

## Characterization of yogurt prepared with kombucha starter culture as inoculum

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### ABSTRACT

In recent years, there is a growing demand for healthy and safe food. In this regard, kombucha products have promising future due to their contents of bioactive compounds that exhibit health-promoting properties. Kombucha yogurt is a novel product with unique quality characteristics. So, in this study, the kombucha extract (KE), 5, 10, and 15% (v/v), was applied as a non-conventional starter for yogurt production, and the effect of kombucha on pH and titratable acidity of yogurt during incubation was investigated. Also, some physicochemical properties and overall acceptability of yogurt samples were determined during 21 days of storage at 8°C. For producing yogurt samples, the amounts of 5, 10, and 15 % (V/V) of concentrate of kombucha layer grown up on black tea, without yogurt starter bacteria, were inoculated to the milk containing 2.2% fat. Meanwhile, the yogurt starter was applied for producing the control sample. The results showed that by increasing the concentration of KE, a slight increase in pH (4-10%) and a decrease (12-35%) in acidity was observed compared to the control sample ( $p < 0.05$ ); syneresis increased and viscosity decreased ( $p < 0.05$ ); the vitamin C (92-200%) and ethanol (0-44%) levels increased ( $p < 0.05$ ) and the overall acceptability decreased ( $p < 0.05$ ) during 21 days of cold storage. The increase of the kombucha concentration in all samples elongated the fermentation period ( $p < 0.05$ ). The best physicochemical and sensory properties were observed in the sample containing 5% KE.

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### 1. Introduction

Kombucha which consists of a consortium of bacteria, *Acetobacter* and *Gluconobacter*, and various yeasts including *Zygosaccharomyces*, *Pichia*, *Saccharomyces*, *Torula*, *Kluyveromyces*, *Schizosaccharomyces*, *Brettanomyces*, and *Candida* (1-7) is a dietary supplement that improves the body's immune system and health-promoting properties for diabetes, high blood pressure, hypercholesterolemia, digestive ailments, combating stress and body vitalization as well as cancer (8-12). This traditional drink with sour and sweet taste can be prepared by inoculation and fermentation of kombucha starter (13-14) on various substrates such as black and green tea, coffee, herbal teas, beer, Coca-Cola, wine, molasses, whey, etc. In addition to sucrose, other sugar sources such as glucose, fructose, maltose, lactose, as well as inulin and oligofructose can be used to grow kombucha. Kombucha is also suitable for fermenting milk because it can ferment lactose (15-16, 2, 4,

13, 17-19). The microorganisms in the kombucha layer can be activated and added to various food compounds during different metabolic processes. The composition of this drink depends on the layer's microorganisms, the culture, and the fermentation conditions. Generally, kombucha contains organic acids (acetic, lactic, gluconic, glucuronic, citric, usnic, oxalic, tartaric, succinic, saccharic, malic, malonic, pyruvic), simple sugars (glucose, fructose), ethanol, lipids, proteins, carbon dioxide, volatile and flavoring compounds (diacetyl acetone, isobutyraldehyde, vanillic acid, methyl ester, acetyl ester, isobutyl ester, isoamyl alcohol), vitamins (C, B<sub>1</sub>, B<sub>2</sub>, B<sub>6</sub>, B<sub>12</sub>), free amino acids (lysine, tyrosine, valine, phenylalanine, leucine, isoleucine), purines, heparin, caffeine and theophylline, tannins, enzymes (amylase, lactase, invertase), antibiotics (nisin), hyaluronic acid, folic acid, polyphenols and catechins, minerals (copper, iron, manganese, nickel, zinc), biogenic amines, anions, and the metabolic products of bacteria and yeasts (20-21, 1, 4, 22, 13, 18). The acetic acid in

