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Encapsulation of Phenolics in Kombucha Tea: Antioxidant Activity of End Products

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Kombucha tea is a beverage rich with phenolic compounds. In this study, encapsulation aims to minimize the degradation of phenolic compounds by using different coating materials namely maltodextrin and gum Arabic. Encapsulated Kombucha tea was obtained by freeze-drying process and the antioxidant activity was assessed by using DPPH and FRAP assays. Gum Arabic also demonstrated consistently higher TPC (254.75 mg GAE/g) and antioxidant activities (141.36 mg GAE/g) values compared to that of maltodextrin. The results of identified compounds showed the catechins of Kombucha tea were successfully encapsulated with the presence of sugar moieties in the chemical structure. The results revealed the presence of of catechins with glucose moiety viz; (−)-epigallocatechin 3-gallate, (−)-epicatechin pentaacetate, epicatechin 3-gallate and gallocatechin(4α→8)-epicatechin in encapsulated Kombucha tea. Kombucha tea exhibited similar trends of pre- and post-encapsulation antioxidant activity. Conclusively, this study presents important information to elucidate the potential actions of organic acids and the activities of Kombucha tea following fermentation and encapsulation process.

Keywords: Kombucha tea; encapsulation; phenolic compounds; antioxidant activity

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Kombucha tea is produced through the fermentation process performed by a symbiotic culture of yeast (SCOBY) and bacteria in a pancake-like shape [1]. It was originated from Northeast China and spread the fermented tea to Japan and eventually to Europe [2]. Kombucha tea has been reported to offer several health benefits such as enhancing the resistance against cancerous cells, promoting digestive functions, preventing cardiovascular diseases, and immune-stimulating activities [3]. Kombucha SCOBY is commonly fermented for 10-14 days with sugar and black or green tea. The tea leaves contain polyphenols, which could be oxidised after a multi-stage enzymatic process [3].

Phenolic compounds are recognised for their sensitivity to high pH, temperature, oxygen, degradation enzymes, and light. Hence, phenolic compounds need to be protected by developing specific strategies to enhance their bioaccessibility and bioavailability while preserving their biological activities [4]. Encapsulation entails a technique used in encapsulating bioactive compounds with coating materials or entrapping them within shells or carriers [5]. The examples of encapsulation are spray drying, freeze drying and vacuum drying. These methods are effective in preservation of bioactive ingredients, phenolic content and retain the antimicrobial activities antioxidant properties, which extends the shelf life [6]. Kombucha tea can only be stored for four months before degradation, where in the fifth month the polyphenol content which is responsible for the antioxidant activity decreases significantly. Only one-third of the initial polyphenol amount is left in the ninth month [7].

Therefore, the present study was designed to employ the common coating materials, viz; maltodextrin and gum Arabic for the encapsulation of phenolic compounds of Kombucha tea. Determination of phenolic content before and after encapsulation is important for understanding the degradation or changes of compounds during the process.

EXPERIMENTAL

Materials

The Kombucha SCOBY was obtained from the Food Biotechnology Laboratory at Faculty of Science and Food Technology of Universiti Putra Malaysia. The black tea (Camellia sinensis) used was bought from Desa Tea Sdn. Bhd. The wall materials used were maltodextrin and gum Arabic purchased from R&M Chemicals and Systerm Chemicals respectively. The Trolox was bought from Acros Organics B.V.B.A. DPPH reagent was purchased from Alfa Aesar.

Fermentation of Kombucha Tea

The ratio for tea to water was 1:10. Twenty grams of tea sample which was used as medium for fermentation was left in 2 litre of boiled water for 15 minutes.

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A total of 140g (70 g per litre) of sugar was added. The prepared tea broth was transferred to glass jars which were sterilized at 121 °C for 20 minutes. After cooled, all samples were inoculated with 14g (7g per litre) Kombucha SCOBY pellicle. Glass jars were covered quickly and left to fermentation at 24 ± 1 °C and in the dark for 14 days.

Encapsulation of Kombucha Tea

Maltodextrin and gum Arabic were used to encapsulate Kombucha tea. The ratio of maltodextrin 30%(w/v) and gum Arabic (25%) to Kombucha tea has been used [8]. The mixtures were then agitated for 30 minutes at 25 degrees Celsius with a magnetic stirrer until homogenised. Prior to freeze-drying, the samples in the falcon tubes samples were stored in a freezer at -80 °C. The frozen samples were then lyophilised in a freeze-dryer (FreeZone 6 Liter Benchtop Freeze Dry System, Labconco) for 72 hours at −50 °C. The freeze-dried powders were collected, wrapped in foil bags, and stored in a desiccator for further analysis

