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# Biological, chemical and antioxidant activities of different types Kombucha

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ABSTRACT

Three types of Kombucha fermented tea, rice, and barley were produced in order to study their changes of biological and chemical parameters. About a 2-fold increase in mushroom dry weight after 8 days of growth was observed with Kombucha tea. The optical density of the Kombucha extracts gave the same trend. Moreover, Kombucha tea recorded the highest specific growth rate and lowest doubling time. The total acidity, ethanol content, and total protein content were incremented with the fermentation process to reach the peak after 6–8 days as in descending order: tea > barley ≥ rice. The scavenging abilities of DPPH were in descending order, tea (89.69%) > barley (76.19%) > rice (36.04%). The total phenolic compounds of Kombucha tea (88.8 ppm) have the same trend, which more than 3-fold of Kombucha rice (26.11 ppm). Results revealed that the Kombucha tea preservation method using heat treatment (at 76 and 100 °C for 10 min) was not successful, resulting in a decrease in antioxidant amounts to 71.0 and 53.42% and total phenolic compounds to 40.22 and 45.69 ppm, respectively.



## 1. Introduction

Kombucha black tea (KT) is an acidic, fermented, tea-based beverage associated with remarkable health benefits (Dickmann et al., 2017), including antioxidant, anti-inflammatory, anti-cancer, hypoglycemic, and antimicrobial effects. KT is typically composed of brewed sugared tea and a symbiotic culture of bacteria and yeast, which forms a carbonated, acidic beverage during a 7–21 days fermentation (Dufresne and Farnworth, 2000; Lee, 1996). Consumption of Kombucha has been shown that many claimed health beneficial effects such as cancer prevention and immunity enhancement. That is related to its antioxidant activities and attributed to the presence of polyphenols and certain organic acids produced during fermentation. These bioactive compounds overcome on arteriosclerosis, toxin excretion, diabetes, nervousness, and aging problems (Bhattacharya et al., 2011; Dufresne and Farnworth, 2000). Kombucha also has various health benefits, such as antibacterial activity against pathogenic bacteria. The high acidity of Kombucha and other biochemical compound provides a novel strategy for control pathogens and food-spoilage bacteria (Shahbazi et al., 2018). Other forms of Kombucha black tea such as green, white and yellow tea and Kombucha fermented with herbal drinks (cinnamon, anise, mint, marjoram, and sage) and leaves extract from *Brassica tournefortii* as suggested by Gramza-Michałowska et al. (2016); Rahmani et al. (2019).

Acetic acid bacteria have formed a floating cellulose layer in the form of cream-colored or light beige, also known as microbial mats and biofilms or mushrooms. It is possible that the name "Kombucha" originates from Japanese words "kombu" which means seaweed and "cha" that stands for tea. The different theory claims that it may have a connection with the name of previously mentioned Korean physician Kombu (Jarrell et al., 2000). Sucrose is a fermented carbon source in the cultivation medium is hydrolyzed by invertase from yeasts. The yeasts ferment glucose and fructose to ethanol, which is then oxidized by acetic acid bacteria to acetic acid. This is the main metabolic path of Kombucha fermentation, and acetic acid, ethanol, and gluconic acid are the main tea fungus products (Sievers et al., 1995). The yeast species in Kombucha consantrum were contains different yeast genera such as *Candida*, *Zygosaccharomyces*, *Pichia*, *Schizosaccharomyces*, *Saccharomyces*, and *Brettanomyces*/Dekkera. The last two genera play a role in acetic acid production with other acetic acid bacteria of species of *Acetobacter*, *Gluconacetobacter* spp. and *Lactobacillus* spp. (Jayabalan et al., 2014; Reva et al., 2015; Trovatti et al., 2011).

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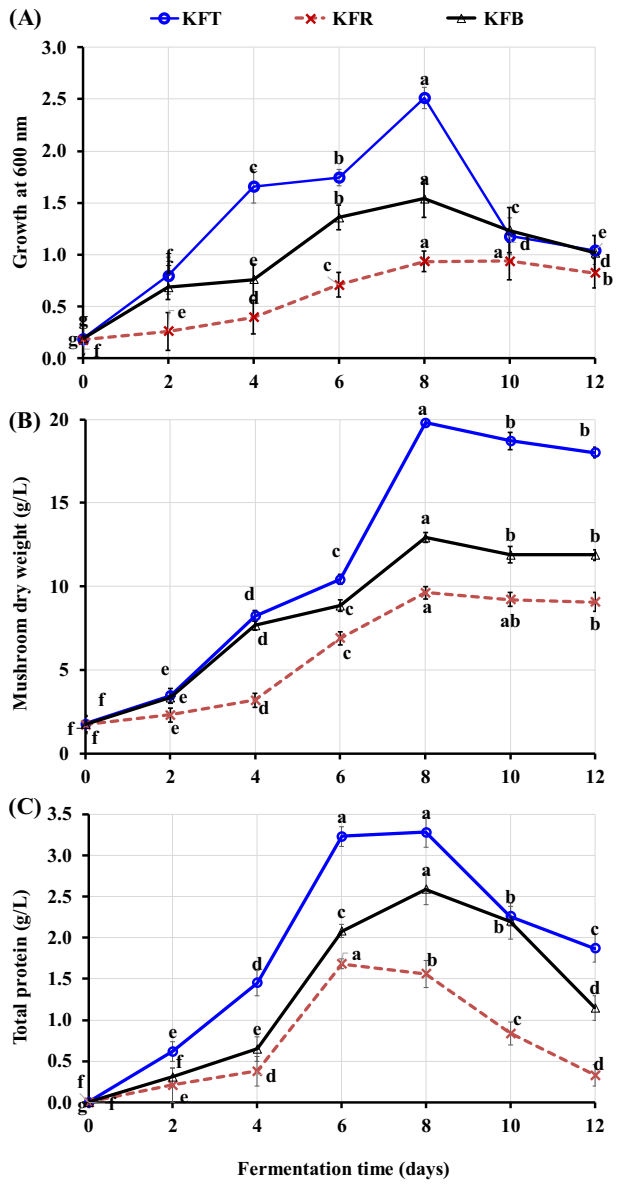


**Fig. 1.** Kombucha liquor (A) and mushrooms dry weight (B) patterns and total protein (C) during Kombucha fermentation on different substrates of tea (KFT), rice (KFR) and barley (KFB). Different letters on top of bars in the same line indicate significant differences, and the same letters do not significantly differ from each other, according to Duncan's (1955) at 5% level. Error bar = ±SE.

