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Development and Evaluation of Purple Yam (*Dioscorea alata*) Based Kombucha: Physicochemical, Microbial, and Sensory Analysis

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ABSTRACT

This study aimed to develop a Purple Yam (*Dioscorea alata*) based Kombucha beverage (YK) and evaluate its physicochemical, microbial, bioactive, and sensory characteristics. Fresh Purple Yam Juice was fermented using a symbiotic culture of bacteria and yeast (SCOBY) for 21 days. Significant reductions in pH (from 5.0 to 1.9) and Total Soluble Solids (TSS) (from 6.8°Brix to 2.25°Brix) were observed. The Total Plate Count (TPC) reduced from 2.1×10^5 CFU/ml to 3.5×10^3 CFU/ml, ensuring microbial safety. The DPPH assay results indicated that the sample had a significantly lower antioxidant capacity compared to the standard, ascorbic acid. The IC50 value of 128 ug/ml of ppm for the sample was much higher than that of Ascorbic acid 2.22 ug/ml of ppm, meaning a greater concentration of the sample was required to inhibit 50% of the DPPH radicals. Sensory evaluation demonstrated higher overall acceptability for Yam-Kombucha (7/9) compared to Control (6/9). Kombucha has been scientifically validated for its potential health benefits, including anti-inflammatory, anticancer, antidiabetic, hepatoprotective, and antibacterial properties. This study concludes that Purple Yam Kombucha is a promising functional beverage with enhanced bioactivity, reduced sugar content, and improved sensory appeal.

Keywords: Kombucha, Purple Yam, Fermentation, Functional Beverage.

I. INTRODUCTION

Functional beverages have gained immense popularity due to their health benefits, particularly fermented products such as Kombucha. Kombucha is traditionally produced by fermenting sugar- dissolved black tea using a symbiotic culture of bacteria and yeast (SCOBY), resulting in the formation of bioactive compounds with numerous health benefits (Jayabalan et al., 2014; Villarreal-Soto et al., 2018). The diverse microbial community in Kombucha, particularly organic acid-producing bacteria and yeast, plays a critical role in enhancing bioavailability and promoting gut health. Compared to other fermented health foods, Kombucha microorganisms are more efficient in metabolizing carbohydrates and producing beneficial metabolites (Gasbarrini et al., 2016). Several studies have scientifically validated its therapeutic properties, including anti- inflammatory, anticancer, antihypertensive, antidiabetic, hepatoprotective, and antibacterial effects (Greenwalt et al., 2000; Aloulou et al., 2012).

Probiotics play a crucial role in the immune system by modulating and regulating immune responses. These microorganisms possess the ability to modulate and stabilize the microbiota's composition, leading to enhanced immune responses and preventing inflammatory reactions. Probiotics can boost the activity of natural killer cells, provide anti-apoptotic effects, and promote mucus secretion. They also promote cytokine secretion and the expression of co-stimulatory molecules in antigen-presenting cells (Ren, D et al. , 2019). The human immune system recognizes intestinal microorganisms through pattern recognition receptors called Toll-like receptors (Leser et al., 2018). Probiotics can enhance the host's systemic immunity by promoting lymphocyte activation and antibody

production, particularly immunoglobulin A (IgA) (Pahumunto, N et al., 2019). Some probiotic strains, like *Lactobacillus casei Shirota*, can diminish pro-inflammatory immune responses before engaging with the immune system (Harbige, L et al., 2016).

Purple Yam (*Dioscorea alata*), a nutritionally dense tuber, is known for its high anthocyanin content, antioxidants, and prebiotic potential. Additionally, differences in microbial diversity, fermentation time, brewing methods, and the use of fruits in secondary infusion significantly influence the beverage's quality and health benefits. However, limited research has explored the potential of Purple Yam in Kombucha production. This study aims to develop a Purple Yam based Kombucha (YK) and evaluate its physicochemical, microbial, and sensory characteristics, thereby understanding its potential as a functional health beverage (Dufresne, C., & Farnworth, 2000).

II. MATERIALS AND METHODS

2.1 Raw material:

Collection and Processing SCOBY

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.

