

REVIEW ARTICLE KOMBUCHA: AN ANCIENT HEALING ELIXIR

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Abstract

"Counting calories becomes completely unnecessary when your food doesn't come with labels". In this busy world of junk food, the value of medicinal based products now has a greater impact to the society. Kombucha is an ancient medicinal beverage which is now one of the rising marketing products in the United States as a part of Global functional beverage.

Kombucha (kombu-seaweed, cha- tea) is nothing but a fermented beverage (an ancient probiotic) consisting of black or green tea which is a substratum and by providing excessive sugar, it prompts a synergistic relationship between bacteria and yeast, later resulting in the formation of a zoogloeal mat. It was once considered as a controversial topic due to the side effects caused by consuming kombucha but now, it is weighed as a topic of research due to some hidden beneficial health effects. It has some water-soluble vitamins, antimicrobial supplements and few hydrolytic enzymes, plays a major role in the microbiome activation in human. It has a unique feature of killing ability i.e., by activation of bacteriocin which firstly lowers the toxicity then it gradually reduces the intracellular ATP production acting against Gram positive and Gram-negative bacteria. The major drawback of kombucha is, it may revert the Carbon tetrachloride (CCl_4) property in hepatic toxicity. This article not only stresses on the proclaimed health effects of consuming kombucha but also on the mystical side that was kept hidden long for about 2000 years and to firmly reveal that it can also be considered as one of the suggested health drinks. *Keywords* : Kombucha, SCOBY, acetic acid, research, antimicrobial, health effects, bacteria, yeast, relationship.

Introduction

According to food microbiologist kombucha is described as one the most ancient probiotic drink due to its unique symbiotic relationship between few strains of yeast and acetic acid bacteria which are the so-called "friendly microorganism" that keeps your gut healthy. It is formed by the fermentation of black tea. Green, Oolong tea are also preferred and by providing sucrose leads to the formation of a thin biofilm (Zoogloeal mat) Leal et al. (2018). The term kombucha, scientifically referred as Medusomyces gisevii primarily derived from the Japanese letter meaning "kelp tea". Kombucha has several other trivial names according to its origin, red tea fungus, champignon de longue vie, ling zhi, kocha kinoko, chainii grib, chainii kvass, kargasok tea, Manchurian mushroom Frazier & Krueger (2009) or Haipao which are marketed under the name of "Fungus Japanicus", "Kombucha Mushroom" (Ernst 2012). The most abundantly found bacteria in kombucha is Acetobacter xylinum, and in case of yeast Kloeckera spp., Schizosaccharomyces pombe. Watawana et al. (2015) Yeasts that is present in the zoogloeal mat utilizes sucrose to produce alcohol, an energy source for bacteria and convert it into acetic acid and gluconic acid that shields the yeast from contaminating microorganisms. A. xylinum primarily helps in the production of SCOBY (symbiotic association of bacteria and yeast) Watawana et al. (2015) is a thin ,slimy, semi permeable membrane, under aerobic condition with optimal temperature 37[°]C, the symbiosis leads to conversion of simple substrate within 7-10 days into a slightly sour, fizzy, acidic, revitalizing beverage containing alcohol about 1.5%-2.0% Villarreal-Soto et al. (2018). This beverage is composed of all necessary substances which makes them antibiotically active. Many Clinical studies were performed on kombucha to specify more about fungal flora but recently the researchers have centralized on functional lactic acid bacteria isolated from it determined to their probiotic benefits. One of

the research studies confirms isolation of 7 strains from the traditional kombucha in which SLG10 strain antibiotically active against both Gram positive and Gram-negative bacteria (S. aureus CICC 10384 and E. coli CICC 10302). The strain of SLG10 was identified as Lactobacillus plantarum which have bacteriocin ability and is also used as a starter culture for fermented foods (Pei et al., 2020). It is not only a tea for pleasure but also an extraordinary medicinal drink preventing microbial infection Battikh, Bakhrouf & Ammar (2012). It is known for its therapeutic and effective curing benefits by eradicating many types of possible toxic agents like pollutants, exogenous chemicals, excess steroid hormones and bilirubin through urinary system Nguyen et al. (2015) The more it is praised for its medicinal effects the more it is disgraced for its adverse effects, as the over consumption of unpasteurized kombucha leads to hepatotoxicity Kovacevic et al. (2014) jaundice, lactic acidosis Gedela et al. (2016).



