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PREPARATION OF ROSEMARY KOMBUCHA BLENDED WITH ORANGE PEEL

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ABSTRACT

The current research work involves the analysis of a practical kombucha drink formulated with dried rosemary leaves and orange peel preferred powder. Kombucha, a traditional fermented tea drink, was enhanced with the medicinal properties of rosemary and the orange, antioxidant-rich profile of orange peel. The base kombucha was prepared by fermenting a sweetened black tea infusion using jaggery (ranging from 2% to 10%) and inoculated with a 10% SCOBY (Symbiotic Culture of Bacteria and Yeast). Three standardizations were developed by altering black tea leaves concentration, rosemary dried leaves and jaggery. And the developed product were of Five treatment variations (T_1–T_5) were developed by altering jaggery concentrations while keeping rosemary leaves (5%) and orange peel powder (1.2%) constant in 250 mL of water. The fermentation was carried out under aerobic conditions at ambient temperature ($25 \pm 2°C$) for 14 days. The pH, titratable acidity, total soluble solids, total sugars, protein content, color, and antioxidant activity were measured as physicochemical properties. The first phase involved a proximate analysis of kombucha pH, titratable acidity, total soluble solids, protein. The Bioactive compounds properties are total antioxidant activity, total phenols and flavonoids. Yeast, acetic acid bacteria and molds, microbial analysis was done periodically to maintain safety and shelf stability. Control and T_3 samples were analysed, showing superior contents, in control pH is 2.9, TSS is 3.9 ° brix, titratable acidity is 1.2, protein is 1.5% and the total antioxidant activity is 73.70µg/100g, total phenols are
35.57mg of GAE/100g, flavonoids content as 14.40mg of QE/100g whereas in T_3 pH is 3.1, TSS is 4.5° brix, titratable acidity is 1.35, protein is 1.7 % and the total antioxidant activity is 75.90µg/100g, total phenols are 43.50mg of GAE/100g, flavonoids content as 19.60mg of QE/100g. Sensory evaluation revealed that T_3 (with 6% jaggery) was most acceptable in terms of appearance, flavor, aroma, and overall acceptability. The addition of rosemary and orange peel were found to improve the bioactive properties and functional health benefits of the kombucha substantially, thereby promising the beverage or health-conscious consumers.

Key words: Kombucha, Rosemary, Orange peel

1. INTRODUCTION

In recent years, people worldwide have become more interested in functional and fermented beverages because they can help improve health, especially those with antioxidant, probiotic and antimicrobial properties. Kombucha is an ancient tea drink that is now considered a good choice for health. It is produced by fermenting sweetened tea with a symbiotic culture of bacteria and yeast (SCOBY), resulting in the production of organic acids, vitamins, and bioactive compounds beneficial to human health (Jayabalan *et al.*, 2014). While kombucha is conventionally brewed using black or green tea, recent innovations have explored the incorporation of herbs, fruit peels, and other plant-based ingredients to enhance its functional properties and consumer appeal (Greenwalt *et al.*, 2000).

Because they contain a variety of beneficial chemicals and are considered healthful, rosemary and orange peel are among the most promising of these additives.

Carosic acid, carnosol and rosmarinic acid are present in rosemary which is why this herb is recognised for its antioxidant and antibacterial benefits. These compounds have been widely studied for their ability to neutralize free radicals, inhibit microbial growth, and contribute to overall health promotion (Bozin *et al.*, 2007). Rosemary, when added to kombucha, can increase the number of antioxidants and give the drink an appealing aroma for many people. Orange peel often isn't used as much as it should be because of its valuable nutrients. It contains a variety of bioactive compounds, including vitamin C, flavonoids and essential oils all of which exhibit antioxidant, antiinflammatory, and antimicrobial properties (González-Molina *et al.*, 2010).

Functional drinks including kombucha have become popular recently due to their probiotic content and possible health advantages. Kombucha, traditionally brewed from sweetened tea and fermented using a symbiotic culture of bacteria and yeast (SCOBY), is known for its antioxidant, antimicrobial, and detoxifying properties (Sathishkumar *et al.*, 2014). Vitamins, organic acids, polyphenols and enzymes found in fermented foods help the digestive system and a healthy balance of metabolic processes. The rise in demand for plantbased and naturally healthy beverages is prompting new innovation in this industry. Adding herbs, spices and fruit peels to products improves both their health benefits and appealing tastes. For instance, herbs like rosemary provide antioxidant and antiinflammatory benefits, while citrus byproducts like orange peel add vitamin C, flavonoids, and essential oils, contributing to immune support and flavour enhancement (Moreno *et al.*, 2010; Anackov *et al.*, 2007).

Kombucha is a tea that is fermented, mildly sparkling and sweetened and many believe it has several health benefits. Traditionally brewed using black or green tea and sugar, kombucha is fermented by a symbiotic culture of bacteria and yeast (SCOBY), which metabolizes the sugars into organic acids, gases, and other bioactive compounds (Lončar *et al.*, 2014). Kombucha is made with the help of Acetobacter, Gluconobacter and Saccharomyces microorganisms, along with others. These microbes work synergistically to produce a range of compounds such as acetic acid, gluconic acid, glucuronic acid, ethanol, lactic acid, B-vitamins, polyphenols, and carbon dioxide (Greenwalt *et al.*, 2000). Kombucha's composition and bioactivity are influenced by several factors, such as tea type, sugar concentration, fermentation time, and temperature, as well as the addition of herbs, spices, or fruit extracts. These components can be controlled to improve the nutritional, performance and taste of the final product.(Beaufort *et al.*, 2018).

