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REVIEW ARTICLE

Production of fermented green tea and its nutrient analysis: a mini review

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ABSTRACT

The most consumed beverage world wide is tea and is grown by approximately more than 60 countries. There are a number of tea's present in the market, out of which "fermented green tea" is one type. The fermented green tea is prepared by the fermentation of green tea leaves by using a consortium of microorganisms which can include bacterial and fungal species like *Bacillus subtilis, Aspergillus oryzae, Pseudomonas savastanoi, Aspergillus lucheunsis* etc. The use of different microorganism leads to different tastes, flavours and textures of the tea. Fermentation enhances the nutritional value of green tea like enhanced levels of polyphenols, flavonoids, catechins, some organic acids etc., and also offer a number of health benefits like reduction in obesity, lower blood pressure, helps in preventing breast cancer and a lot many other benefits. The following article describes the microbial composition, biochemical composition, and methods for the preparation of fermented green tea, effects of fermented green tea and the future prospects

Keywords: Beverage, green tea, health benefits, fermentation, microbial consortium

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INTRODUCTION

Green tea belongs to the Kingdom: Plantae, Order: Ericales, Family: theaceae and Genus: Camellia. Green tea consumption is increasing day by day in the whole world because it offers a wide range of health benefits. With the various studies conducted on green tea, it is found that green tea can be processed with microbes, known as microbial fermentation of green tea. Fermentation of green tea helps in achieving a fruity and mellifluous taste. It also decreases the bitterness and lowers down the astringency. Cho et al. (2019) reported in a study that a high dose of the fermented green tea extracts can reduce weight, cholesterol level, improves blood pressure and lowers down the excess level of lipoproteins in women suffering from Central obesity. During fermentation of green tea, various chemical reaction takes place (Ahandani et al., 2019), out of which, one is oxidation of polyphenol, results in formation of different polymerized compounds, that are responsible for colour and flavour of the green tea. There are several traditional statements regarding the uptake of green tea and use of its plant in medication purpose for headache, digestion, detoxification, fatigue and also as an energizer. The long-term benefits include protection against dental caries, tooth loss etc. In addition, an epidemiological study conducted by Ahandani et al. (2019) revealed that regular intake of green tea has a preventive effect on breast cancers, especially for the women with who drink 10 cups a day. The common products of fermentation are carbon dioxide and alcohol. But in fermented green tea, the level of alcoholic content

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is less than 0.05%, which is nearly negligible and hence the fermented green tea can be consider as non-alcoholic beverage. The problem of obesity is increasing day by day worldwide, which is also resulting in various kinds of metabolic diseases such as chronic inflammation, hyperglycaemia, hypercholesterolemia etc. Less physical activities, eating too much junk food etc, are the usual causes for obesity. Fermented green tea can be used as the solution to address the above problems. The cardioprotective potential of green tea is not only attributed to antioxidants, anti-inflammatory and antithrombogenic properties but also enhances the coronary flow of blood. The main difference lies between fermented green tea and non-fermented green tea is the process of fermentation that totally changes the taste, colour and nutritive value of the green tea. Different methods are used to prepare fermented green tea and many of them include a same important step that is of drying the green tea leaves.

Table 01: Difference between fermentation and oxidation of green tea (Graham and H. N. 1992; Tu et al., 2005)

Fermentation	Oxidation
This process involves breakdown of complex substances into simpler ones using microbial metabolism	This process involves enzymatic and bio chemical activities
In this process, with the help of microbial activities, the compounds in tea leaves are broken down and sugar is converted to gases, acid and alcohol	In this process, the tea leaves absorb oxygen which in turn results in changed colour, texture and taste of the tea leaves
Process of fermentation involves microorganisms which acts as catalysts	Does not include any microorganisms
Process of fermentation requires multiple steps	Oxidation is a single step process
Example: Fermentation of green tea using lactic acid bacteria (LAB)	Example: browning of oolong and black tea due to oxidation process

Because of increasing awareness in people about living a healthy life style, people are looking for a product that can help them wisely in maintaining their organ health. With the increasing awareness the consumption of this beverage is also increasing worldwide.

COMPARISON BETWEEN FERMENTED AND NON-FERMENTED GREEN TEA

People consume green tea world wide as it gives numerous health benefits against obesity, cancer growth, diabetes, has antioxidant properties and also helps in treatment against many neurodegenerative disorders. Fermented green tea is made by using sugar (as substrate) and culture of yeast and bacteria (many of which have symbiotic relationship) for the fermentation of tea. After fermentation, due to the bacterial actions and activities, the end products also consist of various essential elements, vitamins (Vit C, Vit B), antibiotic substances, acids such as lysine, enzymes, sugars with organic acids, polyphenols, fibres, ethanol, hence is having multifunctional properties with added benefits of antioxidant and anti-inflammatory properties. Presence of these extra beneficial compounds makes fermented green tea more beneficial. It provides human body with various health benefits such as it helps in improved gastrointestinal functions such as regulating intestinal, gastric and glandular activities, it consists of antibiotics properties, it provides with great healing effects on cholesterol levels, it helps in relieving haemorrhoids, joint rheumatism and gout, it helps in relieving anxiety, nervousness, diabetes and also helps in reducing aging signs and issues, its consumption helps in toxin excretion from body and blood cleansing. It strengthens our immune system and reduces cancer cells propagation.

