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RESEARCH ARTICLE

Evaluation of the Glucuronic Acid Production and Antibacterial Properties of Kombucha Black Tea

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Abstract: ***Background*:** Kombucha beverage is considered as a dietary supplement and drinking it strengthens the body's immune system which prevents diseases.

***Objective*:** The purpose of this study was to determine the amount of glucuronic acid and antibacterial activity of Kombucha black tea drink during its production at different storage temperature.

***Methods*:** The extent of glucuronic acid at temperatures of 20 °C and 30 °C was explored by the use of the HPLC system for 21 days. To analyse the antibacterial property, the influence of Kombucha black tea supernatant on the growth of *Salmonella* Typhimurium, *Staphylococcus aureus*, and *Lactobacillus rhamnosus* bacteria was examined via the two procedures of the disc and agar well diffusion.

***Results*:** The production of glucuronic acid underwent a variation at 20°C from 17.0 mg/L on day 1 to roughly 27.2 mg/L on day 21, and the difference was significant. Furthermore, the quantity of this acid at 30°C increased from 42.2 mg/L on day 1 to 48.0 mg/L on day 21. The amount of glucuronic acid produced at 30°C was significantly greater than that at 20°C ($p<0.05$). This study indicated that the Kombucha black tea has antibacterial activity against *Salmonella* Typhimurium and *Staphylococcus aureus*, but not against *Lactobacillus rhamnosus*. However, there are no statistical differences in antibacterial activity of Kombucha between incubation at 20°C and 30°C ($P>0.05$).

***Conclusion*:** This study offers a perspective on glucuronic acid production (especially in 30°C rather than 20°C) and antibacterial activity of Kombucha black tea beverage.

Keywords: Kombucha, antibacterial activity, glucuronic acid, tea, HPLC.

1. INTRODUCTION

Functional foods, for instance, probiotic, prebiotic and symbiotic foods are nutrients, which have a potentially positive influence on health beyond basic nourishment. Proponents of functional foodstuffs help in maintaining optimum health and diminishing the hazards of disease. Kombucha has a wonderful array of functions similar to probiotic and prebiotic foods [1-4].

The Kombucha culture looks like a white rubbery layer. The Kombucha beverage is a fermented product obtained by the Kombucha culture. This simple and almost cheap drink is a combination of black or green tea (*Camellia sinensis* L.) and sucrose or glucose, which is fermented using the Kombucha culture changing its chemical structure. Researchers believe that Kombucha beverage is considered as a dietary supplement and drinking it strengthens the body's immune system and prevents diseases. The most beneficial effects of Kombucha include its antibiotic, antiviral and antifungal properties; regulating the physiologic function of the digestive system and the glands, relieving rheumatism, gout, and hemorrhoids, reducing blood cholesterol, removing toxins and purifying the blood as well as therapeutic effects on diabetes and stress. It is also used externally for skin and hair disorders [5-7].

The Kombucha culture is a homogenous colony of yeast and bacteria belonging to the family of fungi and has the shape of a flat, smooth and viscous plate (*SCOBY*). With each fermentation process, a new layer is formed on this plate and can be isolated from the previous layer. This culture is first

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placed as a thin sheet on the surface of the tea and then becomes thickened. Therefore, different microorganisms have been isolated from the Kombucha culture. The bacteria like *Acetobacter xylinum*, *Acetobacter acetic* spp. xylinum, *Acetobacter xylinoides*, *Corynebacterium glutamicum*, *Acetobacter pasteurianus* and yeasts like *Torulopsis* sp., *Pichia* sp., *Brettanomyces* sp., *Saccharomyces bisporus*, *Zygosaccharomyces bailii*, *Saccharomyces cerevisiae*, *Saccharomyces ludwigii*, *Candia krusei*, *Schizosaccharomyces pombe*, *Issatchenkia orientalis occidentalis*, *Candida kefyer* have been isolated from this biotic colony [5, 6, 8].

As the Kombucha culture consumes the sugar, it yields an array of organic acids like glucuronic acid, gluconic acid, citric acid, acetic acid, lactic acid, butyric acid, folic acid, carbonic acid, oxalic acid, malic acid, nucleic acid, usnic acid; vitamins particularly B (including B1, B2, B6, and B12) and C; antibiotics; as well as amino acids, ethanol, carbon dioxide and some enzymes [5, 8-10].