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this drink inhibits the activity of many pathogenic microorganisms. The antioxidant activity is related to the ability of ascorbic acid to scavenge free radicals (23-24). This drink is considered as an inhibitor for genes mutation and cancer cell proliferation and also as an anti-cancer agent (1). The glucuronic acid acts as a detoxifying and anti-cancer compound. The detoxifying properties of kombucha are probably due to the binding of glucuronic acid to toxic molecules and increased excretion by the kidneys and intestines. The butyric acid protects cell membranes and also strengthens the intestinal walls and protects against parasites in combination with glucuronic acid (22). The usnic acid in this drink has antimicrobial properties (5, 19). So far, researches have been done on the preparation of fermented beverages based on milk or whey using kombucha (5, 13, 17-19, 21-22, 25-32). There are also reports of making kombucha yogurt (33) and kombucha cheese (34) by adding kombucha to traditional starters, but there is no available research on yogurt production using kombucha exclusively. Since yogurt is an important fermented dairy product all around the world and also the health-promoting properties of kombucha have been proven, a new functional yogurt was produced by fermenting milk and using kombucha extract (KE) as a non-conventional starter to improve public health. So, based to the above-mentioned gap of research, this study was conducted to evaluate some physicochemical properties and overall acceptability of yogurt produced with KE; also, compare the incubation time and pH and acidity changes of kombucha yogurt samples with control yogurt (prepared by traditional yogurt starter bacteria) during incubation.

## 2.2. Materials and methods

### 2.1. Materials

Frozen culture of YF-L904 (including yogurt-starter bacteria *Streptococcus thermophilus* and *Lactobacillus bulgaricus*) from Christin Hansen (Denmark), skim milk powder from NZNP (New Zealand), raw milk from Damdaran company (Tehran, Iran), sugar from Livar (Tehran, Iran), Black Tea from Refah (Lahijan, Iran), Stabilizer, Carboxymethyl Cellulose, Perigel, Menu and Fatty Acid Diglyceride from Lactoprote (Denmark) were purchased. Kombucha was prepared by the Iranian Research Organization for Science and Technology (Tehran, Iran). All materials required for the experiments were purchased from Merck company of Germany.

### 2.2. Kombucha extract (KE) preparation

One liter of boiled water with 1.5 g of black tea leaves and 70 g of sucrose was heated (100°C for 5 minutes) and after filtration (to remove the tea leaves), the resulting extract was cooled to room temperature. The kombucha starter was inoculated and the fermentation vessel was covered with a thin, clean cotton cloth. Incubation was performed at 25-29°C

for 6 days (25) and then concentrated (to 20%) by a rotary (Heidolph, Germany).

### 2.3. Preparation of yogurt samples

3% skimmed milk and 1% stabilizer were added to raw milk (2.2% fat) and homogenized (70 bar at 70°C) and heated at 95°C for 5 min. After cooling up to 42°C, KE was inoculated in 5 (K5), 10 (K10), and 15% v/v (K15). Yogurt starter culture without KE was used to prepare the control treatment (YK0). After packing and incubating the samples at 42°C (pH= 4.6), the samples were refrigerated at 8°C. It should be noted that according to the results of Kanuric and co-workers (35), the highest lactose fermentation rate is performed by the kombucha starter at 42°C; Therefore, this temperature was selected as the heating temperature. pH and titratable acidity were determined during incubation. Also, the physicochemical properties and overall acceptability were evaluated in 1, 7, 14, and 21 days of storage.

### 2.4. Physicochemical analysis

pH values were determined by using a pH meter (WTW, Germany) during storage. Titratable acidity was evaluated by titration method using NaOH (0.1 N) and phenolphthalein as an indicator and calculated in g LA/Liter (36). For syneresis determination in yogurt samples, 25 g of sample was filtered by filter paper (Whatman No. 41). The amount of extracted water was evaluated after 120 minutes at 4°C (37). The viscosity of the samples was measured using a viscometer (Brookfield, USA). All tests were performed at 4°C and 10 rpm after 10 secs (38). The amount of vitamin C in the samples was measured by the iodometric titration method. 20g of sample, 100 ml of sulfuric acid (0.1 N), and then 15 ml of iodine solution (0.1 N) and 100 ml water were transferred into a 250 mL volumetric flask. The content of the Erlenmeyer flask was stirred for about 20 seconds; then, 5 ml of starch solution (0.5%) was added and titrated sodium thiosulfate (0.1 N). The end of the titration was the blue color of the solution (15). The ethanol content of samples was determined by GC (Agilent, USA); at the injection temperature of 250°C; flame ionization; detector temperature of 20°C; the initial column temperature of 50°C for 30 min; the rising temperature slope of 30°C per min up to 180°C and holding time of 5 min. The standard solutions of ethanol (2%) and butanol (2%) were prepared in distilled water. 5 g of the sample was transferred to the balloon and diluted with the standard solution. Then, the sample was filtered through a filter (0.5µ) and injected into the GC (39).

