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PREPARATION OF FLAVOURED KOMBUCHA TEA USING SCOBY CULTURE

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***Abstract*:** Kombucha is a low alcoholic beverage with high content of bioactive compounds derived from plant material (tea, juices,) and metabolic activity of microorganisms (acetic acid bacteria, lactic acid bacteria and yeasts). Kombucha may be a yeast and bacterially fermented tea that's often described as having an acetic, fruity and sour flavor. Flavor kombucha tea was prepared. Orange Flavor were used for preparing kombucha tea. Flavor kombucha tea was prepared with two different concentrations i.e.,1% conc., 0.1% conc. Biochemical analysis of kombucha tea was performed. TSS of kombucha tea was done. The TSS of flavor kombucha tea was found to be in a range of 3-4 °Brix. The pH of flavor kombucha tea ranges from 3 to 4.8. Scoby culture was used for fermentation of kombucha tea. Biochemical and phytochemical analysis of different flavor kombucha tea was done. The main antioxidants in tea are polyphenols, the consumption of which is proven to be beneficial for human health, e.g., protecting from reactive oxygen species (ROS).

Keyword: kombucha tea, Scoby culture, phytochemical analysis and Biochemical analysis, green tea, antioxidant, polyphenols, sensory value.

I. INTRODUCTION

1.1 Kombucha Tea

Kombucha Tea's actual origins are unknown in the history of kombucha tea, while Manchuria is frequently considered as a potential location of origin. It could have begun as early as 200 years ago or as far back as 2,000 years. The drink is said to have been drank in east Russia as early as 1900, and then spread throughout Europe. Its popularity grew in the early twenty-first century in the United States. Kombucha is not a federally regulated beverage in the United States because it contains less than 0.5 percent alcohol. Prior to 2015, it was discovered that certain commercially available kombucha varieties included alcohol levels that exceeded this limit, prompting the creation of new testing methods. In the early twenty-first century, as kombucha became more popular in industrialized countries, it was promoted as a healthier alternative to beer and other alcoholic beverages in restaurants and pubs (Jayabalan et al., 2014).

According to new research, microbial fermented tea, which has become a popular drink in various European and American countries, is gaining popularity. Despite the fact that kombucha, a natural microbial fermentation product, has a long history in China, scientific research on it has only recently begun. Kombucha tea is traditionally made with a tea leaf brew, sugar, and living microbes and their metabolites (Blanc, 1996). The kombucha tea beverage is comparable to apple cider in that it is effervescent, acidic, and refreshing. The kombucha fermentation strain is made up of various combinations of acetic acid bacteria, yeast, and lactic acid bacteria. In the kombucha drink, however, yeast (Brettanomyces, Zygosaccharomyces, Saccharomyces, and Pichia)and acetic acid bacteria (Acetobacter xylinum) form a mutually beneficial symbiotic relationship. Acetic acid bacteria cannot or only consume a little amount of sugar at the start of the kombucha fermentation process. The yeast, on the opposite hand, will breakdown sucrose into glucose and fructose before continuing

to ferment and make ethanol (Sievers et al., 1996). control and a lot more green tea consumers also have a lower incidence of prostate, breast, and colon cancers, according to studies. (Kurahashi and colleagues, 2003).

1.2 Kombucha is high in antioxidants

Antioxidants are compounds that protect cells from free radicals, which are reactive molecules that can cause cell damage. Antioxidants found in foods and beverages, consistent with several scientists, are better for your health than antioxidant pills. Kombucha appears to possess antioxidant properties within the liver, especially when brewed with tea. Drinking kombucha on a regular basis decreases liver damage caused by toxic substances by at least 70% in rats, according to research. Although there are no human studies on the subject, it appears to be a potential field of research for persons with liver illness (Murugesan et al., 2003).

