Production of Fermented Tea from a Variety of Sources as A Soft Functional Beverage

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Abstract

With the global trend of different hospitality venues providing natural foods and beverages to support the immunity of the guests, fermented tea (kombucha tea) comes as one of these beverages. Kombucha tea is a refreshing beverage that is widely consumed around the world. It has anti-oxidant and anti-bacterial properties.In this study, kombucha tea was produced using extracts of some herbs such as black tea, green tea, mint, anise, roselle, and ginger, which are commonly used as natural herbal beverages. Whereas a significant decrease in the pH of all samples occurred as a result of the fermentation processes and the percentage decrease ranged between 60.4% to 70.4%, except for the fermented ginger beverage. The content of antioxidants and total phenols of these beverages was studied, and it was found that antioxidants increased by rates ranging from 28.56% to 54.17% compared to natural beverages without fermentation, and the rosellebeverage recorded the highest percentage in the increase, as well as high percentages of total phenols were obtained with increased rates ranging between 21.73 % to 31.49%, and mint beverage recorded the highest percentage of increase in phenols, while the lowest percentage change was recorded for a ginger beverage about both antioxidants and total phenols.The antibacterial activity of different kombuchabeverages was studied through its effect on the growth of pathogenic microbes and it was found that the most effective effect of these beverages was on both*Escherichia coli*& *Salmonella enteritidis*.The beverages that most affected the growth of these microbes were rosellebeverages and green tea.Finally, a sensory evaluation was conducted for the produced beverages, and the mint beverage obtained the highest sensory evaluation for each of the taste, aroma, color, and general acceptance.From the above, it is clear that the health benefits of kombuchabeverages are increased and considered functional beverages due to their high content of antioxidants and total phenols and the ability of most of them to act as antimicrobials.Accordingly, we recommend the inclusion of kombuchabeverages as functional beverages in various hospitality places in Egypt, especially in light of the spread of epidemics worldwide.

Keywords: Kombucha tea; herbal teas; Functional beverage; antioxidant; antibacterial activity Sensory evaluation

1- Introduction

Kombucha tea is a kind of fresh beverage that is prepared both homemade and commercially prepared by a lot of companies and is widely consumed in the world. Its consumption is increasing day by day due to the delicious taste, antioxidant characteristics of tea extracts, and beneficial effects of fermentative bacteria which have

antioxidant characteristics. Phenolic compounds found in the leaves of tea used in kombucha tea preparations have also antimicrobial effects on microorganisms. **(Juyoung and Koushik, 2020) .**

Kombucha is a sweet, slightly acidic beverage produced by fermenting tea with added sugar by a symbiotic culture known as SCOBY (Symbiotic Culture of Bacteria and Yeast) **(Zhou *et al.*, 2019**). Kombucha beverage is mainly composed of yeast and acetic acid bacteria in addition to lactic acid bacteria in smaller proportions. These microorganisms, through the fermentation process, generate metabolites such as amino acids, polyphenols, organic acids, vitamins, microelements, and antibiotics, which give the kombucha an antioxidant, anti-hyperlipidemic, anti-hyperglycemic, antimicrobial, and anticarcinogenic effect **(Magalhães-Guedes *et al.*, 2019).**

Some of the useful effects of Kombucha tea are anti-microbial, antioxidant, anti-carcinogenic, and anti-diabetic properties that have positive effects on stomach ulcers and high cholesterol. It is likewise recommended for use in the medication ofvarious diseases like AIDS, arthritis, atherosclerosis, cancer, constipation, gallbladder diseases, haemorrhoids, hair growth, hypertension, and indigestion. It has also been shown to be functional in immune responses and liver detoxification (**Chakravorty *et al.*, 2016**).

Kombucha tea is commonly prepared from black tea and green tea. Also, a variety of substrates have been already tested for the production of kombucha, including some dairy products, fruits, vegetables, herbs, coconut water, and coffee. (**Xia *et al.*, 2019**).

