Trop J Nat Prod Res, May 2023; 7(5):2904-2910 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Development of Kombucha Tea with Gac and Mango Fruits: Sensory, Nutritional, Phytochemical, Physicochemical and Antioxidant Evaluation

Nurus S.A. Aziz[1], Mohd F.A Bakar[1]*, Dzulkarnain F.M. Farid[1], Tri B. Julianti[1,2]

[1]*Faculty of Applied Sciences and Technology, Universiti Tun Hussein Onn Malaysia (UTHM) Pagoh Campus, 84600 Muar, Johor, Malaysia*
[2]*Faculty of Pharmacy, Universitas Muhammadiyah Kalimantan Timur, Samarinda, Kalimantan Timur 75124, Indonesia*

ARTICLE INFO

Article history:
Received 13 March 2023
Revised 04 May 2023
Accepted 09 May 2023
Published online 01 June 2023

ABSTRACT

Kombucha tea is a sweetened black tea beverage that undergoes fermentation with a starter culture of bacteria and yeast (SCOBY). In this study, gac (*Momordica cochinchinensis*) and mango (*Mangifera indica*) fruits were primarily chosen for infusion into kombucha due to their health benefits. Thus, this research aims to develop kombucha black tea with gac and mango, evaluate the sensory evaluation of the kombucha tea, and analyse the nutritional composition, physicochemical, phytochemical, and antioxidant activity of the kombucha tea. In this study, kombucha was prepared by fermenting black tea with sugar at different concentrations (70, 80, and 100 g) and SCOBY for 10 days before infusing it with gac and mango fruits. The sensory evaluation was evaluated using a hedonic 9-scale and another while using the standard methods. The result showed that kombucha gac and mango fruit with100 g of sugar (KGM100) represents the highest score on all attributes with a value of colour 7.5±1.55, aroma 6.47±1.74, taste 6.6±1.77, and overall, of 6.6±1.77. The nutrient of KGM 100 was higher than other concentrations with a value content of carbohydrates 6.9% and vitamin C of 2.70 mg/kg. The phytochemical of all samples showed increment during fermentation time and the antioxidant activity is higher in KGM100 (95.88%) while the physicochemical properties showed an increase in acidity and a decrease in colour. These findings show that kombucha black tea with gac and mango may impact health when consume and future investigation is worthy to determine its potential health benefits.

Keywords: Kombucha, Black tea, Gac fruit, Mango

Introduction

Kombucha is a millennial drink with a lot of health benefits.[1] Kombucha tea is a delightful beverage created by fermenting sugared black tea manufactured from *Camellia sinensis* (L.) leaves with a consortium of yeast and bacteria that mainly produce acetic acid.[2] The available carbon supply is first turned into ethanol by the yeast in the system, which the bacteria then transform into acids.[3] The outcome after fermentation generally had similar modifications, specifically a shift in colour to become lighter.[4] Previous study reported that the greater the degree of color change in kombucha beverages, the longer the fermentation period.[5] As a result, the kombucha biofilm, which forms on a liquid-air interface due to the activities of both bacteria and yeast, has aroused scientific attention throughout history.[2] The beverage is famous for its many prophylactic and therapeutic benefits, including aiding digestion, acting as a laxative, avoiding bacterial contamination, countering tension and disease, providing relief from hemorrhoids, lowering cholesterol levels, facilitating toxin excretion, and disinfecting blood.[6–9] Gac contains lycopene and β-carotene in its greasy seed skin.[10] Gac fruit has a natural concentration of carotenoids compared to other popular fruits and vegetables.[11]

*Corresponding author. E mail: fadzelly@uthm.edu.my
Tel: +606-9745146

Citation: Aziz NSA, Bakar MFA, Farid DFM, Julianti TB. Development of Kombucha Tea with Gac and Mango Fruits: Sensory, Nutritional, Phytochemical, Physicochemical and Antioxidant Evaluation Trop J Nat Prod Res. 2023; 7(5):2904-2910 http://www.doi.org/10.26538/tjnpr/v7i5.10

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

In the last few years, gac fruit has been nicknamed 'super fruit' or 'heaven's fruit' because of their high phytonutrient content in all fractions (aril, seeds, pulp, and peel), as well as their therapeutic and pharmacological characteristics.[10,11] In the other hand, mangoes contain a mixture of sugar ranging from 16–18% w/v, acids, and significant levels of polyphenols, carotene as vitamin A, and ascorbic acid. These compounds are potent antioxidants.[12] Essential bioactive substances such as mangiferin, quercetin, catechin, and kaempferol are present in all portions of the mango.[12] The advantages of mango includes anti-inflammatory, antioxidant, anti-cancer, anti-diabetic, anti-microbial, anti-hyperlipidemic and immunomodulatory properties.[13,14] It can be said that mango has abundant health benefits for humans. Although kombucha comes in various flavours, there has not been much exploration of kombucha made with gac and mango fruit. Therefore, this study aims to produce kombucha black tea with gac and mango that might boost the beverages' nutritional, antioxidant, and phytochemical content while providing desired sensory features.