Kombucha beverage is a nutritious and refreshing drink as the only known nutrition source containing glucuronic acid so far. Glucuronic acid plays an important role in body detoxification by the liver, and several studies have been conducted on this issue. Nowadays, people live under severe environmental pollution, with their livers exposed to the heavy burden by the food and water contaminated with dangerous chemicals, alcohol, tobacco, nitrite, nitrate, and other preservatives, some antibiotics, microbial toxins, heavy metals (such as lead and mercury) and so on. In addition, due to the inability of the liver to produce enough glucuronic acid, the contaminations created in the body cause various and fatal diseases. Glucuronic acid found in this beverage and produced by fermentation is also used to produce important polysaccharides such as hyaluronic acid, which is useful for binding tissues [5, 6, 11].

Black tea components, including catechins, have antibacterial properties intensified by the fermentation effect. Catechin has antioxidant, anti-cancer, anti-diabetes and anti-atherosclerosis properties, becoming more effective in an environment containing acetic and glucuronic acid [5, 6, 12, 13]. Since glucuronic acid plays a crucial role in the metabolism, especially liver function, the higher the glucuronic acid amount in the Kombucha beverage, the greater its nutritional and medical value. Also, the beneficial activities of black tea may become more effective in an environment containing glucuronic acid. The purpose of this study was to determine the amount of glucuronic acid production and antibacterial activity of Kombucha black tea drink during its production and storage.

2. MATERIALS AND METHODS

2.1. Preparing Kombucha Black Tea

In all experiments, a fixed amount of 10% sugar (sucrose sugar) was used. To prepare the Kombucha beverage, the necessary amount of sugar was added to one liter of boiling water in fully sterilized conditions. After the mixture was boiled for 5 minutes, the container was removed away from the flame and 10 grams of black tea were added, then it was given time for tea to be completely brewed. After separating the pulp, the Kombucha culture sheet along with a cup of pre-prepared Kombucha fluid was added to it (Pre-prepared Kombucha fluid that has already been prepared in the same way and some of it is added to the fresh culture like other fermented products for instance yogurt). The mixture was poured in a dish covered with sterile linen to allow air to enter the container for the culture to breathe [10]. The liquid was stored in a dark environment at two temperature levels of 20°C and 30°C for 21 days.

2.2. Determination of pH

The pH of Kombucha fluid samples was determined using an electronic pH meter (AZ-8601, Taiwan).

2.3. Glucuronic Acid Measurement Method

The amount of glucuronic acid at two temperature levels of 20 °C and 30 °C was measured over a period of 21 days on days 0, 7, 14 and 21 with a one-week interval. After completion of fermentation and centrifugation (at 1000 rpm for 3 minutes), the glucuronic acid level was measured using the High-performance liquid chromatography (HPLC) method. In this method, the diluted sample (1 to 10) was placed in the HPLC apparatus after passing through the Millipor filter (0.45 µ). The glucuronic acid analysis was then performed by Reverse Phase HPLC (RP-HPLC). In the next step, 20 µL of the filtered sample was injected into a system equipped with a UV detector. The column analysis Nucleocil C-18 (4 mm ID × 250 mm, 5 µm) and single pump Bischoff HPLC system were used for the analysis. The mobile phase was 50 milli-mole of sodium dihydrogen phosphate with a pH of 2.58. The flow rate was adjusted to 1 ml/min and the column was placed at room temperature. Detection was conducted at 210 nm. The analysis of the recorded peaks was performed on the HPLC chart according to the standard glucuronic acid storage time, and the concentrations were calculated from the standard curves (R2) multiplied by the dilution factor [14].

2.4. Antibacterial Property

To investigate the antibacterial property of Kombucha black tea, the supernatant effect of this beverage on the growth of *Salmonella* Typhimurium (PTCC 1709), *Lactobacillus rhamnosus* (PTCC 1637) and *Staphylococcus aureus* (PTCC 1112) bacteria was tested. Suspensions of every bacterium (100 µl) cultured for 18 h were spread on the plates equivalently. On days 1, 7, 14, and 21, the concentrated Kombucha fluid was used to the superficial of the MRS cultivation medium for *Lactobacillus rhamnosus,* [15-17] and to the superficial of Müller-Hinton cultivation medium for *Salmonella* Typhimurium and *Staphylococcus aureus* [10, 18] using the two methods of the disc and agar well diffusion.