Rosemary is a Mediterranean herb that has long been used in health care, cooking and is now being added to nutritional products. Its medicinal value is largely attributed to its high content of bioactive compounds, especially phenolic diterpenes, flavonoids, and essential oils (Borra *et al.*, 2011). The majority of rosemary's antioxidant power comes from compounds such as carnosic acid, carnosol and rosmarinic acid. These polyphenols help neutralize free radicals and reduce oxidative stress, thus potentially lowering the risk of chronic diseases such as cancer, cardiovascular disorders, and neurodegenerative conditions (López-Jiménez *et al.*, 2016).

This herb shows anti-inflammatory properties which may be related to its management of prostaglandins and cytokines. Additionally, studies suggest that rosemary extracts may offer neuroprotective benefits, potentially improving memory, cognitive function, and protecting against neurodegenerative diseases like Alzheimer's. These effects are often associated

with the compound rosmarinic acid and its ability to inhibit neuronal damage and oxidative stress (Perry *et al.*, 2003). Some studies have found that rosemary may help with digestion, support the liver, reduce blood sugar and support the heart.

Orange peel has a lot of antioxidants, so it can help decrease oxidative stress and protect against chronic illnesses such as cancer, diabetes and heart diseases. Its high fibre content also supports digestive health by promoting satiety and regulating bowel movements (Bocco *et al.*, 1998). Orange peel's natural substances may function to reduce cholesterol and lower the risk of diabetes and cancer, having been tested both inside and outside living organisms. Because of these attributes, orange peel is perfect for building functional beverages since it brings value nutritionally and by its taste and aroma. If not handled well, the big amount of citrus peel waste from the industry can cause environmental problems.

However, incorporating orange peel into value-added products aligns with the principles of sustainable food production and circular economy, by transforming agro-industrial waste into functional food ingredients (Ajila *et al.*, 2007). Adding orange peel to kombucha is helpful as it lower waste, creates less impact on the environment, offers more health benefits, adds flavour and aroma and makes the product more acceptable.

Another additive is orange peel, a nutrient-rich by-product of the citrus industry. Often discarded as waste, orange peel contains dietary fibre , vitamin C, essential oils (especially limonene), and flavonoids such as hesperidin, which exhibit powerful antioxidant and antimicrobial effects (González-Molina *et al.*, 2010). The utilization of orange peel not only boosts the functional quality of food products but also contributes to waste reduction and sustainability, aligning with the principles of the circular economy (Verma *et al.*, 2007).

2. MATERIALS AND METHODS

2.1 Microbial cultures:

Microbial Cultures of Black tea leaves: Despite undergoing withering, rolling, fermentation, and drying during processing, black tea leaves may still harbor trace amounts of microorganisms, particularly those introduced from the environment during harvesting and handling. The most sporadic microbial species found in the dried black tea is spore-forming bacteria

- Bacteria such as *Bacillus subtilis*, *Bacillus cereus*, *Micrococcus luteus* and *Pseudomonas fluorescens.*
- Yeasts such as *Saccharomyces cerevisiae*, *Candida spp*.
- Molds such as *Aspergillus niger*, *Penicillium chrysogenum*.

And other possible microorganisms which in small quantities may grow especially when the tea is stacked in humid or filthy environments.

Microbial Cultures of Dried Rosemary Leaves: Rosemary is known for its strong antimicrobial properties, dried rosemary leaves may still harbor naturally occurring microbial populations, especially if not sterilized. Those are common microorganisms that most likely will be on dried rosemary.

- Bacteria such as *Bacillus subtilis*, *Micrococcus luteus*, *Pseudomonas spp*.
- Yeasts (Non filamentous fungi) such as *Saccharomyces spp*., *Candida spp.*
- Molds such as *Aspergillus* and *Penicillium spp*.

Microbial Cultures of Orange Peel: Orange peels, being agricultural byproducts, are natural carriers

of diverse microbial communities. These microorganisms primarily originate from the fruit's surface (phyllosphere), soil, and post-harvest handling.

- Bacteria such as *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Micrococcus luteus*, and *Staphylococcus epidermidis*.

- Yeasts such as *Candida spp.*, and *Saccharomyces cerevisiae*.

- Molds such as *Aspergillus niger*, *Penicillium spp.*, and *Fusarium spp*.

Microbial Cultures of SCOBY: The SCOBY (Symbiotic Culture of Bacteria and Yeast) is microbial consortium responsible for kombucha fermentation. It consists of a cellulose-based biofilm that harbors a dynamic population of acetic acid bacteria (AAB) and various yeast species in symbiotic association. The most common bacterial genera identified in SCOBY include *Acetobacter*.

- Bacteria such as *Acetobacter xylinum*, *Gluconobacter*, and *Komagataeibacter*, which are primarily responsible for oxidizing ethanol into acetic acid and producing the characteristic sour taste and biofilm (cellulose mat).