Table 2: Comparison between fermented and non- fermented green tea (Ansari et al., 2017)

Fermented green tea	Non fermented green tea
Goes through the process of fermentation using a symbiotic culture of bacteria and fungi known as SCOBY	Does not goes through the process of fermentation
Vitamin B, antibiotics and probiotics	Antioxidants
Provides an environment rich in acetic acid and gluconic acid which helps in better functioning of catechins	Does not consists of environment rich in acetic acid and gluconic acid
It has higher bioactivity	The range of bioactivity tends to be average
Not suitable for people having imbalance of gut flora	Suitable for all body type
Example: Kombucha, Jun	Example: Green tea

THE MICROBES AND THEIR EFFECTS IN FERMENTATION OF GREEN TEA:

Fermented green tea also named as post-fermented tea or sweetened dark tea, is a classification of tea that has been undergone microbial fermentation. The most famous fermented tea is kombucha which is often homebrewed and utilized as a traditional remedy in China, Russia and Germany, similarly puerh produced in Yunnan Province. (Frank, 1991). As the presence of active microorganisms which possess antimicrobial properties, antioxidants, probiotics properties, manufacturing peptide, polyglutamic-acid, degeneration as for anti-nutritive complex etc. (which proved as crucial criteria for the preference of starter) and various health benefits imparted by fermented foods. It is constantly being developed and modified by food technologist and industries. It also has been reported from pre-existing scientific studies that various fermented food has nutritive as well as non-nutritive components, which are having the potential for the modulation of particular targeted function in body to the relevant for the well-being and good health of the consumer.

MICROBIAL COMPOSITION

The fermented green tea is produced by the working of a surface appearing colony of microbes that consist aerobic bacteria i.e. LAB (Lactic acid bacteria) which includes *Lactobacillus, Enterococcus* that belongs to heterogeneous group of *Bifidobacterium* genus, however yeast is also been deployed as a potential culture. Some functional microorganisms are mentioned in (table 03) with their characteristics. Primarily, fermentation is carried out by molds itself. Earlier *Aspergillus niger* was implicated as the main microbial organism, but later on the species identification has been challenged by PCR-DGGE analysis, which points out to *Aspergillus lucheunsis* as the primary agent of fermentation. The colony's presence extensively corresponds to the gradient mold but it is actually a surface colony of cellulose that produced throughout the growth of microbes. The exhibition of inflated mat promotes the aeration especially for aerobic microorganisms (Cani et al., 2008). Along with every series of Kombucha, a new biofilm is formed and this can be used later on in the preparation of subsequent Kombucha batches.

During fermentation process, microorganisms produces several enzymes for the break down complex components to simpler biomolecules to perform various biological activities for example: cellulose, amylase, catalase, proteinase, amylase and mannase, by *Bacillus spp.* cellulose biosynthesis stimulators are biologically producing in the plant infusions. Caffeine and other related compounds such as theobromine and theophylline, both were known to enhance the bacterial potential for producing cellulose, (Fontana et al., 1991). In addition, the methylxanthines inhibit the normal switch-off procedure of the cellulose synthase. As the result, O_2 accessibility is increased for colony in the caffeinated tea. However, caffeine quantity is maximized by 4 to 16 times than that of normal caffeine levels (i.e. 40 mg) which confirmed the prohibited fermentation of Kombucha and don't trigger it. Acetic acid bacteria (*Acetobater aceti, Acetobacter cerevesiae, Acetobacter molorum, Gluconacetobacter liquefaciens*) and bacteria producing gluconic acid (*Pseudomonas savastanoi, Acetic acid)* bacteria are prevalent prokaryotic

species present in Kombucha culture. *Acetobacter xylinum* had been exhibited as a chief bacterium in their colony that produces gluconic acid, acetic acid, and cellulose by utilization of carbon sources i.e., glucose and ethanol. In addition, *A. xylinum* is known as a starter culture in Natto yield (Tsubura, 2012) and ‘‘Mother of Vinegar’ (Gallardo-de Jesus, 1981)

