Original Research Article

HPLC characterization and sensorial analysis of kombucha based synbiotic beverage infused with chicory root powder *(Cichorium intybus)*

Abstract

Probiotic and prebiotic drinks are promoters of the growth and maintenance of healthy intestinal microbiota composition. In spite of that, a product that gathers the medicinal attributes of symbiotic multiple yeast- bacteria colonies, like Kombucha, has not yet been explored and appears to be potentially profitable for human. "Kombucha" is a beverage created by brewing black or green tea and sugar using a SCOBY (Symbiotic Culture of Bacteria and incentive), generally for 7 – 21 days. Quantification of the many constituents that make up our food, whether they are desirable (vitamins, antioxidants, nutrients) or undesirable (pesticides, toxins), is one of the most practical applications of chemistry. A low- sugar kombucha beverage using chicory root powder as fermentation substrate with sensorial acceptability compatible with the traditional Kombucha was developed in the present study and analyzed using high-performance liquid chromatography (HPLC). Kombucha produces acetic acid in addition to a variety of other organic acids and vitamins. The aim of this study was to analyze the acid content of kombucha over a 21-day fermentation period in order to characterize the fermentation kinetics. The organoleptic characteristics such as appearance, odor, flavour, and over all acceptability of the sample were analysed using 9-point hedonic scale. Advancements in the aroma and taste are necessary for acceptable Chicory infused Kombucha beverage.

Keywords: *Kombucha, Sensory analysis, HPLC, Chicory root, SCOBY, Chromatography*

1. Introduction

One of the most well-known low-alcohol fermented beverages in the world, kombucha is reportedly the product with the fastest rising market share in the functional beverage category. A symbiotic colony of bacteria and yeast (SCOBY) ferments sugar-dissolved black tea to make kombucha, a beverage that has been manufactured using this fermentation process for centuries. Another name for this bell-shaped cellulose SCOBY culture is "tea fungus." The beverage referred to as "kombucha" is made by fermenting (either black or green) and sugar using a SCOBY (Symbiotic Culture of Bacteria and Yeast), usually for 7 to 21 days. [1] The SCOBY, a biofilm of bacteria that resembles a mushroom, is used as a starter for additional batches of brew. Numerous acetic acid bacteria, including *Acetobacterxylinum*, *Acetobacteraceti*, *Acetobacterpasteurianus*, and *Gluconobacteroxydans*, are found in the SCOBY, along with yeasts including *Saccharomycessp*., *Zygosaccharomyces kombuchaensis*, *Torulopsissp*.,*Pichiaspp*., *Brettanomycessp*. [2] “Additionally, a number of lactic acid bacteria have been identified. Components of kombucha after fermentation include sugars, polyphenols, organic food acids, fiber, ethanol, and amino acids like lysine, essential elements such as Cu, Fe, Mn, Ni, and Zn; water-soluble vitamins such as vitamin C, and several B vitamins; carbon dioxide; antibiotic substances; and hydrolytic enzymes”. [3]

“When freshly made, this fermented drink has a pleasant, sparkling apple cider flavor. An extended fermentation causes the effervescent beverage to develop an acidic flavor that is similar to vinegar. The microorganisms most related with the probiotic function are bacteria like *Lactobacillus*, *Bifidobacterium*, *Bacilluscereus*, *Propionibacterium freudenreichii*, and yeasts of the genus *Saccharomyces*. These microorganisms present benefits in terms of the microbiome, but they also produce other relevant mixtures, like amino acids, organic acids, sugars, polyphenols, and vitamins. Some other micronutrients, also formed during the fermentation process, foster Kombucha’s implicit benefit. Several in vivo or in vitro studies, especially with animals and, more lately, with humans, have reported some benefits of this product. There are numerous health promoting results reported in the literature, such as anti-inflammatory, antioxidant, anti-bacterial, anti-diabetic, anticarcinogenic activity; reducing the levels of cholesterol; and enhancing the liver metabolism, the immune system, and gastrointestinal functions”. [4,5]