- Yeast side, typical species include *Saccharomyces cerevisiae*, *Zygosaccharomyces bailii*, *Brettanomyces bruxellensis*, and *Candida stellata*. Yeasts break down sugars (like sucrose and glucose) into ethanol and carbon dioxide, which are then used by the bacteria, completing the mutualistic cycle.

2.2 Microbial Composition of SCOBY in Kombucha

Microorganism Group	**Common Species**	**Percentage**
Yeasts	*Saccharomyces cerevisiae*, *Zygosaccharomyces bailii*, *Brettanomyces spp.*, *Candida spp.*	40–60%
Acetic Acid Bacteria	*Gluconacetobacter xylinus*, *Acetobacter aceti*, *Komagataeibacter europaeus*	30–50%
Lactic Acid Bacteria	*Lactobacillus spp.* (variable, not always present)	5-10%
Other Microorganisms	Wild microbes from environment or ingredients	<5% (trace)

2.3 Preparation methods of Kombucha

The preparation of kombucha involves a two-phase process: the creation of a sweetened tea base followed by fermentation using a symbiotic culture of bacteria and yeast (SCOBY). The process occurred

under sanitary laboratory facilities so as to preserve the microbial integrity of the culture.

2.4 Preparation of Tea Base

To ferment the kombucha, a liter of cold distilled water was first boiled until all the water was in a rolling boil. After boiling, 2 grams of black tea leaves were put and left to simmer between 5 to 10 minutes. This was essential in organized elimination of polyphenolic compounds, tannins, and flavouring agents which are essential in kombucha fermentation process. Following the infusion, 80 to 100 grams of jaggery (as an unrefined natural sugar) were added to the hot tea and stirred thoroughly to dissolve. The sugar served as the primary carbon and energy source for the symbiotic culture of bacteria and yeast (SCOBY). The sweetened tea was then filtered using clean muslin cloth to remove solid residues and was allowed to cool naturally to room temperature (25–28°C). This is a significant cooling procedure to prevent heat effects on the SCOBY and in maintaining the microbial culture in a truly viable state prior to propagation.

2.5 Inoculation with Starter Culture

The sweetened tea was filtered through sterile muslin cloth so as to extract the finer particles of tea and to clarify the medium and then cooled at the ambient temperature. The solution was poured into a container and 100 ml of previously fermented kombucha was added to begin fermentation. This acidic starter not only stabilizes the initial pH (to ~4.5 or lower) but also creates a difficult environment for potential contaminants. Subsequently, a SCOBY (Symbiotic Culture of Bacteria and Yeast), measuring approximately 5–10 mm in thickness, was carefully placed on the surface of the tea. The SCOBY provides the essential microbial consortium - primarily acetic acid bacteria (e.g., Acetobacter xylinum) and yeast species (e.g., Saccharomyces cerevisiae) - required for kombucha fermentation. The inoculation was now blended with the mixture, which would be incubated at a controlled environment.

2.6 Fermentation Process

The inoculated tea was kept in fermentation jars and covered with clean and stretched muslin cloth of two layers and sealed by rubber bands. This furnishes a similar degree of aerobic conditions sufficient to preserve bacteria of acetic acid, but excludes any admission of dust and insects, and other pollution. The jars were then incubated at ambient temperature (25–30°C) in a dark, undisturbed environment for a period of 7 to 14 days, depending on ambient conditions and the targeted level of acidity and flavor development. During yeast fermentation, the yeast organisms such as *Saccharomyces cerevisiae* and *Zygosaccharomyces bailii* broke down sucrose in the medium into fructose and glucose and subsequently obtained ethanol and carbon dioxide. This ethanol created was utilized by the acetic acids bacteria which are largely classified as Bacteria and genus Acetobacter among others including bacteria *Acetobacter xylinum* and *Gluconobacter oxydans* to oxidize the ethanol and produce acetic acid, gluconic acid and glucuronic acid. This is a biochemical reaction that resulted in the gradual lowering of the pH, which provided a tangy and somewhat acidic beverage. When the fermentation process is in a progress, it will easily show a new layer of scoby at the surface and this is a very good indication that the microbes are in good health and that synthesis of cellulose is taking place by the acetic acid bacteria.

2.7 Post-Fermentation Handling

Once the process of fermentation had been conducted, using sterilized tongs, the SCOBY was slowly removed to a waiting vessel containing the finished kombucha. It was immediately sealed in a vessel with fresh batch of sweetened tea to retain and be used to

undergo further fermentation. The final fermented kombucha was filtered on a sterile muslin cloth to eliminate possibly remaining tea particles, microbial masses, or debris of the biofilm. The beverage that was clarified was squeezed in a sterile and sealed glass bottles under hygienic conditions. To enhance natural carbonation and flavour complexity, the kombucha could optionally undergo a secondary fermentation, wherein sealed bottles were kept at room temperature (25–28°C) for an additional 2–3 days. The process allows more CO to be generated by even the surviving yeast, resulting in a slightly carbonated beverage. All bottles will then be stored in a refrigerator at 4°C to desist further growth of microbes, to fix acidity and lengthen shelf life until further testing or use.

