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EVALUATION OF JAMBOLAN EXTRACT AS AN ALTERNATIVE SUBSTRATE FOR KOMBUCHA BEVERAGE PRODUCTION

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ABSTRACT

The use of alternative substrates for the development of kombucha beverages has gained great interest. Jambolan is a fruit known for its high nutritional value and bioactive compounds content. The present study aimed to evaluate the potential of jambolan extract as a new substrate for the elaboration of kombucha-type beverages. Jambolan extract was prepared by immersing freeze-dried jambolan pulp powder in hot water. Green tea extract was used as a control. Fermentation occurred under aerobic and static conditions, at room temperature, for 10 days. Samples were collected on days 0 and 10 of fermentation, and analyses of pH, total acidity, °Brix, viable cell count, as well as antioxidant capacity by the DPPH and ABTS radical were carried out. After 10 days of fermentation, jambolan kombucha showed a low decrease in pH and low concentration in total acidity, indicating possible inhibition of acetic acid bacteria by the extract. Besides that, it was observed biological activity in jambolan kombucha by °Brix reduction, as well as the growth of viable cells. Antioxidant activity was reduced after fermentation in both kombuchas. Jambolan kombucha reached radical scavenging activity values of 35% for the DPPH radical and 47% for the ABTS radical, after 10 days of fermentation. Despite the high potential to add nutritional value to beverages, jambolan extract was not a good substrate for kombucha beverage elaboration.

Keywords: *Syzygium cumini (L.), Camellia sinensis*, Antioxidant, Fermentation, Nutraceutical, Tropical Fruits

1 INTRODUCTION

The current interest by consumers in functional beverages has inspired the food industry to develop new products. In this context, Kombucha has been gaining popularity due to its potential health benefits and sensory acceptance.[1] The beverage is obtained through the fermentation of sweetened teas, commonly infusions of *Camellia sinensis* and sucrose, by a symbiotic culture composed of acetic acid bacteria, yeast, and occasionally lactic acid bacteria.[2] The inoculum is composed of a combination of fermented liquid and cellulose biofilm, obtained in previous fermentation, or just fermented liquid, which plays an important role in reducing pH and inhibiting pathogenic microorganisms. The fermentation process is carried out under static and aerobic conditions, at average temperatures of 28° C and fermentation time between 8 to 15 days.[3]

Kombucha fermented tea has a chemical profile mainly composed of sugars, organic acids, alcohols, as well as micronutrients and antioxidant molecules.[4] The functional properties of kombucha is mainly related to tea polyphenols (epicatechin, epicatechin3-gallate, epigallocatechin and epigallocatechin-3-gallate) and metabolities produce during fermentation, such as organic acids (glyconic and glucuronic acid) and vitamins.[5,6] Because of that, kombucha has been known to promote a wide range of health benefits, including antimicrobial, anti-inflammatory, detoxifying effects, among others.[7] Although green tea and/or black tea are tradicionally used to prepare Kombucha, the preparation of Kombucha beverages using alternative raw materials, such as fruits and vegetable co-products has been proposed. [8, 9,10]

Jambolan (*Syzygium cumini* (L.)), also known as Indian black plum and java plum, is a member of Myrtacea family. The fruit is known for its high nutritional value, nutritionally rich in sugars (glucose, raffinose, fructose), vitamins (vitamins A and C), phenols, flavonoids and organic acids.[11,12] Because of that, jambolan has received recognition for its health-relevant biological effects, such as antioxidant activity, antimicrobial activity, antidiabetic, diuretic, among others.[13] Despite its high nutritional value and nutraceutical potential, jambolan is still a fruit with modest consumption.[14] In this context, the objective of this work was to evaluate the potential of jambolan extract as a new substrate for kombucha beverage fermentation, evaluating the physicochemical and microbiological characteristics as well as antioxidant activity after 10 days of fermentation.

