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Formulation and Antioxidant Activity of Corn Silk Kombucha as a Functional Beverage

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Abstract

Degenerative diseases such as cardiovascular, stroke, hypertension, diabetes mellitus, and cancer are among the leading causes of mortality? in Indonesia. These diseases are associated with unhealthy lifestyles, poor dietary habits and exposure to free radicals resulting from air pollution. One preventive strategy is the consumption of functional foods rich in antioxidants. Corn silk contains bioactive compounds that function as antioxidants, such as flavonoids, phenols, and saponins. However, the processing of corn silk still faces challenges, particularly regarding consumer acceptance of its sensory characteristics. This research aims to analyze the organoleptic characteristics of Corn Silk Kombucha and its antioxidant activity. The study uses an experimental Completely Randomized Design (CRD) method with three types of additives: ginger, butterfly pea, and roselle. The analysis results show that the type of additive significantly influences ($p<0.05$) the color, aroma, taste, and overall liking of Kombucha. The most preferred Kombucha is the one with added ginger. The pH values of the control kombucha, butterfly pea flower, ginger, and roselle are 2.78; 3.40; 3.43; and 2.82; respectively. The total titratable acidity ranges from 0.11% to 0.48%. The highest total phenol content is found in the control kombucha (412.250 µg/ml GAE), and the lowest is in the kombucha with roselle addition (317.433 µg/ml GAE). The highest antioxidant activity is found in the kombucha with ginger addition (88.37%) and the control kombucha (78.58%) Corn Silk Kombucha with ginger addition has good acceptability in terms of color, aroma, and taste, as well as higher antioxidant activity and total phenol content compared to other additives.

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Introduction

Changing lifestyles and dietary patterns, particularly among residents of major cities in Indonesia, are major contributors to the increasing prevalence of degenerative diseases. The frequent consumption of fast food that contain high levels of saturated fats, trans fats, sugar, and salt have been associated with an increased risk of cardiovascular disease, diabetes, and other metabolic disorders (WHO, 2023; WHO, 2026). Moreover, exposure to free radicals and other Reactive Oxygen Species (ROS), derived either from normal physiological metabolic perocess in the human body or from external sources such as pollutants and industrial chemicals, can lead to oxidative stress (Chaudhary et al., 2023; Hassan et al., 2024). Oxidative stress is defined as an imbalance between free radicals and antioxidants in the body, resulting in cellular and tissue damage. This condition contributes to the development of various degenerative diseases, including cardiovascular disease, hypertension, diabetes mellitus, and cancer (Leyane et al., 2022; Chaudhary et al., 2023).

Degenerative diseases are the primary cause of worldwide mortality. According to the World Health Organization of the 57 million deaths reported globally, approximately 36 million were attributed to degenerative diseases, with 9 million occurring in individuals younger than 60 years of age. In 2014, the Health Research and Development Registration System reported a 71% rise in degenerative disease fatalities. Additionally, the Indonesian Ministry of Health reported in 2018 that degenerative diseases, including cardiovascular disease, stroke, diabetes mellitus, and cancer, dominate as the leading cause of death. (Kementerian Kesehatan, 2018)

The increasing prevalence of degenerative diseases highlights the importance of adopting healthier lifestyle and dietary patterns through regular physical activity and balanced nutrition. However, the demands of

modern life have led consumers to seek practical and convenient food choices. Consequently, there is a growing demand for functional foods that are not only convenient and nutritious but also provide health benefits beyond basic nutrition.

Corn silk is a rich source of bioactive compounds, particularly flavonoids and polyphenols, including quercetin, rutin, luteolin, apigenin, kaempferol, maysin, and their glycoside derivatives, which contribute significantly to its antioxidant activity. Recent phytochemical studies have identified flavonoids as the predominant secondary metabolites in corn silk, with luteolin- and apigenin-based compounds accounting for a substantial proportion of its antioxidant potential (Lapčík et al., 2023; Wang et al., 2024). In addition to flavonoids, corn silk contains polysaccharides, phenolic acids, tannins, saponins, alkaloids, terpenoids, and sterols, which collectively contribute to its biological activities (Zhang et al., 2024; Singh et al., 2023). Owing to these bioactive constituents, corn silk has been reported to exhibit antioxidant, anti-inflammatory, antihyperglycemic, antihyperlipidemic, nephroprotective, and antihypertensive properties. Emerging evidence suggests that corn silk and its bioactive compounds may play a role in the prevention and management of hypertension, diabetes mellitus, kidney disorders, urinary tract diseases, and other oxidative stress-related chronic conditions (Singh et al., 2023; Wang et al., 2024; Zhang et al., 2024).

