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PRODUCTS OF BIOTRANSFORMATION OF TEA INFUSION – PROPERTIES AND APPLICATION

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A b s t r a c t

Fermented tea broth (known as Kombucha) has been used for ages in many countries, especially in Japan, Russia, China and Eastern Europe. Nowadays, this beverage is generally regarded a universal natural medicament having a strengthening effect on the human body. Kombucha beverage is popular because of its favourable effect on human health. Its composition includes: B vitamins, C vitamin, mineral components and organic acids. It is believed that Kombucha decreases the risk of cancer, prevents circulation disorders, improves the function of the digestive system, mitigates inflammatory conditions and has a favourable effect on the skin, hair and nails.

The composition and properties of tea are well documented. Regrettably, the scientific information on the composition, effect on human body and properties of Kombucha is sparse. The goal of this paper is to present the properties and composition of Kombucha beverage as well as its biological activity and potential favourable effect on the human body.

PRODUKTY BIOTRANSFORMACJI NAPARU HERBATY – WŁAŚCIWOŚCI I ZASTOSOWANIE

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S ł o w a k l u c z o w e: Kombucha, grzyb herbaciany, biotransformacje, fermentacja, skład chemiczny.

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Abstrakt

Napój sfermentowanej herbaty (znany jako Kombucha) stosowany był od wieków w wielu krajach, szczególnie w Japonii, Rosji, Chinach i Europie Wschodniej. W dzisiejszych czasach uznawany jest jako środek naturalny wzmacniający organizm.

Kombucha zyskała popularność ze względu na potencjalny korzystny wpływ na organizm. Do głównych składników sfermentowanej herbaty należą: witaminy z grupy B, witamina C, związki mineralne i kwasy organiczne. Przypuszcza się, że Kombucha może obniżać ryzyko występowania nowotworów, zapobiegać zaburzeniom układu krążenia, poprawiać funkcjonowanie układu pokarmowego, łagodzić stany zapalne oraz korzystnie wpływać na skórę, włosy i paznokcie.

Skład i właściwości herbaty są dobrze udokumentowane. Doniesienia naukowe dotyczące składu, działania i właściwości Kombuchy są nieliczne. Celem pracy jest prezentacja właściwości i składu Kombuchy, jak również aktywności biologicznej oraz potencjalnego korzystnego wpływu na organizm.

Introduction

Tea has been known and appreciated in many countries and cultures for millennia. Presently, it is one of the most popular drinks worldwide. In China, it has been used already five thousand years ago, mainly as stimulating and detoxicating elixir (DUFRENSE and FARNWORTH 2000, BALENTINE et al. 1997). Recently many scientific reports have shown that tea has beneficial influence on human health and well-being (YEN et al. 1997). Tea brew regulates proper function of digestive system, strengthens walls of blood vessels, improves physical abilities and concentration (KUNTZE 2003).

Kombucha is acquired by fermentation process utilizing tea and sucrose. Despite common names, such as: Manchurian mushroom, tea fungus, tea beer, tea cider or Chinese mushroom, Kargasok tea (JARRELL et al. 2000, JAYABALAN et al. 2008, KURTZMAN et al. 2001) it is not fungus, but a symbiotic colony of multiple nonpathogenic bacteria and yeast (TEOH et al. 2004). Acetic acid bacteria synthesize bacterial cellulose (Fig. 1) in form of cream-colored or light-beige layers, also named microbial mats and biofilms (JARRELL et al. 2000, STAHL and CAUMETTE 1994).

The latter product of biotransformation occurs in form of slightly carbonated consumable broth and has specific sweet and sour taste (JAYABALAN et al. 2008). Its potential therapeutic properties have become the field of interest and research (MURUGESAN et al. 2009).

Development of knowledge about tea is a starting point for better understanding of Kombucha beneficial action. In popular medicine fermented tea drink was used by many cultures. It was claimed to be a universal wonderful drug, helping in many illnesses and strengthening organism. It was known as a potion which improves awareness and concentration, slimming, also purifying, regenerating and life extending (JARRELL et al. 2000, DUFRENSE and FARNWORTH 2000).