1.3 Kombucha can kill harmful microorganisms

Acetic acid, which is plentiful in vinegar, is one among the key chemicals created during the fermentation of kombucha. Acetic acid, just like the polyphenols in tea, has the power to kill a good range of probably hazardous microbes. Kombucha brewed from black or tea appears to possess antibacterial qualities, especially against microbes that cause infections and Candida yeasts. These antimicrobial effects stop dangerous bacteria and yeasts from growing, but they have no effect on the healthy, probiotic bacteria and yeasts found in kombucha fermentation. The significance of these antibacterial properties in terms of human health is unknown (Perronet al., 1999).

1.4 Kombucha may reduce heart disease risk

Heart disease is that the biggest explanation for death worldwide. Rat studies show that kombucha can considerably improve two heart disease markers, "bad" LDL and "good" HDL cholesterol, in as little as 30 days. Tea (especially green tea) also helps to prevent LDL cholesterol particles from oxidation, which is thought to play a role in heart disease. Green tea drinkers, for example, had a 31% lower risk of heart disease, which may also apply to kombucha consumers (Shimazuet al., 1998).

1.5 Kombucha may help protect against cancer

Cancer is among the most frequent cause of death around the world. It's characterized by uncontrolled cell proliferation and mutation. Kombucha's high concentration of tea polyphenols and antioxidants inhibited the development and spread of malignant cells in test tubes. Tea polyphenols' anti-cancer potential is unknown. Polyphenols, on the other hand, are thought to limit cancer cell proliferation and point mutations while boosting cancer cell death. As a result, tea drinkers are less likely to develop cancer (Huxley et al., 1999).

II. MATERIALS AND METHODS

2.1 Sample Collection:

The SCOBY were ordered from AMAZON.IN The kombucha was supplied in 400 mL Plastic bags and kept refrigerated at 4◦C, which is how they are sold commercially.

2.2. Physiochemical Analysis:

2.1.1 Requirements:

Instruments	Glass wares	Chemicals	Other requirements
1. Incubator 2. Autoclave 3. Weighing balance 4. Laminar air flow 5. Refrigerator 6. pH meter 7. Colony counter 8. Refractometer	1. Screwcap bottles 2. Measuring cylinder 3. Conical flasks 4. Beakers 5. Glass bottles 6. Test tube 7. Pipettes 8. Petri plates 9. Micro pipettes 10. Spreader 11. Glass jar	1. Wagner's Reagent 2. Chloroform 3. Sulfuric acid 4. Ferric chloride 5. YGC Agar 6. MRS Agar 7. Macconkey Agar 8. 70% Alcohol 9. Phenolphthalein indicator 10. Sodium Hydroxide 11. Hydrochloric Acid	1. Distilled water 2. SCOBY Culture 3. Green tea 4. Sugar 5. Muslin cloth 6. Orange 7. Whatman no.1 filter paper 8. Cutter 9. Peeler 10. Utensils

Table No.1: Requirements

2.2 Kombucha Production:

Take 1 litter filter water was taken and boiled and add 25gm of sugar and dissolve it in water then gas Turn off and green tea bags were placed in water (4-5 tea bags in 1 lit. water). Kept it at room temperature for cooling. Tea bags were removed from the water and discarded. After that tea was filtered and green tea was prepared. filtered green tea in a bottle and added 1 teaspoon starter tea in green tea was added and then mixed properly. SCOBY Culture layer was added in green tea and covered bottle with a muslin cloth. kept the bottle at room temperature for fermentation (the fermentation period is 10-12 days). after fermentation, the Coculture was removed and fermented kombucha tea was filtered. kombucha tea was filled in a bottle and stored in a bottle at 4℃ temperatures.