### 2.5. Overall acceptability Evaluation

The panelists were requested to evaluate the sensory properties (flavor, color, odor, texture, and overall acceptability) according to a five-point hedonic scale on days 1, 7, 14, and 21 during storage at 8°C. Since the final index

assessment is overall acceptability; therefore, only overall acceptability results have been reported.

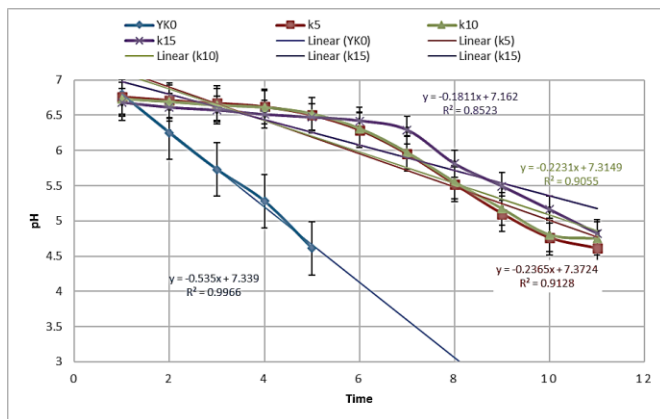
## 2.6. Statistical method

Statistical analysis on a full factorial design was performed for ANOVA using SAS software (version 9.2; NC, USA). All experiments were in 3 replicates. Duncan's multiple range tests were used to compare means at the significant level of 0.05 ( $p < 0.05$ ). Kruskal-Wallis nonparametric test was used to analyze the overall acceptability.

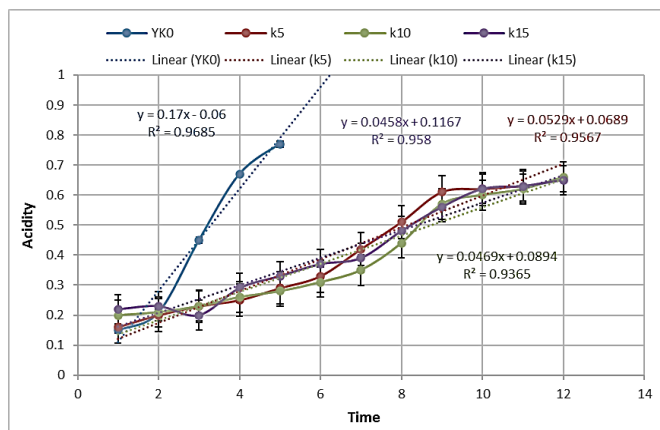
## 3.3. Results and discussion

### 3.1. Changes in pH, titratable acidity, and fermentation time of yogurt samples during incubation

In the control sample, the decrease in pH (Fig. 1) followed by the increase in acidity (Fig. 2) during fermentation was linear. In the control sample, the lag phase was very short.



**Fig. 1.** Changes in pH of yogurt samples during fermentation (h). Y: % Yogurt starter; K: % Kombucha extract [YK0: Y=2.5; K=0. K5: Y=0; K=5. K10: Y=0; K=10. K15: Y=0; K=15].



**Fig. 2.** Changes in titratable acidity (g LA/Liter) of yogurt samples during fermentation (h). Y: % Yogurt starter; K: % Kombucha extract [YK0: Y=2.5; K=0. K5: Y=0; K=5. K10: Y=0; K=10. K15: Y=0; K=15].