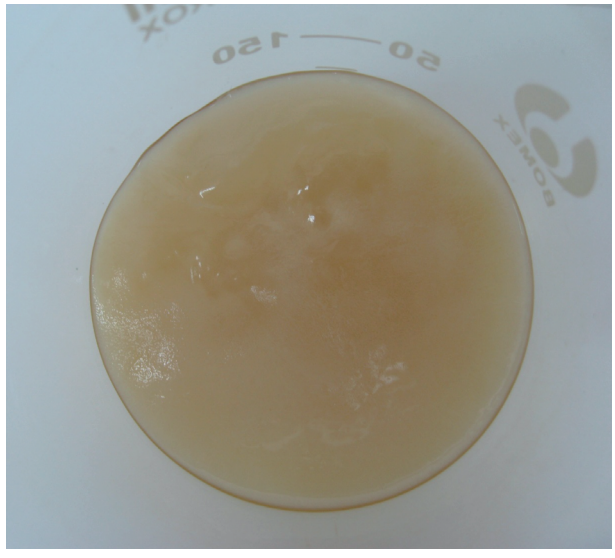

Fig. 1. Bacterial cellulose layer

There are theories in literature describing the origin of the tea mushroom. Kombucha drink is supposed to originate from the Far East (China) and the information on it dates back to 220 BC. According to some reports, Kombucha was brought from Korea to Japan in year 414 AD on the request of the Emperor by a Korean doctor named Kombu in order to treat the digestive problems of the Emperor (DUFRENSE and FARNWORTH 2000). It was brought to Russia by merchants from the Far East, later it was brought to Europe (HARTMANN et al. 2000).

It is possible that the name "Kombucha" originates from Japanese words "kombu", which means seaweed and "cha" that stands for tea. Different theory claims that the it may have connection with the name of previously mentioned Korean physician Kombu (JARRELL et al. 2000).

Kombucha mushroom and its beneficial therapeutic effect was again appreciated as late as at the beginning of the 20th century (HARTMANN et al. 2000). Presently, the tea mushroom is cultured in domestic conditions and passed from family to family.

The composition and properties of the tea are well documented. Regretfully, scientific reports on the composition, effect on the human body and properties of Kombucha are sparse. The aim of this paper is to review the properties and composition of Kombucha beverage as well as its biological activity and possibly favourable effect on the body.

The process of brewing Kombucha

The most common substrates used for Kombucha brewing are black tea and sucrose. The preparation is carried out in the following manner: base tea infusion usually made from black tea is enriched by sucrose. Next, the tea is cooled to room temperature and acidized by adding vinegar or readymade Kombucha beverage. Kombucha "mushroom" is inoculated into the surface area. The solution is left to ferment in room temperature for at least a week. The product contains carbohydrates, organic acids, vitamins and mineral components. Acetic acid bacteria is the most popular microorganism found in Kombucha. Through the process of yeast fermentation, sucrose is transformed into glucose and fructose (SIEVERS et al. 1995). Acetic acid stimulates production of ethanol which, in turn, may facilitate the growth of acetic acid bacteria and production of acetic acid (LIU et al. 1996). Acetic acid bacteria initially oxidises ethanol to acetic aldehyde and then to acetic acid. This bacteria also activate glucose oxidation to gluconic acid (DUFRENSE and FARNWORTH 2000).

Both ethanol and acetic acid (TEOH et al. 2004) have an antimicrobial and anti-pathogenic bacteria effect, providing protection against contamination of the tea mushroom. Besides, the fungi and bacteria present in Kombucha beverage as strong symbiotes are capable of inhibiting the growth of many potentially contaminating bacteria (LIU et al. 1996).

Despite the fact, that Kombucha drink is manufactured mainly in domestic conditions, its potential beneficial action brought much attention in scientific societies. More professional brewing methods with many variants have been incorporated.

