

ANTI CARIOGENIC EFFECTS OF GREEN TEA

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ABSTRACT

Green tea is considered a healthful beverage due to the biological activity of its polyphenols namely catechins. Among the polyphenols EpigalloCatechins 3 gallate and Epicatechin 3 Gallate are the most predominant Catechins. It can play an important role in fighting bacteria in the mouth, freshening the breath, and warding off cavities. Green tea Catechins can help fight the cavity producing effects of *S.mutans* by destroying them, making tooth surface more slippery, so that bacteria have harder time to cling to teeth, inhibiting production of plaque and reducing ability of bacteria to produce the enamel-eating acid. In addition antioxidant, antimicrobial, anticollagenase, antimutagenic and chemopreventive properties of these Catechins provide to be helpful in the treatment of chronic diseases like periodontal diseases. The present review concentrates on the effect of green tea on teeth.

Keywords: Catechins, Green Tea, Teeth, Periodontal Health.

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No of Figures: 14

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INTRODUCTION

Tea comes from the top leaves of the plant *Camellia sinensis*. In 1958, a British botanist J. R. Sealy classified all plants in the genus *Camellia* and tea was given the name it has today. The tea that today is commonly consumed in countries all over the world was once revered for its curative powers. Some of the earliest mentions of tea in Chinese literature refer to it as a remedy for a diverse range of complaints. Gradually though, tea became more and more commonly consumed and its role in society started to shift from that of a highly esteemed panacea to one of being simply a refreshing and habitual beverage.

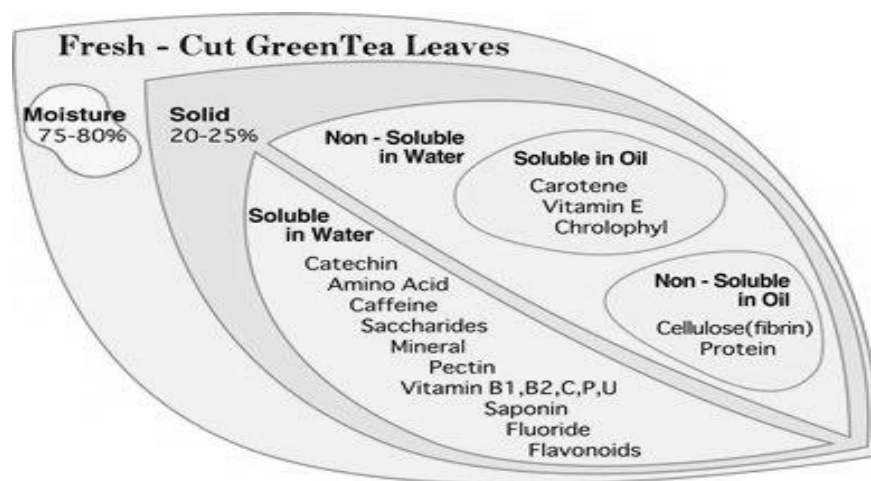
What does a tea leaf contain?

Fresh-cut tea leaves consist of 75-80% water. A variety of green tea flavors are formed through the combination of three main taste components. Catechin–Bitterness & Astringency ; Caffeine–Bitterness Theanine& Amino Acids– Flavor& Sweetness

The chemical history of tea

While the history of tea drinking is ancient, investigation into the chemical components

of tea is in comparison quite recent. Tea is composed of unique constituents among other plants. Caffeine is found only in a few other plants other than tea. Theanine, which is unique to tea, is a kind of amino acid constituting more than half the total amount of amino acids in tea. Major catechins in tea are also unique to tea. Vitamin C was found to be contained in tea after it was discovered in lemons. Tea aroma is an area that attracted the interest of scientists who had been seeking one single compound that represents tea, a search which has yet been in vain. In 1827 caffeine was discovered in tea. At that time it was given the name theanine, but when it was proven that the structure and properties of this substance were exactly the same as caffeine that was identified in coffee in 1820, the name theanine was dropped. In 1924, vitamin C was discovered in green tea by two Japanese scientists, M. Miura and M. Tsujimura, under Professor U. Suzuki. The astringency of tea, too, was investigated extensively by Tsujimura. In the years 1927 to 1935, Tsujimura isolated epicatechin, epicatechin gallate, and epigallo catechin.



Biosynthesis of Tea Catechins

The tea plant contains many kinds of polyphenols, Catechins being particularly prolific. Catechins belong to those groups of compounds generically known as flavonoids, which have a C₆-C₃-C₆ carbon structure and are composed of two aromatic rings. Currently, the tea plant is known to contain seven kinds of major catechins and traces of various other catechin derivatives.

They are divided into two classes:

- The free catechins: (+)-catechin, (+)-gallocatechin, (-)-epicatechin, (-)-epigallocatechin;

And,

- The esterified or galloylcatechins, (-)-epicatechingallate, (-)-epigallocatechingallate, (-)-gallocatechingallate.

While the galloylcatechins are astringent (EGCg, ECg) with a bitter after taste (ECg), free catechins have far less astringency (EGC, EC), leaving a slightly sweet aftertaste (EGC) even at 0.1% aqueous solutions.