Extraction of Phenolic Compound

Using a vortex, the pre-and post-encapsulated dried Kombucha tea samples (0.25 g) were mixed with 10 mL of 90% methanol for 5 minutes. The mixture was sonicated for 30 minutes. The mixture was centrifuged at 3,000 rpm for 15 min. The supernatant was then separated for analysis

Determination of Total Phenolic Content (TPC)

A method proposed by AH Ishak, NH Shafie, NM Esa, H Bahari and A Ismail [9] with slight modifications of the volume of solutions was employed to determine the total phenolic content. The samples or standard (20 µL) were mixed with the dilute Folin-Ciocalteu reagent (1:10, v/v in distilled water) in a 96-well plate. Then, each well was filled with 80 µL of 10% sodium carbonate (Na2CO3) after 5 minutes, followed by measuring the absorbance at 765 nm against a reagent blank. Using gallic acid (100, 200, 300, and 500 µg/mL), a standard calibration curve was plotted and expressed as mg gallic acid equivalent (GAE)/g extract.

Antioxidant Assay

DPPH Free Radical Scavenging Assay

The method described by AH Ishak, NH Shafie, NM Esa, H Bahari and A Ismail [9] was used to determine the DPPH radical scavenging capacity. A 50 µL of various concentrations of samples, ranging from 100 to 1000 µg/mL, was mixed with 195 µL of 100 µM DPPH solution in a 96-well plate. The mixture was left in the dark at room temperature for 30 minutes, followed by measuring the absorbance of the reaction mixture at 517 nm. Trolox was used as standards, whilts 50% methanol solution and DPPH solution were used as controls.

Ferric Reducing Antioxidant Potential Assay (FRAP Assay)

A mixture of 10 mM TPTZ (2, 4, 6- tripyridyl-s-triazine), 300 mM acetate buffer (3.1 g $C_2H_3NaO_2.3H_2O$ and 16 mL $C_2H_4O_2$), pH of 3.6, 20 mM $FeCl_3.6H_2O$ solution and a solution in 40 mM HCl were used to prepare the stock solution. Meanwhile, a fresh preparation of 2.5 mL of 10 mmol/L TPTZ in 2.5 mL $FeCl_3.6H_2O$ and 25 mL acetate buffer were warmed at 37 ºC before use. Then, 20 µL of the samples were mixed with 2800 µL FRAP solution and left to react. Kombucha tea (150 mL) was allowed to react within 15 minutes in the dark before measuring the absorbance at 593 nm.

Identification of Phenolic Compounds

The profile of the phenolic compounds was characterised using Waters ACQUITY UPLC I-Class systems (Waters, Milford, USA) equipped with an autosampler, a binary pump, a diode-array detector (DAD), and a degasser. Meanwhile, the column UPLC HSS T3 C18 (2.1 mm×100 mm, 1.8 µm) was employed with a linear gradient elution of A (0.1% formic acid in deionized water) and B acetonitrile (ACN) for 16 minutes at a flow rate of 0.5 mL/minute. The volume of sample injection was 3 µL. The Xevo G2-S QTOF/ MS (Waters, Milford, USA) fortified with an ion mobility system, an electrospray ion source, and a quadrupole-time-of-flight (Q-TOF) mass spectrometer was used to record all the data. The system was operated in a positive ion mode between m/z 100 and 1000 Da, whereas Waters UNIFI Vion software was used for the system control. The MS fragmentation data were match automatically by the phenolic compounds group in Traditional Medicine Library.

RESULTS AND DISCUSSION

Antioxidant Capacity

The phenolic contents of Kombucha tea before and after encapsulation with different coating materials are shown in Table 1. As can be seen, the TPC of Kombucha tea and encapsulated samples with gum Arabic was not much different with the values of 254.25 ± 0.66 and 254.75 ± 0.76 respectively. The phenolic content was expressed in gallic acid equivalent (GAE) had shown that Kombucha tea encapsulated with maltodextrin was lower than gum Arabic with the value of 241mg GAE/g sample. The result was significant as the $p < 0.05$. The results consistent with the finding by [8] showed that TPC of encapsulated propolis with maltodextrin were significantly lower when compared to gum Arabic with IC_{50}, 241.36 compared to 254.75. Maltodextrin has exceptional properties such as low viscosity, high solubility in water, biodegradable, flavourless, and cost-effective, however, its marginal retention of volatility and low emulsifying capacity are the critical limitations [10].

Table 1. TPC and IC_{50} values of DPPH and FRAP assays of kombucha tea before and after encapsulation.