The aim of the current research was to cultivate Kombucha on multiple substrates and to assess their biological and chemical character as well as to determine their antioxidant activity.

## 2. Material and methods

### 2.1. Samples and Kombucha culture collection

Black tea (*Camellia sinensis*), rice (*Oryza sativa*), and barley (*Hordeum vulgare*) collected from market in Cairo, Egypt. Kombucha culture used as a starter of the fermentation process obtained from Agricultural Microbiology Department, Agriculture Faculty, Ain Shams University.

### 2.2. Kombucha preparation and fermentation process

Kombucha produced on tea (KT), rice (KR), and barley (KB) substrates. It prepared using the technique proposed by Abou-Taleb et al. (2017). Four grams of the substrate (black tea, rice, or barley) soaked in 1 L of boiling tap water for 15 min, then filtered into a sterilized glass jar (length: 12 cm and radius: 3 cm) using filter paper Whatman No. 1. The commercial sucrose (7%) dissolved in the resulting clear filtrate. This filtrate inoculated with 10% of the fermentation broth resulting from the earlier fermentation of black tea produced (one milliliter of standard inoculum containing 5.95, 6.6 and 4.7 log colony-forming unit (CFU)/mL of each total bacteria, yeast, and acetic acid bacteria, respectively and their chemical composition was previously measured by Ahmed (2018) as pH, 2.37; total acidity, 6.52 g/L acetic acid, and 4.52 g/L ethanol) under the same conditions, then jars were covered with a clean piece of cloth. The fermentation of 3 Kombucha kinds was performed at room temperature (28 ± 2 °C), and one jar was withdrawn every 2 days until the 12th day of the fermentation period. At the end of the fermentation period, the Kombucha mushroom (formed by microorganisms and cellulose floating pellicle layer) was separated from surface culture using tongs. In the culture, the growth density (as the optical density (O.D)) and pH were determined. Both Kombucha mushroom dry weight and O.D were determined as biological parameters as subsequently described. The relationship plotted between time (days) and each of O.D and Kombucha mushroom dry weight. During the logarithmic phase, the growth parameters of specific growth rate (μ), doubling time ($t_d$), multiplication rate (MR), and the number of generations (N) were calculated. The Kombucha fermented solutions (supernatants) were obtained after the culture centrifugated at 10,000 rpm for 15 min and used to measure the chemical parameters as described below.

### 2.3. Biological (growth) determination

The growth density (O.D) of fermented culture was determined at 600 nm by using a spectrophotometer (Unico S2100 series UV/–Vis). Kombucha mushroom dry weight was determined by separating the mushroom from the culture into filter paper and washed three times with distilled water, then dried at 80 °C until the weight had been constant (Harta et al., 2004; Malbaša et al., 2008).

### 2.4. Chemical determination

**pH values** were measured using an electronic pH meter (Hanna) calibrated at pH 4.0 and 7.0. **Titratable acidity (TA)** was determined, according to Jacobson (2006). After removing $CO_2$ from the fermentation broth at 100 °C in a water bath for 10 min, a 20-mL aliquot was taken and titrated with 0.1 mol/L NaOH. The TA was expressed in grams of acetic acid per liter of the sample. **Ethanol concentration** was assayed using the redox back titration method, according to Iland (2000). **The total protein concentration** was determined by the method of Bradford (1976) using bovine serum albumin (BSA) as standard. **2,2-Diphenyl-1-picrylhydrazyl (DPPH)** free radical-scavenging ability of Kombucha fermented solutions (KFSs) from tea (KFTS), rice (KFRS) and barley (KFBS) were measured according to the method with some modifications described by Marques et al. (2012). Two milliliters of 160-fold diluted Kombucha fermented solutions were mixed with 2 mL of 0.1 mmol/L DPPH methanolic solution. 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. **The total phenol content** of Kombucha fermented solutions was measured according to the Folin-Ciocalteu method (Singleton et al., 1999). About 0.1 mL of the test sample transfer to an Erlenmeyer flask (100 mL) and the final volume was adjusted with distilled water up to 46 mL. Afterward, 1.0 mL of Folin-Ciocalteu reactive solution was added and incubated at room temperature for 3 min. Three