However, in samples containing kombucha extract, decrease in pH and increase in acidity represent three separated phases of microbial growth: lag, growth, and stationary phases; the observation of the lag phase can be attributed to the time required for the kombucha to adapt to the existing substrate; kombucha has been reported to fermented milk (4, 13, 15, 17-19). It should be noted that the incubation time to reach pH=4.6 in different treatments differs significantly ( $p < 0.05$ ). This time for samples containing kombucha extract K5 ( $601.67 \pm 7.09^c$  min), K10 ( $651.00 \pm 4.58^b$  min) and K15 ( $711.00 \pm 3.00^a$  min) was 2.6, 2.8 and 3 times that of the control sample (YK0=  $230.33 \pm 3.51^s$  min), respectively; the increase is due to the fact that the starter in kombucha extract need more time to adapt to the existing substrate; therefore, the delay phase becomes longer, which in turn increases the fermentation time. Differences in fermentation rates can also be attributed to differences in the metabolic activity of the applied starter cultures (19). The increasing acidity and the incubation time with higher inoculation concentration of kombucha extract was in agreement with the results of other researchers (27). Contrary to the present results, Ilicic and co-workers (40) and Milanovic and co-workers (13, 19) concluded that the concentration of kombucha extract had no significant effect on the fermentation rate.

### 3.2. pH and acidity of kombucha yogurt samples during refrigerated storage

In the control sample, the pH decreased and subsequently, the acidity increased during 21 days of storage (Table 1), indicating the post metabolic activity of starter and excess production of lactic acid due to lactose fermentation (41, 42). In samples containing kombucha extract, decrease in pH and increase in acidity was insignificant compared to the control sample, so the acidity in the control sample increased by 28.79% after 21 days of storage; whereas the percentage of increased acidity in samples of 5K, 10K, and 15K was estimated to be less and was 0.66, 0.73 and 0.84, respectively, which can be due to the relative inactivation of kombucha due to the production of organic acids during refrigerated storage (19, 35). In general, the increase in acidity and decrease in pH in samples containing kombucha during storage can be attributed to the fermentation of sugar and producing acid by a microbial consortium of kombucha (22, 26, 30, 35, 43). The partial trend of increasing acidity during the storage samples incorporated with kombucha extract in the present study was in agreement with the results of other researchers (19, 33).

### 3.3. Syneresis of kombucha yogurt samples during refrigerated storage

Syneresis of yogurt samples increased during storage ( $p < 0.05$ ) as shown in Table 2, which can be attributed to the hydrolysis of proteins by bacteria during storage. After hydrolysis, the proteins lose their properties of binding to water that pH changes and increased acidity are also contributed syneresis (44, 45). Along with decreasing pH and

increasing acidity during storage, the three-dimensional structure of the protein damages and shrinks which lessen the binding ability of proteins and syneresis happens (46). Also, the increasing syneresis in yogurt during storage is due to the structural rearrangement of the casein network, which is associated with whey out (17, 46, 47). The syneresis in the control sample on the first day of storage was much less than other samples and by increasing the amount of KE, syneresis increased ( $p < 0.05$ ). The syneresis in the control sample was 0.092% on the first day of storage and 39.55% at the end of storage. In K5, it was 18.56 and 56% on the 1st and 21st day, respectively; and in K10, they were 27.74 and 49.93%; finally, in 15K, 22.22, and 51.06% on the first and last day of storage, respectively. Nurliyani et al. (32) reported kombucha tea

contained a large amount of water; so, it increased the water content of the final product (goat milk fermented with kombucha and *Lactobacillus casei*). Also, Milanovic et al. (19) reported that the total solid in the fermented milk samples containing KE (10, 15, and 20%) was less than the control sample and by increasing KE, the syneresis in the samples increased. Abdel-Salam and Galal (33) also found that the total solid of kombucha yogurt was less than control yogurt; so, the syneresis was more. In fact, by adding KE, the serum phase increased and the gel network was not able to maintain the entire serum; so, the serum was released and syneresis increased (48). During longer fermentation time, the protein bonds and gel network became more unstable and syneresis increased (49).