Banyumas regency, one of the major corn-producing regions in Central Java, generates a substantial amount of corn silk waste as a by-product of its extensive corn production. If left untreated, this agricultural waste can contribute to environmental pollution through unpleasant odors and the attraction of pests. Therefore, corn silk has considerable potential as a functional food ingredient. Although corn silk has traditionally been consumed as a boiled herbal beverage, this study proposes an innovative approach by processing it into a fermented tea beverage well known as kombucha. Fermentation is expected to enhance the availability of bioactive compounds, improve antioxidant activity, and increase consumer preferences.

Materials and Methods

The materials used in this study included corn silk obtained from local farmers in Banyumas Regency, Central Java, Indonesia. The SCOBY (Symbiotic Culture of Bacteria and Yeast) was obtained from the Biology Laboratory of Jenderal Soedirman University. Additional ingredients used for kombucha production included ginger, butterfly pea flower, roselle flower, granulated sugar, mint leaves, black tea, water, and analytical-grade reagents. The equipment used in this study included a digital weighing scale, glass fermentation jars, a thermometer, an analytical balance (Ohaus, USA), a vortex mixer, a centrifuge (Ohaus, USA), a UV-Vis spectrophotometer (Thermo Scientific Genesys 50 UV-Vis, USA), and other laboratory equipment required for sample preparation and analysis.

The research was conducted at the Basic Science Laboratory and the Food Microbiology Laboratory of Telkom University, Purwokerto. The study focused on the production of Kombucha followed by analysis of organoleptic evaluation, and pH, total titratable acidity, total phenolic compounds, and antioxidant activity.

Kombucha Preparation Process

The preparation of Kombucha began with the sterilization of all equipment used in the fermentation process. Corn silk, black tea, and granulated sugar were weighed and then boiled in water for 15 minutes. The mixture was subsequently filtered and cooled to room temperature. The cooled infusion was then transferred into sterile glass jars, followed by the addition of starter liquid at a ratio of 1:1 (tea infusion : starter liquid) and SCOBY at 10% (w/v). The jars were then covered with a clean cloth and fermented at room temperature for 10 days. After fermentation, the kombucha was pasteurized at 73°C, followed by the addition of mint leaves. The mixture was then filtered and packaged for further analysis. The addition of ginger, butterfly pea flower, and roselle flower was adjusted according to the Kombucha variant being produced, with each additive incorporated at a concentration of 0.05% (w/v). The formulation of corn silk Kombucha is presented in Table 1.

Chemical Analysis of Kombucha

The chemical properties of kombucha samples analyzed in this study pH, alcohol content, total phenolic content (TPC), and antioxidant activity. The pH was measured using a pH meter (Eutech Instruments pH 700, Singapore). Total titratable acidity was determined by alkalimetric titration using a standardized sodium hydroxide (NaOH) solution with phenolphthalein (PP) as the indicator, following the method described by Susanto

Table 1. The formulation of corn silk Kombucha

Ingredient	Formulation Variants		
	Ginger	Butterfly Pea Flower	Roselle
Water (mL)	300	300	300
Starter liquid (mL)	300	300	300
Sugar (g)	30	30	30
Scooby (g)	30	30	30
Corn silk (g)	15	15	15
Ginger (g)	30	-	-
Butterfly pea flower (g)	-	30	-
Roselle (g)	-	-	30
Black tea (g)	12	12	12
Mint leaves (g)	2	2	2

(2006). Total phenolic content was determined using the Folin–Ciocalteu method according to Pure and Monir (2016). Antioxidant activity was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay with a UV–Vis spectrophotometer (Shimadzu, Japan), following the method of Baba and Malik (2014).

pH Determination

The pH of kombucha samples was measured using a calibrated digital pH meter (Eutech Instruments pH 700, Singapore). Prior to measurement, the instrument was calibrated using standard buffer solutions at pH 4.00 and 7.00. Approximately 10 mL of sample was placed in a beaker, and the electrode was immersed directly into the sample until a stable reading was obtained. Measurements were performed in triplicate at room temperature (25 ± 2°C) (AOAC, 2023).