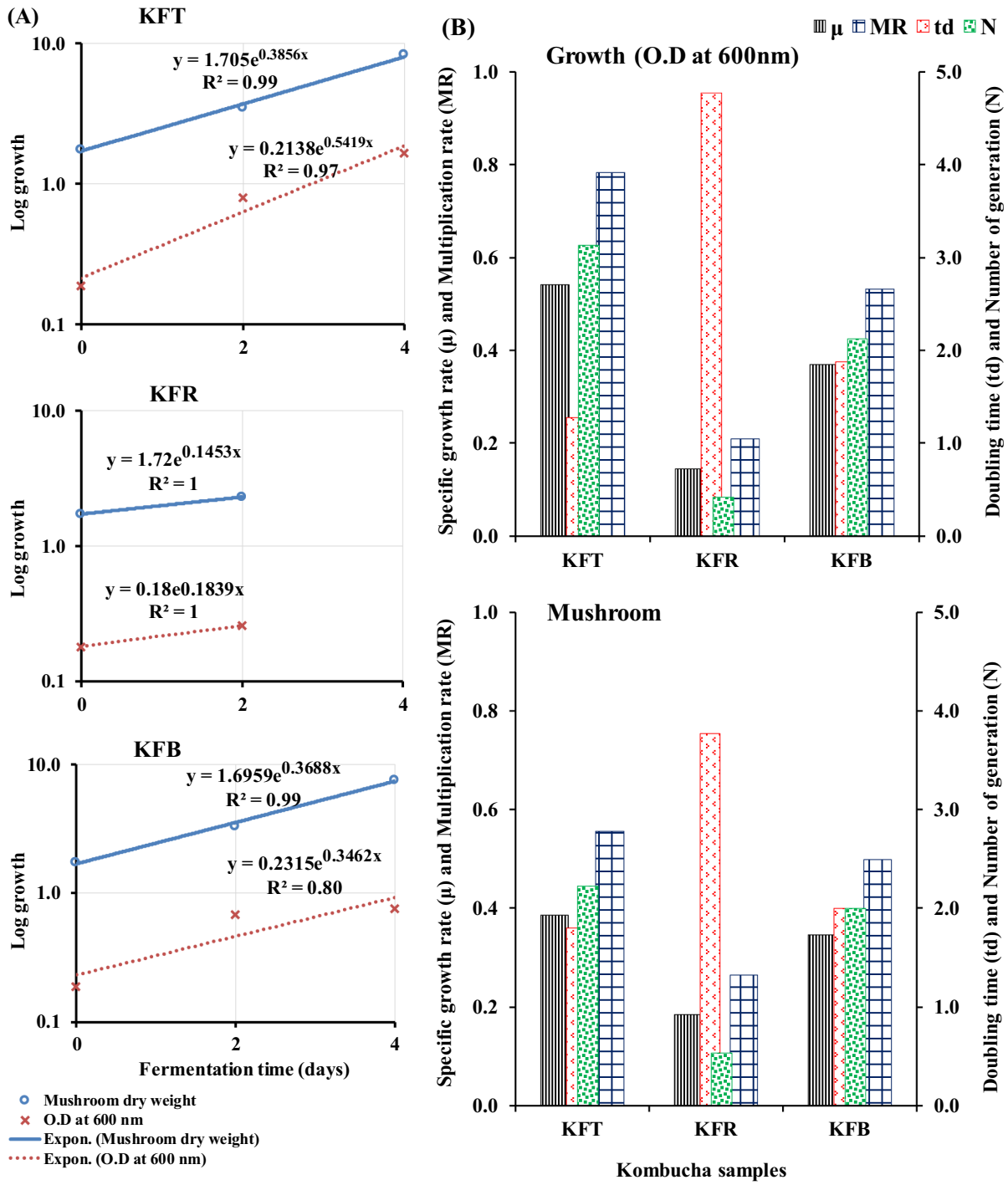


**Fig. 2.** (A) Growth in the extract and mushrooms dry weight in the log phase and determination coefficient (R2). (B) Growth and mushroom kinetics of Kombucha samples calculated during the log phase. KFT = Kombucha fermented tea, KFR = Kombucha fermented rice, and KFB = Kombucha fermented barley. μ = specific growth rate, $t_d$ = doubling time, MR = multiplication rate and N = number of generations.

milliliters of sodium carbonate (2% *w*/*v*) was mixed with the above solution. The absorbance at 760 nm was then measured after 30 min. The total phenol was expressed as gallic acid equivalents from the calibration curve.

### 2.5. Parameters calculations

According to Doelle (1975), the specific growth rate (μ), doubling time ($t_d$), and multiplication rate (MR) were calculated. The number of generations (N), effective yield, ethanol or acetic acid productivity and ethanol or acetic acid yield coefficient relative to biomass were calculated according to Grothe et al. (1999); Lee (1996); Ramadan et al. (1985); Stanier et al. (1958) respectively. Their equations were the following:

$$\text{Specific growth rate } (\mu)\left(d^{-1}\right) = (\ln X - \ln X0)/(t - t0),$$