2.2.1 preparation of flavor kombucha tea:

Prepare tea with two different concentrations 1% and 0.1% taken 500ml filtered water and boiled then 12.5g sugar was added and dissolved in water. For 1% - 5gm orange cubes added in water. for 0.1% - 0.5gm orange cubes added in water. then gas was Turn off the gas and place green tea bags in water (4-5 tea bags in water). kept it at room temperature for cooling. tea bags from removed from water and discarded. tea was filtered and green tea was prepared. filtered green tea was filled in bottle and 1 tea spoon starter tea was added in green tea and mix properly. SCOBY culture layer was added in green tea and bottle covered with muslin cloth. kept bottle at room temperature for fermentation (fermentation period 10-12 days). after fermentation the SCOBY culture was removed and fermented kombucha tea was filtered. kombucha tea was filled in bottle

and stored in bottle and stored in bottle at 4°c temperature.

2.3 Physicochemical analysis:

2.3.1 Total Soluble Solids (TSS):

TSS of all samples was recorded by using hand Refractometer having a range of 3-4.5, 0 Brix. Normally 12.5gm of sugar was added in kombucha tea for fermentation. The Refractometer was taken by placing the prepared sample of kombucha on the prisom.

2.3.2 pH:

pH of the sample was recorded by using refractometer. pH of flavor kombucha tea was different. Normal changes find in flavor kombucha tea. While kombucha is fermenting, the pH continues to lower. The target pH of kombucha is right around 3.0. In this kombucha generally ranges from 2.95 to 3.15.

2.3.3 Total Titrable Acidity.

Acidity was determined by taking known quantity of prepared sample homogenized in the distilled water and filtered known quantity of aliquot was transferred in a conical flask and titrated against 0.1N standard NaoH solution using few drops of 1% phenolphthalein solution was indicator. The total acidity was then calculated in turns of acidity percentage as citric acid (W/V)

Calculation*:*

$$\text{Total acidity as citric acid\% (W/V)} = \frac{T \times N \times V1 \times E}{V2 \times W \times 100} \times 100$$

2.4 Phytochemical analysis:

2.4.1 Saponin test:

Saponins compounds were detected by using the 2.5 ml of the tea was added to 10 ml sterile distilled water in the test tube, then shaken vigorously about 30 second, then allow to stand half an hour.

2.4.2 Tannins test:

To 0.5 ml of test solution, l ml of water and 1-2 drops of 0.1% ferric chloride solution was added. Occurrence of a blue-black, green or blue green precipitate indicates the presence of tannins.

2.4.3 Terpenoid test:

Each extract was dissolved in chloroform, then 3 ml of concentrated sulfuric acid was added carefully and examined reddish brown coloration indicates the presence of terpenoid.

2.4.4 Alkaloids test:

To the 2-3ml of filtrate, 1ml of dil. HCl and a few drops of Wagner's reagent was added and shake well. Formation of reddish-brown precipitate showed the presence of alkaloids.

2.5 Microbial analysis:

Microbial analysis included the total number of bacteria (broth with 2% of agar, temp. 30°C, 48–72 h); mold and yeast (YGC medium, temp. 20°C, 120 h); lactic acid bacteria (MRS-agar medium, 37°C, 48–72 h); and Escherichia coli (MacConkey medium, temp. 37°C, 24–48 h). The microorganism number was presented as an arithmetic mean of the total colony-forming units [CFU] in 1 g of the product. 1 ml of the sample of Flavored kombucha tea was taken in a sterile pipette and transferred it to the first true of diluents 9 ml. rotate the test tube between palms of the hand to complete the mixing. This makes a dilution of 1:10 Similarly, a series of dilution was prepared by transferring 10g of the first dilution 1:1 into another 9ml dilution blank to get 1:100 dilution and so on. Incubate the all-Petri plate in inverted position at 37°C for 48 h. Plates was removed after 48 hrs. number of colonies counted with the help of a colony counter and determine the average of the counts in the two plates and multiply this the dilution factor.

Formula:

Cfu/ml = (`no. of colonies x dilution factor) / volume of culture plate

2.6 Organoleptic evaluation:

The different flavor kombucha tea with normal flavor as a reference check was serving for sensory evaluation. The average value of score given by of 6 professors. The kombucha with score range of 2 to 3 out of 10 was regarded as commercially acceptable wine, those with a score range of 4 to 6 out of 10 as standard wine and with a score range above 7 to 10 out of 10 were regarded as superior quality wine as per the rating given by Ough and Baker (1961). The kombucha with score below 2 were rated as unacceptable.