Total Titratable Acidity (TTA)

Total titratable acidity was determined according to the AOAC method (AOAC, 2023) with slight modifications. A 10 mL aliquot of kombucha sample was diluted with 50 mL of distilled water and titrated with 0.1 N NaOH using phenolphthalein as an indicator until a persistent pale pink color was observed for 30 s. The results were expressed as percentage of acetic acid and calculated using the following equation:

$$\text{TTA (\%)} = \frac{V\ X\ N\ X\ 0.060\ X\ 100}{W}$$

where V is the volume of NaOH used (mL), N is the normality of NaOH, 0.060 is the equivalent weight of acetic acid, and W is the sample volume (mL).

Total Phenolic Content (TPC)

Total phenolic content was determined using the Folin–Ciocalteu colorimetric method as described by Pure and Monir (2016) with modifications. Briefly, 0.5 mL of sample extract was mixed with 2.5 mL of 10% Folin–Ciocalteu reagent and allowed to react for 5 min. Subsequently, 2.0 mL of 7.5% sodium carbonate solution was added, and the mixture was incubated in the dark for 30 min at room temperature. Absorbance was measured at 765 nm using a UV–Vis spectrophotometer (Shimadzu, Japan). Gallic acid was used as the standard, and the results were expressed as mg gallic acid equivalents (GAE) per liter of sample based on the calibration curve:

$$\text{TPC (mg GAE/L)} = \frac{C\ X\ DF\ X\ V}{m}$$

where C is the concentration obtained from the calibration curve, DF is the dilution factor, V is the sample volume, and m is the sample mass or volume analyzed.

Antioxidant Activity (DPPH Assay)

Antioxidant activity was evaluated using the DPPH radical scavenging assay following the method described by Baba and Malik (2014) with modifications. A 0.1 mM DPPH solution was prepared in methanol. Then, 1 mL of kombucha sample was mixed with 3 mL of DPPH solution and incubated in the dark for 30 min at room temperature. The absorbance was measured at 517 nm using a UV–Vis spectrophotometer (Shimadzu, Japan). Methanol containing DPPH solution was used as the control. Antioxidant activity was calculated as percentage inhibition using the following equation:

$$\text{DPPH scavenging activity (\%)} = \frac{Ac-As}{Ac}\ x\ 100$$

where Ac is the absorbance of the control and as is the absorbance of the sample.

Sensory Analysis

The parameters evaluated in the organoleptic analysis of corn silk Kombucha with the addition of ginger, butterfly pea flower, and roselle flower included color, aroma, and taste. Sensory analysis was conducted using a preference test on a 1–5 scale, where 1 = strongly dislike, 2 = dislike, 3 = neutral, 4 = like, and 5 = strongly like. The hedonic test involved 76 untrained panelists aged 18–44 years from Telkom University, Purwokerto. According to Howard et al. (2011), a minimum of 75 untrained panelists is recommended for consumer acceptance testing. In addition to the hedonic evaluation, a ranking test was conducted to determine the overall preference of the panelists among the different kombucha formulations.

Results and Discussion

pH of Corn Silk Kombucha

Measurement of pH in fermented products is essential as an indicator of fermentation progress and the completion of the fermentation process. During Kombucha fermentation, two sequential fermentation stages occur: alcoholic fermentation, in which yeasts convert sugars into etanol, followed by acetic acid fermentation, in which acetic acid bacteria oxidize ethanol into organic acidsThe fermentation process begins with the hydrolysis of sucrose into glucose and fructose by the enzyme invertase. These monosaccharides are readily utilized by microorganisms during fermentation (Rahayu & Rohaeti, 2014). Easts primarily metabolize glucose and fructose to produce ethanol and carbon dioxide, while acetic acid bacteria subsequently oxidize ethanol into acetic acid. In addition, glucose is converted into gluconic acid through the metabolic activity of *Acetobacter* spp., whereas fructose is utilized to a lesser extent and partially remains in the fermented beverage (Rahayu & Rohaeti, 2014). As a result, the major metabolites produced during kombucha fermentation include acetic acid, gluconic acid, and bacterial cellulose (Greenwalt et al., 2000). The accumulation of these organic acids lowers the pH of the beverage, giving kombucha its characteristic sour taste. Generally, the pH of kombucha ranges from 2.5 to 3.5.