Materials and Methods

Preparation of kombucha tea and infusion

Black tea was obtained from Malaysia with the type of product from PT. Unilever food solutions in January 2023. Kombucha tea was prepared at three different concentrations by adding 70 g, 80 g, and 100 g of sugar into 250 mL of hot water.[15] Then, 4 g of tea, or two bags of black tea (Lipton), were added to the hot water. When the solution was cooled down to 20 to 25 °C, the tea bag was discarded, and then the sample was transferred into a clean jar in a sterilized condition. Then, 750 ml of cold water was added to the solution, and 250 ml of kombucha tea was added to the jar. One layer of SCOBY was added, muslin cloth was used to cover the mouth of the pot, and a rubber band was used to tie the muslin cloth. The fermentation process of the tea took ten days to complete at 20 to 25°C. The kombucha infusion was prepared as in

Table 1 and was added together in a bottle-neck glass bottle. Afterward, the beverage was fermented at room temperature for four days.

Preparation of gac fruit
Gac fruit was collected from Johor, Malaysia in January 2023 and the fruits were identified by plant botanists. The method by Zubaidah *et al*.[16] was used to prepare the gac fruit juice. The peel of the gac fruit was removed after being rinsed with tap water. The gac fruit's aril and pulp were gathered. The aril and pulp were then weighed at 200 g. The aril and pulp were mixed with 400 mL of water in a 1:2 (w/v) ratio (aril + pulp: water) and homogenized. After the mixture had been filtered through a muslin cloth, the juice was collected and kept in the fridge for further experiments.

Preparation of mango puree
Mango fruit was obtained from Johor, Malaysia in January 2023 and the fruits were identified by plant botanists. The mango puree was prepared based on the method by Oluyemisi Elizabeth Adelakun *et al*.[17] The mango's skin was washed with tap water and removed. The pulp of the mango was collected and weighed. Then, the mango pulp was blended. The puree was collected and filtered through muslin cloth and stored in the refrigerator for future use.

Sensory evaluation
Sensory evaluation was conducted on 30 untrained panellists with ages ranging from 20 to 30 years old, using the hedonic scale 9-level.[18] The scale run from 1 to 9, with 1 and 9 indicating extremely dislike and extremely like, respectively. Aspects assessed were colour, aroma, taste and general acceptance.

Determination of nutritional composition
In this study, the nutritional composition that was determined were protein, fat, carbohydrate and vitamin C. Protein was determined using the Hashem method.[19] The fat content was determined using the acid hydrolysis method described by Abeyrathne *et al*.[20] The determination of the total carbohydrate content of samples was referred to BeMiller using the method total carbohydrate by differences.[21] The vitamin C was measured based on AOAC International Method Analysis.[22]

Determination of total phenolic content (TPC)
Folin-Ciocalteu colourimetric technique was used to determine the total phenolic content (TPC).[22,23] Two (2) mL of 2% sodium carbonate (Na_2CO_3) (Merck, Germany) was added to 0.1 mL of sample and incubated for 2 min. Then, 0.1 mL of 50% Folin-Ciocalteu reagent (Merck, Germany) was added to the mixture and incubated for 30 min at room temperature. The absorbance of the samples were read at 750 nm (T60u, PG instrument, USA). Gallic acid was used as a standard. The results were presented in milligrams of gallic acid equivalent per millilitre (mg GAE/mL).

Determination of total flavonoid content (TFC)
Total flavonoid content was measured according to a method by Torre *et al*.[24] Briefly, 0.25 mL of kombucha sample and 1.25 mL of pure water was mixed in a beaker. Then, 0.3 mL of 5% sodium nitrite ($NaNO_2$) (Merck, Germany) was added to the mixture. After 5 min, 150 µL of 10% aluminium chloride hexahydrate (Sigma-Aldrich, Germany) was added to the mixture. After 6 min, the mixture was neutralised with 0.5 mL of 1 M sodium hydroxide (Merck, Germany). The absorbance was read using a UV-Vis spectrometer (T60u, PG Instrument, USA) set at 420 nm. Quercetin was used as a standard. The total flavonoid content was expressed as milligrams of quercetin equivalent (QE/mL).

Determination of physicochemical properties
Total soluble solid (TSS), pH, colour and alcohol content were analysed in the kombucha drink. The pH values of the sample were determined using an electronic pH metre (EUTECH Instrumentals, Singapore) based on AOAC modification methods by Barbosa *et al*.[25] A refractometer was used to analyse the total soluble solid (TSS) (ATAGO, USA). A colourimeter (Miniscan EZ, Hunter Lab, USA) was used to analyse the colour of the sample. Lastly, the alcohol level was determined using headspace gas chromatography with flame ionisation detection (GC-FID) based on AOAC International single-laboratory validation guidelines.