This research was carried out considering that it would be valuable to prepare fermented tea (kombucha tea) with black tea, green tea, mint, anise, roselle, and ginger which are highly valuable for health.

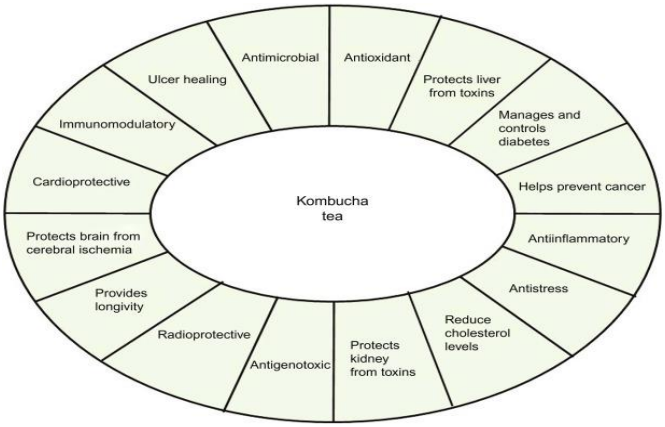

Fig. 1: Properties of KombuchaTea **(Chakravorty *et al.*, 2019)**

2- Materials and methods

2.1 Materials

Black tea, Green tea, Mint, Anise, Roselle, and Ginger used for making kombucha tea were obtained from local markets in Cairo,Egypt.

2.2 Preparation and fermentation of herbal teas

To extract the teas, 10 g of each tea pattern that's used as a bacterial medium for fermentation become left in 1 litre of boiled water for 15 mins.**(Battikh *et al.*, 2012).** After extraction, a suitable medium became prepared by adding 70 g/liter of sucrose. The prepared tea samples were transferred to glass jars which were sterilized at 121 °C for 20 minutes. when we waited until those samples reached room temperature, all samples have been inoculated with 7 g/liter Kombucha mushroom culture. After the glass jars were protected with cheesecloth, left for fermentation at 24 ± 1 °C and inside the dark for 10 days.Tea samples had been taken from the incubator at 3-day intervals and analyzes have been accomplished.

2.3. Pathogenic strains

Four pathogenic bacteria strains used in this investigation were *Salmonella enteritidis*, *Staphylococcus aureus*ATCC3536, *Escherichia coli*ATCC8739,and *Bacillus cereus* were collected from the Department of Agric. Microbiology, Fac. of Agric., Ain Shams University and These strains were maintained on nutrient agar **(Difco Manual, 1984)** or Sabouraud dextrose agar **(BAM, 1998)** slants at 4°C.

2.4 Inoculum preparation

From 4 to 5 colonies of a pure tested microbial culture were collected from an agar plate after 24h of incubation period and subculture into a tube containing 4 ml of Müller-Hinton broth then incubated at 37°C until it achieves the turbidity of 0.5 MacFarland standard after 24 h incubation according to **NCCLS (1998)**. The inoculum was standardized by measuring the optical density using a spectrophotometer at 625nm which ranged from 0.08-0.12. Standardized inoculum has a concentration of $1\text{-}2\times10^8$ CFU/ml for bacteria and is diluted to1:10 in sterile saline solution to obtain the coveted concentration of 10^6cfu/ml.

2.3 Chemical tests

pH values were measured using an electronic pH meter (Hanna). Titratable acidity (TA) became determined in keeping with **Jacobson (2006)** after disposing of CO2 from the fermentation broth. about 10 mL aliquot was taken and titrated with 0.1 mol/L NaOH. The TA was expressed in grams of acetic acid per litre.Produced Ethanol became assayed by using the method of a redox back titration recommended by **Iland (2000).**
The antioxidant activity of kombucha herbal teas and herbal extracts was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical-scavenging ability according to **Marques *et al* (2012)**. Two millilitresof 160-fold diluted Kombucha fermented solutionswere mixed with 2 ml of 0.1 mmol/L DPPH (Diphenyl-2-picrylhydrazyl) methanolicsolution. The mixture was shaken vigorously and allowed to stand in the dark for 20 min,after which the absorbance was measured at 517 nm using a spectrophotometer, BHA{3(2)-t-Butyl-4-hydroxyanisole} was used as a standard curve.
The total phenol content of Kombucha fermented extracts was measured according