The most common scenario can be found in (DUFRENSE and FARNWORTH 2000). As carbon source 50 g dm^{-1} of sucrose is used. Initial acidification can be achieved by either use of vinegar or previous Kombucha sample. Environmental conditions are observed, temperature is maintained between 20°C and 30°C for one to eight weeks of fermentation time.

Most researchers prove that fermentation cycle lasts about 7–10 days; after that time most of carbon sources are used up and all processes slow down (MALBAŠA et al. 2008, GOH et al. 2012(I), GOH et al. 2012(II)). On the other hand there are meaningful arguments towards sustaining prolonged fermentation even up to 60 days (CHEN and LIU 2000), (JAYASUNDARA et al. 2008).

Chemical composition and properties

Detailed knowledge of the composition and properties of tea is crucial for better understanding of Kombucha effect. Tea as a plant belongs to *Camel-*

liaceae family. The leaves are picked up from evergreen shrubs and may be processed using different methods.

Black tea is made from undeveloped leaf buds (*Theae nigre folium*) and the first (two or three-day) leaves which, after picking, are dried in the air and next, rolled or crushed in order to release enzymes, next left for fermentation and then dried. Green tea (*Theae viridis folium*) is obtained through the process of quick drying of the leaves after picking them, in order to inactivate enzymes (KUNTZE 2003).

The main tea components include: purine alkaloids (caffeine, theine, theobromine, theophylline); proantocianidinas and their esters; flavan derivatives (catechins, epicatechins); galotannins (esters of gallic acid with glucose); phenol acids; triterpene saponins (tea saponins); tea flavin yellow in colour, teaflagalin i tearubigenin red in colour (compounds formed during fermentation, absent in green tea); flavonoids (quercetin, kemperol, myricetin and their glycosides); mineral compounds (fluorine, potassium, magnesium and aluminium); aminoacids (theanine); volatile aromatic compounds (mainly teaspiran and other monoterpene, aldehydes, alcohols, ketones); carbohydrates; vitamins (ascorbic acid, B vitamins) (DUFRENSE and FARNWORTH 2000, action KUNTZE 2003).

The presence of alkaloids in black and green tea has influence on their stimulating effect, tannins however, cause constipating effect. Polyphenol compounds express an antioxidant, antivirus and antibacterial (KUNTZE 2003).

Kombucha beverage is consumed because of its favourable effect on health. The most important components, identified in the fermented beverage include: organic acids, mainly acetic acid, L-lactic acid, gluconic acid, glucuronic acid. The rich acid content is one of the most potent and desirable properties of Kombucha. Table 1 presents several measurement results acquired by different authors. Moreover, Kombucha contains B vitamins (B_1, B_2, B_3, B_6, B_{12}, folic acid), vitamin C, mineral components (ions of zinc, copper, iron and manganese), glucose, fructose, ethanol – summarized in Table 1 – (0,5–1,5%), glycerol, carbon dioxide, enzymes, gluconolactone, as well as tea catechines and caffeine (JARRELL et al. 2000, FRANK 1994, REISS 1987). The comparison of main mineral components between teas and Kombucha drink is presented in Table 2.

The main detoxicating compound present in Kombucha drink is gluconic acid. Its beneficial terapeutic action was described by many researchers (FRANK 1994, LONČAR et al. 2000). The antimicrobial effect of the drink is accredited to lactic acid (GREENWALT et al. 1998). Taste of Kombucha and its sensory properties derive from presence of alcohols, aldehydes, ketones, esters and amino acids (TEOH et al. 2004, JAYASUNDARA et al. 2008). Despite many studies and experiments conducted over the drink, there are many unidenti-

Table 1
Most common acids and ethanol contents for Kombucha tea in different days of fermentation cycle

Compound	[g/l]	Day	Growth conditions		Author
			sucrose [g/l]	black tea content [g/l]	
Acetic Acid	4.74	7	70	2.0	Velićanski A. (2013)
	2.44	9	100	1.2	Jayabalan R. et al. (2007)
	5.80	20	70	1.5	Sievers M. et al. (1995)
	5.00	14	100	4.0	Chen C. and Liu B. Y. (2000)
Ethanol	4.07	7	70	2.0	Velićanski A. (2013)
	5.50	14	100	4.0	Chen C. and Liu B. Y. (2000)
Glucuronic acid	1.69	9	100	1.2	Jayabalan R. et al. (2007)
	0.57	14	50	17.0	Talawat S. (2006)
	6.00	20	70	1.5	Sievers M. et al. (1995)