Fig. 1 : SCOBY with extended yeast strands.

Descent of Kombucha

Kombucha is a traditional drink brewed at home, originated from china which dates back 1000 of years to the Qin Dynasty (221 BC). It is believed Emperor Qin Shi Huangdi was the first person to brew and drink kombucha. It is a well-known fact that china is popular for its tea and medicinal food but later gradually got recognized by other territories due to the battle where they ended up exchanging their cultural ethics and elements under which one of them was fermentation (Fermenters no date). Kombucha later extended to America during World War I as a sharing of culture between United States, Russia, and Germany. In the year 1944 Kombucha gets its first major notification in an American publication which later emphasized in research oriented learning and in the year 1945 America's first ever kombucha brand was found, GT Dave authorized the largest kombucha brand in the industry Greenwalt, Steinkraus & Ledford (2000).

Microbial association in Kombucha

Bacteria and yeast found in SCOBY Nutritional and chemical composition

The fermented tea is produced due to the dynamic symbiotic association between a set of prokaryote and eukaryote originated organism leading to the formation of a thin slimy matt or SCOBY on the liquid medium which is a cellulose network providing aerobic condition to it. The predominantly found bacteria on SCOBY is Acetobacter xylinum, mainly yields Acetic-acid, Gluconic acid and cellulose from carbon sources like ethanol and glucose. Lactobacillus bulgaricus, Streptococcus thermophilus, Lactobacillus plantarum are the mainly found lactobacillus strains which are isolated from kombucha Pei et al. (2020). There are several studies that confirm the microbial interaction in SCOBY made are different according to variation in the source. It is analyzed that ethanol production by the Yeasts is also participating in the production of Acetic acid by Bacteria, this synergistic effect between them

Bacteria + Yeast> Breakdown sugar into glucose and fructose		
Yeast \rightarrow Utilizes glucose to give ethanol and CO ₂		
Formation of organic acid		
• Ethanol gets oxidized to form acetaldehyde		
• Acetaldehyde further gets oxidized to acetic acid		

Fig. : 2 Primary biochemical reaction (Sievers et al. 1995)

In the secondary biochemical reaction of Acetobacter, glucose gets utilized and transformed to gluconic acid. Residue of fructose in the ferment broth is taken up by the microorganism in an insignificant amount resulting in production of acetic acid, gluconic acid and cellulose by the bacteria.

Nutritional components				
Essential elements		Na, k, Ca, Fe, Mn, Ni, Zn		
Water soluble vitamins		Vitamin B ₁ , B ₂ , B ₆ , B ₁₂ , Vitamin C Microdoses (2011)		
Sugars	1	Sucrose, Fructose, Glucose		

BACTERIA	
Acetobacter xylinum	Greenwalt et al. (2000)
Acetobacter xylinoides	Battikh et al. (2012)
Bacterium gluconicum	Battikh <i>et al.</i> (2012)
Acetobacter ketogenum	Kovacevic et al. (2014)
Lactic acid bacteria	Villarreal-Soto et al. (2018)
Gluconoacetobacter intermedius	Nilgün ÖZDEMİR & Ahmet Hilmi ÇON (2017)
Acetobacter aceti	Battikh <i>et al.</i> (2012)
Gluconoacetobacter spp.	De Filippis et al. (2018)
Gluconobacter oxydans	(2019)
Acetobacter nitrogenifigend sp.	Dutta & Gachhui (2006)
YEAST	
Saccharomyces cerevisiae	Villarreal-Soto et al. (2018)
Schizosaccharomyces pombe	Villarreal-Soto et al. (2018)
Lutheran S.luwigiii	Pei et al. (2020)
Candida tropicalis	Jayabalan, Marimuthu & Swaminathan (2007)
Candida krusei	Battikh <i>et al.</i> (2012)
Saccharomycodes	Malbaša <i>et al.</i> (2011)
Schizosaccharomyces	Malbaša <i>et al.</i> (2011)
Brettanomyces	Of <i>et al.</i> (2019)
Pichia	Greenwalt et al. (2000)
Mycotorula	Greenwalt et al. (2000)
Mycoderma	Greenwalt et al. (2000)