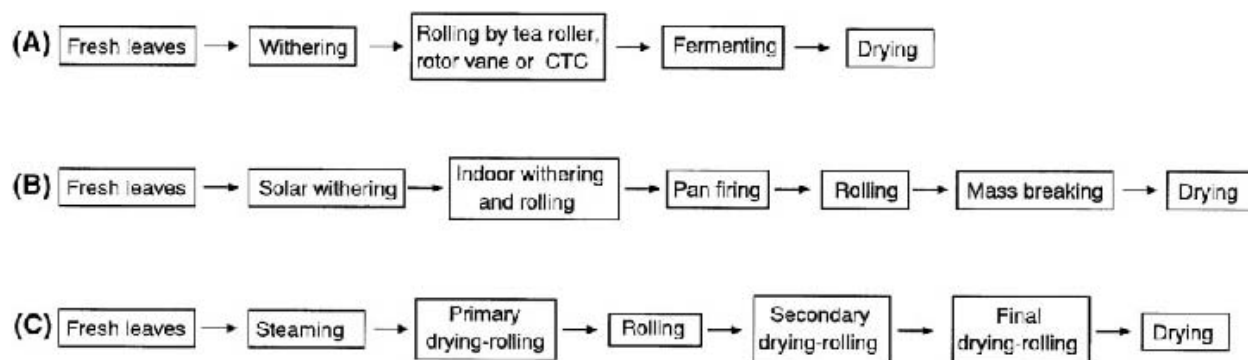


Fig 1 Fermentation of Tea: The manufacturing processes of tea: (A) black tea; (B) oolong tea; and (C) green tea.

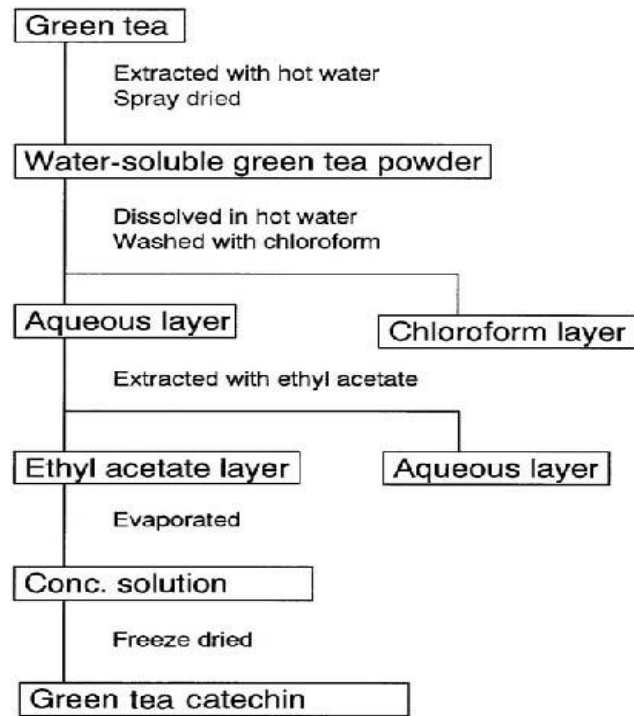


Fig 2 Extracting Polyphenolic Constituents of Tea Leave: The preparation of green tea Catechins

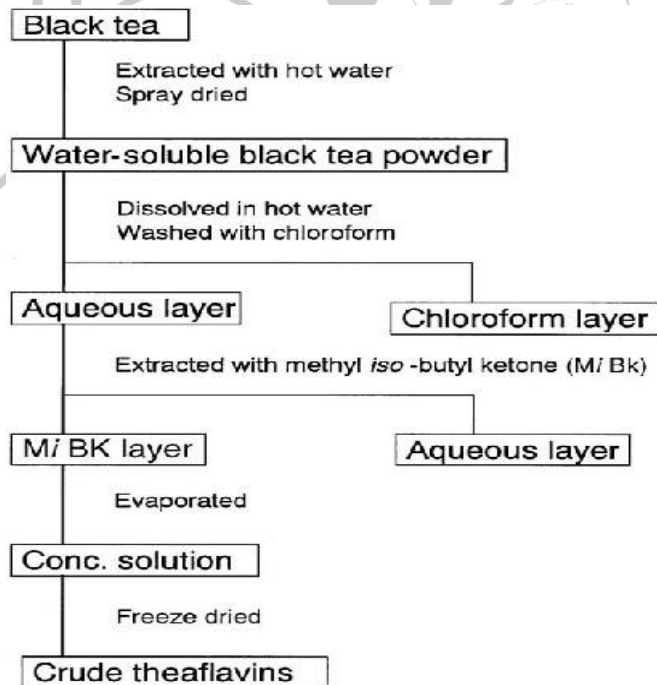


Fig 3: The preparation of crude theaflavins.

Antibacterial Action

It seems that prescribing a cup of tea for an upset stomach can no longer be regarded as merely an old-fashioned remedy. Some people may remember being served up cups of tea to treat a bad cold or flu. Although it cannot be called a miracle cure, there is now some scientific data to support the idea that tea can have a calming effect when bacterial infections are present. People all over the world drink tea and many cultures appear to have incorporated it into various customs. In Japan, green tea traditionally accompanies almost every meal, and particularly strong green tea is always drunk after eating sushi. Somehow, it seems that people have always known that tea can fight bacteria. The antibacterial action of tea is attributed to the poly phenolic components it contains. It could potentially be exploited at all different levels: from protection of bacterial infection on an individual basis by consumption of polyphenols to the large-scale commercial use of polyphenols in order to prevent contamination of food products by pathogenic bacteria.