Determination of antioxidant 2,2-diphenyl-1-picrylhydrazyl (DPPH) method
The DPPH assay was conducted based on the procedure written by Gao *et al*.[26] Briefly, 1.5 ml of each sample and 3 ml of DPPH solution (200 µM) was mixed. After incubation for 30 min in a dark place at room temperature, the decrease in absorbance at 515 nm was measured and the free-radical scavenging activity was calculated as follows:

$$Scavenging\ activity\ (\%) = \left(\frac{Absorbance\ of\ control - Absorbance\ of\ sample}{Absorbance\ of\ control}\right) \times 100$$

Determination of antioxidant activity using 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS)
The ABTS assay was carried out following the procedure described by Xia *et al*.[27] Briefly, 7 mM of ABTS solution was mixed with potassium persulfate solution (2.45 mM), and the mixture was kept in the dark for 12–16 h before being used. The mixture was then diluted to an absorbance of 0.70 ± 0.02 at 734 nm. Afterwards, 4 mm of ABTS dilute solution and 1 ml of each sample were mixed. After the mixture was kept for 6 min in dark at room temperature, the decrease in absorbance at 734 nm was measured. The ABTS•+ radical scavenging activity (%) was calculated using formula:

$$ABTS\ scavenging\ activity\ (\%) = \left(\frac{Absorbance\ of\ control - Absorbance\ of\ sample}{Absorbance\ of\ control}\right) \times 100$$

Statistical analysis
The statistical analysis was conducted using Microsoft Excel 2019. Results were expressed as mean ± standard deviation (SD). The data were analysed using one-way analysis of variance (ANOVA). $P < 0.05$ were used to determine the statistical significance. The data was presented in triplicate.[22]

Table 1: Formulation used to make kombucha black tea and kombucha gac and mango

Parameter	Sample			
	KBT	KGM70	KGM80	KGM100
Volume of black tea decoction (mL)	1000	600	600	600
Mass of sugar (g)	80	70	80	100
Starter culture (mL)	250	250	250	250
Volume of gac juice (mL)	0	300	300	300
Volume of mango juice (mL)	0	100	100	100
Total volume of kombucha (mL)	1000	10000	1000	1000

*KBT= kombucha black tea, KGM70= kombucha gac and mango + 70 g sugar, KGM 80=kombucha gac and mango + 80 g sugar, KGM100= kombucha gac and mango + 100g sugar

Results and Discussion

Table 2 shows the result of sensory evaluation and the results showed that KGM100 (kombucha plus mango and gac fruit with sugar content of 100g) represents the highest score on all attributes as compared to other samples. Among all attributes, the aroma has the lowest score considering that all kombucha samples gave a strong aroma of vinegar after 14 days of fermentation. Long fermentation times that allowed acetic acid bacteria to produce a lot of acetic acids can affect the flavour of kombucha beverages.[24] Therefore, the infusion of gac and mango fruits helps to increase the pH levels and reduces the sour taste of kombucha drinks, thereby making them more acceptable among panellists. Thus, adding gac and mango into the kombucha gives a sweet and balanced taste to the kombucha beverage. The statistical analysis for all attributes showed no significant difference ($p > 0.05$).

The nutritional composition of kombucha gac and mango fruits on the 14th day of fermentation is shown in Table 3. Among all the samples, KGM100 has the highest content of carbohydrates which is 6.9% and the lowest was KGM70 (5.8%). The higher the amount of sugar in kombucha, the higher the total carbohydrate content. The amount of protein and fat in all kombucha samples is less than 0.2 as the amount of protein and fat were found in minimal amounts in gac fruit.[28,29] For the vitamin C content, KGM100 has the highest content of vitamin C (2.70 mg/kg) because the vitamin C amount significantly increased during fermentation.[30] Vitamin C is a well-known antioxidant where it supports healthy teeth and bones, repairs damaged tissue, stimulate collagen in the skin that is still in good condition and strengthens the immune system.[31,32]

As shown in Table 4, the concentration of phenolic content in kombucha gac and mango samples increased proportionally as fermentation day increased. Phenolic compounds are considered high-level antioxidants because of their ability to scavenge free radicals and active oxygen species, and their antioxidant activity and phenolic compounds rose steadily with fermentation periods.12 The amount of polyphenols present determines how deep the blue colour and the deeper blue denotes a higher absorbance and phenolic acid concentration.[7,33] The phenolic content of KBT was higher on the 11th day of fermentation and decreased on the 14th day of fermentation, meanwhile, the phenolic content in all KGM samples steadily increased during fermentation periods because of the infusion of gac fruit. According to Abdulqader et al., gac fruit has higher phenolic content in its aril (30.8 ± 2.7 mg GAE/g) and pulp (28.9 ± 2.4 mg GAE/mg) which was utilised in this beverage.[34]

As shown in Table 4, the total flavonoid content for KGM 100 was greater than KBT during the 11th day of fermentation because of the infusion of gac fruit. On the 14th day of fermentation, the reading for the total flavonoid content of all samples increased. The highest total flavonoid content was 3.43 ± 0.05 mg QE/mL for KGM100 and followed by KGM80 (3.07 ± 0.06 mg QE/mL), KGM70 (3.05 ± 0.00 mg QE/mL), and lastly, KBT (0.57 ± 0.01 mg QE/mL). According to Abdulqader et al., the flavonoid content in the gac fruit was high in aril (24.5 ± 3.3 mg QE/g) which this part of gac fruit was utilised in these studies.[34] Thus, existing flavonoid content contributed to the higher amount in the kombucha that was enriched with gac fruit.