The pH values of corn silk Kombucha ranged from 2.78 to 3.43 (Figure 1). Statistical analysis showed no significant differences ($p > 0.05$) in pH among the control kombucha and the formulations supplemented with butterfly pea flower, ginger, or roselle. The highest pH values were observed in the ginger-supplemented kombucha (3.43) and butterfly pea flower-supplemented kombucha (3.40), whereas the lowest pH values were recorded in the control kombucha (2.78) and the roselle-supplemented kombucha (2.82).

The addition of butterfly pea flower, ginger, and

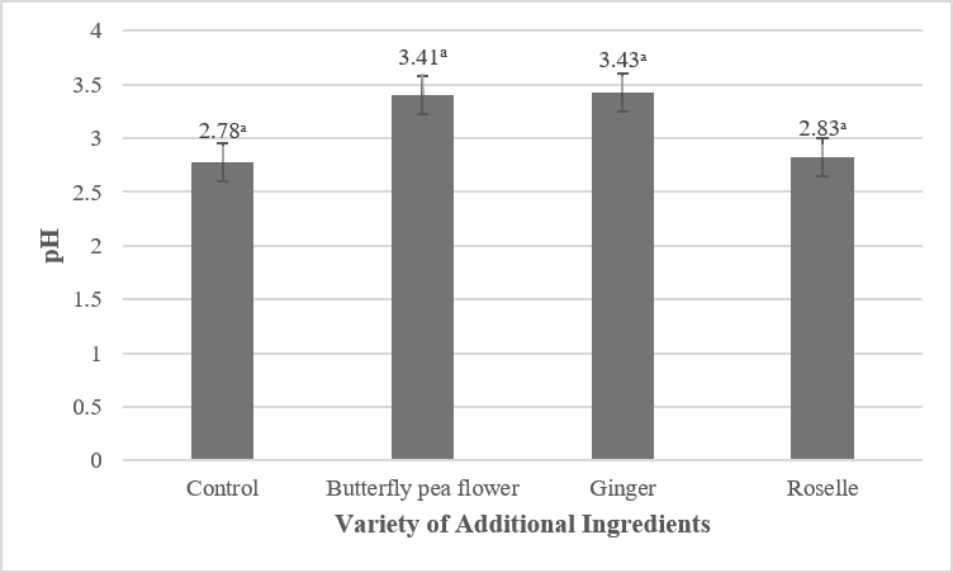


Figure 1. pH values of corn silk kombucha

roselle tended to increase the pH value, indicating a reduction in acidity level of Kombucha. This phenomenon can be attributed to the high bioactive compound content in these additives, which have antimicrobial properties and consequently slow down the conversion of substrates into organic acids. Bioactive compounds act as antibacterial agents through mechanisms such as cell wall degradation, cytoplasmic membrane and membrane protein damage, nucleoid leakage, cytoplasmic coagulation, and proton motive force depletion (Karaca & Newman, 2015).

Sensory Evaluation

The sensory evaluation results demonstrated that the addition of different herbal ingredients significantly affected the colour, odor, and flavour attributes of corn silk kombucha (Table 2). Ginger- and roselle-supplemented kombucha generally received higher sensory scores than butterfly pea flower kombucha. For colour, ginger (3.83 ± 0.06) and roselle (3.75 ± 0.00) showed significantly higher acceptability than butterfly pea flower (3.32 ± 0.03). This result is likely associated with the characteristic pigments of ginger and roselle, which produce more appealing yellowish-brown and red hues, respectively. Ginger contains phenolic compounds, including gingerols and shogaols, that contribute to its characteristic yellowish-brown colour, whereas roselle is rich in anthocyanins responsible for its attractive red pigmentation (Contreras-López et al., 2020; Riaz & Chopra, 2018). In contrast, butterfly pea flower contains ternatin anthocyanins that produce a distinctive blue colour. However, these pigments undergo structural changes in response to acidic conditions during kombucha fermentation, resulting in colour shifts and pigment degradation that may reduce visual appeal and consumer preference (Gamage et al., 2021).

Similarly, ginger (3.43 ± 0.00) and roselle (3.32 ± 0.03) achieved significantly higher odor scores than butterfly pea flower (2.95 ± 0.14). The pleasant spicy aroma of ginger, attributed to volatile compounds such as gingerol, shogaol, and zingiberene, together with the fruity and floral aroma of roselle, may have masked the acidic odor produced during fermentation, whereas butterfly pea flower possesses a relatively mild aroma. For flavour, ginger kombucha obtained the highest score (3.83 ± 0.04), followed by butterfly pea flower (3.68 ± 0.07), while roselle kombucha received the lowest score (3.51 ± 0.01). The lower flavour acceptability of roselle may be attributed to its naturally high organic acid content, which enhances sourness when combined with the organic acids produced during kombucha fermentation. Overall, ginger supplementation provided the most favourable sensory profile, indicating that the selection of herbal additives plays an important role in improving the consumer acceptability of corn silk kombucha (Villarreal-Soto et al., 2023).