2.8 Standardization of black tea leaves in preparation of kombucha beverage

Ingredients used for the preparation of the beverage are water (250 ml),black tea leaves, jaggery, rosemary dried leaves, orange peel powder, starter tea and SCOBY.

The standardization of the black tea leaves was carried out by changing the concentration of the same in 250 ml of water and maintaining the constant jaggery and SCOBY inoculum. The tea leaves were soaked at boiling hot water and left to rest 10 minutes, filtered, then mixed with jaggery followed by the addition of SCOBY.

2.9 Standardization of black tea leaves in preparation of kombucha beverage

Treatment No	**Water**	**Black Tea Leaves**	**Jaggery**	**SCOBY**
T_1	250 ml	4%	10%	10%
T_2	250 ml	3.6 %	10%	10%
T_3	250 ml	2.4 %	10%	10%
T_4	250 ml	1.6 %	10%	10%
T_5	250 ml	1%	10%	10%

Design - Completely random design

Replications - Three

Incubation Temperature - 28°C

Fermentation temperature - room temperature 25°C for 7 days

Storage temperature - from 4°C

Sensory evaluation of the standardized product:

Sensory properties including color, taste, flavor, aroma, alcoholic nature and acceptance. The treatments were assessed and measured as first treatment tastes highly fermented tea flavor followed by treatment 2 which was slightly strong, treatment 3 tasted good and balanced and the last two treatments were mild according to tea taste.

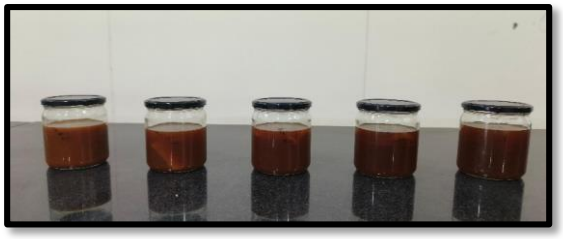


Fig 2.1 Standardization of black tea leaves

2.10 Standardization of dried rosemary leaves and orange peel in the beverage

Dried rosemary leaves and orange peel powder were standardized by varying their concentrations in the kombucha tea base (prepared using black tea, jaggery SCOBY). Steeping of rosemary leaves, filtering and fermentation of the leaves were used to prepare tea, and orange peel powder was added. The two were added in the brewing of tea and strained prior to fermentation. The base kombucha formulation consisted of 3% black tea, 10% jaggery and 10% SCOBY. The following trials were conducted to standardize rosemary dried leaves and orange peel powder as flavoring agent.

2.11 Standardization of dried rosemary leaves and orange peel in the beverage

Treatment No	Water	Dried Rosemary Leaves	Orange Peel Powder
T_1	250 ml	5.2 %	1.2%
T_2	250 ml	4.4 %	1.2%
T_3	250 ml	3.6 %	1.2%
T_4	250 ml	2.8 %	1.2%
T_5	250 ml	2 %	1.2%

Design - Completely random design

Replications - Three

Incubation Temperature - 25°C

Fermentation temperature - room temperature 25°C for 7 days

Storage temperature - from 4°C

Sensory evaluation of the standardized product:

The treatments were assessed and measured as strong initially, first two treatments tastes very strongly herbal, at treatment 3 herbal and citrus balances tastes fine, at treatment 4 mild rosemary flavor and citrus predominant, and at last treatment moderately light infusion and low aroma.

2.12 Standardization of jaggery substrate in the beverage

Jaggery is the basic substrate as it provide basic sweetness to the beverage and the taste. All treatments were prepared using a constant base of 2.4% black tea leaves, 5% rosemary dried leaves, 1.2% orange peel powder, 10% SCOBY, and 250 ml water.
Only jaggery concentration was varied.

2.12 Standardization of jaggery substrate in the beverage

Treatment No	Jaggery
T_1	2%
T_2	4%
T_3	6%
T_4	8%
T_5	10%

Design - Completely random design

Replications - Zero

Incubation Temperature - 25°C

Fermentation temperature - room temperature 25°C for 7 days

Storage temperature - from 4°C

2.13 Sensory evaluation of the standardized product:

The treatments were assessed and measured as weak fermentation initially, first two treatments tastes mild sweetness, at treatment 3 balances tastes fine, at treatment 4 slight too sweet, and at last treatment excessive sweetness and off flavor.



Fig 2.2 Standardization of dried rosemary leaves, orange peel powder and jaggery

2.14 Process of Rosemary Kombucha blended with orange peel

Procurement of Ingredients

⬇

Weighing of Ingredients (as per standardizations)

⬇

Boiling of Water (250 ml) and Brewing of Black Tea (90–95°C for 10–15 min)

⬇

Addition of Rosemary Leaves and Orange

⬇

Peel Powder (Steep together, then filter)

⬇

Dissolution of Jaggery in Warm Infusion

⬇

Cooling to Room Temperature (~25°C)

⬇

Inoculation with 10% SCOBY

⬇

Fermentation (7 days at 25 ± 2°C, covered with muslin cloth)

⬇

Removal of SCOBY and Filtration

⬇

Bottling in Sterile Glass Containers

⬇

Storage at 4 ± 1°C

2.15 Sensory Evaluation

A fermented beverage was prepared using the standard protocol, involving the use of a Symbiotic Culture of Bacteria and Yeast (SCOBY). The primary microorganisms responsible for the fermentation process include acetic acid bacteria (such as *Acetobacter xylinum*, *Gluconobacter spp*.) and various strains of yeast (such as *Saccharomyces cerevisiae*, *Zygosaccharomyces bailii*, and *Brettanomyces spp*.). The combination of these organisms acts collectively to ferment sugar to produce organic acids, carbon dioxide and bioactive compounds hence a working and healthy promoting fermented drink. The ongoing development of the products necessitated an organoleptic evaluation based on various parameters, including flavour, taste, colour, , and overall acceptability. This assessment involved 60 untrained panellists from the School of Agricultural Sciences at Malla Reddy University, Maisammaguda, Hyderabad.