Table 2
Anion content comparison for Kombucha tea, Black tea and Green tea

Anion [mg g^{-1}]	Kombucha Tea	Black Tea	Green Tea	Authors
F^-	–	0.08	–	Alcazar et al. (2003)
	3.2	1.2	–	Kumar et al. (2008)
	–	0.06	–	Spiro et al. (1995)
Cl^-	–	0.6	–	Alcazar et al. (2003)
	–	–	1.78	Ding et al. (1997)
	0.96	3.12	–	Kumar et al. (2008)
	–	0.9	0.53	Spiro et al. (1995)
Br^-	0.04	0.04	–	Kumar et al. (2008)
NO_3^-	0.18	0.34	–	Kumar et al. (2008)
HPO_4^{-2}	–	2.93	–	Alcazar et al. (2003)
	–	–	7.88	Ding et al. (1997)
	0.04	0.08	–	Kumar et al. (2008)
	–	1.18	0.9	Spiro et al. (1995)
SO_4^{-2}	–	–	4.58	Ding et al. (1997)
	1.02	4.2	–	Kumar et al. (2008)
	–	1.45	2.13	Spiro et al. (1995)
I^-	1.04	0.44	–	Kumar et al. (2008)

fied compounds that have antibiotic properties (Jarrell et al. 2000). Full identification of ingredients of Kombucha is a difficult task because of multiple differences in substrate selection, way of preparation and routine of fermentation process (Blanc 1996).

The studies on the effect of Kombucha were mainly conducted by the scientists who performed chemical analysis of this beverage using high performance liquid chromatography (HPLC) (Chen and Liu 2000) and mass spectrophotometry. The results indicated that fructose, acetate and gluconic

acid were the basic components of the fermented tea. Researchers also found that the amount of vitamins present in Kombucha beverage was insufficient for dietary suppementation of the human body. Glucuronic acid was not detected in the studied samples. Steinkraus et al. however, stress that the results cannot be directly compared to other study results due to a significantly lower level of tea in the sample (STEINKRAUS et al. 1996). Total acid content was detected using titration with standard solution of sodium hydroxide with phenolphthalein as indicator (MALBAŠA et al. 2006).

The composition and concentration of the substances present in Kombucha beverage depend on the origin of tea mushroom, the amount of sucrose and the duration of fermentation process. Optimal concentration of ethanol and lactic acid in Kombucha is obtained after adding 50 g of sugar per one litre of the beverage (REISS 1994). It was also proved that the process of fermentation improves the synthesis of B vitamins and folic acid (DUFRENSE and FARNWORTH 2000).

Sucrose is metabolized into organic acids by bacteria and yeast which increases acidity of the beverage. The pH level decreases accordingly to increase of total organic acids content during fermentation (JAYABALAN et al. 2007, MALBAŠA et al. 2008, JAYASUNDARA et al. 2008).

One of the most promising and potent product of tea broth biotransformation is bacterial cellulose synthesized by *Gluconacetobacter xylinus* (former *Acetobacter xylinum*) strain (JARRELL1 et al. 2000). The cellulose has recently been used in production of medical dressing, known as "artificial skin" or "water mantle". Such dressing are utilized in case of hard healing wounds, for instance burnings (KUBIAK et al. 2009, FONTANA et al. 1991).

The US Food and Drug Administration had tested several samples of commercially available Kombucha. In result, neither pathogenic microorganisms nor other contamination were found (KURTZMAN et al. 2001, JAYABALAN et al. 2007, CDC Editorial Note 1996).