where

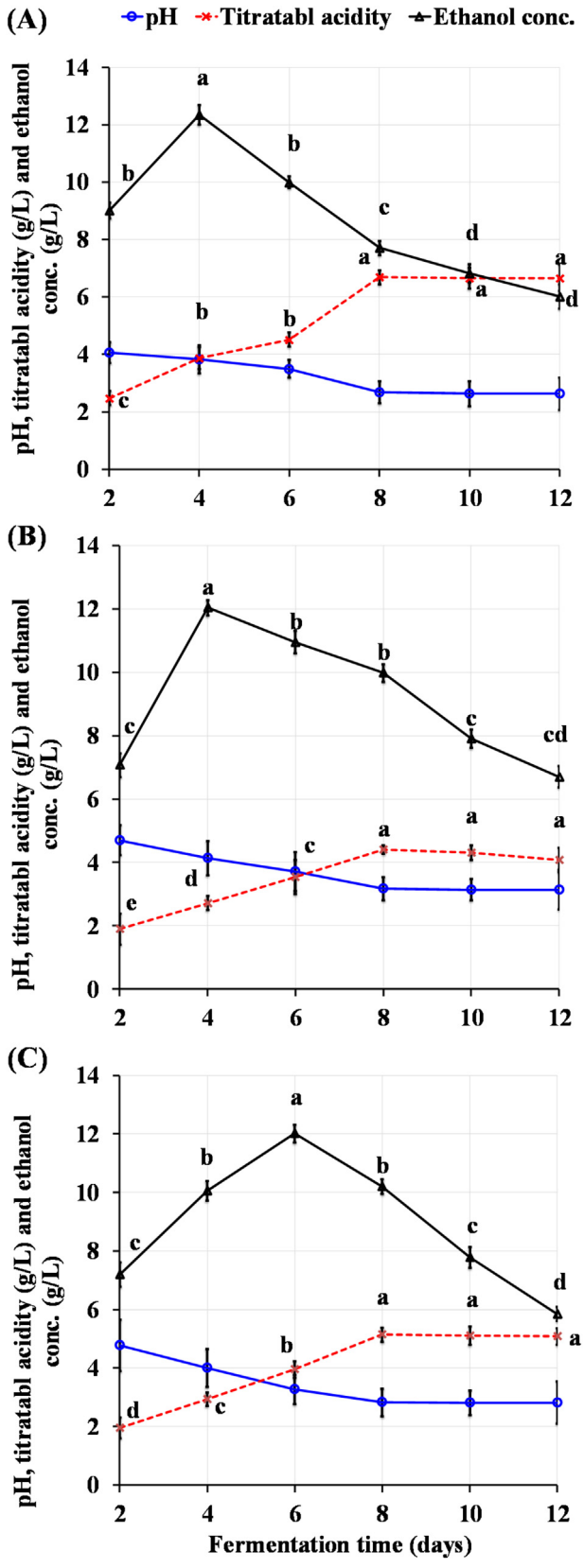


**Fig. 3.** Biochemical properties of Kombucha fermented solutions of KFTS (A), KFRS (B), and KFBS (C) during 12 days of fermentation time. Conc. = concentration, KFTS = Kombucha fermented tea solution, KFRS = Kombucha fermented rice solution, and KFBS = Kombucha fermented barley solution. Different letters on top of bars in the same line indicate significant differences, and the same letters do not significantly differ from each other, according to Duncan's (1955) at 5% level. Error bar = ±SE.

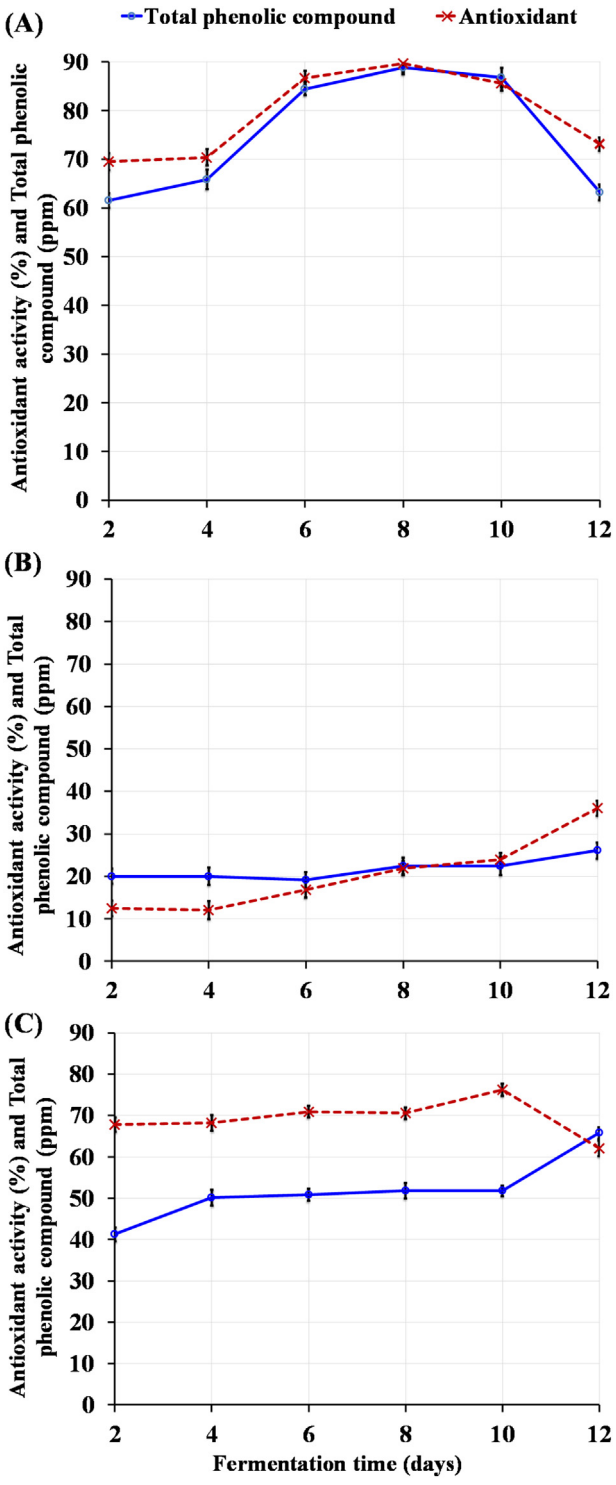


**Fig. 4.** Antioxidant activity and total phenolic compound of Kombucha fermented solutions (KFSs) on various substrates of tea (A), rice (B), and barley (C) during 12 days of fermentation time. Error bar = ±SE.

X = Amount of growth after t time (t) and X0 = Amount of growth at the beginning time (t0).

Doubling time ($t_d$) (d) = $\ln 2/\mu$.

Multiplication rate (MR) = $1/t_d$.