In American culture collection a type of yeast is isolated from the microbial content of Kombucha colonies taken from numeral German households (Mayser et al., 1996). The research signified that yeast compositions found in colony is extremely variable but some yeast i.e. *(Saccharomyces*, *Brettanomyces and Zygosaccharomyces*) are most often found in studies of German household samples. In a study done by Hesseltine, has reported the presence of *Zygosaccharomyces* and *Pichia* in Kombucha green tea. The yeast, *Brettanomyces bruzellensis, Zygosaccharomyces bailii* and *Saccharomyces cerevisiae*, (Liu et al., 1996) were also isolated from the Taiwanese samples. *Candida famata, Pichia, Brettanomyces intermedius*, *membranaefaciens*, *S. cerevisiae* subsp. *cerevisiae*, *S. cerevisiae* subsp. *Aceti, Torulaspora delbrueckii*, *Zygosaccharomyces rouxii* and *Z. bailii,* (Herrara et al., 1989) were isolated from fungus of Mexican tea. *Schizosaccharomyces, Torulopsis, Pichia, Saccharomyces, Mycotorula, ,* were there in the Jankovic and also in Stojanovic’s list of yeasts found in Kombucha. From the demonstration given above it is supposed that configuration of yeast colony is different. The Table 03 given below summarizes about the microbial distribution and their various functional characteristics in colonies.

Table 3: Microorganisms with their functional characteristics. (Tamang et al., 2016)

Group	Genera/species	Characteristics	Function
Bacteria (LAB)	*Lactococcus lactis*	Gram-positive cocci bacterium, first GM organism used for treatment of human disease	Produce lactic acid from sugars
	Lactobascillus	Gram-positive, rod-shaped, non-spore forming bacteria	Able to produce lactic acid as a by-product of glucose metabolism
	Leuconostoc	Gram-positive, ovoid cocci, catalase-negative and contrary to vancomycin (distinguishes them from staphylococci), often do chain formation	Ability to produce dextran from sucrose
	Pediococci	Gram-positive, anaerobic, non-motile, spherical, catalase negative	Enhance flavour defect due to diacetyl production and ferment high salt concentrated food.
	Weissella	Gram-positive, non-spore forming, rod-shaped, heterofermentative	Glucose is fermented via hexose monophosphate and phosphoketolase pathways
Yeast	*Candida*	Opportunistic fungi, high mortality rate	Ferment glucose and maltose to acids and gas; sucrose to acid, and does not ferment lactose.
	Zygosacchromyces bailli	Osmotolerant, resistant to high concentration of acetic acid and ethanol, highly fermentative	Produce various flavour compounds, crude fibre and lysine, rich in crude protein
	Pichia	Teleomorph, halt-shaped, hemispherical or round ascospores	Ability to reach high cell density during fermentation leads to high protein yield
	Torulopsis	Asporogenous yeast, highly osmotolerant, strong biochemical activities	Produce surface active extra-cellular lipids
	Sachharomyces cerevisiae	Single-celled fungus, facultative anaerobic, Flat, smooth and moist or dull cream in colour	Ability to ferment various carbohydrates

The Kombucha can be prepared carefully at home (Mayser et al., 1995) by keeping aseptic conditions (i.e., low level of impurities from the spoilage and by keeping away contagious microorganisms). The ability of some other contaminants and organisms are limited by the acidic nature of the by-product having pH 2.5. However, the unwanted molds, *Mucor, Aspergillus niger* and

Penicillium notatum were found in some samples. The utilization of sterilized equipment help in fast cooling of tea and the addition of Kombucha starter leads to rapid decrease in pH of each batch. It can lower down the possibilities of contaminates from these deleterious organisms.

MICROBIAL SYMBIOSIS

The microbial distribution in cellulose colony is very complex. Anken and Kappel have taken the cytochrome oxidase activity for the characterization of symbiotic relationship in networks of cellulose. The above study explained that yeasts and bacteria of Kombucha colony are organized in the form of layers and bands inside the cellulose network (Anken et al., 1992). The relationship between Kombucha organisms i.e., SCOBY known for (Symbiotic culture of bacteria and yeast) studied by (Liu et al., 1996), found that two processes taking place back and forth. First, alcoholic fermentation through which the yeasts transformed sugar to alcohol under administered conditions. Second, in which bacteria transformed this alcohol to glucuronic acid, other organic acids and acetic acid. Eventually the simultaneous yielding of acetic acid and ethanol hinders the contest between other microorganisms. The above relationship represents the specific degree of symbiosis and compatibility within organisms present in colony of tea.