Biological activity and effect on the body

Kombucha is consumed not only because of its sensory values but also for multiple favourable effects on health (JAYABALAN et al. 2008). Kombucha is believed to decrease the risk of various types of cancer, prevent circulation disorders, improve digestive system function (in case of metabolic diseases), strengthen the immune system, mitigate inflammatory conditions and to have a favourable effect on skin, hair and nails (JAYABALAN et al. 2007). On the other hand however, only few among the described properties have been proved by the scientists in experimental studies (DUFRENSE and FARNWORTH 2000).

Starting from 1852 scientists, mainly from Europe, began more significant trials. The first meaningful reports come from Russia, from the beginning of the 20th century and the World War I when it was announced that the "Russian secret homemade remedy" also called a "Wonder drink" helped in headaches and digestive tract diseases and regulated intestinal function, often impaired due to exposure to stress in the army. In the years 1925–1950, several medical studies conducted by recognised physicians confirmed the traditional opinions about Kombucha beverage and its favourable effect was presented including: antibiotic properties, regulating digestive system and intestinal function and gland activity, bringing relief in rheumatism, arthritis and haemorrhoids, decreasing cholesterol level, preventing atherosclerosis, facilitating release of toxins and purifying blood, helping in neurosis and ageing-related problems (DUFRENSE and FARNWORTH 2000). Regretfully, methods used for these studies still remain unexplained. In 1951, the diagnostic poll conducted in the former Soviet Union by the Central Oncological Research Unit and the Russian Academy of Sciences in Moscow showed that there was a positive correlation between consumption of Kombucha and exceptionally high resistivity to cancer (JAYABALAN et al. 2007). Moreover, the study performed in 1960 confirmed the properties of Kombucha connected with cancer prevention, or its detoxicating effect; additionally, it was confirmed that regular consumption of Kombucha within a long period of time strengthens the immune system and increases interferon production. The properties of Kombucha presented by the Russians were next confirmed by the scientists from Switzerland, Germany and Holland (DUFRENSE and FARNWORTH 2000).

It has been suggested that consumption of Kombucha may regulate blood pressure, bring relief in rheumatoid arthritis, strengthen the immune system (popular with HIV-positives and sick with AIDS (TIMMONS 1994)), prevent cancer, mitigate hair greying, smooth wrinkles, reduce haemorrhoids, (JARRELL et al. 2000, FRANK 1994, STAMETS 1994–95, PETRO 1996). There are reports in literature on antibacterial properties of Kombucha beverage and its favourable effect on bacterial microflora, present in the human digestive tract. The antibacterial activity of Kombucha against *Helicobacter pylori* (being a frequent reason of digestive system disorders and gastric ulcers), *Escherichia coli, Staphylococus aureus* and *Agrobacterium tumefaciens* is supposed to be connected with acetic acid produced during fermentation process (STEINKRAUS et al. 1996), (TEOH et al. 2004). Black tea extracts, used in the same amount, did not show antibacterial effect.

Acetic acid is capable of inhibiting and destroying microorganisms if applied in proper concentration. It is assumed that acetic acid in the amount of only 1 g dm^{-1} inhibits the growth of pathogenic bacteria (ADAMS 1985). Many properties of Kombucha are connected with the acidic nature of the beverage.

The detoxicating effect probably results from the potential of binding toxin particles by glucuronic acid and increased excretion of these toxins by kidneys or intestines. Thanks to this, Kombucha may contribute to the elimination of redundant metabolic products and bring relief in conditions associated with accumulation of toxins in the organism, such as: rheumatism, arthritis or renal calculus (kidney stones) (DUFRENSE and FARNWORTH 2000). However, the presence of glucuronic acid in Kombucha beverage and formation of glucuronide complexes (glucuronic acid glycoside) is still open to discussion.

Recent studies indicate that the substance identified in Kombucha beverage as glucuronic acid is probably 2-keto-gluconic acid. A high level of glucuronides is formed in the urine of individuals drinking Kombucha, thus two explanations are possible. The first one suggests that the increase in glucuronide level is dependent on the consumption of glucuronic acid itself. The second suggestion indicates that the presence of glucuronides may be connected with the inhibition of strong β-glucuronidase by saccharic acid 1.4-lactone which is also present in Kombucha beverage (ROUSSIN 1999, WANG et al. 2010). This indicates that it is not glucuronic acid, but uridine diphosphate (UDP) glucuronic acid, the active form produced in the liver, that plays a role in detoxication processes (DUFRENSE and FARNWORTH 2000).