Torulospora	Sievers et al. 1995)
Zygosaccharomyces spp.	Nilgün ÖZDEMİR & Ahmet Hilmi ÇON (2017)

Catalase, Biogenic amines, Purines, Pigments, Lipids, proteins, Hydrolytic enzymes. The chemical components of kombucha are also caused by the preparation of black tea rather than the fermentation process. Metabolites of kombucha tea ∞ sweetener added. Jayabalan *et al.* (2014)

Chemical name	Concentration
Alcohol	Less than 10g/l
Acetic acid	<10g/l (if allowed to ferment up to 30 days level raises to 30g/l) 3%
Gluconic acid	20g/1 (2%)
Lactic acid	<0.6g/l
Saccharic acid	<1g/l
Oxalic acid	< 1g/l
Carbonic acid	<1g/l
Ketogluconic acid	<1g/l

Fig. 3: Source Greenwalt et al. (2000)

Contribution of flavor-active organic acid

The process of kombucha fermentation is tracked by range of pH and the amount of ethanol produced in it. Gluconic acid, citric acid, formic acid, acetic acid, lactic acid is the chiefly found organic acids in which acetic acid predominant. Ethanol produced by saccharolytic yeast get oxidized by acetic acid bacteria. This series of reaction gives a detectable pungent sourness in flavor and aroma in the beverage, followed by the production of lactic acid during the fermentation of carbohydrates by fructophillic lactic acid bacteria (LAB). Giving a tang sour taste with no aroma. Comparatively less amount of citric acid gives a tart sour taste. The contribution of flavor-active organic acid regulates the taste and smell of kombucha Sievers *et al.* (1995).

Adverse effects of consuming Kombucha

Even though nowadays fermented beverages are accepted worldwide, still it is necessary to know a lot more about its biological properties Villarreal-Soto *et al.* (2018). Favorable health effects were claimed since thousands of years but none of them were scientifically proven till date. However there was a considerable outbreak linked to kombucha consumption in a small community named Idaho in the year 1995 which lead to a public alert Greenwalt *et al.* (2000) and say 'NO' to kombucha. There are several adverse health effects observed after consuming kombucha on daily basis and few have been reported scientifically:

A women with heavy alcohol consumption developed jaundice, in the further investigation it is been reported that she was consuming 2 glasses of kombucha per day. Her LFT reports were abnormal and hepatitis serology was negative. After she discontinued taking kombucha, her reports came to be normal. Another case study disclosed a person experienced severe abdominal cramp, chest tightness, dry cough and rashes on his thorax after taking half a cup of kombucha tea twice daily for 2 weeks. He was diagnosed with toxic hepatitis. He was fully recovered after the discontinuation of kombucha tea Ernst (2012). After reading several cases reports it is being commonly interpreted that metabolic acidosis, DIC (disseminated intravascular coagulopathy), elevation in hepatic enzymes and bilirubin level, jaundice, hyperthermia were the major symptoms, although none of the cases proved that consuming kombucha was the only direct source for the above adverse effect. The distinctive key-point from the above mentioned cases was, all the individual were already immunocompromised and were found to be continuously consuming unpasteurized home brewed kombucha tea SungHee Kole *et al.* (2009).