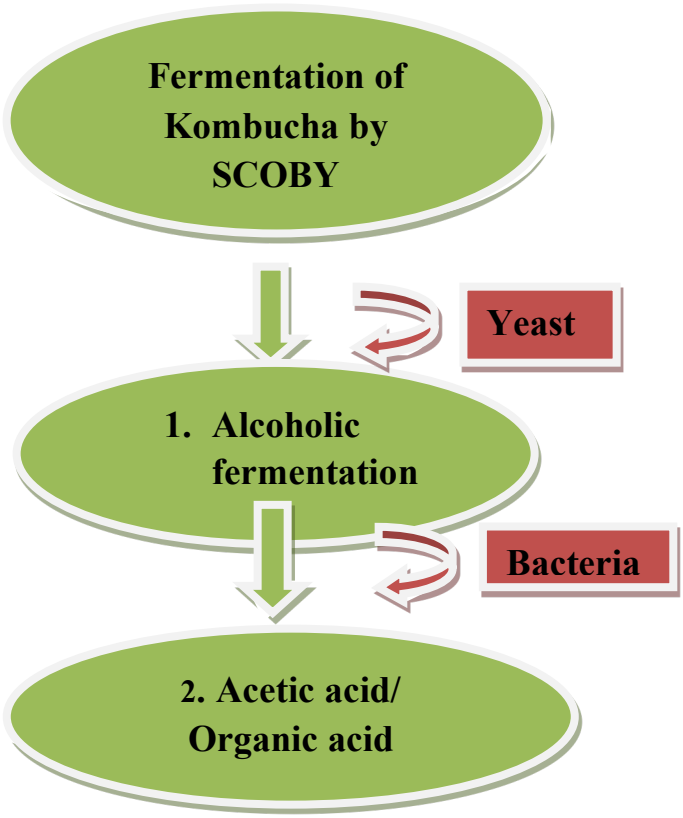


Figure 1: Steps involved in Kombucha fermentation (Greenwalt et al., 2000)

MICROBIAL ACTION IN KOMBUCHA

The yeast initiates sucrose transformation through disintegrating the sugar into two parts i.e. fructose and glucose also known as carbon conversion. Primarily glucose is consumed by yeasts to harvest CO_2 and ethanol. Then, the bacteria oxidized ethanol

to produce acetaldehyde and then into acetic acid. Which results in the alcohol concentration of Kombucha is thought to never increase more than 10 g/liter and conc. of acetic acid will may increase up till 30 g/liter, if Kombucha is placed to ferment for 30 days. Whereas, the acetic acid concentration is less than 10 g/liter. While in the secondary bio-chemical process of *Acetobacter*, glucose is consumed and then changed to into gluconic acid which is usually exist in the considerable amounts, around 20 g/liter, fructose is still continues as a part of the fermented broth and then ingested by the microorganisms. As an outcome, the bacteria firstly produce cellulose, acetic acid and gluconic acid.

BIOCHEMICAL COMPOSITION OF FERMENTED GREEN TEA

During the fermentation of green tea, the parameters that greatly influence the antioxidant and chemical property of the final product are: duration of fermentation, temperature, sucrose concentration, quality of tea and microbees used in the process of fermentation. Due to these parameters, the biochemical composition of green tea like sugar, organic acids, polyphenol, flavonoids and catechins concentration gets affected. Table 04 depicts the concentration of biochemical compounds present in the final product (fermented green tea):

Table 4: Composition of fermented green tea (Gaggìa et # 2019)

Sugars (mg/ml)	Glucose	15.89
	Sucrose	26.21
	Fructose	6.92
Organic acids (mg/ml)	Glucuronic acid	1.96
	Acetic acid	7.65
	Ethanol	4.18
Polyphenols (mg/g DW)		67.40
Flavonoids (mg/g DW)		15.11
Catechins (mg/g DW)	(+)catechin	0.019
	(-)epicatechin	1.769
	(+) gallocatechin	0.110
	(-) epigallocatechin	9.650
	(-) epigallocatechingallate	0.296

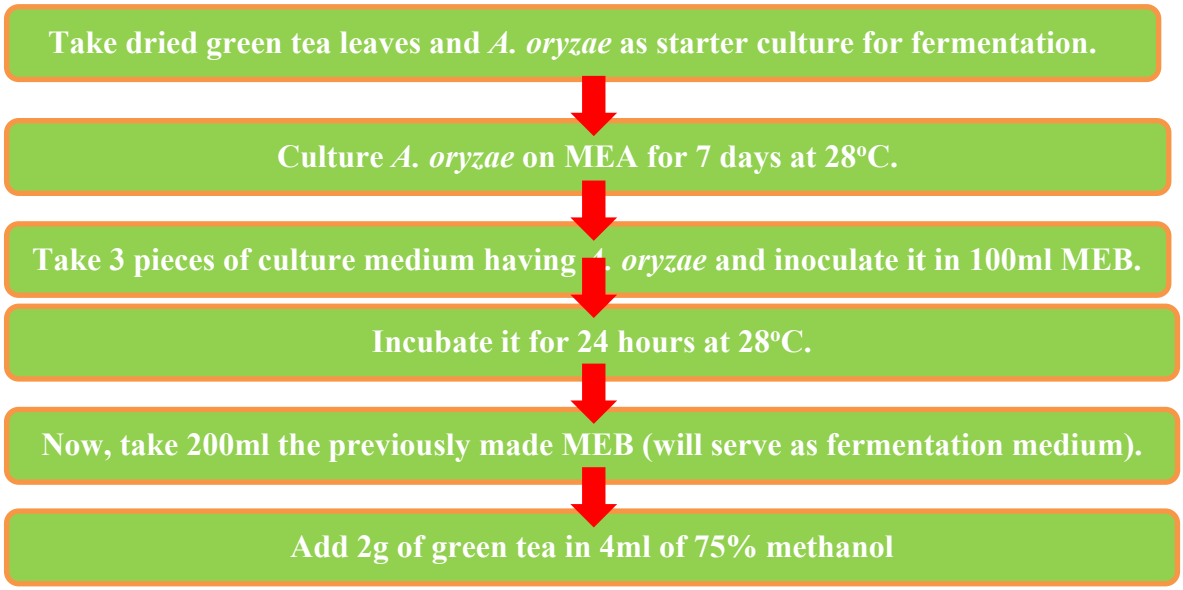
METHODOLOGY FOR PREPARATION OF FERMENTED GREEN TEA

Several methods for the preparation of fermented green tea have been suggested by many scientists, out of which some are as follows:

According to the method used by Seo et al. (2016) in which they used *Bacillus subtilis* as a starter culture for fermentation. For carrying out the fermentation process, they took green tea leaves and then, they kept those leaves for drying at 150^{o}C for 10 minutes. Following which they added 1% sucrose (which acted as a substrate) and $5*10^{7}$ colony forming unit *Bacillus subtilis* after which it was kept at 50 ^{o}C for 72 hours, so that fermentation could take place. After the completion of fermentation process, they again incubated the sample at 90 ^{o}C for 96 hours for the post maturation to occur. After post maturation process, they dried the fermented green tea leaves and extracted it with the help of 50% ethanol for 2 hours at 70 ^{o}C.

Figure 2: Procedure for the preparation of fermented green tea (Seo et al., 2015)

Another group of scientists (Kim et al., 2013) used a different methodology in which they used *Aspergillus oyzae* as the starter culture instead of *Bacillus subtilis*. They took the dried green tea leaves and the culture. After this, they cultured *Aspergillus oryzae* on Malt extract agar (MEA) at 28 ^{0}C for 7 days. Then they out 3 pieces of MEA medium and inoculated those pieces into the 100 mL of MEB (i.e., Malt extract broth). They incubated it for 24 hours in shaking (150 rpm) at 28°C. After incubation they took 6 millilitre of the previously prepared culture and added it in 200ml freshly prepared Malt extract broth medium (which acted as a medium for fermentation of green tea). After the preparation of fermentation medium, they added 2g of green tea powder into the 4 mL of 75% of methanol. Then, 2mL out of the prepared mixture was taken out and was kept for fermentation at 28 °C with shaking at 150 rpm for 8 days. Then, they filtered the medium by using 110-mm Whatman filter and then extracted it by using n-butanol and ethyl acetate in ratio 1:4. Green tea extracts were obtained by recovering ethyl acetate and vacuum drying.



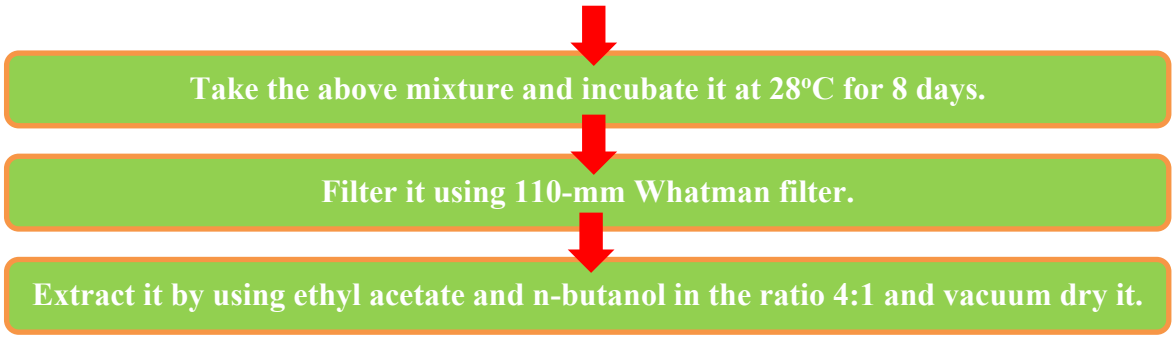


Figure 3: Procedure for the preparation of fermented green tea by using *Aspergillus oryzae* (Kim et al., 2013)

Another method for the preparation of fermented green tea using tea fungi was used by Jayabalan et al., 2006, for which they took 1.2% of waste generated by green tea manufacturing and added it to the water at boiling state. They infused it for nearly 5 minutes and then sieved the infusion using sterile sieve. 10% sucrose was dissolved in the preparation and allowed it to cool down. After that, they took 500 ml glass jar (which was sterilized using autoclave at 121 ºC for 15-20 min. at 15 psi) and added 200 ml of the previously made preparation in it. The solution was inoculated using 3% (w/v) cultured tea fungus and liquid tea which was previously fermented 10% (v/v) stock aseptically. After that, a disinfected cloth was used to cover the jar. Fermentation was allowed to take place in dark incubator for 18 days at 24 ± 3 ºC.