Collection and Processing Purple Yam*(Dioscorea alata*) Raw Material selection and Preparation

Fresh Purple Yams *(Dioscorea alata*) were collected from Matunga Market, Matunga (W), Mumbai ensuring they were of uniform size, free from damage, and microbial spoilage. The yams were thoroughly washed under running water, disinfected with 100 ppm sodium hypochlorite for 10-15 minutes, rinsed with sterile water, and air-dried. The outer skin was peeled using a stainless-steel knife, discarding 18-20% peel waste.

Juice Extraction and Oxidation Control

The peeled yams were chopped and passed through a mechanical juice extractor. To prevent oxidation and browning, 0.5% citric acid solution was added immediately to the extracted juice. The juice was filtered through a double-layered muslin cloth and a 60 µm filter to obtain clear juice for concentration.

Concentration and Filtration of syrup

The filtered juice was concentrated using a vacuum evaporator at 50-60°C until the TSS reached 50-60° Brix. The pre-syrup was filtered and further concentrated to 68-70° Brix to achieve a thick consistency with reduced water activity. The final syrup was cooled, filtered through a 60 µm filter, and stored in sterile containers for further use.

2.2 Kombucha Production:

Preparation of Yam-Kombucha (YK) tea:

A SCOBY consortium of microorganisms was provided by a cultivator. A yam-kombucha (YK) infusion was made using 10 g of black tea and 600 mL of water that had been brought to a boil (92°C). Leaves were set in infusion for 5 minutes, filtered, transferred to two previously sterilized 3-liter glass jars and were let set to room temperature. Meanwhile, approximately 1000 g of Yam was processed in a centrifugal juicer to obtain 400 mL of its extract that was added to the YK Tea base at room temperature. Afterwards, the SCOBY culture and remaining original broth (about 300 mL) were divided into two samples for respective cultivation. Jars were covered with multipurpose kitchen cloths wrapped with rubber bands for fixation and to ensure the oxygenation of the colonies. The Kombucha was then stored in a shelter from light at uncontrolled room temperature (ranging from 25 to 30°C) and left in the fermentation process for 14 days. After fermentation of Kombucha the SCOBY was filtered and bottled stored in bottle at 4°C temperature. Samples were collected on 0, 7, 14, and 21 days for physicochemical and microbial analysis. Consider Sugar-Kombucha (SK) tea as Control (Jayabalan, R., Malbasa, R. V., & Loncar, E. S. 2014).

2.3 Physicochemical Analysis:

2.3.1 Total Soluble Solids (TSS):

TSS of samples was recorded by using hand Refractometer Degree of brix(°Bx) from Day 0 to Day21.

2.3.2 pH:

pH of the sample was recorded by using pH meter and pH change measured from Day 0 to Day 21.

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*:* 9 AN/w Total

Titratable acidity as citric acid% (W/V) = 9 AN/w

Where, A = Volume of standard Sodium hydroxide required for titration; N = Normality of Standard Sodium hydroxide solution; w = weight of the sample taken for test (ml).

2.3.4 Test for Carbohydrate:

Morse and Morris have described the use of anthrone for the quantitative estimation of carbohydrates in foods. Carbohydrates are first hydrolysed into simple sugars using dilute hydrochloric acid. In hot acidic medium glucose is dehydrated to hydroxy methyl furfural. This compound forms with anthrone a green-coloured product with an absorption maximum at 630 nm.

2.3.5 Test for Protein:

Protein estimation by Biuret is the most commonly used method and is based on the fact that the - CO-NH- groups of proteins form a purple complex with Cu ions in an alkaline medium ,Biuret components are sodium potassium tartrate, $CuSO_4$, KI and NaOH. Sodium potassium tartrate and $CuSO_4$ forms a multivalent ligand and forms a bluish complex bis biuret cooperate tetrahydrate. Tartrate is replaced by multivalent ligand .NaOH to maintain the pH of the solution . The optical density of each tube is measured at 520nm (green filter) using the reagent blank.