**Table 1.** pH and titratable acidity of kombucha yogurt samples during storage (Mean  $\pm$  Standard deviation) †.

Sample	1 <sup>st</sup> day	7 <sup>th</sup> day	14 <sup>th</sup> day	21 <sup>st</sup> day
<b>pH</b>				
YK0	4.58 $\pm$ 0.02 <sup>a</sup>	4.39 $\pm$ 0.03 <sup>h</sup>	4.23 $\pm$ 0.03 <sup>k</sup>	4.09 $\pm$ 0.02 <sup>l</sup>
K5	4.58 $\pm$ 0.01 <sup>a</sup>	4.55 $\pm$ 0.02 <sup>bc</sup>	4.51 $\pm$ 0.02 <sup>ef</sup>	4.46 $\pm$ 0.02 <sup>g</sup>
K10	4.57 $\pm$ 0.01 <sup>ab</sup>	4.55 $\pm$ 0.01 <sup>bc</sup>	4.52 $\pm$ 0.02 <sup>de</sup>	4.49 $\pm$ 0.02 <sup>f</sup>
K15	4.57 $\pm$ 0.01 <sup>ab</sup>	4.56 $\pm$ 0.01 <sup>abc</sup>	4.54 $\pm$ 0.01 <sup>cd</sup>	4.51 $\pm$ 0.01 <sup>ef</sup>
<b>Titratable acidity (Lactic acid%)</b>				
YK0	0.77 $\pm$ 0.01 <sup>d</sup>	0.85 $\pm$ 0.01 <sup>c</sup>	0.98 $\pm$ 0.01 <sup>b</sup>	1.08 $\pm$ 0.00 <sup>a</sup>
K5	0.65 $\pm$ 0.01 <sup>k</sup>	0.67 $\pm$ 0.01 <sup>ij</sup>	0.68 $\pm$ 0.01 <sup>h</sup>	0.70 $\pm$ 0.01 <sup>g</sup>
K10	0.67 $\pm$ 0.01 <sup>j</sup>	0.68 $\pm$ 0.01 <sup>h</sup>	0.71 $\pm$ 0.00 <sup>g</sup>	0.73 $\pm$ 0.00 <sup>f</sup>
K15	0.68 $\pm$ 0.01 <sup>hi</sup>	0.70 $\pm$ 0.01 <sup>g</sup>	0.72 $\pm$ 0.00 <sup>f</sup>	0.75 $\pm$ 0.00 <sup>e</sup>

†Different superscript letters differ significantly in treatments during storage ( $p \leq 0.05$ ).

Y: % Yogurt starter, K: % Kombucha extract, YK0: Y=2.5; K=0. K5: Y=0; K=5. K10: Y=0; K=10. K15: Y=0; K=15.