On days 1, 7, 14, and 21, we took a certain amount of Kombucha black tea beverage and placed it in a rotary machine to concentrate the Kombucha fluid (30 min, in 45°C). Sterile supernatant was achieved via filtering the supernatant through a 0.2 µm sterilized microfilter. One hundred microliters of sterile sample were then transferred into the wells (wells of 10 mm diameter were prepared by a warm and disinfected pipe) of agar plates inoculated with target strains (MRS agar for *Lactobacillus rhamnosus,* and

Müller-Hinton agar for *Salmonella* Typhimurium and *Staphylococcus aureus*). Alternatively, in the analogous technique, the submerged discs into the same sterile samples were placed on the similar target plates. Initially, the entire plates were placed at 5°C for 1 h to form a pre-dispersion of Kombucha black tea samples into the agar. Finally, the samples were incubated at 37°C for 24 h and the formation of halos around the discs and wells was surveyed [10, 18-20].

Halo formation indicated the antimicrobial activity of this beverage, where the increase in the diameter of the halo showed the more antibacterial effect of Kombucha beverage. Aimed at the justification of control and comparison, unfermented black tea samples (sterilized and filtered) at the equivalent concentration as that of Kombucha black tea were prepared for antibacterial trials (Similar to preparing the Kombucha beverage; See section 2.1., the amount of sugar was added to one liter of boiling water, and after the mixture was boiled for 5 minutes, the container was removed away from the flame and 10 grams of black tea were added, then it was given time for tea to be completely brewed). Standard discs of Gentamicin (10 UI) offered as positive antibiotic controls according to CASFM 2005 standards [10]. All antibacterial tests were done triplicate.

2.5. Statistical Analysis

The study data obtained were analyzed by IBM SPSS version 21, and the effect of time and the test temperature on the two investigated response variables, i.e. antibacterial activity and glucuronic acid concentration, were measured by Repeated Measures ANOVA and the outcome was reported as P-value. All tests were done triplicate.

3. RESULTS AND DISCUSSION

3.1. Glucuronic Acid and pH Measurement

The outcome of this study indicated that the pH reduced slightly through the storing period from day 1 to day 21 at both temperatures of 20°C and 30°C. Although a slight increase was observed on the 21 day at 30°C, the trend towards the first day was a downward trend. Generally, in two groups at two different temperatures and over a period of 21 days, the reduction of the pH at 30°C was more than 20°C (Fig. **1**). Throughout these days, at 20°C, the production of glucuronic acid increased significantly from 17.0 mg/L on day 1 to roughly 27.2 mg/L on day 21. Furthermore, the quantity of this acid at 30°C increased from 42.2 mg/L on day 1 to 48.0 mg/L on day 21. The amount of glucuronic acid produced through storing at 30°C was significantly greater than that at 20 °C ($p<0.05$) (Fig. **2**).

In similar studies, Beigmohammadi *et al.* (2010) studied the production of the high glucuronic acid level in the Kombucha drink in a particular environmental situation. According to their results, optimal situations for Kombucha and production of the high level of glucuronic acid (44.5 mg/L) were reported in 30°C with sucrose at pH=6. [21] In similar study by Jayabalan *et al.* (2007), the amount of glucuronic acid production was studied in Kombucha and the highest amount of acid production (2.33 g/L) was obtained after 12 days of fermentation. [22]. Also, Chen and Liu (2000) determined the amount of glucuronic acid to be about 39 g/L after 60 days of fermentation. They showed that that with the increase in the length of the Kombucha storage period, the amount of glucuronic acid production increased [23] In another study, Franco *et al.* (2006) established the existence of glucuronic acid (0.07 to 9.63 g/L) in a product acquired after Kombucha cultivation on black tea sweetened by glucose [24].