**Table 1**
Ethanol and acetic acid productivity and yield coefficient relative to mushroom biomass and effective yield.

| Kombucha fermented solutions | Parameters | Fermentation time (days) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 2 | 4 | 6 | 8 | 10 | 12 |
| KFTS | Ethanol productivity (g/L/d) | 4.51 | 3.09 | 1.67 | 0.96 | 0.68 | 0.50 |
| | $Y_{E/M}$ | 1.30 | 0.37 | 0.16 | 0.05 | 0.04 | 0.03 |
| | Acetic acid productivity (g/L/d) | 1.24 | 0.97 | 0.75 | 0.84 | 0.67 | 0.56 |
| | $Y_{A/M}$ | 0.72 | 0.47 | 0.43 | 0.34 | 0.36 | 0.37 |
| | Effective yield (%) | 4.94 | 11.76 | 14.89 | 28.30 | 26.73 | 25.73 |
| KFRS | Ethanol productivity (g/L/d) | 3.54 | 3.01 | 1.83 | 1.25 | 0.79 | 0.56 |
| | $Y_{E/M}$ | 3.07 | 3.76 | 1.59 | 1.04 | 0.86 | 0.74 |
| | Acetic acid productivity (g/L/d) | 0.95 | 0.68 | 0.59 | 0.55 | 0.43 | 0.34 |
| | $Y_{A/M}$ | 0.83 | 0.85 | 0.51 | 0.46 | 0.47 | 0.45 |
| | Effective yield (%) | 3.29 | 4.57 | 9.86 | 13.74 | 13.16 | 12.94 |
| KFBS | Ethanol productivity (g/L/d) | 3.60 | 2.52 | 2.00 | 1.28 | 0.78 | 0.49 |
| | $Y_{E/M}$ | 2.16 | 1.32 | 1.36 | 0.79 | 0.65 | 0.49 |
| | Acetic acid productivity (g/L/d) | 0.98 | 0.73 | 0.66 | 0.64 | 0.51 | 0.42 |
| | $Y_{A/M}$ | 0.59 | 0.38 | 0.45 | 0.40 | 0.43 | 0.43 |
| | Effective yield (%) | 4.76 | 10.93 | 12.63 | 18.46 | 17.00 | 16.97 |

KFTS = Kombucha fermented tea solution, KFRS = Kombucha fermented rice solution, KFBS = Kombucha fermented barley solution, Y = yield, M = mushroom dry weight, E = ethanol concentration, and A = acetic acid concentration.

The number of generations (N) = $(t-t0)/t_d$

where:

t = Ending time and t0 = Beginning time.

Effective yield (%) = (Mushroom dry weight (g/L)/Initial sugar (g/L)) × 100.

Ethanol or acetic acid productivity (g/L/d)
= Ethanol or acetic acid concentration (g/L)/Fermentation time (day).

Ethanol or acetic acid yield coefficient relative to biomass ($Y_E$ or$_{A/M}$)
= Ethanol or acetic acid concentration (g/L)/Mushroom dry weight (g/L).

### 2.6. Statistical analysis

Data were shown as the mean ± standard error (SE). An analysis of variance test (ANOVA), IBM® SPSS® Statistics Server Version 23.0 (2015) was used to evaluate the statistical significance of the difference suggested by Duncan's (1955) at $p \leq 0.05$. All the analyses were conducted in a triplicate.

## 3. Results and discussion

### 3.1. Biological activity of different types of Kombucha extracts

In this experiment, Kombucha was cultivated on different substrates (tea, rice, and barley) and produced three different types of Kombucha; KFT, KFR, and KFB, respectively.

Three different types of Kombucha cultures and mushrooms showed exponential growth in the case of KFR, or both of KFT and KFB, respectively, during the first 2 or 4 days of the fermentation period (Fig. 1).

Data presented in Fig. 1 indicated that the growth expressed as O. $D_{600}$ of the tested Kombucha cultures increased significantly ($p \leq 0.05$) by increasing the fermentation time to peak after 8 days for both KFB and KFT at 1.54 and 2.51, respectively, while KFR peaked significantly ($p \leq 0.05$) by 0.94 after 8 and 10 days. The dry weight of the Kombucha mushroom provided the same pattern too. After eight days of fermentation, it reached a significant peak output of 9.62, 12.92 & 19.81 g/L for KFR, KFB, and KFT, respectively. KFT produced about 2-fold of the maximum dry mushroom weight relative to KFR. The same trend of Kombucha mushroom dry weight incremental with fermentation time was stated by Amarasinghe et al. (2018), who reported that the enlarge of Kombucha mushroom is related to a cellulosic matrix that composed of acetic acid bacteria growth.

**Table 2**
Antioxidant and total phenolic compounds of Kombucha fermented tea (KFT) after 6 and 8 days of fermentation as affected with heat treatments at 76 and 100 °C for 10 min.

| Fermentation time (days) | Control | | Heat treatment for 10 min at | | | |
|---|---|---|---|---|---|---|
| | | | 76 °C | | 100 °C | |
| | Antioxidant activity (%) | Total phenolic compounds (ppm) | Antioxidant activity (%) | Total phenolic compounds (ppm) | Antioxidant activity (%) | Total phenolic compounds (ppm) |
| 6 | 86.65 | 84.61 | 63.00 | 46.33 | 50.23 | 36.22 |
| 8 | 89.37 | 88.10 | 71.25 | 40.22 | 53.42 | 45.69 |

**Table 3**
Chemical analysis of Kombucha fermented tea (KFT) after 6 and 8 days of fermentation as affected by heat treatments at 76 and 100 °C for 10 min.

| Chemical analysis | Fermentation time (days) | Heat treatment for 10 min at | |
|---|---|---|---|
| | | 76 °C | 100 °C |
| pH | 6 | 3.52 | 3.46 |
| | 8 | 2.66 | 2.60 |
| Total acidity g/L | 6 | 4.66 | 4.92 |
| | 8 | 6.23 | 6.11 |
| Total protein g/L | 6 | 4.60 | 4.29 |
| | 8 | 5.21 | 4.98 |

These data are in line with Abou-Taleb et al. (2017); Talawat et al. (2006), who noticed that the Kombucha black tea increased by $O.D_{600}$ within the first four days. After that, the O.D gradually increased until the fermentation finished (14 days). Moreover, Neffe-Skocińska et al. (2017) found that total yeast and acetic acid bacteria count increased with fermentation time to reach the peak after 10 days. As well as, other studies found that the total bacterial count in Kombucha liquor was more than in the Kombucha mat (Watawana et al., 2016).