toFolin-Ciocalteau method **(Singleton *et al.*, 1999).** About 0.1 ml of kombucha extract wastransferred to a 100 ml Erlenmeyer flask and the final volume was adjusted to 46 ml by the addition of distilled water and added 1.0 ml of Folin-Ciocalteau reagent. Afterwards, 1 ml of Folin-Ciocalteau reactive solution was added and incubated at room temperature for 3 min.3 ml of 2% sodium carbonate was mixed with the above solution. The absorbance at 760nm was then measured after 30 min. The total phenol was expressed as gallic acidequivalents from the calibration curve.

2.4 Microbiologicaltest

The antimicrobial activity of Kombucha was tested by agar well diffusion assay **(Mo *et al*, 2005)** against pathogenic strains. Müller-Hinton agar (MHA)medium was poured into Petri dishes. Suspensions (100µl) of the target strain previously incubated for 24 h were spread on the plates, and wells of 7 mm in diameter were made with a sterile cork borer **(ShahidiBonjar, 2004).**Kombucha samples were centrifuged at 10,000 rpm for 10 min to remove cell debris and supernatant samples (100µL) were then transferred into the wells in the agar plates previously inoculated with the target strain. The plates were allowed to stand until Kombucha samples were completely absorbed and then incubated at 37°C overnight. The growth inhibition was observed by the naked eye and the inhibition zone diameter (IZD) was measured using a ruler after 24 h of incubation. The antimicrobial activity was evaluated by measuring the growth inhibition zone surrounding the wells.

2.5 Sensory analysis

Within the sensory assessment, the fermented tea (kombucha) samples were filtered and placed to serve as light cold to 30 Egyptian guests (volunteers) contributors. The hedonic scale become used within the sensory assessment of kombucha tea samples. The ratings given on this scale range between 1 and five (five is superb, four is right, 3is ideal, 2 isn't sufficient, and 1 is terrible). The samples had been evaluated in terms of flavor, odor, and standard acceptability in sensory evaluation**(Meilgaard *et al*., 1999).**

2.6 Statistical analysis

To test the differences between the parameters analyzed before and after fermentation in tea samples, two ways analysis of variance (ANOVA)IBM® SPSS® Statistics Server Version 23.0 (2015) was performed. Significant differences between means were determined by Duncan's multiple range tests at a $P < 0.05$ significance level.

3- ResultsandDiscussion

3.1. Bio-activity of kombucha tea

The data from the analyzes carried out on the kombuch as ample produced with black tea, green tea, mint, anise, Roselle, and ginger were evaluated to compare the interaction between the physical-chemical and microbiological results after obtaining the developed product.

Fig.2: Kombucha teas after 8 days of fermentation

pH values

A statistically significant difference was observed between the pH values before and after fermentation in all tea samples. There was also a significant difference among the initial pH values of the tea samples. Initially, the lowest pH value was measured in the ginger sample at 5.74, and the highest pH value was measured in the roselle at 7.64. The pH values of all tea samples decreased after fermentation and there were statistically significant differences among them. After fermentation, the lowest pH value was found in anise at 2.22 and the highest pH value was found in ginger at 4.83. The decrease in pH was the highest in kombucha tea prepared with anise tea with 70.4%

It has been reported that the pH value decreases over time in kombucha tea samples that have been fermented for 10 days using black tea and sucrose sugar, and this decrease is due to organic acids resulting from fermentation **(Lončar *et al.*, 2006)**. Due to kombucha tea fermentation carried out for two weeks on sucrose sugar the use of black tea and green tea, it changed stated the pH value rapidly reduced from 5 to 3-3.5 **(Kallel *et al.*, 2012)**. Like results were obtained in this research.

Table 1.pH values of kombucha tea samples before and after fermentation.