III. Results

3.1 Total Soluble Solids:

Sr no	Name of Samples	1% Conc.	0.1% Conc.
1	kombucha tea	3°	3°
6	Orange flavored of kombucha tea	3°	3.1°

Table No.2: Total Soluble Solid

3.2 pH:

Sr no	Name of sample	1% Conc.	0.1% Conc.
1	kombucha tea	4.16	4.16
6	Orange flavored of kombucha tea	3.33	3.38

Table No.3: pH

3.3 Total titrable acidity:

Sr no	Name of sample	1% Conc.	0.1% Conc.
1	kombucha tea	0.3	0.3
6	Orange flavored of kombucha tea	0.33	0.33

Table No.4 Total titrable acidity

3.4 Saponin and tannin test:

SR NO	SAMPLE	SAPONIN TEST		TANNIN TEST	
		1% Conc.	0.1% Conc.	1% Conc.	0.1% Conc.
1	Kombucha tea	+	+	_	_
5	Orange flavored kombucha tea	+	+	_	+

Table No.5 Qualitative analysis of Flavored kombucha tea (Saponin test & Tannin test)

3.5 Terpenoids test & Alkaloid test:

SR NO	SAMPLE	TERPENOID TEST		ALKALOID TEST	
		1% Conc.	0.1% Conc.	1% Conc.	0.1% Conc.
1	Kombucha tea	_	_	+	+
5	Orange flavored kombucha tea	+	+	+	+

Table No.6 Qualitative analysis of Flavored kombucha tea (Terpenoids test & Alkaloid test

3.6 Microbial Analysis:

The results of microbiological analysis showed that acetic acid bacteria and yeast are the dominant ones in the kombucha. Standard acetic acid bacteria work well with yeast in the media, and tolerate the alcohol produced, which usually inhibits bacteria growth (McDonnell and Russell, 1999).

SR No.	Sample	YGC Agar	MacConkey's Agar	MRS Agar
1	Kombucha Tea	2.1×10^5	4.9×10^7	2.5×10^6
2	Orange Flavor Kombucha Tea	2.9×10^5	3.8×10^6	00

Table No.7: Microbial analysis

3.7 Organoleptic Evaluation:

Sr No.	SAMPLE	FLAVOUR		COLOUR		TASTE		OVERALL ACCEPTABLITY
		1%	0.1%	1%	0.1%	1%	0.1%	
1	Kombucha tea	7	6.5	6.5	6.7	6.9	6.5	6.6
4	Orange flavoured kombucha tea	6	6.5	6.5	8	7	7	6.8

Table No.8: Score Card for Sensory Evaluation of Flavored Kombucha Tea

IV. CONCLUSION:

Kombucha has a diverse microbial community that is rich in organic acid-producing bacteria, establishing the groundwork for a wide range of health benefits. The dominance of yeast and bacterial strains, as well as their interactions, are critical for bioavailability in a healthy human gut. In comparison to other fermented health foods, kombucha microorganisms are more efficient in metabolising carbohydrates and producing important metabolites. More research is needed to determine how differences in microbial diversity, brewing procedure, fermentation period, and fruits in a secondary infusion affect the quality of kombucha and its health effects.

Home-scale fermentation of sugar-dissolved black tea by a SCOBY is traditionally used to make kombucha. For a long time, people in various regions of the world have claimed that the fermented beverage has numerous health-promoting and antibacterial benefits. Because of its long history of use as a health-promoting beverage around the world, kombucha is always being researched for its intrinsic characteristics, which has confirmed a number of commonly held beliefs. Antiinflammatory and anticancer capabilities, as well as antihypertensive, antidiabetic, hepatoprotective, antibacterial, and other therapeutic and health-promoting properties of kombucha beverage, have all been studied and scientifically proven.

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