Although no nitrogen source added to the tested substrates prior to fermentation, the results in Fig. 1 also showed a significant improvement in protein concentration with fermentation time and peaked for KFT (3.23 and 3.28 g/L) and KFR (1.68 and 1.56 g/L) after 6 and 8 days, respectively, but for KFB (2.59 g/L) after 8 days. Moreover, KFT recorded the highest protein content, approximately 1.3 and 2-folds of KFB and KFR, respectively. Protein existence in Kombucha cultures was probably owing to yeast and bacteria releasing extracellular protein throughout the fermentation process and/or initially present in the extract of substrates as has been suggested by Sreeramulu et al. (2000). The growth parameters (μ, $t_d$, MR, and N) for both Kombucha mushrooms and extracts calculated during the exponential phase. The data presented in Fig. 2 showed that 0.54/d, 0.78, 3.13, and 1.28 d of O.D and 0.39/d, 0.56, 2.23, and 1.80 d of Kombucha tea mushroom were the highest μ, MR & N and lowest $t_d$ recorded by KFT, respectively. Followed by KFB achieved 0.37, 0.53, 2.13, and 1.88 d of O.D and 0.35/d, 0.50, 2.00, and 2.00 d of Kombucha barley mushroom. On the other hand, KFR (Kombucha rice mushroom and O.D) had the lowest values of growth parameters.

### 3.2. Chemical composition of Kombucha fermented solutions (KFSs)

Data presented in Fig. 3 and Table 1 showed that pH of different KFSs decreased with increasing fermentation time to give the lowest values after 10 days being 2.63, 2.81, and 3.14 for tea, barley, and rice, respectively, and remained stable at the end of fermentation. On the other hand, total acidity (as acetic concentration) of KFSs was incremented with fermentation to reach the peak after 8 days to give the highest significant values being 6.66 g/L (with productivity and acetic acid yield coefficient relative to biomass being 0.84 g/L/d and 0.34 g/L, respectively) for KFTS then being to decline with fermentation. Data were in line with Goh et al. (2012); Velićanski et al. (2013) they noticed that Kombucha pH dropped gradually as the fermentation proceeded to reach ranged from 2.5 to 2.95 at the end of fermentation. This regarded to the presence of sucrose, which metabolized into organic acids by bacteria and yeast, led to increasing the acidity of the beverage. The pH level decreases accordingly to increase of total organic acids content during fermentation (Jayasundara et al., 2008). Furthermore, there was great attention to pH dropped and their harmful effect on the human body (Amarasinghe et al., 2018).

As well as, ethanol content of KFSs were raised during fermentation to reach the maximum significantly ($p \leq 0.05$) after 4 and 6 days then the ethanol content decline to give the lowest concentration at the end of fermentation time being 5.84, 6.02 and 6.7 g/L (with productivity and ethanol yield coefficient relative to biomass ranged from 0.49 to 0.56 g/L/d and 0.03 to 0.74 g/L for barley, tea and rice respectively). These data in line with Neffe-Skocińska et al. (2017) who found that the content of ethanol increased with the fermentation time reaching the maximum value of 1.10% on the 10th day at 25 °C, while the expected decrease of ethanol due to the conversions into acetic acid was not observed (Chakravorty et al., 2016), probably because of the short period of fermentation. It could be stated that ethanol trend inversion with a total acidity of Kombucha trend, this can be explained with the observation of Mo et al. (2008), who found that yeast converted sucrose into glucose and fructose and produce ethanol through glycolysis then acetic bacteria fermented glucose into gluconic acid and ethanol and producing acetic acid.

In addition, after 8 days of fermentation for each of KFTS, KFRS, and KFBS, the highest effective yield of dry mushrooms related to initial sugar was observed in Table 1 at 28.30%, 13.74%, and 18.46%, respectively. The effective yield after this period had been a slight decline.

### 3.3. Antioxidant activity and total phenolic compound of Kombucha fermented solutions (KFSs)

Data presented in Fig. 4 shown that antioxidant activity of different Kombucha types were increased with fermentation time to reach the highest values after 8, 10, and 12 days of fermentation for KFSs of tea, barley, and rice, respectively. These findings were in line with Amarasinghe et al. (2018), who found a significant enhancement of Kombucha antioxidant with fermentation period. DPPH's scavenging capabilities were in decreasing order, KFTS > KFBS > KFRS, KFTS recorded the highest antioxidant activity of 89.69% (after 8 days of fermentation) approximately 2.5-fold of KFRS, giving the lowest antioxidant activity of 36.04% (12 days of fermentation). With respect to total phenolic compounds, the fermentation time increased to a maximum of 88.8 ppm at the end of the fermentation time of both KFRS and KFBS, whereas KFTS gave 88.8 ppm of the highest phenolic compound after 8 days of fermentation, which was more than 3 times of the KFRS (lowest, 26.11 ppm). These data are supported by Lobo et al. (2017) found that the metabolic conversion of tea constituents during fermentation by microbial enzymes may contribute towards the increase in antioxidant activity of Kombucha when compared to tea. Furthermore, many health beneficial effects of Kombucha such as the alleviation of inflammation and arthritis, cancer prevention and immunity enhancement may be associated to its antioxidant activities, and these effects are attributed to the presence of polyphenols, certain organic acids too which produced during fermentation (Vijayaraghavan et al., 2000). As well as, Srihari and Satyanarayana (2012) found that the antioxidant activity and phenolic compounds were increased gradually with fermentation time, phenolic compounds are regarded as high-level antioxidants because of their ability to scavenge free radical and active oxygen species. Kombucha fermentation of ethyl acetate *B. tournefortii* leaves extract had significantly increased both antioxidant and total phenolic content (Rahmani et al., 2019).