Foodborne Pathogenic Bacteria

The polyphenolic components of tea have been found to be extremely effective against various strains of foodborne pathogenic bacteria that can be harmful and, in some incidences, even fatal. Tea polyphenols have shown an antibacterial effect against a number of such destructive bacteria, namely: *Clostridium perfringens*, *Vibrio parahaemolyticus*, *Vibrio fluvialis*, *Vibrio metchnikovii*, *Staphylococcus aureus*, *Bacillus cereus*, *Plesiomonasshigelloides*, and *Aeromonassobria*. Such an immediate

susceptibility was not seen with bacteria such as *Aeromonas hydrophila* subsp. *hydrophila*, *Salmonella enteritidis*, *Salmonella typhimurium*, enteropathogenic *Escherichia coli*, entero invasive *E.coli*, *Yersinia enterocolitica*, *Campylobacter jejuni*, and *Campylobacter coli*, although they were inhibited after a longer period of contact with tea polyphenols.

Anticariogenic Action

Green tea's ability to help reduce symptoms of periodontal disease may be due to the presence of the antioxidant catechin. Previous research has demonstrated antioxidants' ability to reduce inflammation in the body."These results suggest that EGCg is effective in reducing acid production in dental plaque and mutans *streptococci*."Now science has shown that this idea makes sense, as green tea can play an important part in fighting bacteria in the mouth, freshening the breath, warding off cavities and even protecting against the need for that dreaded procedure known as the root canal.

Luckily, green tea catechins can help fight the cavity-producing effects of *Streptococcus mutans* by: destroying a good percentage of the *S.coccus* mutans bacteria themselves; making the tooth surface more slippery, so the bacteria have a harder time clinging to the teeth; inhibiting the production of plaque; reducing the ability of bacteria to produce the enamel-eating acid. Surprisingly, it doesn't take quarts of tea to produce these benefits. Green tea catechins have destroyed *Streptococcus mutans* bacteria even when Catechins have been shown to kill mouth bacteria associated with

tooth decay and gum disease, so the researchers suspect this is what gives green tea its dental benefits. Previous research has indicated that regular consumption of green tea may lead to a lower instance of periodontal disease, a leading cause of tooth loss in adults, said Samuel Low of the University of Florida, College of Dentistry and President of the American Academy of Periodontology in a statement to Discovery News. Maintaining healthy teeth and gums is part of maintaining a healthy body, low said. "That is why it is so important to find simple ways to boost periodontal health, such as regularly drinking green tea something already known to possess certain health-related benefits." Green tea's ability to fight bacteria isn't just limited to the digestive system. It can also play an important part in fighting bacteria in the mouth, helping to freshen the breath, ward off cavities and even protect against root canals! *Streptococcus mutans* is the strain of bacteria primarily responsible for causing cavities. It clings to your teeth and uses the sugars in your mouth to produce a sticky, water-insoluble substance called plaque that coats your teeth. *S mutans* and other bacteria then hang on to the plaque and convert sugar to lactic acid, which eats away at your tooth

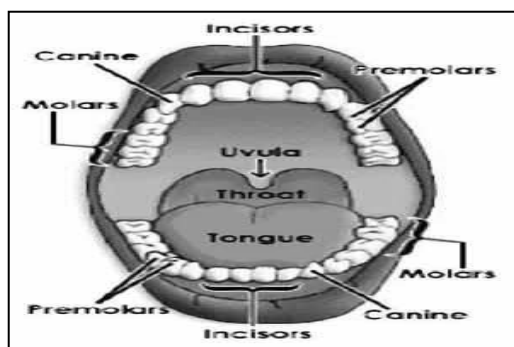


Fig 4: Anatomy of the Human Mouth

enamel. The combination of plaque and acid causes cavities. Green tea catechins help fight dental cavities in three ways: they make it harder for the bacteria to cling to the teeth, inhibit the production of plaque, and actually destroy the *S. mutans* bacteria themselves. Studies have shown these effects can occur with as little as one cup of green tea. No wonder that the Japanese people have long held that drinking green tea makes the mouth clean.

Tooth Decay

By the time the human mouth reaches maturity, most adult humans have thirty two teeth as is seen in Fig. 4. At the front of the mouth are incisors, the sharpest teeth, which are used to bite food and direct the food into the mouth. The canine teeth are located behind the incisors on either side of the mouth. They have long roots and grasp incoming food. The pre molars are located even farther back into the mouth. These teeth are wide and flat, equipped for grinding food before it is fully digested. Finally, the molars grind food into particles small enough to be swallowed and broken down in human's digestive tracts. It is very important to keep all of these teeth clean, and cavities are a real concern for humans in today's society.