The changes in pH values of all four samples were presented in Table 5. There is a significant difference ($p < 0.05$) in each of the samples in these studies. When pH values were analysed, all of the tested samples' pH values fell as fermentation time. The initial pH of all kombucha tea samples was around 3.20, and it dropped during the fermentation period. The increasing concentration of organic acids produced during the fermentation process by bacteria and yeast in the tea affects the decrease in pH value. The increase if pH value on day-11 was caused by the infusion of gac and mango into the kombucha making the pH value slightly increase but as fermentation time passed, the value of pH dropped back into 2.8 and 2.9 in all KGM samples.

Based on the results from Table 5, before the fermentation process, all the kombucha samples had the highest concentration of sugar. When it comes to KBT, the content of sugar decreases as fermentation time, thereby achieving the lowest value on the 14th day of fermentation (5.13º Brix). However, in the case of KGM, the content of sugar decreased on the first 7 days of fermentation but increased on the 11th day because of infusion of gac and mango juices and decreased on the 14th day of fermentation. The decrease in sugar content in all kombucha samples can be due to the yeast and bacteria in the kombucha. When yeast hydrolyses sucrose into glucose and fructose during the fermentation stage, ethanol is produced and the total soluble solid are reduced.[15]

Colour is a crucial factor in deciding which beverage's physical quality is more important.[11] Table 6 showed the L* value for each sample of kombucha gac and mango increases at the end of the fermentation. This indicates that all the samples become lighter after the 14th day of fermentation. However, KBT was lightest in colour (61.57 ± 0.04) as compared to three samples of kombucha gac and mango. Positive a* value corresponds to colour red, whereas a* negative value corresponds to green.[35] The colour of all KGM samples increases on day 11 because of the addition of gac and mango juice on day 10, particularly b* values. This is because the colour of gac juice is orange while mango is yellow and the combination of these two fruits will give yellowish colour to kombucha tea.

Table 2: Sensory analysis of kombucha black tea and kombucha gac and mango fruit

Samples	Attributes			
	Colour	**Aroma**	**Taste**	**Overall**
KBT	6.89 ± 1.73	5.45 ± 1.79	5.10 ± 2.23	5.53 ± 1.74
KGM70	7.13 ± 1.63	5.17 ± 2.16	5.8 ± 2.14	6.03 ± 2.11
KGM80	7.03 ± 1.51	5.4 ± 1.79	5.53 ± 1.94	5.73 ± 1.94
KGM100	7.5 ± 1.55	6.47 ± 1.74	6.6 ± 1.77	6.6 ± 1.77

*KBT= kombucha black tea, KGM70= Kombucha gac and mango + 70g sugar, KGM80= Kombucha gac and mango + 80g sugar, KGM100= Kombucha gac and mango + 100g sugar

Table 3: Nutritional value of kombucha tea enriched with gac and mango fruit

Nutritional composition	Sample		
	KGM70	**KGM80**	**KGM100**
Carbohydrate	5.8 %	6.4 %	6.9 %
Protein	ND (<0.2)	ND (<0.2)	ND (<0.2)
Fat	ND (<0.2)	ND (<0.2)	ND (<0.2)
Vitamin C	2.63 mg/kg	2.68 mg/kg	2.70 mg/kg

*KGM70= Kombucha gac and mango + 70g sugar, KGM80= Kombucha gac and mango + 80g sugar, KGM100= Kombucha gac and mango + 100g sugar

Table 4: Result of TPC and TFC of kombucha sample on different fermentation time

	Sample	Fermentation time (days)			
		1	7	11	14
Total phenolic content (mg GAE/mL)	KBT	0.27 ± 0.00	0.46 ± 0.00	1.27 ± 0.00	1.04 ± 0.00
	KGM70	0.18 ± 0.04	0.20 ± 0.01	0.32 ± 0.01	0.57 ± 0.01
	KGM80	0.21 ± 0.03	0.32 ± 0.01	0.58 ± 0.01	0.82 ± 0.01
	KGM100	0.28 ± 0.18	0.58 ± 0.02	1.11 ± 0.03	1.38 ± 0.03
Total flavonoid content (mg QE/mL)	KBT	1.38 ± 0.03	0.10 ± 0.01	0.15 ± 0.01	0.57 ± 0.01
	KGM70	0.18 ± 0.00	0.75 ± 0.01	1.58 ± 0.02	3.05 ± 0.00
	KGM80	0.21 ± 0.01	1.11 ± 0.01	1.59 ± 0.01	3.07 ± 0.06
	KGM100	0.28 ± 0.02	1.17 ± 0.01	2.39 ± 0.01	3.43 ± 0.05