Positive influence of Kombucha on nervous system is probably connected with the presence of B vitamins (ROCHE 1998).

Laxative action can be caused by lactic acid content (REISS 1987). There are also assumptions that lactic acid bacteria may act in immunostimulatory way. However, according to our knowledge, there is no evidence of successful colonization of human digestive system by organisms derived from Kombucha (MARTEAU and RAMBAUD 1993).

Tea and Kombucha are presented in literature as two different beverages, having different properties. However, some effects of tea and Kombucha may be similar. The studies conducted after year 1945 by Russians among the population drinking Kombucha allowed them to observe a decreased prevalence of cancerous diseases (ROCHE 1998). These findings are probably not due to the presence of anticancer substances, not only in tea, but also in the fermented Kombucha beverage. However, the issue of transformation of tea components during the process of fermentation remains unexplained. Catechins, present in tea extract, are increasingly often known as the substances with a strong antioxidant, anti-atherosclerotic, anti-inflammatory, anti-cancerous and anti-diabetic effect (JAYABALAN et al. 2008, JAYABALAN et al. 2007). Therefore, we may assume that the above mentioned benefits of both tea and Kombucha consumption may be due to the presence of catechins in tea alone. However, catechin activity may be also modified by the chemical environment of the fermented beverage. Specific properties of the Kombucha beverage may

also result from the synergistic effect of its compounds. The described properties of Kombucha beverage, connected with strengthening of the immune and gastrointestinal systems, and metabolic function improvement, may be thus connected both with tea properties and the transformations occurring during the process of fermentation (DUFRENSE and FARNWORTH 2000).

Consumption of Kombucha drink usually does not cause harmful side-effects, but several intolerance cases were reported, such as digestion problems, allergic reactions (most likely connected with individual sensitivity to acids) and even kidney failure (DUFRENSE and FARNWORTH 2000). There are two reported cases of severe metabolic acidosis after admission of Kombucha (SRINIVASAN et al. 1997) one case of hepatotoxicity (PERRON et al. 1995), one case of skin reaction (SADJADI 1998), several cases of toxic gastrointestinal reactions and one probable fatal case (PERRON et al. 1995). However these reactions mechanisms have not been met and explained. There is an assumption that all above can be caused by domestic grow contamination (SRINIVASAN et al. 1997, KURTZMAN et al. 2001).

Conclusions

Despite the dynamic development of contemporary medicine, people still search new methods to improve their health and physical condition as well as to strengthen their bodies. The trend of healthy lifestyle and nutrition contributes to the growing interest in natural medicine. Kombucha beverage is consumed due to its favourable effect on health. Its composition includes B vitamins, C vitamin, mineral components (ions of zinc, copper, iron and manganese), acetic acid, lactate, gluconic acid and glucuronic acid.

Kombucha is described by its enthusiasts as a "miraculous medicament that cures every condition". It is supposed to eliminate grey hair, improve vision, purify the body and the strengthen immune system. It also provides protection against cancerous diseases. Consumption of Kombucha usually does not produce adverse side effects, however, some cases of intolerance were reported. These included digestive problems or allergic reactions (partly due to the predisposal to sensitivity to acids) and renal insufficiency. However, the mechanisms of these adverse reactions have not been explained so far. It should be emphasised that more studies are needed on the biological activity and safety of Kombucha consumption. All the components of the beverage should be identified, both tea components and the components of the fermented Kombucha beverage. Further studies are required as more information is also necessary for the explanation of the effect of Kombucha components within the human body. Although we need more studies on Kombucha

beverage effects, there are reasons to believe that its effect on the human organism is beneficial. Probably, the process of Kombucha beverage fermentation involves more complex processes, yet they have not been known and explained so far.

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