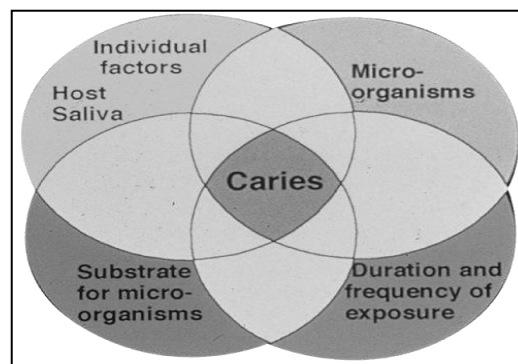


Fig 5: Dental Caries

After food enters the mouth, particles and bacteria are able to cling to the surface of the teeth. Many times, if the teeth are not brushed soon enough after eating, the bacteria can grow and form a film on the teeth. It is important to understand that cavities are not formed by the sugars consumed by humans, but instead by the bacteria that grow in the mouth if food particles remain on and between teeth for an extended period of time, Fig 5. Certain bacteria thrive in the conditions found in the human mouth. The warm environment and constant source of food both make the teeth and gums ideal locations for bacterial cultures to grow. *Streptococcus mutans* and *Streptococcus sobrinus* are two such bacteria commonly found in dental cavities. When they grow on the surfaces of teeth, they eat at the food particles and release acid as a waste product. This acid eventually builds-up, and breaks down the minerals in the teeth. Over time, when large amounts of acid have been released surrounding teeth, cavities begin to form. The acid initially leaves the surface of the tooth intact, while breaking down the enamel lying beneath the surface. When the tooth has enough damage, the surface also breaks down and a cavity is formed. The most common means to prevent tooth decay are to consistently brush and floss the teeth. Fluoride is usually present in toothpaste as a means to breakdown bacteria and prevent acid build-up. Fluoride is also present in green and black tea, one reason why drinking tea can prevent tooth decay. In some areas, fluoride is also present in the public drinking water, although this is a topic of much debate recently. Many

Americans support the use of fluoride in their drinking water. This provides a way for humans to protect their teeth without making much of a personal effort, beyond drinking the water provided them from their faucets.

Bacterium Causing Tooth Decay

Ancient Japanese folklore tells how drinking tea leads to long life and clean teeth. At least the second part of this fable seems to be true. Recent research indicates that tea is able to counter some of the microorganisms, *Streptococcus mutans*, *Streptococcus sobrinus*, and *Lactobacillus* that can form plaque and bio-films on teeth, resulting in tooth decay. Microorganisms from the genus *Streptococcus* are gram-positive bacteria. They have a round shape and frequently grow in chains. They are anaerobes that thrive in a complex culture. *Streptococcus* can cause many diseases, ranging from strep throat, to necrotizing fasciitis (flesh-eating disease) to the more mild tooth biofilm build-up. Although not the most dangerous infliction caused by this genus, here the focus is on the latter condition and how to prevent it. *Streptococcus mutans* (Fig. 6) is a species of *Streptococcus* that usually resides in the human mouth. It was discovered in 1924 by J K Clark and has recently been a topic of study due to several hundred unique genes that the bacteria has. These genes could potentially be a targeted in an attempt to kill the bacteria that cause tooth decay. *S mutans* is able to cling to the surface of teeth and feed on food particles, especially carbohydrates, that become trapped on and between teeth. The acid released by this

bacteria is the leading cause of tooth decay in the world. *Streptococcus sobrinus* is

closely related to *S. mutans*, although it is less common in human beings.

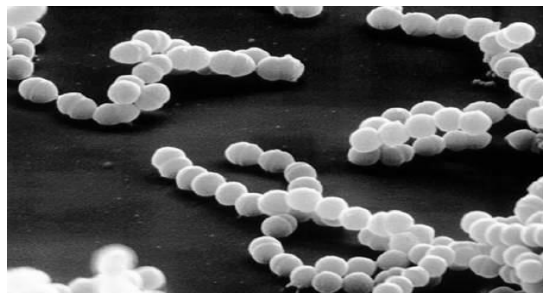


Fig 6: Scanning electron microscope image of *Streptococcus* bacteria

Inhibition of Plaque Formation

The inhibition of the plaque-forming enzyme glucosyl transferase (GTF) by tea polyphenols was investigated in vitro. The enzyme GTF, sucrose, and tea polyphenols were mixed and incubated at 37°C for an hour. The sucrose carbon was labeled so that the fate of sucrose could be traced. Without tea poly-phenols, the enzyme catalyzes the formation of insoluble glucan, i.e., plaque.

In the solutions containing tea polyphenols a dose-dependent inhibition of insoluble glucan formation was noticeable. EGCg (and its isomer, GCg) and all theaflavins inhibited the glucan formation almost completely at the concentration of 10 mM. At 1 mM, about the drinking concentration of tea polyphenols, more than 50% inhibition was observed (Table 1).