*KBT= kombucha black tea, KGM70= Kombucha gac and mango + 70g sugar, KGM80= Kombucha gac and mango + 80g sugar, KGM100= Kombucha gac and mango + 100g sugar

Table 5: Result of pH and total soluble solid (TSS) kombucha samples on different fermentation time

	Sample	Fermentation time (days)			
		1	7	11	14
pH	KBT	3.27 ± 0.03	2.45 ± 0.02	2.42 ± 0.02	2.36 ± 0.01
	KGM70	3.28 ± 0.07	2.4 ± 0.02	4.37 ± 0.10	2.48 ± 0.04
	KGM80	3.26 ± 0.04	2.41 ± 0.03	4.52 ± 0.01	2.88 ± 0.03
	KGM100	3.24 ± 0.02	2.47 ± 0.02	4.54 ± 0.04	2.94 ± 0.04
TSS (ºBrix)	KBT	7.19 ± 0.01	6.13 ± 0.06	5.7 ± 0.01	5.13 ± 0.06
	KGM70	6.57 ± 0.02	5.12 ± 0.06	6.41 ± 0.08	6.11 ± 0.02
	KGM80	7.18 ± 0.01	5.57 ± 0.06	7.03 ± 0.05	6.70 ± 0.04
	KGM100	8.49 ± 0.01	6.36 ± 0.06	7.80 ± 0.05	7.50 ± 0.02

*KBT= kombucha black tea, KGM70= Kombucha gac and mango + 70g sugar, KGM80= Kombucha gac and mango + 80g sugar, KGM100= Kombucha gac and mango + 100g sugar

All the samples showed that the colour tends to be yellow because the b* is a positive value. The reading of the b* value for KBT was also the highest 60.92 ± 0.13 while the lowest b* value was held by KGM100 (29.79 ± 0.24). Given the ability of microorganisms to use sugar as energy, the colour of kombucha generally becomes lighter. As the fermentation process continues, kombucha's polyphenol content rises, which is another factor contributing to its lighter colour.[22]

The result of alcohol content was presented in Table 7. Among the three samples, KGM100 has the highest amount of alcohol content with 0.49%, followed by KGM80 and KGM70 which are 0.45 and 0.32% ABV, respectively. High-energy molecules such as ethanol and higher alcohol form when there is a shortage of oxygen.[35,36] When sugar was added as the primary ingredient, higher levels of ethanol were produced. As shown in Table 7, the percentage of alcohol is lower than 0.05% ABV. This is because long storage in the refrigerator would make the alcohol content reduced. According to Wang *et al*, chilling the beverage too long can significantly decrease alcohol levels.[37] Meanwhile, based on Malaysia Food Regulation 1985, Regulation 36(1), an alcoholic beverage must contain more than 2% alcohol by volume; hence, kombucha with an alcohol content of 0.5% or more can be classified as a non-alcoholic beverage.

The anti-radical capabilities of kombucha were influenced by the length of fermentation. In fermented kombucha tea drinks, polyphones and tea fungal metabolites such as vitamins and organic acids were the principal antioxidants and because of this, fermented kombucha tea typically exhibits a stronger antioxidant potential than unfermented tea.[22] Based on the study of the analysed sample antioxidant potentials, the percentage of antioxidant compounds was between 27.86% and 64.38% which inhibited DPPH (Table 8). KGM100 was the highest scavenging effects followed by KGM80, KBT and KGM70. Analysis of variance revealed a significant difference ($p < 0.05$) for scavenging free-radical activity for these three samples or formulations.

The pre-formed radical monocation of 2,2'-azinobis- (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS•+) is produced by oxidising ABTS with potassium persulfate, and it is reduced in the presence of such hydrogen-donating antioxidants. Consideration is given to the effects of both the antioxidant concentration and reaction time on the inhibition of radical cation absorption when calculating the antioxidant activity. Based on Table 8, KGM100 showed the highest value of ABTS assay with 95.88 ± 0.00% followed by KGM80, KBT and KGM70 with 2.97 ± 0.05%, 70.92 ± 0.05% and 67.49 ± 0.02%, respectively. Analysis of variance revealed a significant difference ($p < 0.05$) between the four samples. The antioxidant capacity determined in ABTS was much higher than as determined by the DPPH assay. Thus, it is suggested that the ABTS assay better reflects the antioxidant contents than the DPPH assay.[24]

Conclusion

The present study showed that kombucha black tea with gac and mango with the highest sugar content (100g) showed the highest carbohydrate and vitamin C content, was most preferred by the panelists, and possessed significant amounts of phenolic and flavonoids with high antioxidant activity. The result of alcohol content for all samples were presented as less than 0.5% ABV, where all samples were classified as non-alcoholic beverages.

Conflict of Interest

The authors declare no conflict of interest.