**Table 2.** Syneresis and viscosity of kombucha yogurt samples during storage (Mean  $\pm$  Standard deviation) †.

Sample	1 <sup>st</sup> day	7 <sup>th</sup> day	14 <sup>th</sup> day	21 <sup>st</sup> day
<b>Syneresis (%)</b>				
YK0	11.22 $\pm$ 0.03 <sup>o</sup>	15.19 $\pm$ 0.05 <sup>n</sup>	31.43 $\pm$ 0.25 <sup>h</sup>	39.55 $\pm$ 0.89 <sup>f</sup>
K5	18.65 $\pm$ 0.05 <sup>m</sup>	23.19 $\pm$ 0.04 <sup>k</sup>	33.93 $\pm$ 0.04 <sup>g</sup>	45.56 $\pm$ 0.29 <sup>c</sup>
K10	24.74 $\pm$ 0.16 <sup>j</sup>	31.18 $\pm$ 0.05 <sup>h</sup>	43.68 $\pm$ 0.08 <sup>d</sup>	49.93 $\pm$ 0.11 <sup>b</sup>
K15	22.22 $\pm$ 0.079 <sup>l</sup>	29.64 $\pm$ 0.05 <sup>i</sup>	41.17 $\pm$ 0.04 <sup>e</sup>	51.06 $\pm$ 0.05 <sup>a</sup>
<b>Viscosity (cp)</b>				
YK0	17.26 $\pm$ 0.66 <sup>a</sup>	15.80 $\pm$ 0.09 <sup>b</sup>	13.65 $\pm$ 0.04 <sup>c</sup>	12.76 $\pm$ 0.04 <sup>d</sup>
K5	9.24 $\pm$ 0.08 <sup>e</sup>	7.48 $\pm$ 0.16 <sup>f</sup>	6.17 $\pm$ 0.03 <sup>g</sup>	5.61 $\pm$ 0.17 <sup>h</sup>
K10	6.25 $\pm$ 0.05 <sup>g</sup>	4.79 $\pm$ 0.08 <sup>i</sup>	3.80 $\pm$ 0.05 <sup>k</sup>	2.71 $\pm$ 0.03 <sup>m</sup>
K15	5.83 $\pm$ 0.02 <sup>h</sup>	4.50 $\pm$ 0.18 <sup>j</sup>	3.19 $\pm$ 0.03 <sup>l</sup>	1.87 $\pm$ 0.03 <sup>n</sup>

†Different superscript letters differ significantly in treatments during storage ( $p \leq 0.05$ ).

Y: % Yogurt starter, K: % Kombucha extract, YK0: Y=2.5; K=0. K5: Y=0; K=5. K10: Y=0; K=10. K15: Y=0; K=15.

### 3.4. Viscosity of kombucha yogurt samples during refrigerated storage

As shown in Table 2, viscosity decreased with a constant trend in all samples during storage ( $p < 0.05$ ). The decrease in viscosity of the samples can be attributed to the increased syneresis during the storage. The decrease of viscosity at the end of storage would be due to the microorganism's activity in the yogurt (50). The rheological properties of yogurt were related to pH changes (2). The viscosity in the control sample decreased from 17.26 to 12.76 cp during storage. The kombucha yogurt samples had lower viscosity than the control. In K5, the viscosity decreased from 9.24 to 5.61cp; in K10, from 6.25 to 2.71cp and in K15 from 5.83 to 1.87cp during 21 days of storage. Viscosity depends on various factors such as the content of skimmed solids, fat, type of starter culture, type of heat treatment, temperature and time of

fermentation, as well as cooling process and storage conditions (2, 49). In this study, by increasing the amount of KE in yogurt samples, syneresis enhanced and viscosity decreased ( $p < 0.05$ ), it would be due to the decreasing of total solid content as the KE amount increased (19, 32). In previous studies, the viscosity of kombucha yogurt was lower than the control sample (15, 51).

### 3.5. Vitamin C in kombucha yogurt samples during refrigerated storage

A decrease in vitamin C (Table 3) was observed over time ( $p < 0.05$ ). Vitamin C is soluble in water and very sensitive to oxidation. Low temperature and acidic conditions help in preserving this vitamin. The amount of vitamin C in the control sample decreased from 6.6 to 4.18 mg/100 g of yogurt on the 1st to the 21st day. Vitamin C levels increased with

increasing the KE content ( $p < 0.05$ ). The amount of this vitamin in the kombucha yogurt was almost 2 times more than the control sample on the first day. In K5, the vitamin C levels were 12.67 and 11.92 mg/100g; in K10, 12.81 and 12.25 mg/100g and in K15, 12.98 and 12.63 mg/100g of yogurt in the 1st and 21st day, respectively. In K5, K10, K15, 94, 96,

and 97% of this vitamin was preserved during storage, respectively. The vitamin C in fermented dairy products containing kombucha was reported 12.36-31.19 mg/L (52). The average amount of vitamin C in fermented milk with 15 and 20% of KE was 13.47 mg and 82% of this vitamin was retained after 20 days of storage (53).