Total Titratable Acidity (TTA)

Total titratable acidity (TTA) is an important quality parameter in kombucha because it reflects the accumulation of organic acids produced during fermentation and serves as an indicator of microbial metabolic activity and fermentation progress. During kombucha fermentation, yeasts hydrolyze sucrose into glucose and fructose and subsequently convert these sugars into ethanol. The ethanol produced is then oxidized by acetic acid bacteria into organic acids,

Table 2. Sensory score of corn silk kombucha

Sample Variants	Parameter		
	Colour	Odor	Flavour
Butterfly Pea Flower	3.32±0.03^{b}	2.95±0.14^{b}	3.68±0.07^{a}
Ginger	3.83±0.06^{a}	3.43±0.00^{a}	3.83±0.04^{a}
Roselle	3.75±0.00^{a}	3.32±0.03^{a}	3.51±0.01^{b}

Results are mean±standard deviation; Different superscript letters in the same column indicate the significant differences ($p < 0.05$).

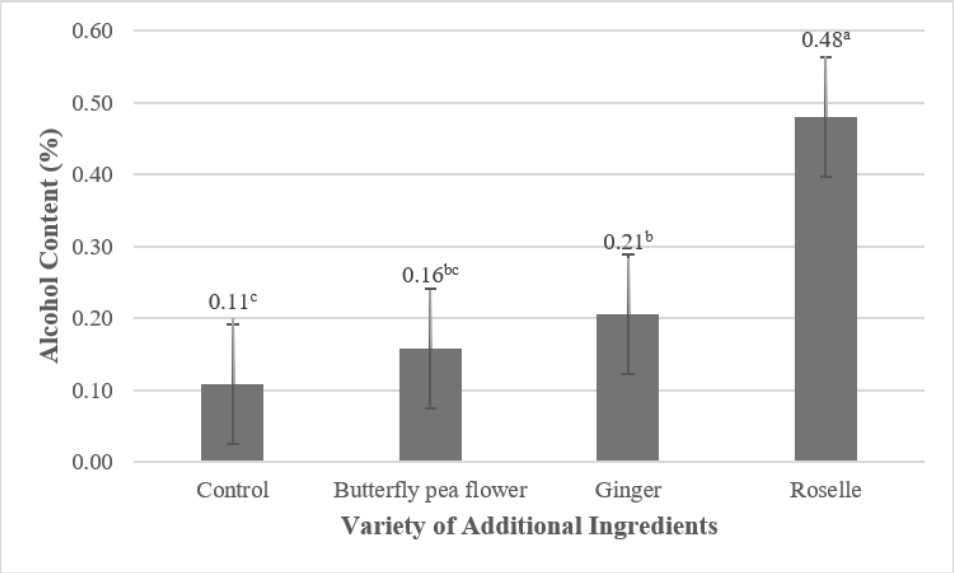


Figure 2. Total Titratable Acidity of Corn Silk Kombucha

primarily acetic acid, gluconic acid, and glucuronic acid, leading to an increase in titratable acidity and the characteristic sour taste of kombucha (Jayabalan et al., 2014; Villarreal-Soto et al., 2023).

The results of this study showed that the total titratable acidity of corn silk kombucha ranged from 0.11% to 0.48% (Figure 2). The highest TTA was observed in the kombucha supplemented with roselle (0.48%), whereas the lowest TTA was found in the control kombucha (0.11%).

The TTA values of kombucha supplemented with butterfly pea flower, ginger, and roselle were 0.16%, 0.21%, and 0.48%, respectively. The higher TTA observed in the supplemented kombucha may be attributed to the additional organic acids and nutrients supplied by the herbal ingredients, which support microbial metabolism during fermentation. These nutrients enhance the activity of yeasts and acetic acid bacteria, resulting in greater production of organic acids.