Due to its high antioxidant content, kombucha has proven health benefits, including helping with digestion, infections, stress, and even cancer. Polyphenols, which are natural antioxidants found in tea, are said to be increased during kombucha's fermentation process. These serve as antioxidants by dissipating free radicals and reducing the harmful buildup of reactive oxygen species. Reactive oxygen species are synthesized in excess due to unhealthy lifestyle choices, severe physical activity, stress, and environmental pollution. Free radicals' disruption of homeostasis causes the development of oxidative stress, which harms the human body's structural components. Atherosclerosis, neurological conditions like Parkinson's or Alzheimer's disease, and even obesity are conditions that can be brought on by free radical problems.[6-9] It is essential to look for readily available sources of antioxidants to maintain the equilibrium between the generation and elimination of reactive oxygen species. The major and most common antioxidants include polyphenolic chemicals and the vitamins E, A, and C.One class of polyphenols found in tea is called flavonoids, and the flavonoids catechin, epicatechin, and epigallocatechingallate (EGCG) are known for their antioxidant and antibacterial activities. Most teas naturally include caffeine, which has antioxidant benefits. [10,11]

In a research published in 2019, Kumar et al. investigated “the effects of chicory root extract addition on the physico-chemical characteristics of multifunctional synbiotic yoghurt-ice cream. The study showed how adding chicory root extract affected the physical and chemical characteristics of yoghurt ice cream with probiotics in capsules and came to the conclusion that it increased the product's therapeutic value. The plant chicory (*Cichorium intybus* L.), which is a member of the Asteraceae family, can be utilized as flowers, leaves, or roots. The main non-digestible dietary component in chicory root is inulin, a fructose polymer with a (2-1) glycosidic bond that feeds probiotic microorganisms. Although inulin and fructooligosaccharides (FOS) have similarities, they have different chemical structures.FOS chains of molecules are shorter than inulin chains. A food product containing properties of both, probiotic and prebiotic is called a synbiotic which offers prophylactic management of gastro-intestinal defects”. [12,13]

“Functional beverages added with live microorganisms (probiotics) and non-digestible food or ingredients that modulate the intestinal microbiome (prebiotics) have been considered as an increasingly important topic on human nutrition, exerting together a synergetic effect (synbiotics) on promotion. Foods with these properties are part of the food and human technology development. Products like yogurt and wine are typically cited as the oldest in the Western world, while soy and are part of the eastern traditional fermented foods”. [12]

The main objectives of this study were; development of chicory infused kombucha and its comparison with the standard kombucha on the basis of physicochemical properties, sensory analysis of both the samples, and HPLC characterization of chicory infused kombucha to analyze its acid content.

2. Materials and methods

2.1 Preparation of beverages

"Two different samples of kombucha were prepared using different tea bases and composition. SCOBY (Symbiotic Culture of Bacteria and Yeasts) was added to both the samples after preparing the sugar- tea base. First sample was prepared by dissolving 10g of black tea and 40g sugar in 500 ml boiling water, labeled as Standard Kombucha (SK). The second sample, labeled as Chicory Infused Kombucha (CK), was prepared with 500 mL boiling water, 5g black tea, 5g of green tea, 10g sugar and 40g of chicory root powder. For both the samples, tea leaves were set in infusion for 5 minutes; filtered, transferred to two previously sterilized glass jars and was left to chill to room temperature. Afterwards, live starter culture of kombucha along with the thin layer of SCOBY was added to both the samples and the jars were covered with the plastic lid. The lid was not tightened all the way to ensure proper growth and oxygenation of the colonies. Both the jars were then stored in a cupboard sheltered from light at uncontrolled room temperature (ranging from 25 to 30°C) and left in the fermentation process for 14 days". [7]

2.2 Physicochemical Analysis

Samples containing 10-30 mL aliquots were taken on 0, 4, 8 and 14th days of first fermentation from both standard kombucha and chicory infused kombucha for physicochemical analysis. Properties like pH, titratable acidity and total soluble solids (TSS) were assessed for both the samples for comparison. [9] "Potential of hydrogen (pH) values were determined by using a calibrated pH meter, read after stabilized. Degree Brix (°Bx) was used to measure the amount of total soluble solids (TSS) in both the samples and was measured in a digital refractometer". [10] For titratable acidity, 10 mL aliquots were taken and titrated against 0.1N NaOH. Titratable acidity was calculated in terms of % acetic acid.