**Table 3.** Vitamin C and ethanol of kombucha yogurt samples during storage (Mean  $\pm$  Standard deviation) †.

Sample	1 <sup>st</sup> day	7 <sup>th</sup> day	14 <sup>th</sup> day	21 <sup>st</sup> day
<b>Vitamin C (mg/100g)</b>				
YK0	6.61 $\pm$ 0.02 <sup>i</sup>	5.63 $\pm$ 0.026 <sup>j</sup>	4.75 $\pm$ 0.03 <sup>k</sup>	4.18 $\pm$ 0.03 <sup>l</sup>
K5	12.67 $\pm$ 0.05 <sup>c</sup>	12.32 $\pm$ 0.045 <sup>e</sup>	12.08 $\pm$ 0.04 <sup>e</sup>	11.92 $\pm$ 0.079 <sup>h</sup>
K10	12.81 $\pm$ 0.03 <sup>b</sup>	12.67 $\pm$ 0.045 <sup>e</sup>	12.42 $\pm$ 0.036 <sup>d</sup>	12.25 $\pm$ 0.04 <sup>f</sup>
K15	12.98 $\pm$ 0.045 <sup>a</sup>	12.82 $\pm$ 0.045 <sup>b</sup>	12.69 $\pm$ 0.036 <sup>c</sup>	12.63 $\pm$ 0.04 <sup>c</sup>
<b>Ethanol (ml/g)</b>				
YK0	0.13 $\pm$ 0.02 <sup>j</sup>	0.13 $\pm$ 0.01 <sup>j</sup>	0.14 $\pm$ 0.02 <sup>i</sup>	0.14 $\pm$ 0.02 <sup>i</sup>
K5	0.13 $\pm$ 0.02 <sup>j</sup>	0.13 $\pm$ 0.01 <sup>j</sup>	0.14 $\pm$ 0.02 <sup>i</sup>	0.14 $\pm$ 0.02 <sup>i</sup>
K10	41.2 $\pm$ 1.8 <sup>h</sup>	42.7 $\pm$ 0.1 <sup>g</sup>	44.2 $\pm$ 0.30 <sup>f</sup>	45.8 $\pm$ 0.36 <sup>e</sup>
K15	55.0 $\pm$ 0.45 <sup>d</sup>	57.4 $\pm$ 0.36 <sup>c</sup>	59.4 $\pm$ 0.36 <sup>b</sup>	61.2 $\pm$ 0.30 <sup>a</sup>

†Different superscript letters differ significantly in treatments during storage ( $p < 0.05$ ).

Y: % Yogurt starter, K: % Kombucha extract, YK0: Y=2.5; K=0. K5: Y=0; K=5. K10: Y=0; K=10. K15: Y=0; K=15.

### 3.6. Ethanol in kombucha yogurt samples during refrigerated storage

The ethanol in the control sample was in very small amount and did not change over time (Table 3). However, in kombucha yogurt, the ethanol level was slightly higher than the control sample, and with increasing the amount of KE, the ethanol amount was also increased slightly. The metabolism of fermentation by kombucha starts with converting sucrose to glucose and fructose by the invertase of the yeasts. In the next stage, these sugars are consumed by yeasts, and ethanol and CO<sub>2</sub> are produced through glycolysis. By producing an adequate amount of ethanol, some of the bacteria that are able to use a carbon source, grow and multiply. Ethanol is oxidized to acetic acid by acetobacter and organic acids, proteins, and other beneficial compounds are produced (30). Also, small amounts of ethanol are produced after fermentation of lactose, compared to sucrose, by kombucha (5, 19). Therefore, the little amount of ethanol in all kombucha yogurt samples is related to the conversion of ethanol to acetic acid by acetic acid

bacteria in kombucha. These results are consistent with the results of other researchers: the ethanol in fermented milk with 10, 15 and 20% of KE was less than 5 g/L (19), which was similar to the reported ethanol content in whey and milk fermented beverages with 10 and 15% of KE (40, 54); also, the low ethanol content (0.005-0.009 g /100g) was reported in fermented beverages with kombucha (30).