Table 6: The L*, a* and b* values of kombucha samples on different fermentation time

Sample	Fermentation time (days)	Colour		
		L*	a*	b*
KBT	1	60.33 ± 0.14	-0.05 ± 0.01	14.06 ± 0.01
	7	43.45 ± 0.08	-2.24 ± 0.01	2.83 ± 0.09
	11	60.71 ± 0.10	-0.47 ± 0.01	10.98 ± 0.10
	14	61.57 ± 0.04	-0.46 ± 0.01	60.92 ± 0.13
KGM70	1	59.46 ± 0.66	-0.03 ± 0.01	7.78 ± 1.47
	7	43.99 ± 0.03	-2.28 ± 0.02	6.29 ± 0.03
	11	57.3 ± 3.18	11.55 ± 0.75	81.46 ± 0.91
	14	60.92 ± 0.13	4.08 ± 0.33	30.36 ± 0.63
KGM80	1	59.42 ± 0.22	-0.29 ± 0.13	3.99 ± 1.17
	7	43.46 ± 0.03	-2.24 ± 0.02	3.42 ± 0.03
	11	58.3 ± 2.13	11.34 ± 0.63	84.50 ± 2.12
	14	59.42 ± 0.11	4.03 ± 0.23	30.27 ± 0.20
KGM100	1	61.23 ± 0.16	-0.39 ± 0.04	12.45 ± 0.11
	7	44.54 ± 0.31	-2.14 ± 0.02	1.83 ± 0.03
	11	56.94 ± 1.44	9.02 ± 0.57	78.57 ± 1.77
	14	60.36 ± 0.08	2.92 ± 0.09	29.79 ± 0.24

*KBT= kombucha black tea, KGM70= Kombucha gac and mango + 70g sugar, KGM80= Kombucha gac and mango + 80g sugar, KGM100= Kombucha gac and mango + 100g sugar

Table 7: Result of alcohol content of kombucha samples

Samples	Alcohol (ABV %)
KBT	0.5
KGM70	0.39
KGM80	0.45
KGM100	0.49

*KBT= kombucha black tea, KGM70= Kombucha gac and mango + 70g sugar, KGM80= Kombucha gac and mango + 80g sugar, KGM100= Kombucha gac and mango + 100g sugar

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgements

This research was made possible by funding from UWG Marketing and Distributor Sdn Bhd and Universiti Tun Hussein Onn Malaysia (UTHM) through RE-SIP matching grant (Vot No: M082). The authors would also like to thank the Faculty of Applied Sciences and Technology, Universiti Tun Hussein Onn Malaysia for its support.

Table 8: Result of antioxidant activity of kombucha black tea and kombucha gac and mango by DPPH and ABTS on different fermentation time.

	Sample	Fermentation time (days)			
		1	7	11	14
DPPH (%)	KBT	36.78 ± 0.01	40.38 ± 0.01	48.77 ± 0.03	53.74 ± 0.02
	KGM70	20.78 ± 0.01	34.38 ± 0.01	38.43 ± 0.01	27.86 ± 0.11
	KGM80	34.38 ± 0.01	35.94 ± 0.01	50.99 ± 0.01	56.04 ± 0.02
	KGM100	39.06 ± 0.01	46.86 ± 0.02	55.63 ± 0.03	64.38 ± 0.02
ABTS	KBT	46.95 ± 0.03	59.02 ± 0.06	64.58 ± 0.02	70.92 ± 0.05
	KGM70	34.98 ± 0.01	48.06 ± 0.01	62.60 ± 0.01	67.49 ± 0.02
	KGM80	45.98 ± 0.03	58.72 ± 0.01	71.02 ± 0.04	72.97 ± 0.05
	KGM100	64.68 ± 0.04	82.80 ± 0.02	92.68 ± 0.01	95.88 ± 0.00

*KBT= kombucha black tea, KGM70= Kombucha gac and mango + 70g sugar, KGM80= Kombucha gac and mango + 80g sugar, KGM100= Kombucha gac and mango + 100g sugar