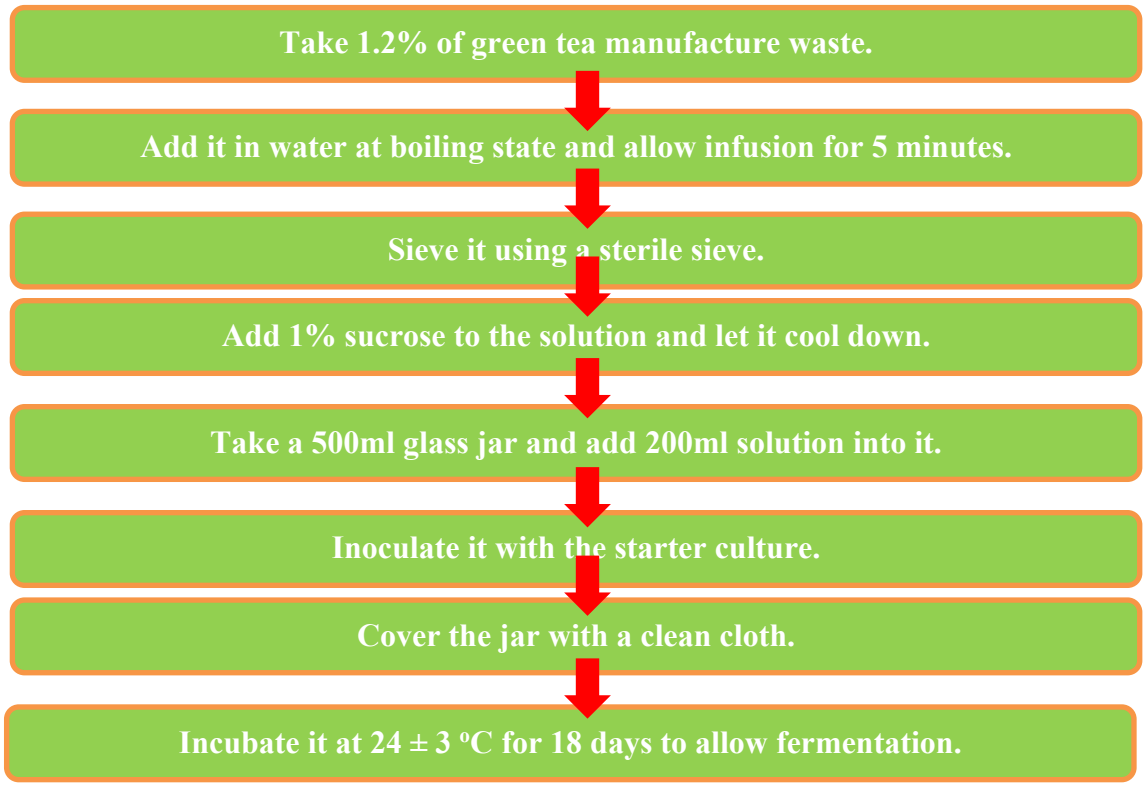


Figure4: Procedure for the formulation of fermented green tea using tea fungus (Jayabalan et al., 2007)

For the formulation of kombucha tea (a type of fermented green tea), Gaggìa et al., 2018, adopted a methodology for which they took green tea and the starter culture and inoculated pellicle (30gm) along with its liquid (100 mL) in green tea, which was prepared by using 1 L of H_2O and sucrose (80 g/L) and add dried leaves (8 g/L) with water at 74 ◦C for 3 minutes. Then they incubated the sample for 14 days at 27 ± 1 ºC.

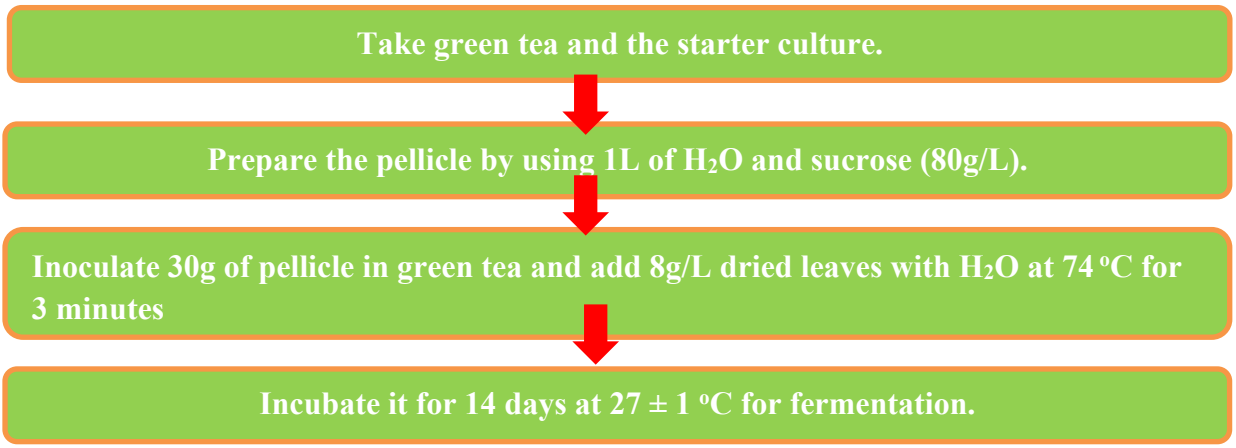


Figure 5: Procedure for the preparation of fermented green tea (Kombucha) (Gaggìa et al., 2019)