Table 1: Effect of Tea Polyphenols on the Insoluble-Glucan Formations Catalyzed by GTF (glucosyl transferase)

Sample	Concentration (mM)	% incorporation of (¹⁴ C) glucose ^a insoluble glucan
(-)-Catechin	1.0	77.7 ± 5.9 ^b
	10.0	38.3 ± 3.0
(-)-Epicatechin	1.0	94.5 ± 3.5
	10.0	57.7 ± 5.0
(-)-Epicatechin gallate	1.0	64.5 ± 6.2
	10.0	17.0 ± 3.2
(-)-Gallocatechin gallate	1.0	52.8 ± 2.6
	10.0	4.6 ± 0.1
(-)-Epigallocatechin gallate	1.0	58.4 ± 3.3
	10.0	25.0 ± 1.9
(-)-Free theaflavin	1.0	43.2 ± 1.4
	10.0	1.7 ± 0.2
Theaflavin monogallate A	1.0	35.5 ± 1.6
	10.0	2.7 ± 0.6
Theaflavin monogallate B	1.0	52.9 ± 7.1
	10.0	2.2 ± 0.4
Theaflavin digallate	1.0	44.1 ± 2.6
	10.0	1.8 ± 0.3

a. Incorporation ratios into insoluble-glucans relative to the respective control are expressed as follows:

$$\% \text{incorporation} = \frac{\text{test}^{(14}\text{C-incorporation})}{\text{control}^{(14}\text{C-incorporation)}} \times 100$$

b. Mean ± S.E. (n=4)

So, the influence of tea beverages on the formation of plaque by *S. mutans* was observed. The method is described in Fig. 1(below). Tea leaves were drawn to make a tea extract of normal concentration (2 g/200 ml hot water) containing about 1,000 ppm of polyphenol concentration and in thereafter diluted. Sucrose at 1% concentration was dissolved in the normal and the diluted brews. After adding drops of bacterial

solution to the test beverage, a cover glass was immersed in the solution and incubated at 37°C for 3 days. The bacterial plaque formed on the surface of the cover glass was observed. All tea beverages (black tea, oolong tea, green tea, and puer tea) at normal concentrations and upto 4 times dilution were found to inhibit plaque formation(Fig7).

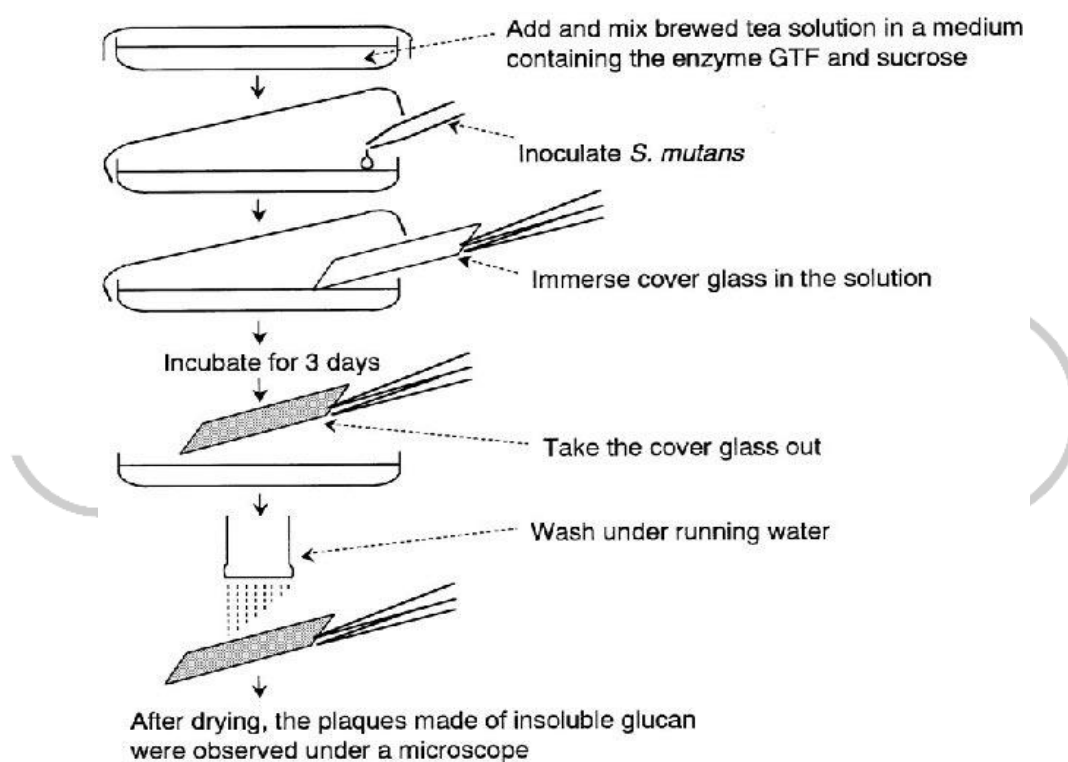


Fig7:Method for microscopic observation of dental plaques (insoluble glucan).GTF, glucosyltransferase.

Inhibition of the proliferation of *S. Mutans*:

It was also confirmed,that the inhibitory potency of various tea beverages extracted at normal drinking concentrations on the growth of *S. mutans*. The results in Fig. 8 show that green tea is most effective in

suppressing the growth of the bacteria. The separation of black tea infusion into polyphenolic and nonpolyphenolic fractions, which were adjusted to normal drinking concentration, acted on the bacteria as shown in Fig. 10. Polyphenolic fraction

suppressed the growth of *S. mutans*, while the non polyphenolic fraction had no effect.