Origin of the kombucha tea	Before the fermentation	After the fermentation	Variation rate (%)
Black tea	6.86 a	2.37 a	-65.5
Green tea	6.94 b	2.75 c	-60.4
Mint	7.26 c	2.75 c	-62.1
Anise	7.51 d	2.22 b	-70.4
Roselle	7.64 e	2.35 d	-69.2
Ginger	5.74 f	4.83e	-15.9

3.2. Antioxidant activity and total phenolic compounds of kombucha tea samples before and after fermentation

Antioxidants examined in kombucha tea extracts were measured before and after the fermentation process.Data inTable(2) show that antioxidant activity w a s raised after brewing with SCOBY(Symbiotic Culture Of Bacteria and Yeasts) for 10 days.The antioxidant activity of

kombucha tea drinks ranged from 28.05 to 89.12% (with antioxidant equivalent to BHA11.71 to 37.34 μgl^{-1})and ranked in ascending order: ginger < mint < green tea < black tea < anise < rosella. Moreover, anise and rosella show the highest percentage of increase in antioxidant activity about 45.70 % and 54.27 %,respectively,

Table2: Antioxidant content of kombucha tea samples before and after fermentation

Origin of the kombucha tea	**Antioxidantactivity**					
	Scavenging activity(%)		Variation rate (%)	**Antioxidantequivalentto BHA(mgL^{-1})**		Variation rate (%)
	before the fermentation	After the fermentation		before the fermentation	After the fermentation	
Black tea	31.32	44.12	+ 40.87	16.23	21.41	+ 31.92
Green tea	30.12	41.63	+ 38.21	14.88	19.13	+ 28.56
Mint	28.92	40.22	+ 39.07	12.21	17.32	+ 41.85
Anise	30.48	44.41	+ 45.70	12.68	18.91	+ 49.13
Roselle	57.82	89.12	+ 54.27	24.22	37.34	+ 54.17
Ginger	27.88	28.05	+ 0.61	11.68	11.71	+ 0.26

Total phenolic compounds were determined using gallic acid as standard, data presented in Table(3), show that total phenolic ranged from 33.52 to 66.12 ppm gallic acid for kombucha tea before fermentation and from 40.93 to 85.42 ppm gallic acid for kombucha tea after fermentation,these values werer eceived by ginger and roselle fermented teas, respectively, also roselle conducted the highest amount of total phenolic production after fermentation with SCOBY which upgraded from 66.12 up to 85.42 ppm gallic acid in total phenolic compound content, this high phenolic content affected the antioxidant activity of kombucha roselle drink which registered as the highest antioxidant activity kombucha herbal drink. Phenolic compounds were suggested as the responsible factor for herbal antioxidants as reported by **Kruawan and Kangsadalampai (2006)**.This action refers to the hydroxyl group in the phenolic compound which donates hydrogen proton and is classifiedas a reducingagent **(Pietta,2000).**The increase in bioactive compounds total phenolic compound and other secondary metabolites with antioxidant activity in kombucha herbal drinks may come from the growth of yeast, acetic and lactic acid bacteria in SCOBY culture, this observation maybe supported by **Zhang *et al.*,(2012)**. The total amount of phenolic content in tea samples showed a significant difference at the beginning and after fermentation. The difference between the tea samples was also important in terms of initial values and the least amount of phenolic content.

Table 3. The total amount of phenolic content of kombucha tea samples before and after fermentation (mgl^{-1}gallic acid).

Origin of the kombucha tea	Before the fermentation	After the fermentation	Variation rate (%)
Black tea	33.52	41.71	+ 24.43
Green tea	51.25	66.42	+ 29.6
Mint	52.31	68.78	+ 31.49
Anise	43.63	53.11	+ 21.73
Roselle	66.12	85.24	+ 28.92
Ginger	44.10	40.93	- 7.19