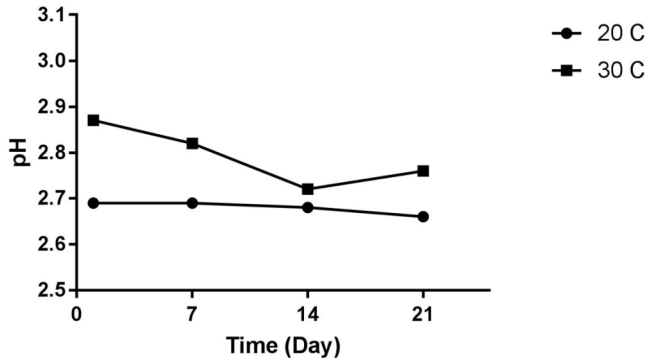

Fig. (1). The amount of pH in Kombucha black tea in 20°C and 30°C.

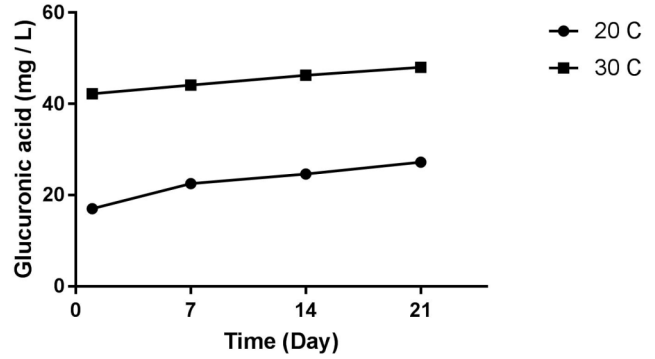

Fig. (2). Glucuronic acid concentration (mg/L) in kombucha black tea in 20°C and 30°C.

3.2. Antibacterial Activity

Inhibition area of the unfermented black tea control (-) and Gentamicin (10UI) control (+) is shown in Table **1**.

Table **2** lists the diameter of the halo formed around the bacteria in millimeters on diverse days subsequent incubation for the two disc and well techniques at both 20°C and 30°C (Mean ± SD).

On the first day of fermentation, after adding concentrated liquid to the wells and discs, no antibacterial activity and no halo formation occurred in the plates. On the 7th day of Kombucha beverage fermentation, halos were formed over time around the wells and discs in *Staphylococcus aureus* and *Salmonella* Typhimurium bacteria. The diameter of the halo formed around the wells and discs in *Salmonella* Typhimurium bacteria was greater than that formed in *Staphylococcus aureus* bacteria. In both bacteria, the halo diameter was greater at 20°C than that at 30°C.

After 14 days of fermentation, the diameter of inhibition zone around the well and discs were significantly increased

Table 1. Inhibition area of the Gentamicin (10UI) control (+) and unfermented black tea control (-). Inhibition area measured in diameter round the disc for Gentamicin and round the well for unfermented black tea.

Bacterial Strain	Unfermented Black Tea Control (-)	Gentamicin (10UI) Control (+)
S. aureus	Φ	22.0±0.0
S. typhimurium	Φ	22.0±5.0
L. rhamnosus	Φ	Φ

Φ No activity, and no growth on the disc or in the well.

Table 2. Antimicrobial activity of Kombucha black tea through incubation at 20°C and 30°C. Inhibition area measured in diameter round the well disc (Mean±SD).

Incubation Day	Temperature	Disk Method			Well Method		
		L. rhamnosus	*S. typhimurium*	*S. aureus*	*L. rhamnosus*	*S. typhimurium*	*S. aureus*
1	20°C	Φ	Φ[a]	Φ[a]	Φ	Φ[a]	Φ[a]
	30°C	Φ	Φ[a]	Φ[a]	Φ	Φ[a]	Φ[a]
7	20°C	Φ	12.0±0.0[b]	10.3±0.6[b]	Φ	13.0±0.0[b]	13.0±0.0[b]
	30°C	Φ	13.3±1.1[b]	10.0±0.0[b]	Φ	11.0±0.0[b]	15.0±0.0[b]
14	20°C	Φ	15.7±0.6[c]	13.0±0.0[b]	Φ	19.3±1.1[b]	19.3±1.1[bc]
	30°C	Φ	15.7±1.1[b]	12.3±0.6[b]	Φ	18.7±1.1[bc]	15.7±1.1[bc]
21	20°C	Φ	16.3±1.1[bc]	14.3±1.1[b]	Φ	20.3±2.1[b]	20.0±0.0[c]
	30°C	Φ	16.3±0.6[b]	12.3±0.6[b]	Φ	22.3±0.6[c]	20.0±0.0[c]

*Statistical significant alteration between incubation days are determined for each bacteria and each temperature distinctly and are showed by diverse lowercase superscript letters ($P<0.05$). There was not any statistical differences between incubation in 20 °C and 30 °C in diameter of halo ($P>0.05$).