The home brewed kombucha gets easily contaminated by other pathogenic bacteria or yeast. An open inspection of home cultivated kombucha declared enormous contamination with *Penicillium* spp., *Candida albicans* in an unsterilized condition Srinivasan, Smolinske & Greenbaum (1997). It is crucial to avoid the storage of the fermented beverage in a ceramic or lead crystal container as toxic elements can permeate into the tea Vijayaraghavan *et al.* (2000).

Why Kombucha is now, considered a boon?

Kombucha is considered to be one of the most venerable fermented beverages, it provides relief from many physical illnesses. Till date its consumption rate has been drastically raised in the U.S community for its refreshing and unproven curative effects in the human system Sai Ram et al. (2000). It is believed to have many prophylactic and therapeutic benefits such as relief against arthritis, hemorrhoids, helps in digestion, increases the immunity, removes toxins and cleanses blood, triggers good cholesterol, also balances the gastrointestinal microbial flora as it is playing the role of probiotic Jayabalan et al. (2014). During the process of fermentation, glucuronic acid is produced which stimulates few beneficial properties. This acid is highly water-soluble carboxylic acid, which later on gets converted into glucosamine and chondroitin-sulphate which are associated with collagen and the fluid that works as a lubricating substance in the joints. Few butyric acids that is produced by the symbiotic association, helps in the shielding of human cellular membrane. These two acids combine together in order to strengthen the walls of gut by protecting against the invasion of parasites Watawana et al. (2015). Inhibits the growth of Helicobacter pylori, Escherichia coli, Pseudomonas Entamoeba cloacae, aeruginosa, Staphylococcus aureus, Agrobacterium tumefaciens, Bacillus Salmonella typhimurium, Shigella sonnei, cereus, Leuconostoc monocytogenes, Campylobacter jejuni, and also some species of fungus like Candida albicans due to the presence of acetic acid, other effective organic acids and catechins Watawana et al. (2015). Further studies demonstrate about DPPH (2,2-diphenylpicrylhydrazyl) scavenging ability against linoleic acid peroxidation and superoxide radical scavenging property also increased simultaneously with the increased acidity during fermentation, Phenolic compounds and the degradation of

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enzymes liberated by bacteria and yeast in the SCOBY Nilgün ÖZDEMİR & Ahmet Hilmi (ÇON 2017). Some experiments confirmed biological activities in kombucha tea: protection against free radical damage, detoxification, influences immune system, energizing capability Vina et al. (2014). It also enhances humoral response, glutathione peroxidase and catalase activities. It can reverse back the chromate inducing changes thus resulting in the presence of high anti-oxidant and immunopotentiation properties Sai Ram et al. (2000). To detect the antibacterial activity in the fermented beverage, an experiment was performed by adding extraction of different plant species where it was confirmed that kombucha made by Lippia citriodora showed the highest antibacterial activity against seven bacterial strains used for testing, with the zones ranging from 12.5mm (S. typhimurium) to 27.5mm (L. monocytogenes) Battikh et al. (2012). Kombucha is not only a beverage to stimulate functions of human body system but is also used as one of the nitrogen fixing agent as it was declared that a nitrogenfixing bacterial strain RG1T was isolated from kombucha tea which was classified under acetobacter species, although RG1T strain differed from Acetobacter acetii. Now it is replaced in the name of Acetobacter nitrogenifigens with the type strain RG1(Dutta & Gachhui (2006). Kombucha tea holds a strong water-insoluble bacteriocin activity against Diphtheria, Scarlet fever, Influenza, Typhoid, Paratyphoid, Dysentery Vina et al. (2014). Ethyl acetate produced from 14 days of fermented kombucha contains catechin and isorhamnetin which regulates polyphenolic activity, inhibiting bacterial swarming motility in case of Vibrio cholera and also prohibits bacterial invasion over the mucin layer, as well as fluid accumulation Bhattacharya et al. (2020).Back then it was believed to heal wounds as it acts as an antibiotic, detoxifies, and is also considered as a lubricant thus used as one of the topical ointment. A small test concluded that on the 4^{th} , 8^{th} , 12^{th} , 16^{th} and 20^{th} days of observation the healing rate was much faster in kombucha group Barati et al. (2016). Consuming kombucha tea can symbolically lessen the development of prostate cancer cells and metastasis by stopping the expression of angiogenesis stimulators Vina et al. (2014). The further study demonstrated that kombucha could restrain HIF-1a, IL-8, VEGF, COX-2, MMP-2 and MMP-9 activation of malignant cells Srihari et al. (2013). In addition to all these it is also used in waste water treatment and removal of heavy metals such as Pb (II) ions from water instead of using Graphene Oxide (GO) or modified GO with Fe₃O₄. Pb (II) metal ions are carcinogenic in nature and stable compounds that cannot be easily degraded. Remarkably two adsorbents were used in the experiment, modified (GO) with magnetite nanoparticles (GO/Fe₃O₄) and kombucha SCOBY. Results came to be GO/Fe₃O₄ showing dosage increased from 1-3 g/l whereas kombucha SCOBY changed from 50-150 g/l indicating that in comparative to GO/Fe₃O₄, kombucha SCOBY is a better adsorbent and has more efficiency Mousavi et al. (2018). Pasteurized or kombucha brewed in a sterile condition is formally assured to be healthy drink that can be consumed on a daily basis except for those who are pregnant (parturition state), young children, with pre-existing discomfort and immunocompromised.

