DEVELOPMENT OF KOMBUCHA POWDER WITH SUGAR SWEETENERS USING FREEZE-DRYING AND SPRAY-DRYING METHODS

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ABSTRACT

Kombucha is a fermented drink produced through the metabolization of sugar by a symbiotic colony of acetic bacteria and yeast. This beverage has well-documented antioxidant and probiotic properties, which are highly beneficial to human health and have been extensively investigated. Its growing popularity has led to an increase in home production. However, the lengthy production process makes it impractical for many people, creating a need for a soluble powder product that is quick and efficient to prepare while maintaining its bioactive properties. One of the major challenges in developing this powdered product was the caramelization of residual sugar during the freeze-drying or spray-drying process. To overcome this, different amounts of sugar and various sweeteners were tested until a suitable combination was found that could be successfully dried without compromising the drink's beneficial properties.

Keywords: Kombucha. Freeze-drying. Spray-drying. Sugar. Caramelization.

1 INTRODUCTION

Kombucha is a fermented drink made from black or green tea, sugar and a colony of bacteria and yeast called a SCOBY (Symbiotic Culture of Bacteria and Yeast). Due to its sweet and sour taste and potential health benefits, Kombucha has gained popularity as a healthy alternative to soda and other sugary drinks [1]. The microbiota that makes up the symbiotic consortium responsible for producing the fermented drink is generally made up of acetic acid bacteria and yeast. The predominant acetic acid bacteria in kombucha are *Acetobacter xylinum, Acetobacter xylinoides, Bacterium gluconicum, Acetobacter aceti and Acetobacter pasteurianus*. The most common yeasts are *Schizosaccharomyces pombe, Saccharomycodes ludwigii, Kloeckera apiculata, Hanseniaspora guilliermondii, Saccharomyces cerevisiae, Zygosaccharomyces bailii, Torulaspora delbrueckii, Brettanomyces bruxellensis, Brettanomyces lambicus* and *Brettanomyces custersii*, in addition to Candida stellate [2].

Studies have shown that Kombucha may have beneficial health properties, including improving immune function, reducing the risk of heart disease, and lowering blood glucose levels in diabetics [3]. Studies the immunomodulation capacity of Kombucha, which is important in defending against pathogenic microorganisms in the gastrointestinal tract. Gut epithelial cells play an important role in regulating the response to microbiota through pattern recognition receptors (PRRs) that recognize pathogen-associated molecular patterns (PAMPs), evolutionarily conserved molecules expressed on microbial surfaces [4]. Immunomodulation can be influenced by the presence of probiotics, such as *Bifidobacterium animalis* and *Lactobacillus casei*. As well as the potential of probiotics present in this microbiota to inhibit pathogenic bacteria. Several studies have investigated the effects of adding yerba mate to kombucha. Evaluating the addition of yerba mate, Kombucha showed that the addition of yerba mate improved the flavor and aroma of the drink and increased its antioxidant activity [5].

Kombucha powder is a convenient form of the drink as it makes preparation, transportation and storage easier. Optimizing the Kombucha powder production process can help reduce costs, improve efficiency and minimize product variation. To do this, it is necessary to evaluate the ideal fermentation conditions, such as temperature, fermentation time, amount of SCOBY and sugar concentration. It is also important to analyze the chemical composition of the drink, including the amount of acetic acid, antioxidants and probiotics present. Recent studies have explored different strategies to optimize the Kombucha powder production process, such as adding different types of sugar, using different types of tea and varying fermentation conditions [6]. In a study to evaluated the effects of fermentation time on the quality and antioxidant activity of Kombucha. The results showed that a longer fermentation period led to greater antioxidant activity and a higher concentration of acetic acid and other compounds beneficial to health. This type of research is important to help determine the ideal fermentation conditions to produce Kombucha powder with the best quality and highest concentration of nutrients. This research is crucial to ensure the safety and quality of the final product and to meet consumers' growing demands for healthy and convenient foods [7].

2 MATERIAL & METHODS

The production of Kombucha was conducted in an artisanal way, following the methodology adopted by [2]. The process was carried out in the standard using a 1.5 liter glass jar with 9cm diameter opening, covered with a porous Perfex-type cloth to allow air passage while preventing contamination by insects and/or physical dirt. The experiments were carried out in duplicate for both yerba mate and green tea substrates, and the jars were kept out of direct light at room temperature (25 to 28 ºC). [2] used a formulation of green tea and yerba mate for kombucha production but did not specify the concentration of each. In the initial tests

of this study, a 50% yerba mate and 50% green tea formulation was used. However, the strong flavor and bitterness of yerba mate led to poor acceptance. Consequently, a new concentration of 70% green tea and 30% yerba mate was tested and adopted for its more palatable and balanced taste, though this formulation may be adjusted based on sensory analyses. Preliminary tests were conducted to define the formulation of the kombucha, with yerba mate. The amounts of sugar in grams will not be disclosed. The tests were carried out in accordance with a full factorial planning 2^2 with three central points (CP), **(Tables 1 and 2)**.