Φ No activity, and no growth on the disc or in the well.

($p<0.05$). Halo diameter in *Staphylococcus aureus* and *Salmonella* Typhimurium bacteria at a temperature of 20°C was significantly different as compared to that at 30°C ($p<0.05$). On the 21st day, the halo diameters were significantly increased at 20°C as compared to those at 30°C in both *Staphylococcus aureus* and *Salmonella* Typhimurium bacteria.

For *Lactobacillus rhamnosus* bacteria, no halos were formed around the discs and wells on days 1, 7, 14, and 21, indicating the resistance of this bacterium to the anti-bacterial property of Kombucha black tea. It appears to be probable to use *Lactobacillus rhamnosus* accompany the Kombucha black tea drink [25, 26]. Nguyen *et al.* (2014) studied the glucuronic acid production and antimicrobial activity of fermented sweeten black tea by Kombucha layer and the co-culture with different *Lactobacillus* sp. Strains. They reported that *Lactobacillus casei* can increase the glucuronic acid production up to 39.6% whereas *Lactobacillus plantarum* can increase the antibacterial activities as a higher level compared to the usual fermented black tea and blended culture with further *Lactobacillus* strains [27]. However, it requires more studies. In our study, unfermented black tea (control group) did not indicate antimicrobial activity against test bacteria.

Deghrigue *et al.* (2013) reported the antibacterial activity of Kombucha tea beverage against a spectrum of Gram-negative and Gram-positive bacteria. Kombucha tea showed an antibacterial activity against *Salmonella* Typhimurium, *Micrococcus luteus*, *Escherichia coli*, and *Staphylococcus epidermidis* strains [10]. Velicanski *et al.* (2014) reported that lemon balm Kombucha has antibiotic activity against some pathogenic Gram-negative and Gram-positive bacteria. They showed that the main antibacterial components are organic acids especially acetic acid, whose concentration in Kombucha drink effect right its antimicrobial potential [18]. Already, Greenwalt *et al.* (1998) had reported that Kombucha tea containing 33 g/L total acid had antibacterial activity against *Salmonella* Typhimurium, *Staphylococcus aureus*, *Bacillus cereus*, *Agrobacterium tumefaciens*, and *Escherichia coli* [28].

CONCLUSION

This study indicated that the Kombucha black tea has antibacterial activity against *Salmonella* Typhimurium and *Staphylococcus aureus*, but not against *Lactobacillus rhamnosus*. Unfermented black tea (control group) did not display antibacterial action in contradiction of the test bacteria. Throughout the storing period of Kombucha black

tea, by time rise, the pH reduced, the extent of glucuronic acid increased and the halo diameter around the disc and well (in all positive cases) increased.

LIST OF ABBREVIATIONS

HPLC = High-performance Liquid Chromatography

RP-HPLC = Reverse Phase High-Performance Liquid Chromatography

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are base of this research.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

FUNDING

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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REFERENCES

[1] Pourjafar, H.; Noori, N.; Gandomi, H.; Basti, A.A. Study of protective role of double coated beads of calcium alginate-chitosan-eudragit S100 nanoparticles achieved from microencapsulation of Lactobacillus acidophilus as a predominant flora of human and animals gut. *J. Vet. Res. (Pulawy),* **2016**, *71*(3), 311-320.

[2] Wang, Y.; Ji, B.; Wu, W.; Wang, R.; Yang, Z.; Zhang, D.; Tian, W. Hepatoprotective effects of kombucha tea: identification of functional strains and quantification of functional components. *J. Sci. Food Agric.,* **2014**, *94*(2), 265-272. [http://dx.doi.org/10.1002/jsfa.6245] [PMID: 23716136]

[3] Pourjafar, H.; Noori, N.; Gandomi, H.; Basti, A.A.; Ansari, F. Stability and efficiency of double-coated beads containing Lactobacillus acidophilus obtained from the calcium alginate-chitosan and Eudragit S100 nanoparticles microencapsulation. *Int. J. Probiotics Prebiotics,* **2018**, *13*(2/3), 77-84.