2 MATERIAL & METHODS

Preparation of jambolan and green tea kombucha

Jambolan kombucha (JBK) was obtained according to the protocol described by Morales[15], with some modifications. Jambolan infusion was prepared using 1,2% (m/v) lyophilized jambolan pulp powder in hot water (85 °C), together with 7% (m/v) of crystal sugar, followed by filtration of mixture in qualitative filter paper. To compare jambolan kombucha with traditional commercial kombucha, a green tea kombucha (GTK, control) was prepared with 0.6% (m/v) of *Camellia sinensis* and 7% (m/v) of crystal sugar, by immersion of leaves in hot water (85 °C) during 5min, followed of filtration. After reaching room temperature, 10% (v/v)

of inoculum, composed of fermented tea kombucha, was added to the sweetened infusions. Fermentation was performed at room temperature (28 °C ± 4) under aerobic conditions in sterilized erlenmeyers (100mL) covered with paper towels, for 10 days. To analyze the effects of fermentation in jambolan beverages, sweetened jambolan extract (JBE) without kombucha inoculum was prepared and kept under the same conditions. The samples were collected on days 0 and 10 of fermentation for further analysis.

pH, soluble solids and total acidity

The pH values of the samples were measured using a pHmeter according to AOAC.[16] The total soluble solids were determined as °BRIX in a digital refractometer (VGS-C10 Abbe Refractometer, Novatech International). Total acidity (TA) was obtained by titration of diluted samples with standardized 0.01N NaOH solution and phenolphthalein indicator, following the analytical standards of IAL.[17] The results were expressed as grams of acetic acid by liter.

Enumeration of viable cells

Viable cell counts were estimated by the CFU method (Colony Forming Unit), using the plating method. Nine milliliters of 0,1% (w/v) peptone water solution were used to dilute the sample. After serial dilution, 0.1mL of the samples were spread, with a Drigalski strap, in Petri dishes containing the growth media. The total number of aerobic bacteria was determined in Plate Count Agar (Oxoid, Basingstoke, UK), with an incubation time of 72h and temperature of 30 °C. The results were expressed in CFU/mL.

Antioxidant activities

Antioxidant activities were assessed by ABTS and DPPH radical scavenging assays, according to protocols described by Embrapa[18], with some modifications. The samples collected on days 0 and 10 of fermentation were filtered (0.20 µm, Sartorius Stedim) before analysis. The ABTS radical reaction was prepared from a mixture of 40 μL of diluted sample and 260 μL of ABTS solution, in a microplate reader (Asys UVM 340, Biochrom, Singapore) with a wavelength of 714 nm. The antioxidant capacity by the DPPH method was carried out by mixing 40 μL of diluted sample and 200 μL of 0.06 mM DPPH methanolic solution, with time reaction of 20 minutes at room temperature. The absorbance was measured with a wavelength of 517 nm. The antioxidant capacity was expressed as the percent inhibition of radicals, determined by difference between absorbance of control and absorbance of samples.

Statistical analysis

Statistical analysis was evaluated using Statistica v 7.1 package (StatSoft, Tulsa, OK, USA). The results were subjected to analysis of variance (ANOVA) and post-hoc test (Tukey, α = 0.05). All measurements were conducted in triplicate.

3 RESULTS & DISCUSSION

According to Table 1, jambolan kombucha showed a lower pH reduction when compared to green tea kombucha. The pH of green tea kombucha significantly reduces after 7 days of fermentation to values above 3.[19] The pH considered safe for human consumption is below 4.2 [20], value reached by both kombuchas. During fermentation, pH reduction and increase of acidity indicate the production of organic acids, metabolized mostly by acetic acid bacteria. This class of microorganisms oxidizes alcohol into acetic acid, and consumes glucose, producing mainly acetic acid. The production of other organic acids, such as gluconic acid, glucuronic acid, lactic acid, among others, by kombucha inoculum is also reported. [21] In this way, the pH reduction is an important parameter to indicate the biological activity of kombucha inoculum, which also prevents the growth of pathogenic microorganisms. [22] The small reduction in pH and total acidity in jambolan kombucha may indicate the presence of antimicrobial compounds in the fruit pulp and inhibition of the metabolism of acetic acid bacteria. Singh[23] showed that jambolan extract performed a positive spectrum of antimicrobial activity against *S. aureus, E. coli, K. pneumoniae* and *C. albican.*

Table 1 Physicochemical and microbiological analysis of kombucha beverages on days 0 and 10.