References

1. Júnior JCdaS, Mafaldo ÍM, Brito, IdeL, Cordeiro, MTdeM. Kombucha: Formulation, chemical composition, and therapeutic potentialities. Curr. Res. Food Sci. 2022; 5: 360–365. Doi: 10.1016/j.crfs.2022.01.023.
2. Chakravorty S, Bhattacharya S, Bhattacharya D, Sarkar S, Gachhui R. Kombucha: A promising functional beverage prepared from tea. Non-alcoholic Beverages: Volume 6. The Science of Beverage, eds. Grumezescu A. M., Holban A. M. (Woodhead Publishing, Elsevier Inc). 2019: 285-327. Doi: 10.1016/B978-0-12-815270-6.00010-4
3. Okolie NP, Falodun A, Davids O. Evaluation of the antioxidant activity of root extract of pepper fruit (*Dennetia tripetala*), and it's potential for the inhibition of lipid peroxidation. Afr. J. Tradit. Complement. Altern. Med. 2014; 11 (3): 221–227. Doi: 10.4314/ajtcam.v11i3.31.
4. Riswanto D, Rezaldi F. Kombucha Tea: A Study on the Halal of Fermented Drink. IJMA. 2021; 1 (2): 71–77.
5. Khaerah A, Akbar F. Aktivitas Antioksidan Teh Kombucha dari Beberapa Varian Teh yang Berbeda. Pros. Semin. Nas. *LP2M UNM*. 2019; 472–476.
6. Massoud R, Jafari-Dastjerdeh R, Naghavi N, Khosravi-Darani K. All aspects of antioxidant properties of kombucha drink. Biointerface Res. Appl. Chem. 2022; 12(3): 4018–4027. Doi: 10.33263/BRIAC123.40184027.
7. Cardoso RR, Neto RO, D'Almeida CTdS, Nascimento TPdo, Pressete, CG, Azevedo, L, Martino HSD, Cameron LC, Ferreira MSL, Barros FARde. Kombuchas from green and black teas have different phenolic profile, which impacts their antioxidant capacities, antibacterial and antiproliferative activities. Food Res. Int. 128, 108782 (2020). Doi: /10.1016/j.foodres.2019.108782.
8. Kitwetcharoen H, Phung LT, Klanrit P, Thanonkoe S, Tippayawat P, Yamada M, Thanonkoe P. Kombucha Healthy Drink—Recent Advances in Production, Chemical Composition and Health Benefits. Fermentation. 2023; 9: 48. Doi: 10.3390/fermentation9010048.
9. Egharevba E, Chukwuemeke-Nwani P, Eboh U, Okoye E, Bolanle IO, Oseghale IO, Imieje VO, Erharuyi O, Falodun A. Evaluation of the antioxidant and hypoglycaemic potentials of the leaf extracts of *Stachytarphyta jamaicensis* (Verbenaceae). Trop. J. Nat. Prod. Res. 2019; 3 (5): 170–174. Doi: 10.26538/tjnpr/v3i5.4.
10. Do TVT, Fan L, Suhartini W, Girmatsion M. Gac (*Momordica cochinchinensis* Spreng) fruit: A functional food and medicinal resource. J. Funct. Foods. 2019; 62: 103512. Doi: 10.1016/j.jff.2019.103512.
11. V LeA, Parks ES, Nguyen HM, Roach DP. Physicochemical Properties of Gac (*Momordica cochinchinensis* (Lour.) Spreng) Seeds and Their Oil Extracted by Supercritical Carbon Dioxide and Soxhlet Methods. Technologies. 2018; 6: 94. Doi: 10.3390/technologies6040094
12. Lebaka VR, Wee YJ, Ye W, Korivi M. Nutritional composition and bioactive compounds in three different parts of mango fruit. Int. J. Environ. Res. Public Health. 2021; 18: 741. Doi: 10.3390/ijerph18020741.
13. Lauricella M, Emanuele S, Calvaruso G, Giuliano M, D'Anneo A. Multifaceted health benefits of *Mangifera indica* L. (Mango): The inestimable value of orchards recently planted in sicilian rural areas. Nutrients. 2017; 9: 525. Doi: 10.3390/nu9050525.
14. Mubarik F, Noreen S, Farooq F, Siddiqa A, Khan M. A Review on Pharmacological and Nutritional Benefits of Mango (*Mangifera indica* Linn): A Remedy for Cancer, Diabetes and Gastrointestinal Infections. Abasyn J. Life Sci. 2020; 3(2): 82–92. Doi:10.34091/ajls.3.2.8.
15. Wang X, Wang D, Wang H., Jiao S, Wu J, Hou Y, Sun J, Yuan J. Chemical Profile and Antioxidant Capacity of Kombucha Tea by the Pure Cultured Kombucha. Lwt. 2022; 168: 113931. Doi: 10.1016/j.lwt.2022.113931.
16. Zubaidah E, Dewantari FJ, Novitasari FR, Srianta I, Blanc PJ. Potential of snake fruit (*Salacca zalacca* (Gaerth.) Voss) for the development of a beverage through fermentation with the Kombucha consortium. Biocatal. Agric. Biotechnol. 2018; 13: 198–203.
17. Adelakun OE, Oke MO, Akande EA, Olabode AOA. Extraction of Mango Juice with Pectinase Influences Quality. Asian Food Sci. J. 2020; 16(3): 43–52. Doi: 10.9734/AFSJ/2020/v16i330174.
18. Ivanišová E, Meňhartová K, Terentjeva M, Harangozo L, Kántor A, Kačániová M. The evaluation of chemical, antioxidant, antimicrobial and sensory properties of kombucha tea beverage. J. Food Sci. Technol. 2020; 57(5): 1840–1846. Doi: 10.1007/s13197-019-04217-3.
19. Hashem MS, El-Lahot MSA, Helal AM, Massoud MI. Evaluation the Phytochemicals and Nutritional Characteristics of Some Microalgae Grown in Egypt as Healthy Food Supplements. Egypt. J. Food Sci. 2021; 49(1): 173-185. Doi: 10.21608/ejfs.2021.66232.1100.
20. Abeyrathne EDNS, Nam K, Ahn DU. Analytical methods for lipid oxidation and antioxidant capacity in food systems. Antioxidants. 2021; 10: 1587. Doi: 10.3390/ antiox10101587.
21. Cintya H, Putra EDL, Muhammad M, Pranata C, Syahputra HD. Analysis of carbohydrate, protein and fat levels using various type rice with different cooking process. IOP Conf. Ser. Earth Environ. Sci. 2022; 977: 012079. Doi: 10.1088/1755-1315/977/1/012079.
22. Zhou DD, Saimaiti A, Luo M, Huang SY, Xiong RG, Shang A, Gan RY, Li HB. Fermentation with Tea Residues Enhances Antioxidant Activities and Polyphenol Contents in Kombucha Beverages. Antioxidants. 2022; 11: 155. Doi: 10.3390/ antiox11010155.
23. Odion, E.E., Falodun, A, Adelusi, SA. Total flavonoid, Total Phenolic and antioxidant potential of root bark extract and fractions of from *Cola rostrata* (Sterculiaceae) K. Schum. Uniben J. Sci. 2013; 1:38–42.
24. Torre LC, Fazio A, Caputo P, Plastina P, Caroleo MC, Cannataro R, Cione E. Effects of long-term storage on radical scavenging properties and phenolic content of kombucha from black tea. Molecules. 2021; 26:5474. Doi: /10.3390/molecules26185474.
25. Barbosa CD, Uetanabaro APT, Santos WCR, Caetano RG, Albano H, Kato R, Cosenza GP, Azeredo A, G´oes-Neto A, Rosa CA, Teixeira P, Alvarenga VO, Lacerda ICA. Microbial–physicochemical integrated analysis of kombucha fermentation. Lwt. 2021; 148: 111788. Doi: /10.1016/j.lwt.2021.11178.
26. Gao Y, Wang J, Fua Y, Yina J, Shic J, Xua J. Chemical composition, sensory properties and bioactivities of *Castanopsis lamontii* buds and mature leaves. Food Chem. 2020; 316: 126370. Doi: /10.1016/j.foodchem.2020.126370.
27. Xia X, Dai Y, Wu H, Liu X, Wang Y, Yin L, Wang Z, Li X, Zhou J. Kombucha fermentation enhances the health-promoting properties of soymilk beverage. J. Funct. Foods. 2019; 62: 103549.
28. Srigley CT, Mossoba MM. Current analytical techniques for food lipids. Food Saf. Innov. Anal. Tools Saf. Assess. 2016; 33–64.
29. Fatmawati S, Yuliana, Purnomo AS, Abu Bakar MF. Chemical constituents, usage and pharmacological activity of *Cassia alata*. Heliyon. 2020; 6: e04396. Doi: /10.1016/j.heliyon.2020.e04396.
30. Candra A, Prasetyo BE, Tarigan JB. Study of vitamin C level of soursop leaves (*Annona muricata* l.) and galactomannan utilization in kombucha during fermentation. AIP Conf. Proc. 2021; 2342. Doi: 10.1063/5.0045669.
31. Gombart AF, Pierre A, Maggini S. A review of Micronutrients and the Immune System-Working in Harmony to Reduce the Risk of Infection. Nutrients. 2020; 12:236. Doi: 10.3390/nu12010236.