Popularity and its marketing spectrum

The main outcome of kombucha's popularity is because it is an ancient fermented beverage with considerable benefits and some drawbacks in it. Fermented foods were long ago considered as one of the natural ways of influencing the microbial interaction to preserve or increase the shelf life of some seasonal harvesting products. There are many foods that are traditionally fermented and have a biological background of health replenishing, balancing the gut flora like yogurt, cottage cheese, cheese are widely accepted and are available mostly all over the world whereas some are not known worldwide, like kimchi (Korean fermented food), kefir (similar to thin yogurt), sauerkraut (raw cabbage that has been fermented by various lactic acid bacteria), clabbered milk (naturally fermented milk product). Currently kombucha has 39.4% of the functional beverages market share in the US. In Many industries the manufacturing rate is profoundly seen increasing by adding quite variety of flavors, which attract the consumers with their own choice of taste. According to SPINS Market research group it is declared to be 29% growth Feb 2013-Feb 2014 with sales range at \$ 122.7 million per year. Kombucha is the most frequently recognized product in the functional beverage market and is roughly estimated to exceed 3.5 dollar industry to 5billion dollar industry by2025 Kim & Adhikari (2020). There is still need for a massive recognition and sustaining a healthy increment in the manufacturing rate in other developing countries too.



Fig. 5: Kombucha market value analysis, 2013-2020 Kim & Adhikari (2020)

Conclusion

In the beginning of World War-I people declared Kombucha; a tea of immortality that was accepted in all communities. Despite having many reports regarding its effect, it is still considered to be one of the beverages with most ancient ideology of fermentation. There are comparatively very less researches done to prove the complete biological activity, still there are many distinctive properties that confirms kombucha and SCOBY to be of a more use in the near future in research field. Kombucha is not only a so called ancient beverage but is also an ecofriendly combination that can be used in waste water treatment, removal of heavy metals that harbors carcinogenic properties which is often considered as one the major threat for the society, few strain isolated from kombucha SCOBY is found to have nitrogen-fixing ability. considering these contribution of kombucha it is necessary to know a lot more about its usage in different field of science, broad spectrum antimicrobial property as kombucha is derived from the symbiotic association of various strains of bacteria and yeasts that are capable of preventing cancerous cell proliferation and many other possible diseases which can lead to the introduction of a new member of antibiotic resulting to be a major stepping stone in the science field.

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