Fig 2.3 Sensory evaluation of developed products

3. REULTS AND DISCUSSION

3.1 Percent standardization of black tea leaves for the development of kombucha beverage

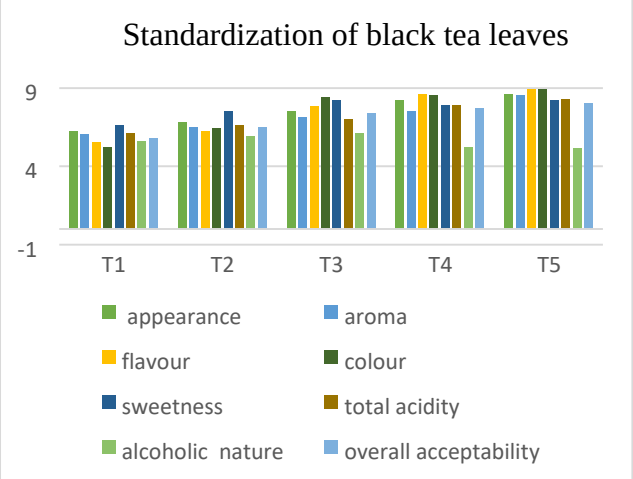
Black tea leaves (200 g) were procured from a commercial source, received in packed form, and stored under cool, dry, dark conditions (15–25°C) to preserve flavour, aroma, and bioactive compounds (polyphenols, catechins). They naturally contain microorganisms such as *Bacillus subtilis*, *B. cereus*,

Micrococcus luteus, *Pseudomonas fluorescens*, *Saccharomyces cerevisiae*, and *Aspergillus niger*.

For the first standardization of rosemary kombucha, varying concentrations of tea leaves (4%, 3.6%, 2.4%, 1.6%, 1%) were tested to balance fermented and alcoholic flavour. Parameters analysed included TSS, Brix, and pH. Initial TSS (10.0–11.5°Bx) decreased to 2.0–4.5°Bx, while pH dropped from 5.1–5.7 to 2.7–3.8, showing sugar utilization and organic acid production.

Treatment T1 (4%) gave highly fermented flavour but least acceptability (5.8), while T3 (2.4%) was superior with overall acceptability (7.4). T4 (7.7) was on par with T3, and T5 (8.0) was most preferred. Critical difference was 0.51, with 5 treatment degrees of freedom and error 8.19.

Nutritionally, black tea provides polyphenols, dietary fibre, potassium, modest protein, caffeine, and theaflavins, supporting its role as a functional kombucha ingredient (Khokhar & Magnusdottir, 2002). Its bioactives (theaflavins, catechins) offer antioxidant, anti-inflammatory, cardiovascular, anticancer, antidiabetic, neuroprotective, gastrointestinal, and antibacterial benefits. Thus, black tea in fermented beverages like kombucha enhances functional and health-promoting properties (Chen & Liu, 2020).



3.2 Percent Standardization of dried rosemary leaves and orange peel for the development of kombucha beverage

Rosemary dried leaves (200 g) were procured from a certified herbal source, cleaned, shade-dried, crushed, and stored under dry, dark conditions (20–25°C) to preserve phytochemical stability, aroma, and antioxidant compounds. They naturally contain microorganisms such as *Bacillus subtilis*, *Micrococcus luteus*, *Pseudomonas* spp., *Saccharomyces* spp., and *Penicillium* spp. Oranges (100 g) were obtained from a commercial source, and fresh peels were washed, shade-dried, coarsely ground, and stored in airtight opaque containers (20–25°C) to retain essential oils, bioactive components, and aroma. These peels harbor microorganisms including *B. subtilis*, *Pseudomonas aeruginosa*, *S. cerevisiae*, and *Aspergillus niger*.