3.3. Antibacterial activity of kombucha tea samples.

The antibacterial activity of fermented herbal drinks was determined using an the data were in Table(4). Obtained results show that the fermentation process of kombucha herbal drinks with SCOYB accelerates the antibacterial activity efficiency for all tested herbal drinks after 10 days of fermentation. The highest figures of the zone were observed by kombucha herbal extracts of black tea, green tea, mint, anise, roselle, and gingerbeing 15, 18, 8, 16, 18 & 14 m.m, respectively, which inhibit the growth of *Escherichia coli*. There are various studies reported that the polyphenols and tannins extracted from tea inhibit a broad spectrum of Gram-positive and negative bacteria **(Sreeramulu*etal.*,2000)**, these bioactive compounds as well as ethanol and acid content were increased with t h e kombucha fermentation process as a result of yeast and bacteria growth which presence in kombucha community. These data were in line with **(Velianski*etal.*,2007)** who found that non-fermented tea and neutralized kombucha have no antibacterial activity. Otherwise, the antimicrobiala ctivity of kombucha tea was increased with the tea concentration used in kombucha preparation. **(Greenwalt*etal.*,1998).**

Table4. Antibacterial activity of kombucha tea samples using agar diffusion method.

Origin of the kombucha tea	**Zoneofinhibition(m.m)**			
	Salmonella enteritidis	*Staphylococcus aureus*	*Escherichia coli*	*Bacillus cereus*
Black tea	6	4	15	4
Green tea	8	4	18	6
Mint	6	6	8	6
Anise	4	6	16	2
Roselle	11	8	18	6
Ginger	6	6	14	4

On the other hand, **Sreeramulu *et al.*(2000)** found that the neutral pH kombucha reported antibacterial activityagainst *E. coli, S. sonnei, S. enteritidis,* and *S. Typhimurium,* this may explain by the presence of other bioactive compounds with

antibacterial activity.

Furthermore,antibacterial efficiency increases after herbals fermentation with kombucha fungus, this may explain by the ability of yeast and bacteria presented in kombucha fungus to produce other compounds with antimicrobial activity like ethanol, acetic acid, and polyphenolic compounds as reported by **Sabel *et al.*, (2017).**

3.4. Sensory evaluation of different Kombucha samples.

In the sensory evaluation of kombucha tea samples, taste, smell, color, and general acceptability parameters were used.

Table 5.Scores of sensory tests of kombucha tea samples.

Origin of the kombucha tea	Taste	Smell	Color	General acceptability
Black tea	2.05 $^{a.b}$	2.16 ^{a}	2.29 $^{a.b}$	2.24 ^{a}
Green tea	2.74 $^{b.c}$	2.58 $^{a.b}$	3.58 ^{c}	2.98 ^{b}
Mint	3.95 ^{d}	3.47 ^{c}	4.05 ^{c}	3.82 ^{c}
Anise	2.24$^{b.c}$	2.31$^{a.b}$	3.27^{c}	2.78 ^{b}
Roselle	3.21 ^{c}	3.00 $^{b.c}$	3.42 $^{b.c}$	3.21 ^{b}
Ginger	1.58 ^{a}	1.79 ^{a}	2.88 ^{a}	2.10 ^{a}

Of all sensory parameters evaluated. In terms of taste, mint tea has the highest score with 3.95 points, while ginger tea has the lowest score with 1.58 points. In the evaluation of smell, mint tea received the highest score of 3.47, while ginger received the lowest score with 1.79 points. In color evaluation, as in odor evaluation, mint tea got the highest score of4.05 points, while black tea got the lowest score of 2.29 points. In sensory evaluation, mint tea received the highest score with 3.82 points, while the lowest score was ginger tea with 2.10 points. When all param

eters were taken into account in sensory tests, it was seen that mint tea had the highest scores and ginger tea has the lowest scores.

As fermentation progresses, the taste of kombucha tea turns from a delightfully fruity, sour, and frothy flavor to a light vinegar-like flavor, thereby increasing the consumer acceptability of the flavor and other sensory aspects of the drink **(Marsh *et al*., 2014).**

4. Conclusions

In this study, kombucha tea was produced by using extracts of some herbs such as Black tea, Green tea, Mint, Anise, Roselle, and Ginger, which are commonly used as natural herbal drinks.