Strengthening Acid Resistancy of Teeth

It is known that tea contains 300–2000 ppm fluoride of which more than 50% is extracted into the tea infusion. Fluoride exists as fluoro apatite in the enamel of teeth, and it is well known that it strengthens the acid resistancy of the enamel. As a model for tooth decay, hydroxy apatite (HA), which has the same composition, as enamel, $\text{Ca}_5(\text{OH})(\text{PO}_4)_3$, was used; and the acidic environment which is produced by lactic acid in the mouth was replaced by acetate buffer (pH 4) in this model. Using the above model, Hayakawa et al. investigated acid resistancy of HA with various components. First, the uptake of fluoride was measured by shaking 100 mg of HA with 2 ml of tea extract or NaF (sodium fluoride) solution for 10 min at 37°C. As long as the concentration of fluoride in the tea solution was the same as that of the NaF solution, the rate of fluoride uptake to HA was the same, as shown in Fig. 5. This shows that fluoride absorption to the teeth will occur not only from NaF (fluoride solution), but also from tea infusion. Next,

acid resistance was determined by adding 10 ml of acetate buffer (pH 4.0) and measuring the phosphate extracted into the solution from the HA. In tooth decay, the dissimilation of phosphate occurs simultaneously with that of calcium, so either phosphate or calcium may be measured to determine the acid resistancy of the solution, but analysis of the former is considerably easier. Acid Resistancy = (Amount of Phosphate in the Control/Amount of Phosphate in the test solution)

Even though the fluoride uptake in tea solution is the same as in NaF solution, acid resistance by tea solution is much stronger (Fig. 12). In the same way aluminum ions in tea as well as polyphenolic components were found to increase the acid resistance. Conclusively, 50g of fluoride and 100g of aluminum ion was added to 2 ml of buffer solution with various amounts of tea polyphenols and acid resistancy was measured as in (Fig. 13). From this graph, it could be said that the combination of tea polyphenols, fluoride, and aluminum ions (these three elements being the most abundant in tea) will increase acid resistancy of tooth enamel.

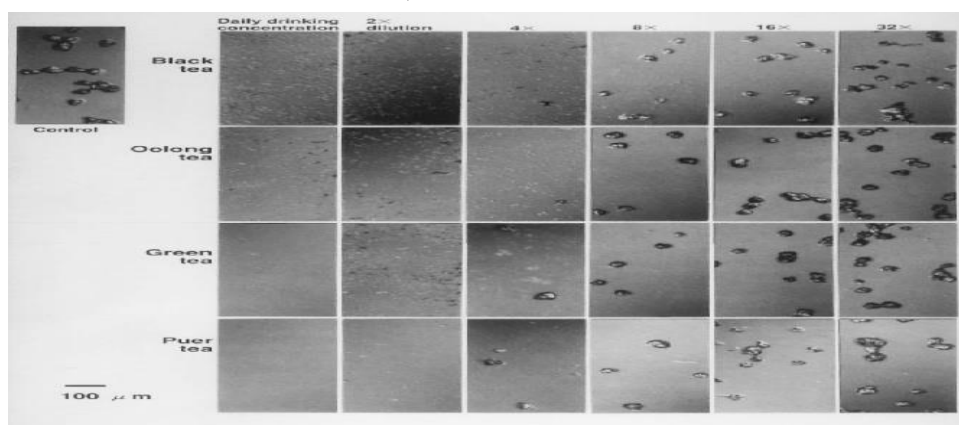


Fig 8: Anti-dental plaque effect of tea beverages.

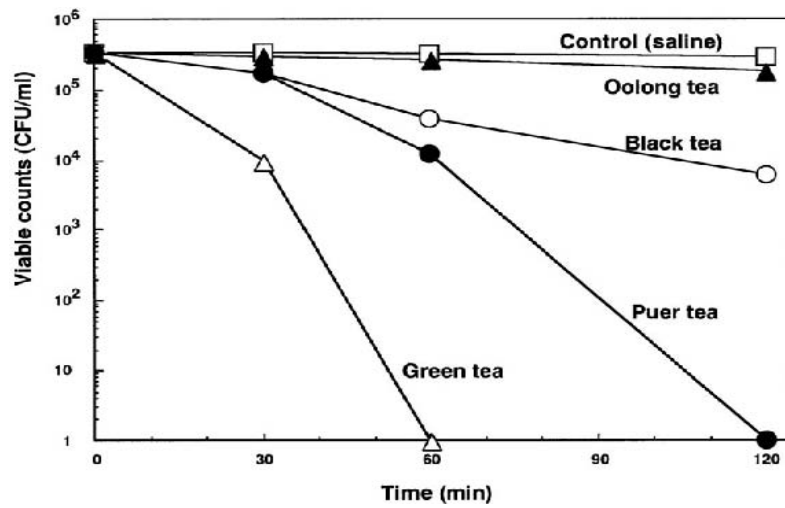


Fig 9:Antibacterial effect of tea beverages against cariogenic bacterium *S. Mutans*