	Day 0		Day 10	
	GTK	JBK	GTK	JBK
pH	$2.82 \pm 0,01^{a}$	$2.95 \pm 0,03^{b}$	$2.47 \pm 0,02^{c}$	$2,83 \pm 0,01^{a}$
°Brix	$7.15 \pm 0,07^{a}$	$8.05 \pm 0,05^{b}$	$6.55 \pm 0,06^{c}$	$6.75 \pm 0,02^{c}$
Total acidity	$1.80 \pm 0,02^{a}$	$1.20 \pm 0,04^{b}$	$6.0 \pm 0,01^{c}$	$2.40 \pm 0,01^{d}$
Viable cells	$6.3x10^{4} \pm 0,2^{a}$	$4.6\ x10^{4} \pm 0,6^{a}$	$1.9x10^{6} \pm 0,8^{c}$	$1.2x10^{6} \pm 0,2^{d}$

GTK (Green tea kombucha), JBI (Jambolan infusion) and JBK (Jambolan kombucha). Different lower cases (a, b, c, d) on the table show statistical differences between the samples ($P < 0.05$).

Despite the low acidity and small pH reduction, the soluble sugars were consumed during fermentation, both in green tea and jambolan kombucha. This consumption may indicate the biological activity of yeast, which hydrolyzes sucrose into glucose and fructose, and can also convert reducing sugars into ethanol and CO_2. This biological activity is observed in the viable cell count, which showed a significant increase of viable cell at the end of fermentation for both kombuchas. The total count of microorganisms in kombucha fermentation generally reaches values between 10^4 and 10^6 CFU/mL after approximately 10 days of fermentation[24].

The radical scavenging activity of a beverage is a demonstration of its antioxidant capacity. [25] According to the results presented in Table 2, the DPPH assay demonstrated a significant difference between the samples on day 0 and after fermentation for 10 days. This demonstrates that the fermentation process that occurs in the kombucha process reduce the loss of the sample's antioxidant capacity when compared to the loss of a common fruit extract (without the fermentation process). In the ABTS radical scavenging assay, the behavior was maintained between the samples about the loss of the percentage of inhibition between day 0 and day 10, varying from 5% to 11.34% for kombuchas and 14.36% (P > 0.05) for jambolan extract. The reduction in antioxidant activity can occur due to the degradation of bioactive compounds, which depends on substrate and fermentation conditions. [26]

Table 2 Antioxidant activity (%) of samples on day 0 and 10.

Radical scavenging activity	Antioxidant activities (%)					
	Day 0			Day 10		
	GTK	JBE	JBK	GTK	JBE	JBK
DPPH	42,5470 ± 0,6140 ^{b}	41,6064 ± 2,3536^{b}	41,6064 ± 0,7163 ^{b}	33,9363 ± 0,5117^{a}	31,1143 ± 0,2047^{a}	35,4559 ± 1,6373 ^{a}
ABTS^{+}	54,5070 ± 0,3984 ^{d}	59,6479 ± 1,4939 ^{c}	58,7324 ± 0,7967 ^{c}	49,5070 ± 1,4939 ^{b}	45,2817 ± 0,2988 ^{a}	47,3944 ± 0,0996 ^{ab}

GTK (Green tea kombucha), JBE (Jambolan extract) and JBK (Jambolan kombucha). Different lower cases (a, b, c, d) on the table show statistical differences between the samples (P < 0.05).

4 CONCLUSION

The elaboration of a fermented beverage using jambolan infusion and kombucha inoculum showed that although the jambolan analogue kombucha is in a safe pH range, there was low total acidity in the end of fermentation, indicating a low concentration of acids, and possible inhibition of the metabolism of acetic acid bacteria. Therefore, the fermentation of the jambolan infusion did not result in a beverage with a chemical profile characteristic of kombucha beverages. The consumption of sugars and viable cell growth indicates biological activity, probably due to yeast metabolism. The antioxidant activity of both kombuchas was affected by fermentation, with a significant reduction of activity at the end of fermentation. Although jambolan extract was not a good alternative substrate for the preparation of fermented kombucha-type beverages, its antioxidant potential and nutritional value still make it a promising source for the preparation of beverages.

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