Among the tested herbal additives, roselle supplementation produced the highest TTA, which is consistent with its naturally high content of organic acids, including citric acid, malic acid, tartaric acid, hibiscus acid, and ascorbic acid. These intrinsic acids directly contribute to the acidity of the fermented beverage in addition to the organic acids produced during fermentation (Riaz & Chopra, 2018). In contrast, ginger contains relatively lower amounts of organic acids than roselle but is rich in carbohydrates, minerals, proteins, amino acids, and phenolic compounds, particularly gingerols and shogaols. These components provide nutrients that support the metabolic activity of microorganisms during fermentation, thereby contributing to the production of organic acids and the increase in TTA (Yang et al., 2024). Similarly, butterfly pea flower contains bioactive compounds and nutrients that can support microbial activity; however, its relatively low organic acid content resulted in a lower TTA than ginger and roselle.

Overall, the increase in total titratable acidity confirms that active fermentation occurred in all kombucha formulations. The differences among treatments indicate that the type of herbal additive influences the accumulation of organic acids through its intrinsic acid composition and nutrient content, thereby affecting the acidity and quality characteristics of the final kombucha beverage (Jayabalan et al., 2014; Villarreal-Soto et al., 2023).

Phenolic Compounds in Corn Silk Kombucha

Phenolic compounds are commonly found in plants and are characterized by the presence of an aromatic ring containing one or more hydroxyl (–OH) groups. In this study, the total phenolic content of corn silk Kombucha was measured using a gallic acid standard curve (µg GAE/g). The results showed that the average total phenolic content of corn silk Kombucha with the addition of butterfly pea flower (373.833 µg GAE/mL), ginger (384.533 µg GAE/mL), and roselle (317.433 µg GAE/mL) was lower than that of the control corn silk Kombucha. Figure 3 presents a direct comparison of the total phenolic content between the control and the additive-enriched Kombucha samples.

The higher total phenolic content observed in the kombucha supplemented with herbal ingredients can be attributed to the naturally occurring phenolic compounds present in the added materials. Roselle is rich in phenolic acids, flavonoids, and anthocyanins, whereas ginger contains abundant phenolic constituents such as gingerols, shogaols, paradols, and zingerone, all of which contribute to its antioxidant properties. Similarly, butterfly pea flower contains high levels of flavonoids and ternatin anthocyanins that contribute to its phenolic content. During kombucha fermentation, microbial enzymatic activity may hydrolyze complex polyphenols and release bound phenolic compounds into simpler forms, thereby increasing the extractable total phenolic content. Consequently, the combination of the intrinsic phenolic compounds from the herbal additives and the biotransformation occurring during fermentation contributed to the higher total phenolic content observed in the supplemented kombucha formulations (Liang et al., 2022; Villarreal-Soto et al., 2023; Jayabalan et al., 2014).