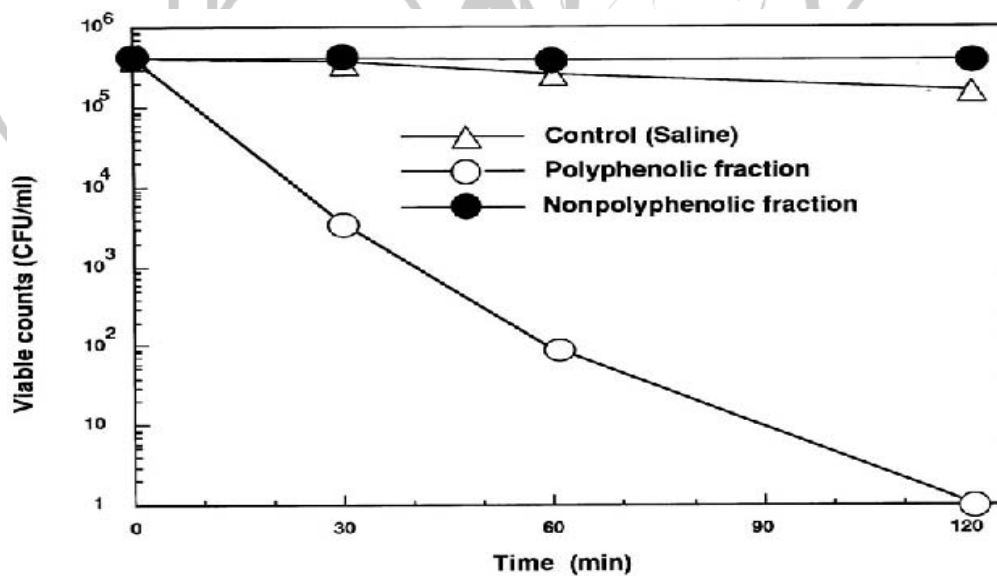


Fig 10:Antibacterial effect of black tea fractions against cariogenic bacterium *S. Mutans*

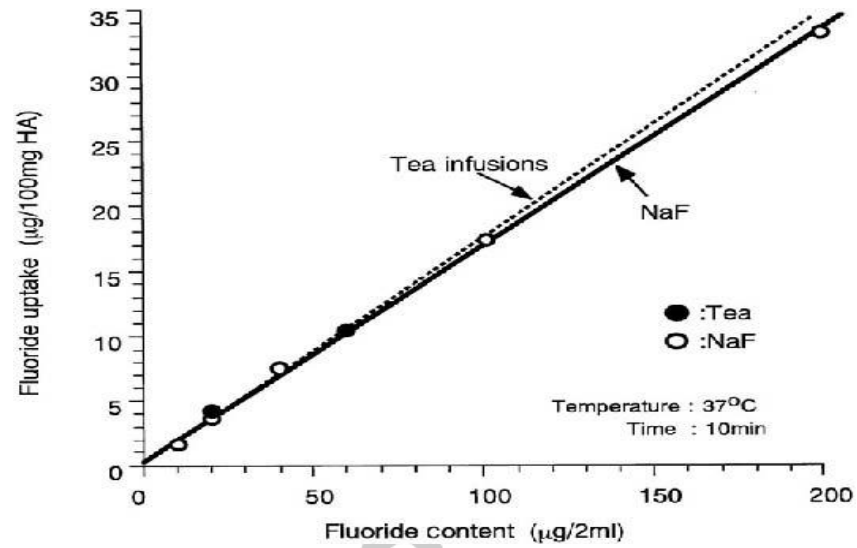


Fig11: Rate of fluoride uptake to HA (hydroxyapatite).

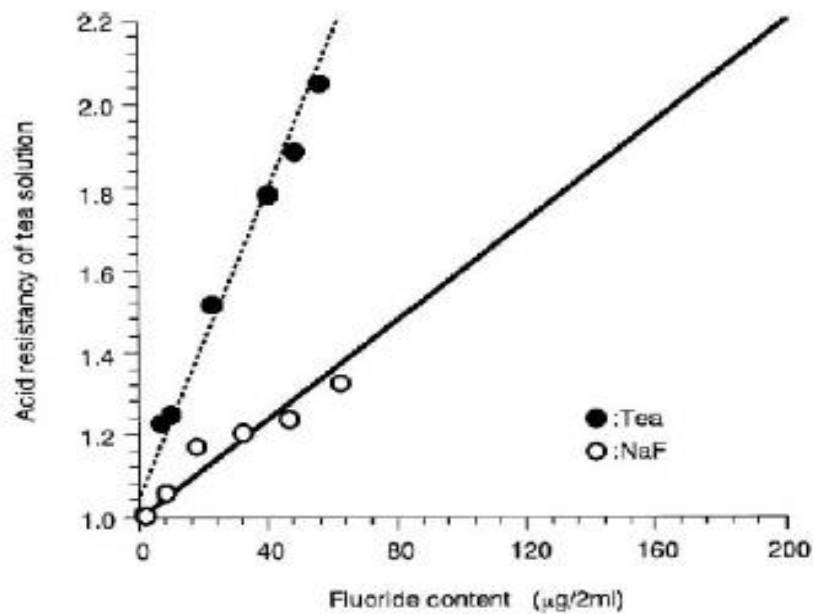


Fig12: Acid resistivity of hydroxyapatite treated with NaF and tea.