[4] Soni, S.; Dey, G. Perspectives on global fermented foods. *Br. Food J.,* **2014**, *116*, 1767-1787. [http://dx.doi.org/10.1108/BFJ-01-2014-0032]

[5] Jayabalan, R.; Malbaša, R.V.; Lončar, E.S.; Vitas, J.S.; Sathishkumar, M. A review on kombucha tea—microbiology, composition, fermentation, beneficial effects, toxicity, and tea fungus. *Compr. Rev. Food Sci. Food Saf.,* **2014**, *13*, 538-550. [http://dx.doi.org/10.1111/1541-4337.12073]

[6] Dufresne, C.; Farnworth, E. Tea, Kombucha, and health: a review. *Food Res. Int.,* **2000**, *33*, 409-421. [http://dx.doi.org/10.1016/S0963-9969(00)00067-3]

[7] Vázquez-Cabral, B.D.; Larrosa-Pérez, M.; Gallegos-Infante, J.A.; Moreno-Jiménez, M.R.; González-Laredo, R.F.; Rutiaga-Quiñones, J.G.; Gamboa-Gómez, C.I.; Rocha-Guzmán, N.E. Oak kombucha protects against oxidative stress and inflammatory processes. *Chem. Biol. Interact.,* **2017**, *272*, 1-9. [http://dx.doi.org/10.1016/j.cbi.2017.05.001] [PMID: 28476604]

[8] Lobo, R.; Dias, F.; Shenoy, C. Kombucha for healthy living: evaluation of antioxidant potential and bioactive compounds. *Int. Food Res. J.,* **2017**, *24*(2), 541-546.

[9] Greenwalt, C.J.; Steinkraus, K.H.; Ledford, R.A. Kombucha, the fermented tea: microbiology, composition, and claimed health effects. *J. Food Prot.,* **2000**, *63*(7), 976-981. [http://dx.doi.org/10.4315/0362-028X-63.7.976] [PMID: 10914673]

[10] Deghrigue, M.; Chriaa, J.; Battikh, H.; Kawther, A.; Bakhrouf, A. Antiproliferative and antimicrobial activities of kombucha tea. *Afr. J. Microbiol. Res.,* **2013**, *7*, 3466-3470.

[11] Nguyen, N.K.; Nguyen, P.B.; Nguyen, H.T.; Le, P.H. Screening the optimal ratio of symbiosis between isolated yeast and acetic acid bacteria strain from traditional kombucha for high-level production of glucuronic acid. *Lebensm. Wiss. Technol.,* **2015**, *64*, 1149-1155. [http://dx.doi.org/10.1016/j.lwt.2015.07.018]

[12] Balentine, D.A.; Wiseman, S.A.; Bouwens, L.C. The chemistry of tea flavonoids. *Crit. Rev. Food Sci. Nutr.,* **1997**, *37*(8), 693-704. [http://dx.doi.org/10.1080/10408399709527797] [PMID: 9447270]

[13] Koch, W.; Kukula-Koch, W.; Głowniak, K. Catechin Composition and Antioxidant Activity of Black Teas in Relation to Brewing Time. *J. AOAC Int.,* **2017**, *100*(6), 1694-1699. [http://dx.doi.org/10.5740/jaoacint.17-0235] [PMID: 28707612]

[14] Yavari, N.; Assadi, M.M.; Moghadam, M.B.; Larijani, K. Optimizing glucuronic acid production using tea fungus on grape juice by response surface methodology. *Aust. J. Basic Appl. Sci.,* **2011**, *5*, 1788-1794.

[15] Abdolhosseinzadeh, E.; Dehnad, A.R.; Pourjafar, H.; Homayouni, A.; Ansari, F. The production of probiotic Scallion Yogurt: Viability of Lactobacillus acidoplilus freely and microencapsulated in the product. *Carpath. J. Food Sci. Technol.,* **2018**, *10*(3), 72-80.