### 3.4. Antioxidant and total phenolic compound of KFT as affected by heat treatment

In this experiment, KFT was exposed to high temperature (at 100 °C and 76 °C for 10 min), and antioxidant activity and total phenolic compounds were determined. Data presented in Table 2 clearly showed that the heat treatment reduced the antioxidant activity and phenolic compound, moreover KFT treated with 100 °C caused a reduction in the antioxidant activity about 36.42 and 35.95% for 6 and 8 days of KFT, respectively. Furthermore, Jayabalan et al. (2008) have the same

observation for Kombucha heat treatment; the heat treatment led to decreases polyphenols and free radical scavenging properties during storage. This observation suggested that heat treatment was not a suitable method for Kombucha preservation. On the other hand, the 8 days KFT recorded the lowest reduction in antioxidant activity after treated at 76 °C, being 18.12%. Also, the total phenolic compound affected with high temperature (100 °C), which reduced the phenolic compound more than 2-fold of 6 days KFT. Furthermore, the lowest heat treatment has a slight effect on the phenolic compound, which reduced about 1.5-fold for 8 days KFT. Heat treatment has slight or no impact on pH, total acidity, and total protein content, data showed in Table 3.

## 4. Conclusions

Data summarized that three types of Kombucha produced from fermented tea (KFT), rice (KFR), and barley (KFB). KFT was more efficient in biological (counting of yeast and acetic acid bacteria, mushroom dry weight, and total protein) and chemical (acetic acid and ethanol content) properties than KFR and KFB, as well as had higher (3-folds increase over) antioxidant activity against DPPH compared to other types. KFT heat treatment was not preferred because it reduced antioxidant activity. In the future, the properties obtained from KFT and KFT will be used as a probiotic in the field of agriculture and fermented food, as well as Kombucha mushrooms, used in the production of cellulose.

## References


Abou-Taleb, K.A., Ebeed, N.M., Abd El-salam, S.S., Amin, S.A., 2017. Antimicrobial and antiproliferative, pro-apoptotic actions of Kombucha fermented solutions against colon and heptao cancer cell lines. World J. Pharm. Life Sci. 3, 120–132.

Ahmed, R.F., 2018. Antioxidant and antibacterial activity of some fermented herbal teas with kombucha culture. Middle East J. Appl. Sci. 8, 1560–1568.

Amarasinghe, H., Weerakkody, N.S., Waisundara, V.Y., 2018. Evaluation of physicochemical properties and antioxidant activities of kombucha “tea fungus” during extended periods of fermentation. Food Sci. Nut. 6, 659–665.

Bhattacharya, S., Gachhui, R., Sil, P.C., 2011. Hepatoprotective properties of Kombucha tea against TBHP-induced oxidative stress via suppression of mitochondria dependent apoptosis. Pathophysiol. 18, 221–234.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254.

Chakravorty, S., Bhattacharya, S., Chatzinotas, A., Chakraborty, W., Bhattacharya, D., Gachhui, R., 2016. Kombucha tea fermentation: microbial and biochemical dynamics. Int. J. Food Microbiol. 220, 63–72.

Dickmann, M., Schneider, R., Armando, S., Seehusen, K., Hager, P., 2017. Analysis of the role of acidity and tea substrate on the inhibition of α-amylase by Kombucha. J. Nutr. Food Technol. 1, 1–5.

Doelle, H.W., 1975. 2-Enzymes, Coenzymes and Bacterial Growth Kinetics. ed 2nd. Academic Press, pp. 38–83.

Dufresne, C., Farnworth, E., 2000. Tea, Kombucha, and health: a review. Food Res. Int. 33, 409–421.

Duncan's, D.B., 1955. Multiple range and multiple F tests. Biometric. 11, 1–42.

Goh, W., Rosma, A., Kaur, B., Fazilah, A., Karim, A., Bhat, R., 2012. Fermentation of black tea broth (Kombucha): I. Effects of sucrose concentration and fermentation time on the yield of microbial cellulose. Int. Food Res. J. 19, 109–117.

Gramza-Michałowska, A., Kulczyński, B., Xindi, Y., Gumienna, M., 2016. Research on the effect of culture time on the kombucha tea beverage's antiradical capacity and sensory value. Acta Sci. Pol. Technol. Aliment. 15, 447–457.

Grothe, E., Moo-Young, M., Chisti, Y., 1999. Fermentation optimization for the production of poly (β-hydroxybutyric acid) microbial thermoplastic. Enzym. Microb. Technol. 25, 132–141.

Harta, O., Iconomopoulou, M., Bekatorou, A., Nigam, P., Kontominas, M., Koutinas, A.A., 2004. Effect of various carbohydrate substrates on the production of kefir grains for use as a novel baking starter. Food Chem. 88, 237–242.

IBM® SPSS® Statistics Server Version 23.0. SPSS Inc., Chicago, Illinois.

Iland, P., 2000. Techniques for chemical analysis and quality monitoring during winemaking. Handbook of Campbelltown. Patrick Iland Wine Promotions, Australia, pp. 1–111.

Jacobson, J.L., 2006. Introduction to Wine Laboratory Practices and Procedures. Springer Science & Business Media, Inc., New York, pp. 257–277.