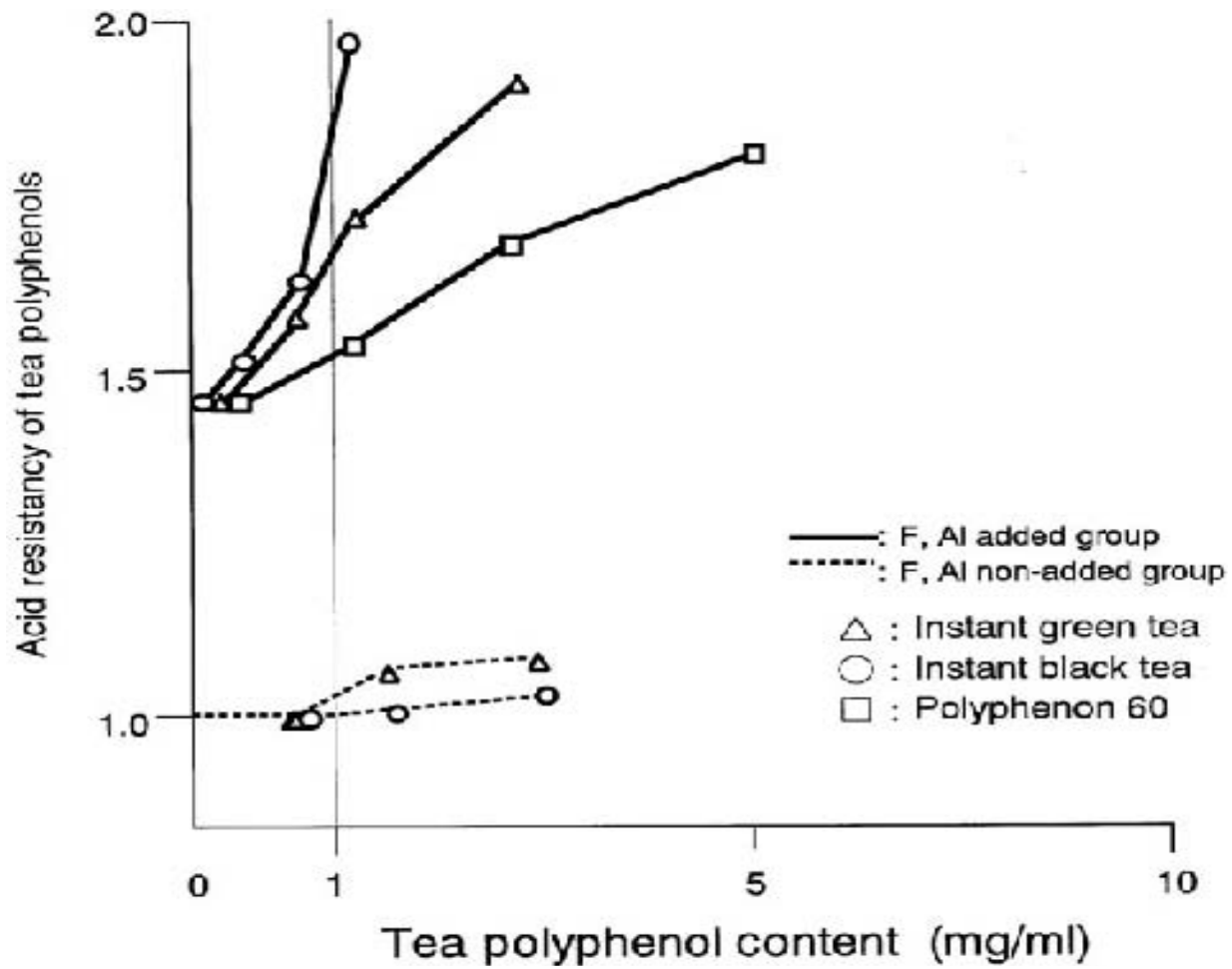


Fig 13: Relationship between the polyphenol concentration and acid resistivity.

CONCLUSION

Finally, in today's world, humans are bombarded daily with high sugar food and drinks. From soda pop to brownies, humans consume more sugar than our bodies want or need. Just one side effect of such consumption is unhealthy teeth. With our teeth being such a prominent aspect of our physical appearance, it is no surprise that oral cleanliness is important. Humans have many ways of keeping teeth looking bright and healthy. Brushing and flossing is common, along with more extreme methods of teeth whitening and bleaching. However, drinking tea is another method that could be employed by humans to prevent teeth decay. Studies have shown that countries in which tea drinking is widespread, such as India, Japan, and China, there is a lower incidence of dental problems. Many major toothpaste

companies have taken the positive effects of green tea into consideration, and products such as green tea infused toothpaste are now on the market, for example the Crest toothpaste seen in figure 14. Additional research is needed to determine the exact methods employed by green and black tea extracts to break down and inhibit the activity of *S. mutans*, *S. sobrinus*, etc. One exciting future research prospect is the ability of *S. mutans* to produce certain genes specific to this species. If these genes could be identified and examined more closely, it is possible that antibodies could be created to target this specifically. This could lead to treatments and prevention of bacterial oral disease that are more effective and less time consuming than brushing and flossing.



Fig 14: Example of Green Tea extracts in Toothpaste

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