Extract	TPC (mg GAE/g sample)	IC_{50} values (µg/mL)	
		DPPH	FRAP
KT	254.25 ± 0.66	185.15 ± 0.65	165.15 ± 0.16
KTGA	254.75 ± 0.76	185.68 ± 0.04	175.25 ± 0.48
KTMD	241.36 ± 0.24	218.11 ± 0.08	201.23 ± 0.09

* Kombucha Tea (KT), Kombucha Tea Gum Arabic (KTGA), Kombucha Tea Maltodextrin (KTMD)

In comparison to the amphiphilic glycoprotein fraction present in the coating material, gum Arabic, the material has the ability of encapsulating hydrophilic and hydrophobic compounds. These properties lead to the formation of a strong protective matrix around the core material, resulting in higher encapsulation efficiency estimates.

The DPPH radical scavenging ability and ferric reducing antioxidant power (FRAP) were used to measure the antioxidant activities of various encapsulated Kombucha tea. As shown in Table 1, the DPPH and FRAP assay results revealed that the antioxidant activity relates to the phenolic contents. DPPH assay expressed in IC_{50} for Kombucha tea and gum Arabic were 185.15 µg/mL and 185.68 µg/mL respectively while with maltodextrin value was 218.11 µg/mL. Similar to the trend of FRAP, the antioxidant of Kombucha tea and gum Arabic were higher than the maltodextrin. The present study proves that the association between antioxidant activity and total phenolic was proportional as evidenced when the inhibition and IC_{50} value are compared. The inhibition increases as the IC_{50} value decreases for higher phenolic contents, thereby denoting greater antioxidant activities.

Table 2. Ion responses detected by UPLC QTOF/MS of phenolic compounds of kombucha tea before and after encapsulation.

Component Name	**Ion Response**		
	KT	**KTGA**	**KTMD**
(−)-Epigallocatechin 3-gallate	-	243	228
(−)-Gallocatechin	143	-	-
(−)-Epicatechin	225	-	-
(−)-Epicatechin pentaacetate	-	146	142
(−)-Epigallocatechin	237	-	-
(+)-Gallocatechin hexaacetate	899	896	884
Catechin-(4α→8)-catechin	395	343	346
Epicatechin 3-gallate	-	215	208
Epigallocatechin(4β,8)gallocatechin	-	338	437
Gallocatechin(4α→8)-epicatechin	-	149	145

Kombucha Tea (KT), Kombucha Tea Gum Arabic (KTGA), Kombucha Tea Maltodextrin (KTMD)

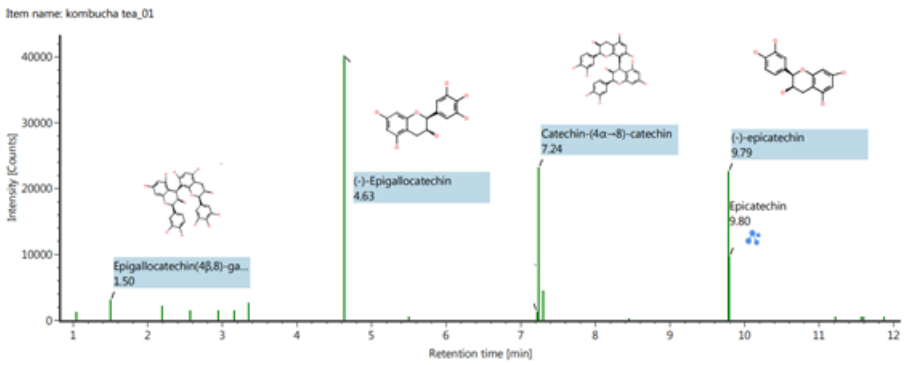


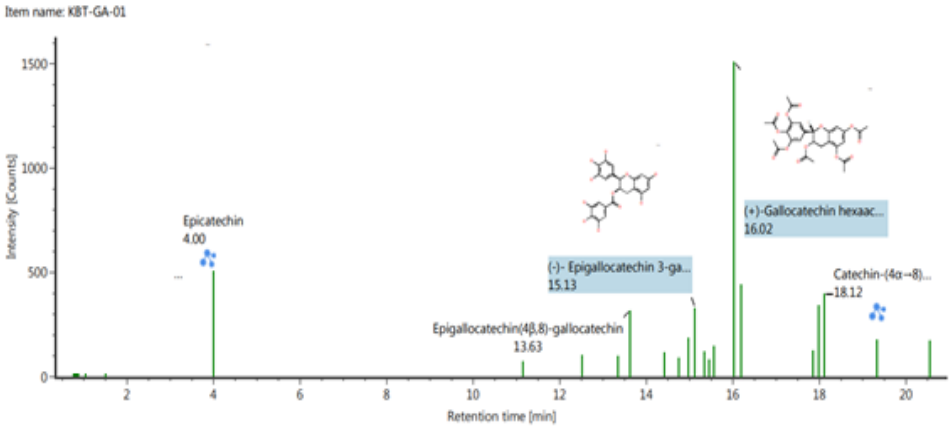
Figure 1. Catechins derivatives identified of the kombucha tea.