2.3 HPLC Analysis of Acetic Acid and Caffeine

"HPLC analysis was performed on samples fermented for 21 days using a Shimadzu DGU-28 series liquid chromatograph system equipped with a SPD-20A photodiode array detector. Samples were separated with a Phenomenex C18 reverse-phase column (5 μm, 150 mm × 4.6 mm) using a low pressure gradient mobile phase of 0.1% H3PO4 in H2O with a 20−40−20 MeOH gradient. The flow rate was 0.75 mL/min, and the total run time was 10 min. The column was operated at room temperature, and the sample injection volume was 20 μL. Standard additions were prepared for acetic acid and caffeine. All samples were filtered prior to HPLC analysis using a 0.45 μm filter". [11]

2.4 Sensorial analysis

"A panel of 22 untrained people was selected from the college for assessing the standard kombucha as well as chicory infused kombucha. The organoleptic characteristics such as appearance, odor, flavour, and over all acceptability of both the samples were analysed using 9-point hedonic scale. The hedonic scale was ranked as follows: like extremely to very much (8-9 scores), like moderately to like slightly (5- 7 scores), neither like nor dislike to dislike slightly-dislike moderately (2-4 scores) and dislike very much to dislike extremely (0-1 score)". [2,13]

2.5 Data Analysis

The data were arranged in a spreadsheet, treated and analyzed using Microsoft Excel. Results are represented as mean ± standard deviation.

3. Results and discussion

3.1 Analysis of physicochemical properties

"The development of Chicory infused Kombucha and its comparison with the standard kombucha on the basis of physicochemical properties was studied. It is known that the fermentation of traditional Kombucha depends on many factors, mostly as temperature, pH, time, quality and concentration of tea and sugar substrates. The physicochemical characteristics of a product are essential for its development and acceptance". [13,14]

Table 1 Comparison between physicochemical properties of Standard Kombucha and Chicory infused kombucha

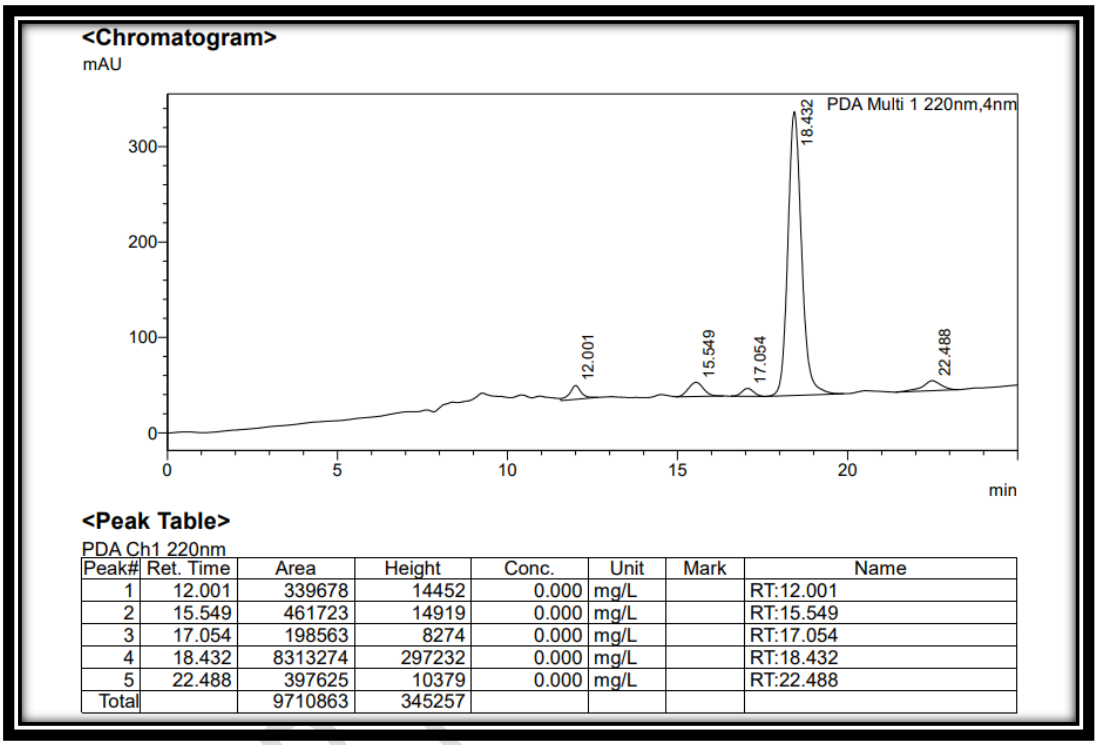
	Standard Kombucha				**Chicory infused Kombucha**			
No. of days	1	4	8	14	1	4	8	14
pH	3.91±0.02	3.63±0.02	3.45±0.02	3.33±0.02	3.96±0.02	3.57±0.02	3.41±0.02	3.21±0.02
Titratable acidity (%)	0.26±0.031	0.34±0.031	0.49±0.031	0.90±0.031	0.45±0.031	0.51±0.031	0.60±0.031	0.72±0.031
Degree Brix	7.8±0.41	7.6±0.36	7.2±0.16	6.0±0.34	7.6±0.42	7.2±0.25	6.4±0.18	5.9±0.47