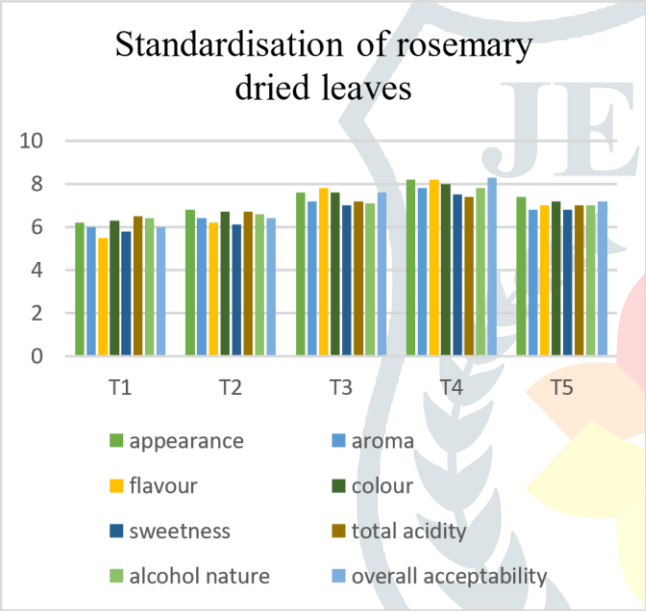
For the second standardization of rosemary kombucha, rosemary leaves (5.2%, 4.4%, 2.4%, 1.6%, 1%) and orange peel powder (1.2%) were used. Parameters analysed were TSS, Brix, pH, and titratable acidity. Initial TSS (10.0–11.3°Bx) increased with higher rosemary content, then decreased to 2.2–4.2°Bx during fermentation, indicating sugar utilization. pH dropped from 5.3–5.8 to 2.8–3.7, while titratable acidity rose from 0.2–0.4% to 0.8–1.3%, correlating with higher rosemary levels and microbial activity. Sensory evaluation (Table 4.2) showed overall acceptability: T1 = 6.0, T2 = 6.4, T3 = 7.6, T4 = 8.3, T5 = 7.2. Treatment mean comparison showed critical difference = 0.05, with 4 treatment degrees of freedom and error = 10.

Rosemary is rich in phenolic compounds (rosmarinic acid, carnosic acid, carnosol) with strong antioxidant activity (Nieto et al., 2018), enhancing nutritional value, shelf life, and safety of kombucha (Burt, 2004; Borrás-Linares et al., 2014). It reduces risks of cardiovascular, neurodegenerative, and cancer conditions (Bozin et al., 2007), exerts anti-

inflammatory effects via COX-2 and cytokine modulation

(Embuscado, 2015), shows broad antimicrobial activity (Nieto et al., 2018), and provides neuroprotective benefits by improving memory, mood, and inhibiting acetylcholinesterase linked to Alzheimer's (Pengelly et al., 2012).

Orange peel powder (*Citrus sinensis*) contains dietary fiber, vitamin C, flavonoids (hesperidin, naringin), phenolic acids, and essential oils with antioxidant, antiinflammatory, antimicrobial, and



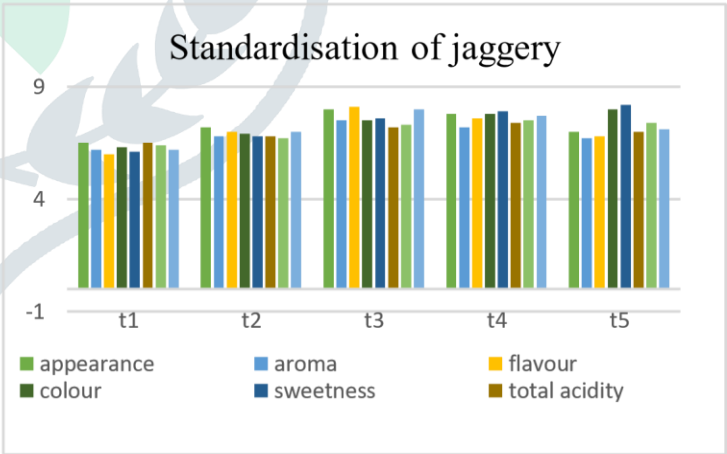
3.3 Percent standardization of jaggery substrate for the development of kombucha beverage

cholesterollowering effects (González-Molina et al., 2010; Tripoli et al., 2007). Its phenolic content supports free-radical scavenging and protection against oxidative stress, contributing to prevention of cardiovascular disease, type 2 diabetes, and cancer (Zhao et al., 2014).

The high polyphenol and limonene content helps combat oxidative stress, supports immune health, and extends food shelf life (Sun, 2007). Its fiber aids digestion and glucose control, while flavonoids contribute to cardiovascular protection, anti-obesity effects, and potential anti-cancer activity (Park *et al.*, 2006; Tanaka *et al.*, 1997).Thus, incorporating orange peel powder into functional products like kombucha enhances both nutritional value and therapeutic potential.

In the first standardization, black tea leaves were tested at varying percentages to identify the suitable fermented taste and flavour, while rosemary dried leaves were used at 5.2%, 4.4%, 2.4%, 1.6%, and 1% across five treatments. Proximate analysis showed

contain added sugars that compromise nutritional value. This study reviews sugar levels in probiotic beverages and their health impacts, noting risks of weight gain, insulin resistance, metabolic disorders, and dental problems despite probiotic benefits. It also examines regulatory standards and labeling practices on sugar content, highlighting the need for consumer awareness and informed choices (Zanirati et al., 2015).



Total

Soluble Solids (TSS) ranging 2–5 °Brix and pH 5.32–5.61. Treatment T1 (5% jaggery substrate) produced a mild beverage with limited fermentation and fruity notes but higher sweetness, altering the flavour. Sensory evaluation (Table 3.3) showed overall acceptability scores: T1 = 6.2, T2 = 7.0, T3 = 8.0, T4 = 7.7, T5 = 7.1. The treatment mean comparison gave a critical difference of 0.05, with 4 degrees of freedom and error = 10.

