



**SYNERGISTIC EFFECT OF *SACCHAROMYCES CEREVISIAE* AND  
*ACETOBACTER XYLINUM* IN THE PRODUCTION ALCOHOLIC  
 BEVERAGES USING TEA POWDER AND SUGAR AS SUBSTRATES**

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**Abstract**

The objective of these studies was to determine the synergistic fermentation using *Saccharomyces cerevisiae* and *Acetobacter xylinum* in the production of alcoholic beverage using tea powder and sugar as substrates for producing high nutritional as well as economic therapeutic valued beverage. The present study was initiated to produce fermented beverage of satisfactory quality, by following several processing conditions. Among the different tea brands viz., brand I (Assam Tea), brand II (Darjeeling Tea), brand III (Nilgiri Tea) were tested for beverage preparation. Brand I yields the maximum amount of the alcohol. Over all acceptability of the brand-I is satisfactory comparing to the remaining two brands. It tastes sweet, mildly alcoholic and slightly acidic. The acidic compound was confirmed to be acetic acid. The beverage stored at refrigerated temperature is fit for the consumption up to 3 months. It also showed good antibacterial activity against gram negative and gram positive bacteria.

**Keywords:** *Acetobacter xylinum*, *Saccharomyces cerevisiae*, Tea, Alcoholic beverages.

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**Introduction**

The second most consumed drink after water is tea compared to coffee, beer, wine and soft drinks<sup>1</sup>. Black tea serve as a good fermentation media which is containing amino acids, proteins, volatile compounds, lipids, polyphenols and enzymes<sup>2</sup> and fermented tea is believed to be containing vitamin A, B- complex and C making it a nutritious and therapeutic agent<sup>3, 4</sup>. Fermentation of tea leads to

addition of taste; flavor and health components such as “Kombu- cha” have been prepared by co-fermentation with yeast and acetic acid bacteria and are known to have health benefits<sup>5,6</sup>. *Saccharomyces cerevisiae* is an industrially important microorganism plays important role in converting carbohydrate sources into alcohol. In the next step alcohol is converted into acetic acid

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by *Acetobacter xylinum*. The medical reports are apprehensive about tea, because of its tannin and caffeine contents. It is said to increase life span, vitality and sex life and prevent cancer, arthritis, heart diseases, blood pressure and skin diseases. However, Fermentation of tea by yeast resulted in reduction of caffeine and excess tannins in significant amounts. After fermentation, the amount of theophylline was increased to make fermented tea a potent bronchodilator<sup>7</sup>. The beverage preparation involves addition of tea leaves/powder as the flavoring agents in order to get characteristic flavor and possible therapeutic benefits. The potential value of tea has been well recognized in Russia but has received scant attention in India. Thus, additional benefits of adding tea powder to beverage need to be explored. Beverage is said to increase life span, vitality and prevent cancer, arthritis, heart diseases, blood pressure and skin diseases. Thus longitudinal studies on different sections of society based on the medicinal values of beverage may be initiated. Little or no attention has been given to the antibiotic effects of tea fungus elsewhere in the world except Soviet Union<sup>8</sup>. The antimicrobial components produced during the fermentation process have shown inhibitory effects against several pathogenic bacteria<sup>9</sup>.

The objective of these studies was to determine the synergistic fermentation using *Acetobacter xylinum* and *Saccharomyces cerevisiae* in the production of alcoholic beverages by using tea powder and sugar as a substrate for developing high nutritional as well as economic therapeutic value beverage.

### Materials and Methods

Pure culture of *Acetobacter xylinum* was obtained from Administrative Management College, Bangalore. *Saccharomyces cerevisiae* was purchased from local market as yeast granules. Three varieties of tea powder; Assam Tea, Darjeeling Tea and Nilgiri Tea were collected from the respective tea garden and sugar was purchased from local general stores.

### Analysis of tea decoction before and after fermentation

In the study of physical properties of tea decoction different parameters like color; pH, odor, taste, specific gravity and density and chemical properties like alcohol content and carbohydrate

content were analyzed. Specific gravity method and Anthrone method were used to estimate alcohol and carbohydrate respectively<sup>1</sup>.

### Starter culture

Starter cultures were prepared in tea decoction. 25ml of the tea decoction was inoculated with *A. xylinum* and *S. cerevisiae* separately and were added to 2 liters of tea decoction, which was prepared freshly and cooled.

### Fermented beverage preparation

About 300 grams of sugar was added to two liters sterile water. 10 gms of tea powder was added and simmered for five minutes on lower flame. The hot decoction was allowed to cool in a 5 liter capacity fermenter. Starter cultures were added and incubated at room temperature for 12 days. After 12 days liquid was decanted into another vessel. The vessel containing the decanted tea decoction was heated in a vessel containing boiling water for about 15 minutes and the beverage was bottled hot in juice bottles and stored for regular drinking usage.