In the year 2020, Jakubczyk et al., used a totally different methodology than the above for the preparation of kombucha tea, in which they used a starter culture known as SCOBY (Symbiotic culture of bacteria and yeast), which comprised of *Acetobacter xylinum*, Gluconobacter, *S. cerevisiae*. The starter culture ccomprised of cellulosic layer and sour broth which was maintained at 4°C in a refrigerator. To prepare kombucha tea they added 100g of sugar and 8g of tea in 1 L distilled hot water (90 ºC). Then the solution was infused in conical flask for 10 minutes. The solution was allowed to cool down and then it was filtered using the nylon filters and was poured in sterile bottles. Then they incubated the kombucha culture for 1,7 and 14 days at 28 ± 1 ºC. After incubation kombucha was obtained by filtration.

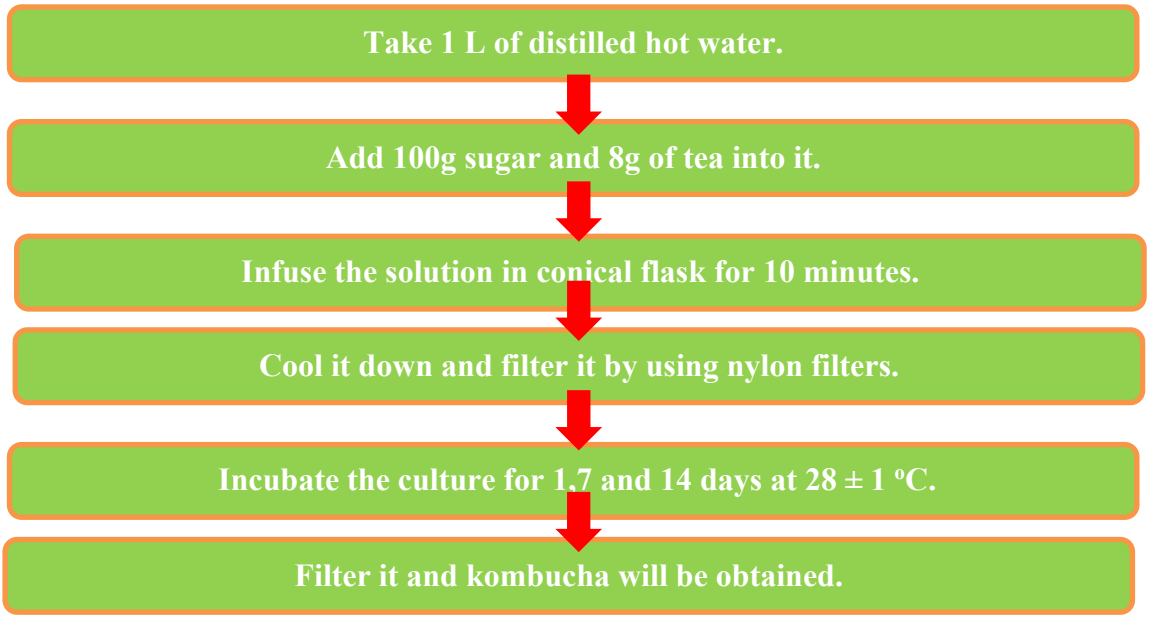


Figure 6: Procedure for the preparation of fermented green tea (Kombucha) (Jakubczyk et al., 2020)

EFFECTS OF FERMENTED GREEN TEA

Fermented green tea acts as a dairy free pro biotic source and hence, can also be consumed by people having lactose intolerance. The caffeine content of fermented green tea is also low which is only up to 25 mg (average) as compared to green tea having caffeine content up to 40- 50mg (Mishra, 2009). Fermented green tea consists of polyphenols and acetic acids and hence, possess strong antibacterial properties against various yeast and bacterial infection while not affecting the beneficial pro biotic bacteria and yeast (Mizuta et al., 2020). In an experiment carried out by Maron et al. (2019) on wistar rats showed that fermented green tea helps in improvement of both good HDL (high-density lipoprotein) and LDL (low-density lipoprotein) cholesterol. Fermented green tea also has properties to prevents cholesterol particles from oxidation hence reduces the risk of heart attack. After a study on diabetic rats (Wister rats), it was found that green tea also helps in liver toxicity (Heikal et al., 2013). Green tea extracts prove helpful in the improvement of serum level in the patients undergoing with non-alcoholic fatty liver disease (NAFLD) (Pezeshki et al., 2017). Fermented green tea has anti-obesogenic effects and hypolipidemic effects (Seo et al., 2017).