There was a significant decrease in the pH of all samples as a result of the fermentation processes. The content of antioxidants and total phenols in these drinks was studied, and it was found that the antioxidants increased compared to natural drinks without fermentation,

The antibacterial activity of different kombucha drinks was evaluated by studying their effect on some microbes such as *Salmonella enteritidis, Staphylococcus aureus, Escherichia coli* and *Bacillus cereus.*

Finally, a sensory evaluation was conducted for the produced drinks, and all drinks were accepted, the mint drink obtained the highest sensory evaluation for each of the taste, aroma, color, and general acceptance. So, it is clear that the health benefits of kombucha drinks are increased and considered functional soft drinks due to their high content of antioxidants and total phenols and the ability of most of them to act as antimicrobials.

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إنتاج شاى متخمر من مجموعة متنوعة من المصادر كمشروبات وظيفية خفيفة

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الملخص:

مع الاتجاه العالمي لإهتمام أماكن الضيافة المختلفة بتوفير الأطعمة والمشروبات الطبيعية لدعم مناعة الضيوف ، يأتي الشاي المتخمر (شاى الكمبوشا) كأحد هذه المشروبات. شاي الكمبوشا هو نوع من المشروبات المنعشة ويتم استهلاكها على نطاق واسع في العالم. حيث يتميز بخواصه المضادة للأكسدة والمضادة لنمو البكتيريا.

فى هذه الدراسة تم إنتاج مشروب شاى الكمبوشا بإستخدام مستخلصات لبعض الاعشاب مثل الشاى الأسود ، الشاى الأخضر، النعناع، اليانسون، الكركديه و الزنجبيل والتى تستخدم بشكل شائع كمشروبات أعشاب طبيعية .

حيث حدث إنخفاض لدرجة الحموضة لجميع العينات بدرجة كبيرة نتيجة عمليات التخمير التى تمت وقد تراوحت نسبة الإنخفاض بين 60.4 % إلى 70.4 % بإستثناء مشروب الزنجبيل المخمر.

تم دراسة المحتوى من مضادات الإكسدة والفينولات الكلية لتلك المشروبات وقد وجد إرتفاع مضادات الأكسدة بنسب تتراوح بين 28.56% إلى 54.17% مقارنة بالمشروبات الطبيعية بدون تخمير وسجل مشروب الكركديه أعلى نسبة فى الارتفاع، كذلك تم الحصول على نسب مرتفعة من الفينولات الكلية بنسب زيادة تراوحت بين 21.73% إلى 31.49% وسجل مشروب النعناع أعلى نسبة من زيادة الفينولات، بينما أقل نسبة تغير تم تسجيلها كانت لمشروب الزنجبيل وذلك بالنسبة لكلاً من مضادات الأكسدة والفينولات الكلية.

تم دراسة النشاط المضاد للبكتريا المرضية لمشروبات الكمبوشا المختلفة وذلك من خلال تأثيرها على نمو بعض الميكروبات المرضية ووجد أن أكثر تأثير لهذه المشروبات كانت على *Escherichia coli*و*Salmonella enteritidis* وكانت المشروبات الأكثر تأثير على نمو تلك الميكروبات هى مشروب الكركديه والشاى الأخضر.

وأخيراً تم عمل تقييم حسى للمشروبات المنتجة وقد حصل مشروب النعناع على أعلى تقييم حسى لكلاً من الطعم والرائحة واللون والقبول العام.

مما سبق يتضح زيادة الفوائد الصحية لمشروبات الكمبوشا واعتبارها من المشروبات الوظيفية نظراً لإرتفاع محتواها من مضادات الأكسدة والفينولات الكلية وقدرة أغلبها على العمل كمضاد للميكروبات.

وعليه فإننا نوصى بإدراج المشروبات المتخمرة(مشروبات الكمبوشا) كمشروبات وظيفية خفيفة فى أماكن الضيافة المختلفة فى مصر خاصة فى ظل إنتشار الإوبئة على مستوى العالم.