystem with complex interactions that are both collaborative and conflicting. Yeast produces an environment that allows both yeast and bacteria to metabolize sugars. Bacteria produce a surface biofilm that acts as an environment to provide protection from invaders, conserve resources, and increase access to oxygen for the microbes that grow in it. Ethanol and acid produced during the enzymatic process (by yeast and bacteria, respectively) can also help protect the system from invasion by microbial competitors from the environment. In this way, kombucha can serve as a model system for exploring important issues for the development of cooperation and conflict in various multi-species systems. In addition, it has the potential to be artificially selected for specific human uses, including the development of antimicrobial ecosystems, preservatives and new materials.

Finally, kombucha is easily propagated, non-toxic and inexpensive, making it an excellent system for research and use. Kombucha is considered a dietary supplement; its intake strengthens the body's immune system, which prevents disease.

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