32. Rahmat A, Edrini S, Md Akim A, Ismail P, Hin TYY, Abu Bakar MF. Anticarcinogenic effects of *Strobilanthes crispus* extracts and its compound in vitro. Intl. J. Cancer Res. 2006; 2(1):47–49.
33. Abu Bakar FI, Abu Bakar MF, Abdullah N, Endrini S, Fatmawati S. Optimization of Extraction Conditions of Phytochemical Compounds and Anti-Gout Activity of *Euphorbia hirta* L. (Ara Tanah) Using Response Surface Methodology and Liquid Chromatography-Mass Spectrometry (LC-MS) Analysis. Evidence-based Complement. Altern. Med. 2020; 4501261.Doi: 10.1155/2020/4501261.
34. Abdulqader A, Ali F, Ismail A, Mohd Esa N. Antioxidant compounds and capacities of Gac (*Momordica cochinchinensis* Spreng) fruits. Asian Pac. J. Trop. Biomed. 2019; 9(4): 158–167. Doi: 10.4103/2221-1691.256729.
35. Zielińska D, Bilska B, Marciniak-łukasiak K, Łepecka A, Trząskowska M, Neffe-skocińska K, Tomaszewska M, Szydłowska A, Kołożyn-krajewska D. Consumer understanding of the date of minimum durability of food in association with quality evaluation of food products after expiration. Int. J. Environ. Res. Public Health. 2020; 17: 1632. Doi:10.3390/ijerph17051632.
36. Abu Bakar MF, Ahmad NE, Suleiman M, Rahmat A, Isha A. *Garcinia dulcis* Fruit Extract Induced Cytotoxicity and Apoptosis in HepG2 Liver Cancer Cell Line. Biomed Res. Int. 2015; 916902. Doi: /10.1155/2015/916902.
37. Wang B, Rutherfurd-Markwick K, Zhang XX, Mutukumira AN. Kombucha: Production and Microbiological Research. Foods. 2022; 11: 3456. Doi: 10.3390/ foods11213456.