Jarrell, J., Cal, T., Bennett, J.W., 2000. The Kombucha consortia of yeasts and bacteria. Mycologist. 14, 166–170.

Jayabalan, R., Marimuthu, S., Thangaraj, P., Sathishkumar, M., Binupriya, A.R., Swaminathan, K., Yun, S.E., 2008. Preservation of Kombucha tea-effect of temperature on tea components and free radical scavenging properties. J. Agric. Food Chem. 56, 9064–9071.

Jayabalan, R., Malbaša, R.V., Lončar, E.S., Vitas, J.S., Sathishkumar, M., 2014. A review on kombucha tea—microbiology, composition, fermentation, beneficial effects, toxicity, and tea fungus. Compr. Rev. Food Sci. F. 13, 538–550.

Jayasundara, J.W., Phutela, R., Kocher, G., 2008. Preparation of an alcoholic beverage from tea leaves. J. Inst. Brew. 114, 111–113.

Lee, S.Y., 1996. Plastic bacteria? Progress and prospects for polyhydroxyalkanoate production in bacteria. Trends Biotechnol. 14, 431–438.

Lobo, R., Dias, F., Shenoy, C., 2017. Kombucha for healthy living: evaluation of antioxidant potential and bioactive compounds. Int. Food Res. J. 24, 541–546.

Malbaša, R., Lončar, E., Djurić, M., 2008. Comparison of the products of Kombucha fermentation on sucrose and molasses. Food Chem. 106, 1039–1045.

Marques, M.R., Paz, D.D., Batista, L.P.R., Barbosa, C.d.O., Araújo, M.A.M., Moreira-Araújo, R.S.d.R., 2012. An in vitro analysis of the total phenolic content, antioxidant power, physical, physicochemical, and chemical composition of *Terminalia catappa* Linn fruits. Food Sci. Technol. 32, 209–213.

Mo, H., Zhu, Y., Chen, Z., 2008. Microbial fermented tea–a potential source of natural food preservatives. Trends Food Sci. Technol. 19, 124–130.

Neffe-Skocińska, K., Sionek, B., Ścibisz, I., Kołożyn-Krajewska, D., 2017. Acid contents and the effect of fermentation condition of Kombucha tea beverages on physicochemical, microbiological and sensory properties. CYTA-J Food. 15, 601–607.

Rahmani, R., Beaufort, S., Villarreal-Soto, S.A., Taillandier, P., Bouajila, J., Debouba, M., 2019. Kombucha fermentation of African mustard (*Brassica tournefortii*) leaves: chemical composition and bioactivity. Food Biosci. 30, 100414.

Ramadan, E., El Sawy, M., Gamal, R.F., Abd El Hady, H.M., 1985. Growth parameters of yeast grown on agricultural residues using shake flasks as a batch culture. Ann. Agric. Sci. 30, 25–45.

Reva, O.N., Zaets, I.E., Ovcharenko, L.P., Kukharenko, O.E., Shpylova, S.P., Podolich, O.V., de Vera, J.-P., Kozyrovska, N.O., 2015. Metabarcoding of the kombucha microbial community grown in different microenvironments. AMB Express 5, 1–8.

Shahbazi, H., Hashemi Gahruie, H., Golmakani, M.-T., Eskandari, M.H., Movahedi, M., 2018. Effect of medicinal plant type and concentration on physicochemical, antioxidant, antimicrobial, and sensorial properties of kombucha. Food Sci Nutr. 6, 2568–2577.

Sievers, M., Lanini, C., Weber, A., Schuler-Schmid, U., Teuber, M., 1995. Microbiology and fermentation balance in a Kombucha beverage obtained from a tea fungus fermentation. Syst. Appl. Microbiol. 18, 590–594.

Singleton, V.L., Orthofer, R., Lamuela-Raventós, R.M., 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. Methods in Enzymology. vol 299. Academic Press, pp. 152–178.

Sreeramulu, G., Zhu, Y., Knol, W., 2000. Kombucha fermentation and its antimicrobial activity. J. Agric. Food Chem. 48, 2589–2594.

Srihari, T., Satyanarayana, U., 2012. Changes in free radical scavenging activity of Kombucha during fermentation. Int. J. Pharm. Sci. Res. 4, 1978–1981.

Stanier, Y., Doudoroff, M., Adelberg, E.A., 1958. General Microbiology. ed 3th. Macmillan & Co., Ltd., London, pp. 302–306.

Talawat, S., Ahantharik, P., Laohawiwattanakul, S., Premsuk, A., Ratanapo, S., 2006. Efficacy of fermented teas in antibacterial activity. Kasetsart Journal-Natural Science. 40, 925–933.

Trovatti, E., Serafim, L.S., Freire, C.S., Silvestre, A.J., Neto, C.P., 2011. *Gluconacetobacter sacchari*: an efficient bacterial cellulose cell-factory. Carbohydr. Polym. 86, 1417–1420.

Velićanski, A., Cvetković, D., Markov, S., 2013. Characteristics of Kombucha fermentation on medicinal herbs from Lamiaceae family. Rom. Biotechnol. Lett. 18, 8034–8042.

Vijayaraghavan, R., Singh, M., Rao, P., Bhattacharya, R., Kumar, P., Sugendran, K., Kumar, O., Pant, S., Singh, R., 2000. Subacute (90 days) oral toxicity studies of Kombucha tea. Biomed. Environ. Sci. 13, 293–299.

Watawana, M.I., Jayawardena, N., Gunawardhana, C.B., Waisundara, V.Y., 2016. Enhancement of the antioxidant and starch hydrolase inhibitory activities of king coconut water (*Cocos nucifera* var. *aurantiaca*) by fermentation with kombucha 'tea fungus'. Int. J. Food Sci. Technol. 51, 490–498.