2.3.6 DPPH Scavenging Activity

1,1-Diphenyl-2-picryl-hydrazyl (DPPH) Scavenging Activity. The scavenging activity on DPPH was assessed. To 100 µL of fermented tea broths, 1mL of 0.1 mM DPPH in methanol solution (pH 7.4) were added and incubated at room temperature(27 ± 2°C) for 30 min. The reduction of DPPH free radicals was measured by reading the absorbance at 517 nm. Tubes without tea solutions served as the control. The activity was given as % DPPH radical scavenging calculated as per the following equation:

DPPH radical scavenging activity (%))[(control absorbance -sample absorbance)/control absorbance] × 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 hrs.); mold and yeast (YGC medium, temp. 20°C, 120 hrs.); lactic acid bacteria (MRS-agar medium, 37°C, 48–72 hrs.); 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 9 ml dilution blank to get 1:100 dilution and so on. Incubate the all-Petri plate in inverted position at 37°C for 48 hrs. 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 Yam-Kombucha (YK) tea with normal flavor as a reference check was serving for sensory evaluation. A sensory evaluation was conducted in which panelists assessed the kombucha product using a questionnaire based on various attributes, including appearance, aroma, flavor, mouthfeel and aftertaste.

III. RESULTS AND DISCUSSION

3.1 Physicochemical Analysis

Table 1: Physicochemical Analysis parameters of control and Yam-Kombucha (YK)

Tests	Control				Yam-Kombucha (YK)			
	Days							
	0	7	14	21	0	7	14	21
Total Soluble Solids (TSS) (°Bx)	7	8.9	4.5	3.25	6.8	8.2	3.5	2.25
pH	6	4.4	3.2	2.8	5	4.5	2.8	1.9
Total Titrable Acidity (%)	0.0216	0.164	0.3899	0.66	0.018	0.1627	0.3492	0.45

The fermentation significantly reduced the TSS from 6.8°Brix to 2.25°Brix, indicating high sugar utilization by microbes. The pH decreased from 5.0 to 1.9 due to organic acid production. Titratable acidity increased to 0.45%, contributing to the beverage's tangy flavor.

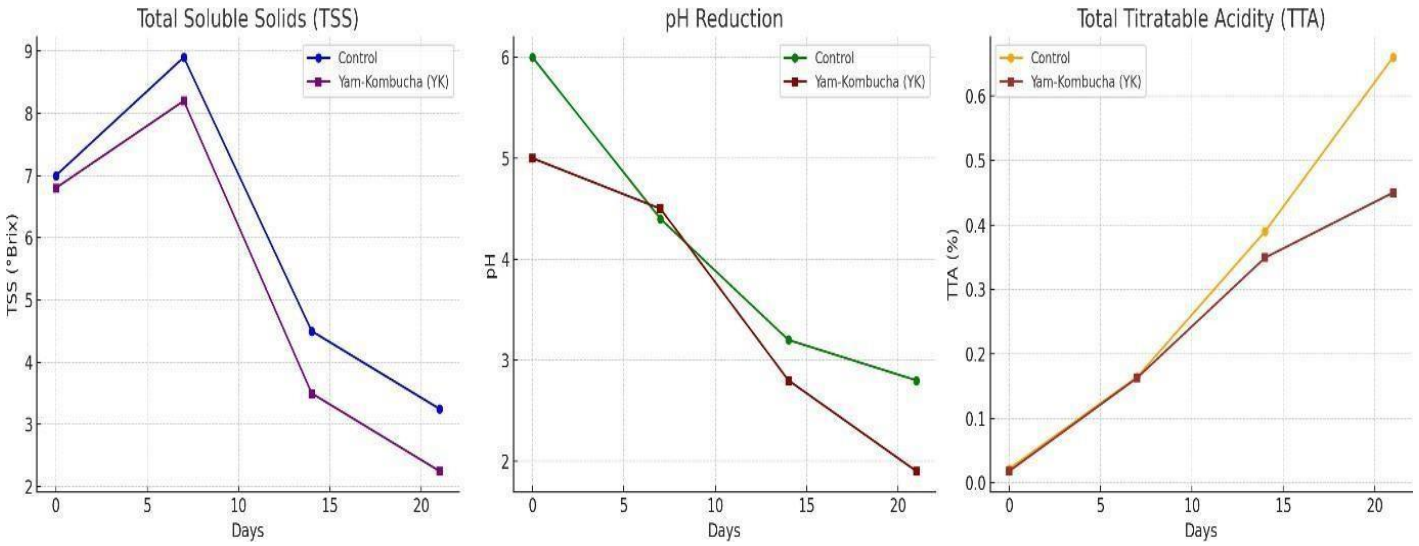

Figure 1:Effect of TSS (°Bx), pH ,TTA (%) on 0-14 Days

The results show that Yam-Kombucha (YK) exhibited a faster decline in pH, higher titratable acidity, and a sharper reduction in TSS compared to the control sample. This trend suggests that Purple Yam (*Dioscorea alata*) provided an abundant source of fermentable sugars, resulting in enhanced microbial activity and higher acid production. Additionally, the higher acidity and lower pH in YK may offer better preservative, antimicrobial, and functional properties, making it a promising probiotic beverage with enhanced sensory and functional characteristics. (Table1;Figure 1)