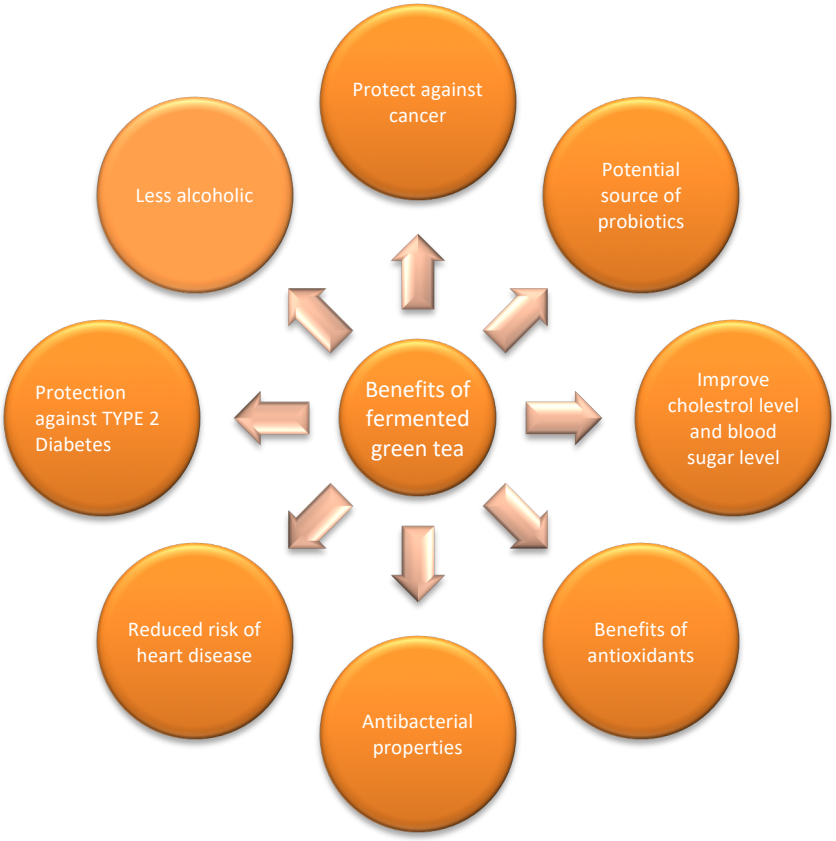


Figure7: Benefits of fermented green tea (Jakubczyk et al., 2020)

Fermented green tea can be differentiated into two different forms that are jun tea and kombucha tea depending on the process and materials used.

Table 5: Difference between Jun and Kombucha (Villarreal-Soto et al., 2018; Mo et al., 2005)

Jun tea	Kombucha tea
Jun is made using only green tea	Both black and green tea can be uutilized in the formation of kombucha
Jun is fermented using honey as a sweetener	Kombucha is fermented using cane sugar
The SCOBY (symbiotic culture of bacteria and yeast) culture used for fermentation of jun is trained to feed on honey as a sugar source.	There is no need of special handling of SCOBY culture for the fermentation of Kombucha
Jun is less sour and much mild	Kombucha is sour in taste

FUTURE PROSPECTS

Tea comes next to water as most consumed drink worldwide, but most people consume black tea which has high caffeine content, which when consumed in long term can lead to adverse health conditions like chronic insomnia, depression, anxiety and stomach problems. These health conditions can be resolved or avoided by replacing black tea with a better and healthier option i.e., fermented green tea. In traditional Chinese medicine, fermented green tea is considered as a healthy beverage since ancient times. Modern studies revealed that fermented green tea contributes towards reduction in the risk of cardiovascular disease and some forms of malignant neoplastic disease. It has also been proved that, it promotes oral health and various other physiological functions such as controlling high blood pressure, enhance body metabolic rate, antibacterial, antioxidant and antivirasic effect, protection against solar ultraviolet rays, increase in density of bone mineral, anti- fibrotic properties. With such wide range of health benefits, fermented green tea proves to be potential of helping people cope up with the increasing busy and stressed life style.

Growing interest in its health benefits has led to the involvement of fermented green tea in the group of beverages with useful properties, hence fermented green tea can also be replaced by alcoholic drinks with high alcohol content. Fermented green tea with such wide range of health and functional benefits becomes a need of future traditional medicine. Hence, more and more studies should be conducted in order to explore the unexplored beneficial properties of fermented green tea and also for the enhancement of its existing nutritional content.

CONCLUSION

The article demonstrates about the benefits of fermented green tea to the consumers because of the presence of beneficial microorganisms, which are having probiotic properties, antioxidants and antimicrobial properties (antibacterial properties for infection causing yeast and bacteria while not harming the good pro-biotic yeast and bacteria). Non fermented green tea's are different form of fermented green tea's, as they do not go through with fermentation process and doesn't need an environment rich in acetic acid and gluconic acid. Different methods are being used to prepare fermented green tea.

Fermented green tea is very useful and can be used as a dairy free probiotic source due to which people with lactose intolerance can also consume. In addition, fermented green tea also consist of low level of caffeine which is only up to 25 mg (average). It also helps in reducing bad cholesterol level and gives good health.

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