Identification of Phenolic Compounds

Despite an extensive documentation of the composition and properties of tea, there is limited scientific information regarding the composition of Kombucha tea. Thus, in the present study, the phenolic compounds of Kombucha tea were identified by UPLC QTOF/MS. Table 2 illustrates the phenolic compounds in Kombucha tea, encapsulated Kombucha tea with maltodextrin (KTMD) and gum Arabic (KTGA). In the fermented Kombucha tea the most abundant ion response detected were (−)-epicatechin and (−)-epigallocatechin (Figure 1) The results show that the fermented Kombucha tea contained important characteristic of tea polyphenols which were catechins and the major ones (−)-epicatechin (EC), (−)-epicatechin gallate (ECG), (−)-epigallocatechin (EGC), (−)-epigallocatechin gallate (EGCG), (+)-catechin (C), and (+)-gallocatechin (GC) [11]. The astringency, bitterness, and sweet aftertaste of tea beverages are significantly impacted by these compounds. The results of Kombucha tea showed that there were no changes of the tea composition by fermentation process. The findings reflected that the strong antioxidant activities were mainly attributed to the catechins. Hence, these benefits ascribed to Kombucha tea might be possibly related to the catechin content of tea itself.

The encapsulation by freeze drying process led to a chemical interaction of the phenolic compounds with the sugar moiety from the wall materials. This fact can be observed in the catechins content and its ion responses. The results revealed the presence of catechins with glucose moiety viz;. (−)-epigallocatechin 3-gallate, (−)-epicatechin pentaacetate, epicatechin 3-gallate and gallocatechin(4α→8)-epicatechin in the encapsulated Kombucha tea with gum Arabic and maltodextrin (Figure 2). The new compounds identified after encapsulation might be explained by the reaction of hydroxyl group in the phenolic compound with the sugar moieties of the coating materials. The backbone structure of gum Arabic is mainly composed of 1,3-linked β-d-galactopyranosyl units while maltodextrin consist of D-glucose units. Thus, the new catechin derivative contained sugar moieties and the results revealed the encapsulation process occur with chemical interaction. The TPC of encapsulated powder of gum Arabic was almost similar to the Kombucha tea and was supported with identified compounds that were synchronize with targeted phenolic compounds in TCM library of UPLC QTOF/MS.

a)



b)

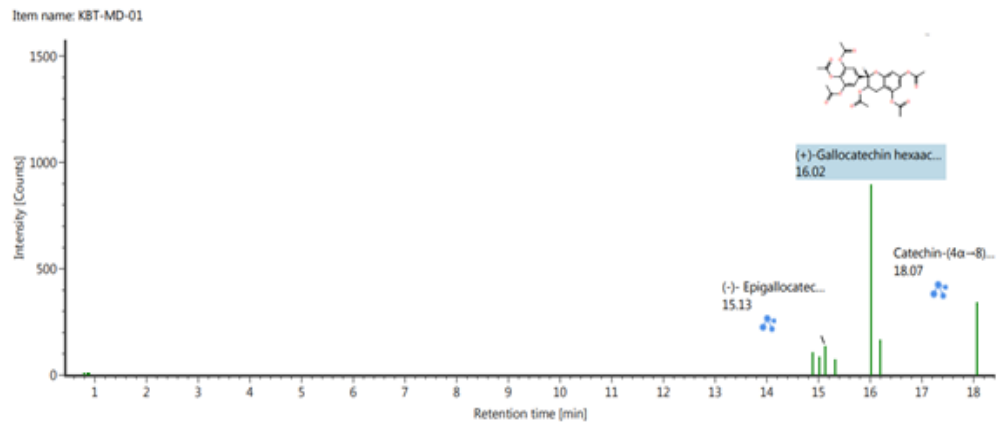


Figure 2. Catechins derivatives identified of the kombucha tea encapsulated with a) gum Arabic b) maltodextrin.

The identified compounds can be related to the antioxidant properties of Kombucha tea before and after encapsulation. In general, antioxidant activity of phenolic compounds depends on occurrence, position, structure, and total number of sugar moieties. The complementary results demonstrated the antioxidant properties of encapsulated Kombucha tea were similar to the non-encapsulated one due to the presence of phenolic compounds.

CONCLUSION

The tea and fermentation products are responsible for the health benefits of Kombucha. The result demonstrated that gum Arabic showed better encapsulation of phenolic compounds of kombucha tea than the maltodextrin. The antioxidant activity showed correlation with TPC. The identification of compounds was limited to derivatives of catechins only. The findings revealed the biological activities and the underlying specific substances while attempting to improve the current understanding of Kombucha tea pre-and post-encapsulation. Results regarding the health-related benefits of tea and fermented tea is relevant given the widespread consumption of beverages worldwide.

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