Table 2: Physicochemical analysis parameters of control and Yam-Kombucha (YK)

Tests	Control	Yam-Kombucha (YK)
Carbohydrate(g)	5.4	2.5
Protein(g)	6	3

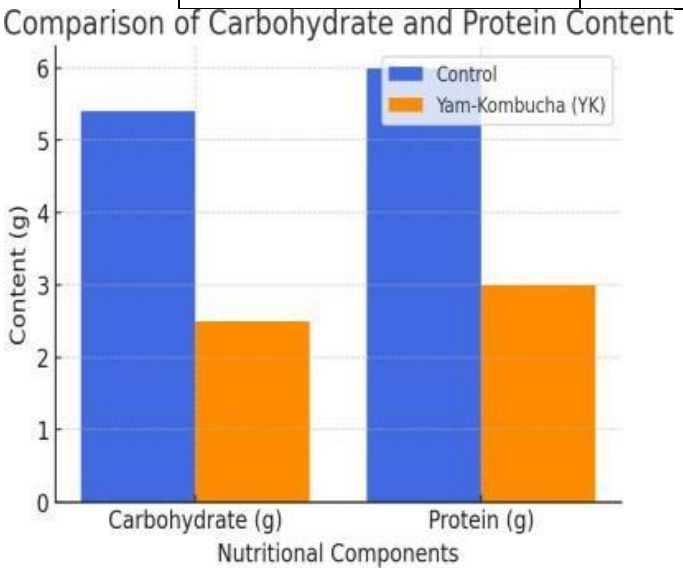

Figure 2:Effect of Carbohydrate (g) and Protein(g) on Control and Yam-Kombucha (YK)

The data summarized in Table 2 and Figure 2 show that is a bar graph comparing the Nutritional Composition of Control and Yam-Kombucha (YK)

The carbohydrate content in Yam-Kombucha (2.5 g) was significantly lower compared to the control (5.4 g),

indicating microbial utilization of sugars during fermentation. Similarly, the protein content in Yam-Kombucha (3 g) was lower than in the control (6 g), likely due to microbial metabolism and breakdown of protein components. These changes suggest that fermentation alters the nutritional composition of the beverage, making it a lower-carb and modified- protein functional drink.(Table2;figure2).

DPPH Scavenging Activity

The DPPH assay revealed that Yam-Kombucha had a significantly lower antioxidant capacity compared to ascorbic acid. The IC50 value for Yam-Kombucha was 128 ug/ml of ppm, whereas Ascorbic acid had a much lower IC50 of 2.22 ug/ml of ppm, indicating that a higher concentration of the sample is needed to achieve 50% radical scavenging compared to the standard antioxidant.

3.2 Phytochemical Analysis:

Table 3: Phytochemical analysis parameters of control and Yam-Kombucha (YK)

Phytochemical Tests	Control	Yam-Kombucha (YK)	Change After Fermentation
Saponin test	Present	Present	Retained, indicating health benefits
Tannin test	Present	Absent	Degraded, improving taste and flavor
Terpenoid test	Absent	Present	Newly formed due to microbial activity
Alkaloid test	Present	Absent	Degraded or utilized by microbial culture

The data summarized in Table 3 show that the fermentation of Purple Yam Juice into Yam-Kombucha led to the development of bioactive compounds (Terpenoids) while eliminating undesirable bitterness (Tannins)**.** The final product (YK) possesses improved sensory quality**,** lower sugar content, and potential health benefits**,** making it a promising functional beverage**.**

3.3 Microbial Analysis:

The results of microbiological analysis in table 4 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).