The results indicated that "the longer the time left in fermentation, the greater the final acidity of Standard Kombucha (SK) and Chicory infused Kombucha (CK), both for pH, and titratable acidity. It was observed that SK and YK started at day zero with nearly the same pH and acidity. As the fermentation process increased, the drinks became more acidic, as expected". [13]

"The titratable acidity was calculated in terms of % acetic acid as it is the predominant acid present in kombucha along with other organic acids like gluconic acid, ascorbic acid, lactic acid etc. In regular sugar-Kombucha fermentation, the initial hydrolysis of sucrose is attributed to the action of yeasts breaking it down into glucose and fructose. As fermentation progresses, fructose is used anaerobically to produce ethanol and, later, acetic acid, while acetic bacteria use glucose and ethanol to produce gluconic acid and acetic acid, respectively". [14] This allows acid concentrations to increase and consequently lower the pH, as observed here for both SK and CK. The sample containing chicory (CK) started with higher acidity percentage and showed a gradual increase in acidity. However, at the end of the 14 days, SK was more acidic than CK.

3.2 HPLC characterization of chicory infused kombucha

"Acetic acid and caffeine were monitored at 210 and 273 nm, respectively. Under these conditions, acetic acid eluted at 12.001 ± 0.01 min and caffeine eluted at 18.432 ± 0.08 min (Figure 1). The method of standard addition was used both to identify the acetic acid and caffeine peaks and to quantify the amounts of acetic acid and caffeine in kombucha samples fermented for 14 days. For quantification, three separate samples (kombucha and two standard additions) were analyzed twice each. The peak heights were plotted as a function of the concentration of the added standard, and the plots were then analyzed". [15]



<Peak Table>
PDA Ch1 220nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	12.001	339678	14452	0.000	mg/L		RT:12.001
2	15.549	461723	14919	0.000	mg/L		RT:15.549
3	17.054	198563	8274	0.000	mg/L		RT:17.054
4	18.432	8313274	297232	0.000	mg/L		RT:18.432
5	22.488	397625	10379	0.000	mg/L		RT:22.488
Total		9710863	345257				

Figure1. HPLC analysis of a kombucha sample fermented for 21 days

3.3 Sensorial analysis

"The aromatic compounds in kombucha can be divided into three categories according to their origin: native from the used matrix (tea constituents and derived ingredients), from the saccharide source substrates, and from the metabolites produced by the microorganisms during fermentation. The composition and concentration of VOCs vary according to the fermentation stages of the kombucha as well as the fermentation temperature. Furthermore, the production of sapid metabolites such as organic acids during fermentation also adds to the overall sensorial experience. The main VOCs reported in the literature are classified into six different families, namely carboxylic acids, alcohols, aldehydes, ketones, esters, and benzenoids". [2,13]

The hedonic scale rating of chicory infused kombucha in terms of appearance, odor, flavor and overall acceptability:

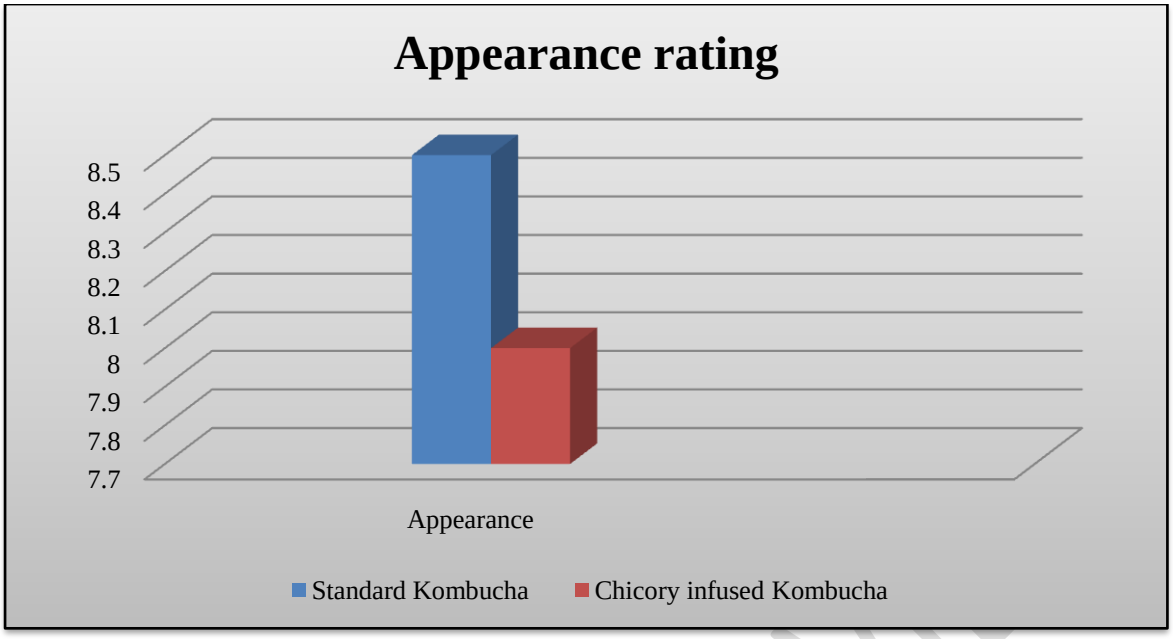


Figure2. Appearance hedonic rating

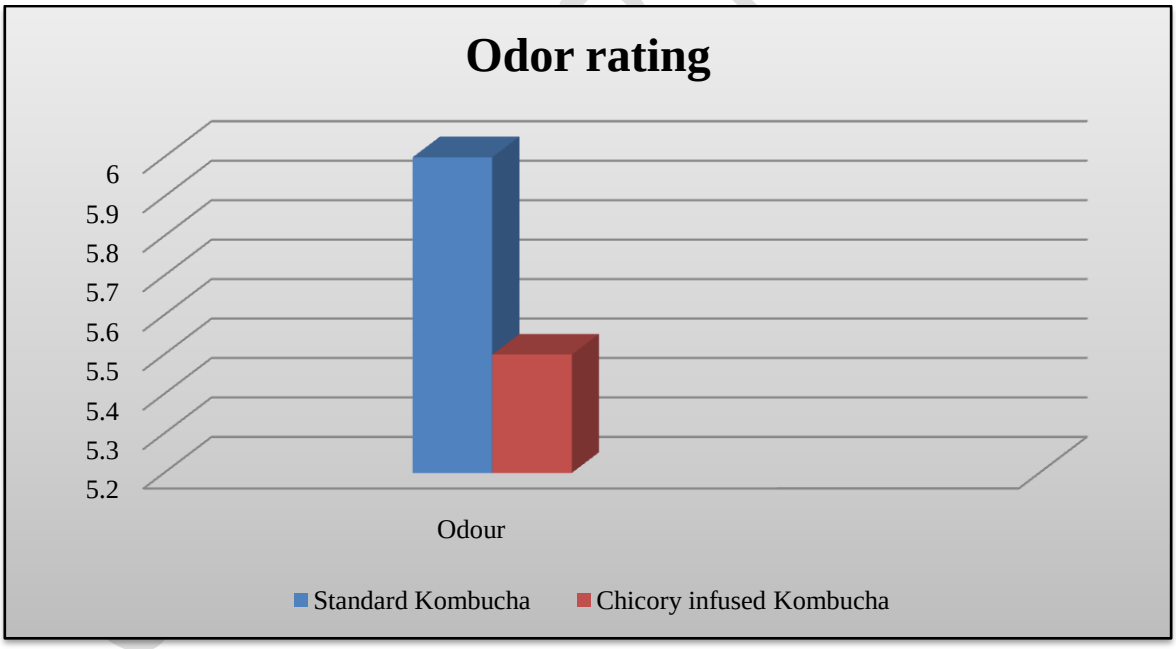


Figure3. Odor hedonic rating

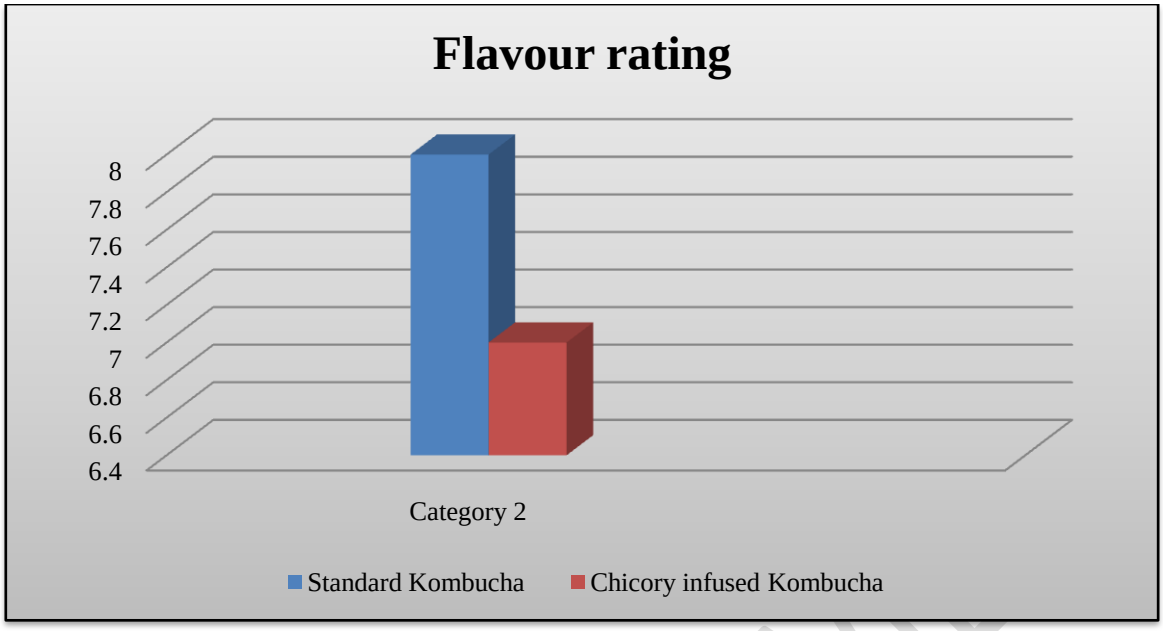


Figure4. Flavor hedonic rating

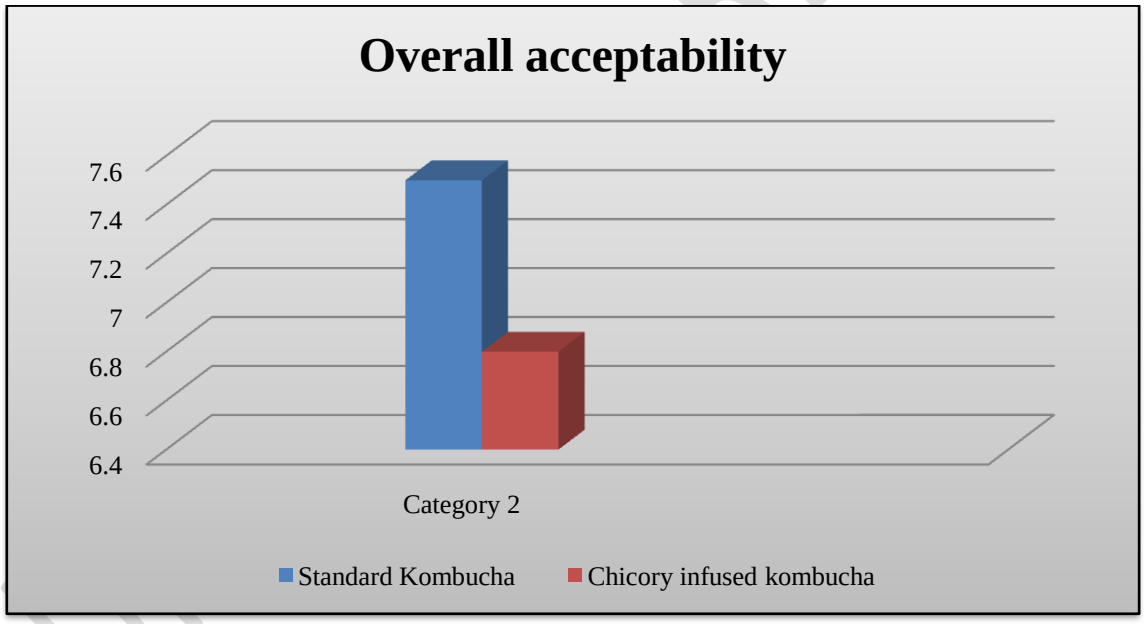


Figure5. Overall acceptability hedonic rating

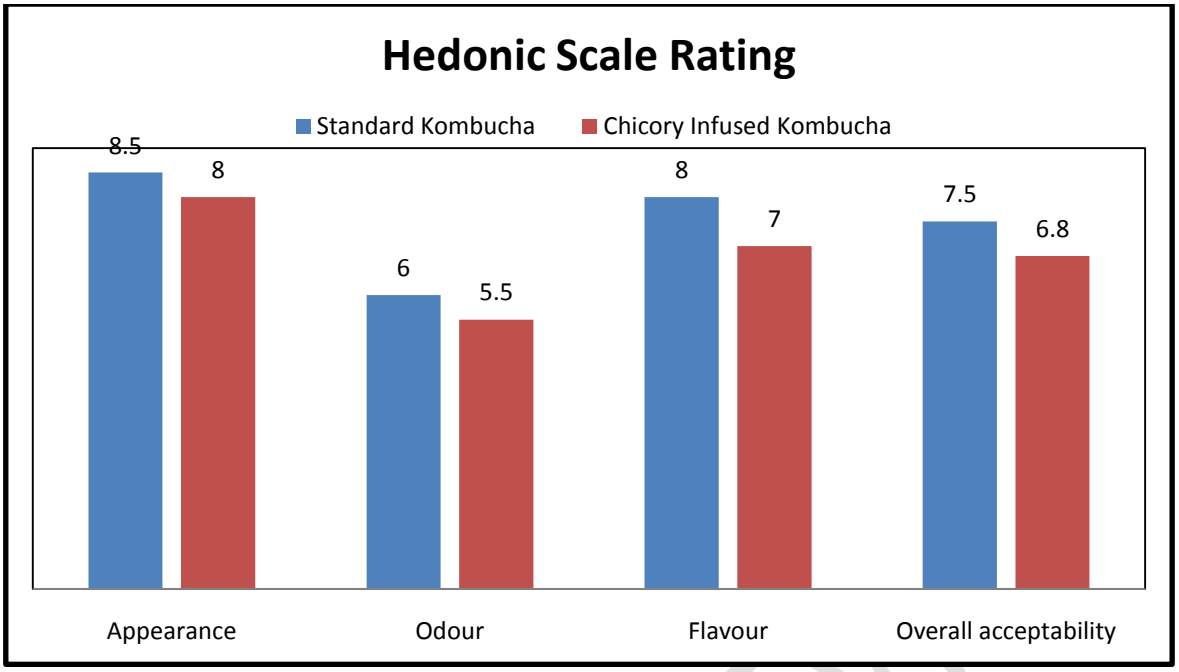


Figure6. Comparison of sensory characteristics between Standard Kombucha and Chicory infused kombucha

4. Conclusion

The development of an acceptable Kombucha drink prepared with Chicory root powder and less amount of sugar was possible due to fructose-rich FOS content, capable of providing conditions for fermentation. The HPLC characterization of acetic acid and caffeine was done in chicory infused kombucha. More initiatives should explore the whole chemical composition of beverages using precise analytical techniques such as mass spectrometry and NMR spectroscopy. This will enable the enhancement of the sensory properties of this drink, which combines the medicinal properties of Kombucha and Chicory in a single synbiotic beverage with the potential to benefit human health.

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