Probiotic drinks are increasingly popular for gut health benefits via live beneficial microorganisms, yet commercial versions often

3.4 Proximate analysis

The proximate analysis which is carried out through the fermented beverage analysis are been done under the supervision in agriculture microbiology lab (SOAS) in Malla Reddy University. As the instruments were properly used and analysis results are been noted.

Analysis which been carried out are as follows:

- Total soluble solids
- Protein
- pH
- Titratable acidity

3.4.1 Total soluble sugars

The experiment was carried out with all the 5 treatments with the help of the instrument which is called as hand refractometer. As it shows the amount of sugar present in the beverage in the form of brix%.

The sensory evaluation which was first conducted for the standardization of the black tea leaves as a small amount of sugar was added to the beverage shows the result as between 2.0

-5.0 ° brix and can pH ranges between 5.6 - 5.3

This shows the beverage contains good amount of sugar which is needed in the 100ml beverage

and can be distinguished as per the treatments such as

TREATMENTS	Total soluble sugars (TSS) (° Brix)
T1	4.4
T2	3.9
T3	3.2
T4	2.7
T5	2.2

3.4.2 Protein content of the developed product

TREATMENTS	Total soluble sugars (TSS) (° Brix)
T1	2.0
T2	2.5
T3	3.8
T4	4.3
T5	4.7

TREATMENTS	PROTEIN
T1	0.32 %
T2	0.38 %
T3	0.44 %
T4	0.40 %
T5	0.36 %

TREAT MENTS	Total soluble sugars (TSS) (° Brix)
T1	2.3
T2	2.6

T3	3.2
T4	3.8
T5	4.2

3.4.3 pH content of the developed product

3.4.4 Titratable Acidity content of the developed product

3.4.5 Bioactive compound analysis

TREATMENTS	pH
T_1	3.80
T_2	3.60
T_3	3.45
T_4	3.35
T_5	3.20

TREATMENTS	Titratable Acidity
T_1	1.10
T_2	1.25
T_3	1.40
T_4	1.55
T_5	1.65

Natural molecules called bioactive compounds can be found in a variety of meals, but they are especially abundant in fruits, vegetables, herbs, and spices. Because of their biological actions, they may have positive health consequences. Different bioactive compounds which are been analyzed are

Total antioxidant activity

Total phenols

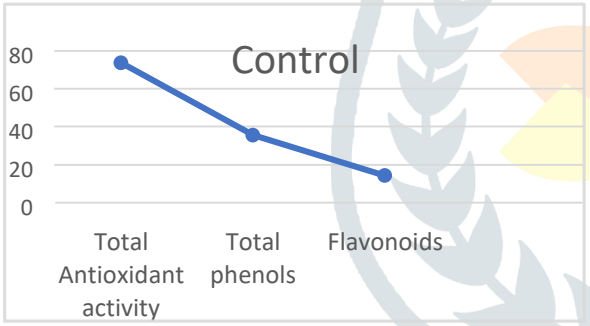
Flavonoids

Treatments	TSS	Protein	pH	Titratable acidity

3.4.5 Proximate analysis carried out for the final product are:

			Initial	Final	Initial	Final
T1	3.2	0.92	0.0	3.65	0	0.65
T2	3.8	1.48	5.22	3.45	0.27	0.80
T3	4.5	1.7	5.25	3.30	0.32	1.05
T4	5.2	1.42	5.28	3.10	0.37	1.35
T5	6.8	1.39	5.30	2.95	0.43	1.60

ACETIC ACID BACTERIA	GYC AGAR	3.1 $\times 10^6$ CFU/ml
YEAST	PDA	4.2 $\times 10^6$ CFU/ml
MOLDS	PDA	0



The graph shows the amount of bioactive compounds present in the fermented beverage.

Shelf life - Microbial

Analysis shows the microbial activity in the fermented beverage as

FOR THE 15th DAY

The acetic acid bacteria found on the 15th day is 3.1 $\times 10^6$ CFU/ml with the help of pour plate method and the GYC Agar media, the number of yeast found is 4.2$\times 10^6$ CFU/ml, molds as 0 in the PDA medium (with 3 replications).

FOR THE 25TH DAY

MICROBE	MEDIA	CFU/ML
ACETIC ACID BACTERIA	GYC Agar	3.6 $\times 10^6$ CFU/ml
YEAST	PDA	4.8 $\times 10^6$ CFU/ml
FUNGI	PDA	0

The acetic acid bacteria found on the 25th day is 3.6×10^6 CFU/ml with the help of pour plate method and the GYC Agar media, the number of yeast found is 4.8×10^6 CFU/ml, molds as 0 in the PDA medium(with 3 replications).

FOR THE 35th DAY

MICROBE	MEDIA	CFU/ML
ACETIC ACID BACTERIA	GYC AGAR	4.2 $\times10^6$ CFU/ml
YEAST	PDA	5.4 $\times10^6$ CFU/ml
MOLDS	PDA	0

The acetic acid bacteria found on the 35th day is 4.2 $\times10^6$ CFU/ml with the help of pour plate method and the GYC Agar media, the number of yeast found is 5.4×10^6 CFU/ml, molds as 0 in the PDA medium (with 3 replications).