[16] Shah, N.P. Probiotic bacteria: selective enumeration and survival in dairy foods. *J. Dairy Sci.,* **2000**, *83*(4), 894-907. [http://dx.doi.org/10.3168/jds.S0022-0302(00)74953-8] [PMID: 10791807]

[17] Ghasemnezhad, R.; Razavilar, V.; Pourjafar, H.; Khosravi-Darani, K.; Ala, K. The viability of free and encapsulated Lactobacillus casei and Bifidobacterium animalis in chocolate milk, and evaluation of its pH changes and sensory properties during storage. *Ann. Res. Rev. Biol.,* **2017**, 1-8. [http://dx.doi.org/10.9734/ARRB/2017/37885]

[18] Velićanski, A.S.; Cvetković, D.D.; Markov, S.L.; Šaponjac, V.T.; Vulić, J.J.; Vulić, J.J. Antioxidant and antibacterial activity of the beverage obtained by fermentation of sweetened lemon balm (Melissa offi cinalis L.) tea with symbiotic consortium of bacteria and yeasts. *Food Technol. Biotechnol.,* **2014**, *52*(4), 420-429. [http://dx.doi.org/10.17113/ftb.52.04.14.3611] [PMID: 27904315]

[19] Velićanski, A.S.; Cvetković, D.D.; Markov, S.L.; Tumbas, V.T.; Savatović, S.M. Antimicrobial and antioxidant activity of lemon balm Kombucha. *Acta Period. Technol.,* **2007**, *38*, 165-172. [http://dx.doi.org/10.2298/APT0738165V]

[20] Ansari, F.; Pourjafar, H.; Esmailpour, S. Study on Citric Acid Production and Antibacterial Activity of Kombucha Green Tea Beverage during Production and Storage. *Annu. Res. Rev. Biol.,* **2017**, *16*, 1-8. [http://dx.doi.org/10.9734/ARRB/2017/35664]

[21] Beigmohammadi, F.; Karbasi, A.; Beigmohammadi, Z. Production of high glucuronic acid level in Kombucha beverage under the influence environmental condition. *J. Food Technol. Nutr.,* **2010**, *7*(2), 30-38.

[22] Jayabalan, R.; Marimuthu, S.; Swaminathan, K. Changes in content of organic acids and tea polyphenols during kombucha tea fermentation. *Food Chem.,* **2007**, *102*, 392-398. [http://dx.doi.org/10.1016/j.foodchem.2006.05.032]

[23] Chen, C.; Liu, B.Y. Changes in major components of tea fungus metabolites during prolonged fermentation. *J. Appl. Microbiol.,* **2000**, *89*(5), 834-839. [http://dx.doi.org/10.1046/j.1365-2672.2000.01188.x] [PMID: 11119158]

[24] Franco, V.G.; Perín, J.C.; Mantovani, V.E.; Goicoechea, H.C. Monitoring substrate and products in a bioprocess with FTIR spec-

troscopy coupled to artificial neural networks enhanced with a genetic-algorithm-based method for wavelength selection. *Talanta,* **2006**, *68*(3), 1005-1012. [http://dx.doi.org/10.1016/j.talanta.2005.07.003] [PMID: 18970424]

[25] Mirzaei, H.; Pourjafar, H.; Homayouni, A. The effect of microencapsulation with calcium alginate and resistant starch on the Lactobacillus acidophilus (La5) survival rate in simulated gastrointestinal juice conditions. *J. Vet. Res. (Pulawy),* **2011**, *66*, 337-377.

[26] Fu, C.; Yan, F.; Cao, Z.; Xie, F.; Lin, J. Antioxidant activities of kombucha prepared from three different substrates and changes in content of probiotics during storage. *Food Sci. Technol.,* **2014**, *34*, 123-126. [http://dx.doi.org/10.1590/S0101-20612014005000012]

[27] Nguyen, N.K.; Dong, N.T.; Le, P.H.; Nguyen, H.T. Evaluation of the glucuronic acid production and other biological activities of fermented sweeten-black tea by kombucha layer and the co-culture with different Lactobacillus sp. strains. *Ijmer,* **2014**, *4*, 12-17.

[28] Greenwalt, C.; Ledford, R.; Steinkraus, K. Determination and Characterization of the Antimicrobial Activity of the Fermented Tea Kombucha. *Lebensm. Wiss. Technol.,* **1998**, *31*, 291-296. [http://dx.doi.org/10.1006/fstl.1997.0354]