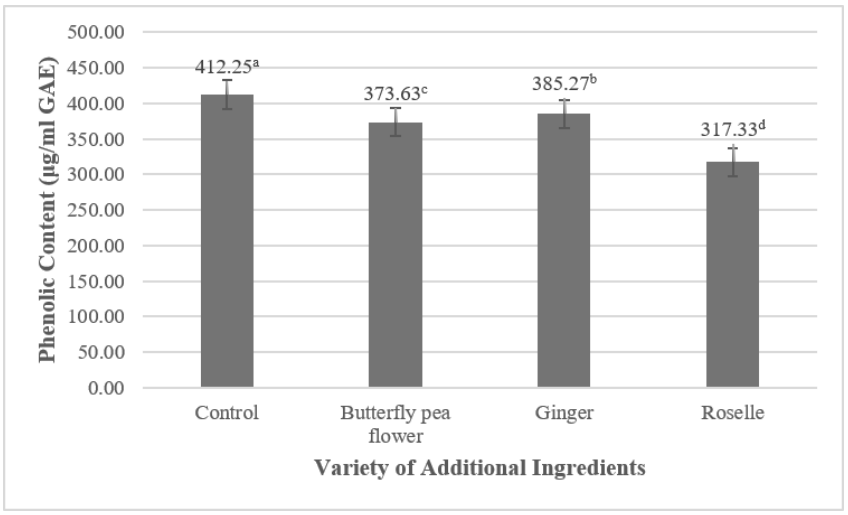


Figure 3. Phenolic Content of corn silk kombucha

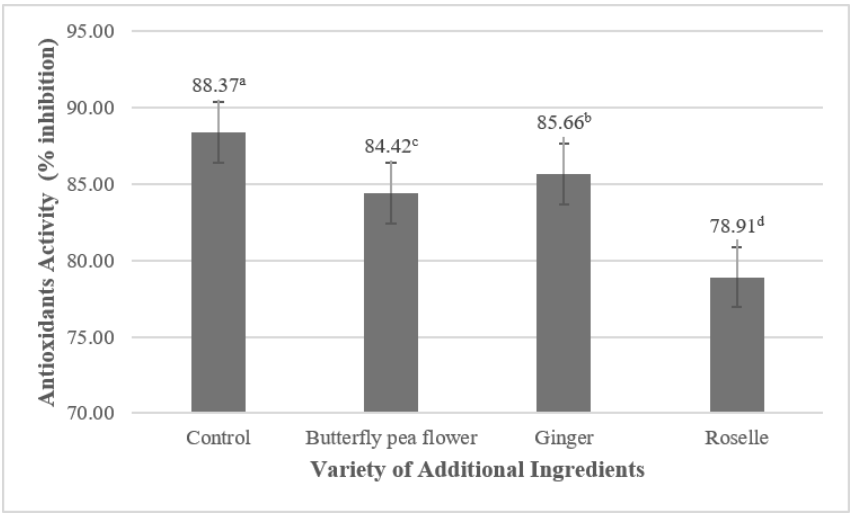


Figure 4. Antioxidants Activity of corn silk kombucha

Antioxidants Activity of Corn Silk Kombucha

The antioxidant activity of corn silk kombucha ranged from 78.58% to 88.37% (Figure 4). The control kombucha exhibited the highest antioxidant activity (88.37%), followed by kombucha supplemented with ginger (85.58%), butterfly pea flower (84.50%), and roselle (78.58%). A similar trend was observed for the total phenolic content (Figure 3), suggesting a positive relationship between phenolic content and antioxidant activity. During kombucha fermentation, microbial enzymes hydrolyze complex phenolic compounds into simpler and more bioavailable forms, thereby enhancing their free radical scavenging capacity (Budiari et al., 2023).

Among the herbal-supplemented kombucha samples, ginger showed the highest antioxidant activity. This result is associated with the presence of stable phenolic compounds, particularly gingerols and shogaols, together with terpenes such as α-zingiberene and β-bisabolene, which exhibit strong antioxidant properties and remain relatively stable during fermentation (Contreras-López et al., 2020). In contrast, the butterfly pea flower kombucha exhibited slightly lower antioxidant activity. Although butterfly pea flower is rich in anthocyanins (ternatins) and flavonoids with recognized antioxidant potential, these pigments are susceptible to degradation under acidic conditions generated during kombucha fermentation, resulting in a reduction of antioxidant capacity (Liang et al., 2024).

Roselle-supplemented kombucha showed the lowest antioxidant activity among the herbal formulations. Roselle contains abundant anthocyanins, phenolic acids, and flavonoids; however, these compounds are also sensitive to prolonged fermentation and acidic environments. The degradation of anthocyanins and oxidation of phenolic constituents during fermentation may reduce their radical-scavenging activity, leading to lower antioxidant inhibition compared with the ginger- and butterfly pea flower-supplemented kombucha (Villarreal-Soto et al., 2023).

Overall, although the incorporation of herbal ingredients influenced antioxidant activity to different extents, all kombucha formulations retained high antioxidant activity (>75%), indicating that corn silk is a promising source of natural antioxidant compounds. The observed differences among treatments suggest that the type and stability of phenolic compounds present in each herbal additive play an important role in determining the antioxidant capacity of the final fermented beverage (Jayabalan et al., 2014).

Conclusion

Based on the findings of this study, Corn silk Kombucha exhibited pH and total titratable acidity that were within safe limits for consumption. Among the tested formulations, corn silk Kombucha supplemented with ginger showed the highest consumer preference based on sensory attributes, including color, aroma, and taste. The pH values of the Kombucha samples were 2.78 (control), 3.40 (butterfly pea flower), 3.43 (ginger), and 2.82 (roselle) While the total titratable acidity ranged from 0.11% to 0.48%. The highest total phenolic content was observed in the control kombucha (412.250 µg GAE/mL), whereas the lowest was found in the roselle Kombucha (317.433 µg GAE/mL). In contrast, kombucha supplemented with ginger exhibited the highest antioxidant activity (88.37%). Overall, the addition of ginger improved consumer acceptability in terms of color, aroma, and taste while maintaining relatively high total phenolic content and providing the greatest antioxidant activity among the tested formula.

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