### Shelf life studies

For the purpose of shelf life studies, the sauce bottles, hot filled with pasteurized beverage with tight lid were aseptically sealed with candle wax. The samples were allowed to cool for an hour at the room temperature and subsequently stored at refrigerated temperature (8 to 10<sup>0</sup>C) and at the ambient temperature. The beverage samples were evaluated for pH, alcohol, sensory attributes and microbiological quality for a period of 3 months in 15 days interval.

### Antimicrobial activity of beverage

Lawn cultures of different pathogens (*Staphylococcus aureus*, *Salmonella typhimurium*, *Pseudomonas aeruginosa* and *Escherichia coli*) were done on Muller Hinton agar medium. Paper discs of 6mm in diameter were dipped in the beverage and kept in the center of the culture. The plates were incubated at 32<sup>0</sup>C for 24 hours. After 24 hours the zone of inhibition was measured.

### Results

The results of tea decoction before fermentation and after fermentation were tabulated in Table 1 and 2 respectively.

**Table No. 01: The physicochemical properties of tea decoction before fermentation**

Parameters	Assam Tea	Darjeeling Tea	Nilgiri Tea
Colour	Dark brown	Dark brown	Dark brown
pH	6.3	6.3	6.0
Specific gravity	1.02	1.02	1.02
Density	1.05	1.05	1.05
Carbohydrate	70 mg/ml	66 mg/ml	68mg/ml

**Table No. 02: The physicochemical parameters of fermented beverage**

Parameters	Assam Tea	Darjeeling Tea	Nilgiri Tea
pH	3.2	2.8	2.9
Density	1.02	1.02	1.02
Carbohydrate	22 mg/ml	26 mg/ml	29 mg/ml
Alcohol content	4.12%	4.08%	4.09%
Titrateable acidity	2.18%	2.06%	2.11%

Among the different tea brands viz., brand I (Assam Tea), brand II (Darjeeling Tea), brand III (Nilgiri Tea) were tested for beverage preparation. Brand I yields the maximum amount of the alcohol. Over all acceptability of the brand-I is satisfactory comparing to the remaining two brands. The

beverage is mildly alcoholic and acidic. It tastes sweet, alcoholic and slightly acidic. The acidic compound was found to be acetic acid. Finally the antimicrobial activity of beverage was carried out and showed in Table 3. The effect of beverage was more on *Escherichia coli* than other bacteria.

**Table No. 03: Results of Antimicrobial activity of beverage**

Organisms	Zone of inhibition
<i>Staphylococcus aureus</i>	7 mm
<i>Salmonella typhimurium</i>	8 mm
<i>Pseudomonas aeruginosa</i>	8 mm
<i>Escherichia. coli</i>	9 mm

Shelf life studies were done by considering the parameters such as percentage of alcohol and pH. Microbiological examination like direct microscopic observations and plating of the sample were done, for every 15 days interval of time.

### Discussion

The present study was initiated to develop fermented beverage of satisfactory quality, by following several processing conditions. The pharmacological action of caffeine in tea is so modified probably by its interaction with polyphenols, that the beneficial properties of the alkaloid may be enjoyed without harmful side effects when used in moderate amounts. The beneficial effects of tea polyphenols acting synergistically with ascorbic acid include the strengthening of capillary blood vessels and anti atherosclerotic action. Similarly, catechin present in tea, particularly epigallocatechingalate, posses' antimicrobial and anti-cancer property. Beverage being a fermented said to supplement diet with substantial quantities of B-Complex vitamins,

essential amino acids, calories, enzymes and other contents. The World Health Organization Technical Committee on Cardiovascular Disease asserted that the relationship between moderate alcohol consumption and reduced death from heart disease could no longer be doubted<sup>10</sup>. The moderate alcohol consumption helps to protect against several diseases like diabetes<sup>11</sup>, peptic ulcers<sup>12</sup>, gallbladder<sup>13</sup>, and other diseases like Alzheimer's disease<sup>14</sup>, angina pectoris<sup>15</sup>, and peripheral artery disease<sup>16</sup>. The production of alcohol from tea and sugar as a substrate yields good quality of alcohol were of good quality, taste and odor above all it is economically cheap. After the fermentation the beverage that is stored at refrigerated temperature will not alter the any parameters such as pH, titrateable acidity, and variation in alcohol content. Mishandling may lead to the contamination and change in the product. The beverage stored at refrigerated temperature is fit for the consumption up to 3 months. The beverage that is stored at the ambient temperature alters the parameters quickly. This is due to the production of acid by bacteria.

Change in pH, titratable acidity and decrease in alcohol content was observed. Beverage stored at ambient temperature is unfit for consumption after 15 to 30 days due to accumulation of acid. Microbiological analysis revealed no contamination during the shelf life studies. It also showed good antibacterial activity against gram negative and gram positive bacteria.

### Conclusion

The present study of fermentation to determine the parameters for industrial scale production of alcoholic beverage from tea and sugar as the pilot scale study gave the good result.

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