Table 1 Levels of independent variables

Levels of independent variables	- 1	0	+ 1
Sugar (%)	X	2X	3X
Fermented liquid (%)	3*	6*	9*

* The levels were confirmed from preliminary tests

Table 2 Experimental planning matrix

Test	Sugar (%)	Previous fermented liquid (%)
1	-1	-1
2	+1	-1
3	-1	+1
4	+1	+1
5	-1	+1
6 (CP)	0	0
7 (CP)	0	0
8 (CP)	0	0

In the adopted formulation (10% previous fermented liquid and X% sugar), refined sugar was used. After 14 days of fermentation, the product was filtered through cotton. One part was frozen at -40°C for 24 h before undergoing freeze-drying, while another part was dried using a spray dryer. Both methods resulted in caramelization of the sample, attributed to unmetabolized residual sugar. To address this issue, a new test was proposed: sugar levels were reduced by 1%, and alternative sweeteners were tested, including xylitol, erythritol, and stevia, each at a 10% concentration. These samples were fermented for 14 days, filtered using a vacuum filtration pump with a qualitative filter, and then subjected to freeze-drying and spray drying. These methods were still insufficient, so a 7-day anaerobic fermentation period was added, which significantly reduced caramelization. Success was finally achieved at an X% concentration of refined sugar, combined with 14 days of aerobic fermentation followed by 7 days of anaerobic fermentation. After optimizing the formulation, it was tested in triplicate, each in a volume six times larger, with a two-week interval between each batch.

3 RESULTS & DISCUSSION

This study aimed to determine an efficient dosage of sugar or sweetener for kombucha production that could be fully metabolized by the microbiota, eliminating residual sugar or sweetener and preventing caramelization during freeze-drying or spray-drying. When testing the sweeteners xylitol, erythritol, and stevia at concentrations of X%, 2X%, and 3X%, it was found that these compounds were poorly absorbed and metabolized, resulting in insignificant growth of the SCOBY. During the drying process, these samples caramelized under both freeze-drying and spray-drying, leading to negative results. Due to the low absorption of these sweeteners by the symbiotic colonies, lower dosages were not tested. For the sugar samples, prepared with concentrations of 2X-1, 2X-2, 2X-3, 2X-4, X, X-1, X-2, and X-3 of refined sugar, the drying process was successful only at the X% dosage. This concentration allowed for complete drying and acceptable growth of the symbiotic colony, while lower doses delayed development.Triplicates of the formulation were prepared and kept at room temperature, allowing for the observation of temperature's influence on sugar metabolization and the drying process. The batch prepared during colder days failed in the drying process but succeeded when incubated in an oven at 37ºC.

Regarding the product's bioactive properties after drying and rehydration, it was found that the rehydrated product had a weaker flavor and lacked acidity, although gas production was observed. Flavor adjustments will be made in stages. Both drying processes preserved the bacteria, although different species were found in each process, while the yeast did not survive. [8] conducted a physicochemical and microbiological evaluation of kombucha and its freeze-dried derivatives, finding that drying reduced microbiological counts compared to the SCOBY and wet precipitate. SCOBY dehydration resulted in a more significant reduction, while freeze-drying was less harmful to microorganisms. The dehydrated precipitate was similar to the SCOBY and the freeze-dried precipitate in terms of total bacteria, acetic bacteria, fungi, and yeast counts. Both freeze-drying and dehydration reduced the counts of total bacteria, acetic bacteria, fungi, and yeast to a viable level for human consumption. Products derived from kombucha SCOBY were considered safe for human consumption, as confirmed by an "*in vivo*" test with the *C. elegans* nematode.

4 CONCLUSION

Both drying processes proved to be efficient in processing the samples within the appropriate formulation and using refined sugar, as well as all the sweeteners tested caramelized and were not efficient in the development of the symbiotic colony. Among the sugar dosages, drying was successful at dosages below X% and this in itself was sufficient for the development of SCOBY. Therefore, it can be seen that the dosage of X% refined sugar is ideal and capable of being transformed into powder after including the period of anaerobic fermentation at temperatures above YºC and filtering.

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