FOR THE 45th DAY

MICROBE	MEDIA	CFU/ML
ACETIC ACID BACTERIA	GYC AGAR	4.9 $\times10^6$ CFU/ml
YEAST	PDA	6.1×10^6 CFU/ml
MOLDS	PDA	0

The acetic acid bacteria found on the 35th day is 4.9 $\times10^6$ CFU/ml with the help of pour plate method and the GYC Agar media, the number of yeast found is 6.1×10^6 CFU/ml, molds as 0 in the PDA medium (with 3 replications).

4. SUMMARY

Kombucha is a non-dairy, fermented functional beverage enriched with a diverse community of beneficial microorganisms that contribute to its probiotic, antioxidant, and digestive health properties. It is completely lactose-free and naturally contains organic acids, enzymes, and bioactive compounds formed during fermentation. In the initial phase, the raw materials including dried rosemary leaves, orange peel powder, and jaggery are carefully procured and stored under low-temperature conditions to maintain their phytochemical integrity. The dried rosemary leaves are selected for their antioxidant and antimicrobial potential, while orange peel powder is incorporated for its fiber, vitamin C, and polyphenolic content. Jaggery acts as a natural fermentable sugar, supporting microbial activity and contributing to the flavor and nutritional profile of the beverage.

The fermented kombucha beverage was prepared under Standardization protocol is for getting the optimum results such as there are total 5 treatments T_1,T_2,T_3,T_4,T_5 with its replications so that we can get optimum results as the moisture content in rosemary dried leaves are as sample 1-9.5. In the second phase the proximate analysis for the standardization pH of the product was found to be T_1 is 3.65, T_2 is 3.45, T_3 is 3.30, and T_4 is 3.10, T_5 is 2.95. TSS(Total Soluble Solids) as 3.2° brix in T_1, 3.8°brix in T_2, 4.5° brix in T_3, 5.2° brix in T_4 and 5.8° brix in T_5. Protein of the product was found to be T_1 is 0.92,T_2 is 1.48, T_3 is 1.7, T_4 is 1.42 and T_5 is 1.39. The highest Titratable acidity found is 1.60.

Treatment 3 was the most efficient compared with the other treatments and higher concentration because effective low pH indicates acidic product in fermented drink. As sensory evaluation was used to determine the most optimal fermented beverage and an incubation period lasting 3648 hours was used in addition to a temperature of 30-32 ℃.

The proximate analysis for the developed product contains a pH of 3.45 and a TSS of 4.4 ° brix with the higher Titratable acidity as 1.65. There are different bioactive compounds found in the kombucha beverage such as Phenols, Flavonoids, Antioxidants. As for the control sample the phenolic content is 35.57mg of GAE/100g, flavonoids content as 14.40mg of QE/100g, and Antioxidant activity as 73.70µg/100g. In the developed product the bioactive compounds found are phenolic content is 43.50mg of GAE/100g, flavonoids content as 19.60mg of QE/100g and Antioxidant activity as 75.90 µg/100g and Ascorbic acid as 1.06 mg/100g.

Compared to control sample the amount of treated sample is more nutritious. The amount of antioxidants is more in the treated sample, and other bioactive compounds are found to be superior in the treated sample such as flavonoids, phenolic content. Ascorbic acid is also found in the treated sample as orange peel powder contains vitamin c in them. The microbial analysis performed for the shelf life of the product contains 3 microbial groups in them such as bacteria, yeast and molds.

The total bacteria count for the first 15 days were found as 3.1 $\times 10^6$ CFU/ml. Yeast was found to be 4.2 CFU $\times 10^6$ CFU/ml . Molds was found to be 0 CFU $\times$ 10^{-5}. The total bacteria count for the first 25 days were found as 3.6 $\times 10^6$ CFU/ml. .Yeast was found to be 4.8 $\times 10^6$ CFU/ml. Fungi was found to be 0 CFU $\times$ 10^{-5}.

The total bacteria count for the first 35 days were found as 4.2 $\times 10^6$ CFU/ml. .Yeast was found to be 5.4 $\times 10^6$ CFU/ml. Fungi was found to be 0 CFU $\times$ 10^{-5}. The total bacteria count for the first 45 days were found as 4.9 $\times 10^6$ CFU/ml. .Yeast was found to be 6.1 $\times 10^6$ CFU/ml. Fungi was found to be 0 CFU $\times$ 10^{-5}.

The result showed that the *nardus* G.) and rosemary (*Rosmarinus* standardization process contained various *officinalis* L.) extracts prepared by results such as the taste, colour, appearance, steam distillation, supercritical and alcoholic nature and its nutritional profile solvent extraction. *Food Chemistry*, between different percentages of the **85**(4), 527–533. treatments and control sample. The difference

in the sensory quality shows the developed Barreca, D., Gattuso, G., Bellocco, E., product probiotic nature in the superior form. Calderaro, A., Trombetta, D., The nutritional content in the treated sample Smeriglio, A., ... & Nabavi, S. M. is also found to be superior under the (2017). Flavanones: Citrus bioactive compound analysis. phytochemical with health-promoting properties. *BioFactors*, *43*(4), 495-

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