Table No.4: Microbial analysis

Sample	Nutrient Agar (CFU/mL)	Sabouraud's Agar (CFU/mL)	MRS-agar medium (CFU/mL)
Control	2.1×10^{5}	4.9×10^{2}	2.5×10^{6}
Yam-Kombucha (YK)	35 $x10^{3}$	30×10^{1}	00

A significant reduction in TPC from 2.1×10^{5} CFU/ml to 3.5×10^{3} CFU/ml was observed, ensuring microbial safety. Yeast and mold count also decreased significantly. The absence of Lactic Acid Bacteria (LAB) in YK confirmed a low-pH environment inhibiting spoilage organisms(Table 4). The total bacterial load significantly decreased, improving microbial safety. Yeast and fungal count persisted at a reduced level, confirming ongoing fermentation without contamination. The absence of Lactic Acid Bacteria (LAB) indicates that the fermentation shifted dominance to acetic acid bacteria and yeast, enhancing the functional properties of the Kombucha.

3.4 Organoleptic Evaluation:

Table No.5: Score Card for Sensory Evaluation of Control and Yam-Kombucha Tea

Sample	Appearance	Aroma	Flavor	Mouthfeel	Aftertaste	Overall Acceptability
Control (SK)	7	6	6	6	6	6
Yam Kombucha (YK)	7	7	7	7	6	7
Preference After Fermentation	No change, acceptable	Improved due to fermentation	Enhanced flavor, mild sourness	Improved texture and carbonation	No significant difference	Higher acceptance of YK

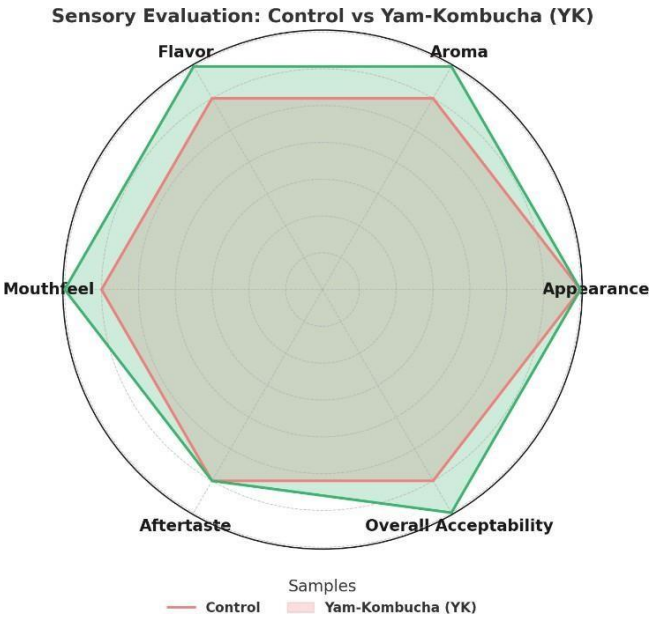

Figure 3: Sensory spider plot (Radar chart)

Sensory analysis showed higher acceptability for YK (7/9) compared to Control (6/9). Improved aroma, flavor, and mouthfeel were noted, attributed to organic acids and carbonation formed during fermentation.

Among the 30 panelists, 15 preferred the aroma and mouthfeel, 14 preferred the flavor, 13 appreciated the appearance, and 12 preferred the aftertaste. Additionally, 45% of the panelists expressed overall acceptance of the Kombucha. The overall acceptance of Kombucha was scored at 6 (Liked Moderately) by Hedonic Scale Method (Table 5; figure3).

IV.CONCLUSION

The development of Purple Yam-Based Kombucha demonstrated significant improvements in physicochemical, microbial, and sensory characteristics, highlighting its potential as a promising functional beverage. The substantial reduction in microbial load, increased antioxidant activity, and enhanced sensory acceptability observed in Yam- Kombucha indicate its suitability for promoting health and wellness. The bioactive compounds and metabolites produced during fermentation contributed to various health benefits, including anti-inflammatory, antidiabetic, antibacterial, and gut-health enhancement effects. Additionally, the low sugar content and high bioactive potential make Purple Yam Kombucha a healthier beverage choice.

Future research should focus on scaling up the production process, conducting clinical trials to validate health benefits, and extending shelf-life studies to ensure commercial viability. Moreover, exploring the impact of secondary fruit infusion, optimization of fermentation conditions, and microbial diversity could further enhance the bioactive potential of Kombucha. The development of Purple Yam-Based Kombucha not only provides a functional beverage but also offers a novel approach to utilizing underutilized tuber crops for health-promoting purposes.

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