### 3.7. Sensory evaluation of kombucha yogurt samples during refrigerated storage

The highest overall acceptability score (5) was related to the control sample and K5 on the first day, this score was reduced to 2.4 and 4.2, respectively, on the 21<sup>st</sup> day of storage. The overall acceptability scores in K10 and K15 were 3.7 and 2.8, respectively, on the 1<sup>st</sup> day. On the 21<sup>st</sup> day, these scores were declined to 2.24 and 1.48, respectively. According to the results of this study, the highest overall acceptability on both the 1<sup>st</sup> and 21<sup>st</sup> day belonged to K5; however, K15 had the lowest overall acceptability among all (Table 4).

**Table 4.** Overall acceptability of kombucha yogurt samples during storage (Mean  $\pm$  Standard deviation) †.

Sample	1 <sup>st</sup> day	7 <sup>th</sup> day	14 <sup>th</sup> day	21 <sup>st</sup> day
YK0	5.00 $\pm$ 0.01 <sup>a</sup>	4.40 $\pm$ 0.05 <sup>bc</sup>	3.40 $\pm$ 0.01 <sup>ef</sup>	2.37 $\pm$ 0.05 <sup>ijkl</sup>
K5	5.00 $\pm$ 0.01 <sup>a</sup>	4.79 $\pm$ 0.04 <sup>ab</sup>	4.69 $\pm$ 0.01 <sup>ab</sup>	4.20 $\pm$ 0.05 <sup>cd</sup>
K10	3.70 $\pm$ 0.04 <sup>e</sup>	3.10 $\pm$ 0.04 <sup>gh</sup>	2.58 $\pm$ 0.01 <sup>ijk</sup>	2.29 $\pm$ 0.05 <sup>kl</sup>
K15	2.80 $\pm$ 0.05 <sup>shii</sup>	2.70 $\pm$ 0.04 <sup>hij</sup>	2.20 $\pm$ 0.01 <sup>kl</sup>	1.48 $\pm$ 0.05 <sup>m</sup>

†Different superscript letters differ significantly in treatments during storage ( $p < 0.05$ ).

Y: % Yogurt starter, K: % Kombucha extract, YK0: Y=2.5; K=0. K5: Y=0; K=5. K10: Y=0; K=10. K15: Y=0; K=15.

According to the results of the study by Milanovic et al. (19), among all milk-based kombucha drinks, the best sensory characteristic was for the one with 15% of KE. In another research, the sensory properties and overall acceptability of fermented dairy products by kombucha were reported to be similar to control yogurt samples (15). The sensory properties of kombucha fermented milk were pleasant and similar to yogurt and kefir; all the samples had better sensory scores than

control ones during storage (16). The taste of kombucha fermented whey was also acceptable to the consumers (31). The fermented goat's milk with 5% kombucha compared to 10% kombucha was more acceptable (32). There was no difference in the overall acceptability score of kombucha yogurt and control yogurt (33) but the kombucha cheese had lower sensory acceptance compared to the control sample (34).

#### 4. Conclusion

In this study 5, 10, and 15% (v/v) of KE were used and some physicochemical properties and overall acceptability of yogurt samples were evaluated during 21 days of storage. Inoculation size of KE was effective on fermentation time; as the inoculation concentration increased, the fermentation time became longer. The fermentation rate in milk samples inoculated with traditional yogurt starter was higher than that in milk samples inoculated with kombucha starter. also, during the cold storage, the decrease of pH (1-3%) and increase of acidity (8-10%) in samples were minor; and with raising the KE, these changes were significantly less than the control sample. Based on the results of our study, the yogurt sample with 5% of KE was the best sample among all. Many additives especially functional ingredients can be used to improve the taste, texture, and nutritional value of kombucha yogurt. This study confirmed that kombucha yogurt would be considered as a novel dairy product in the food industry; also, kombucha, a symbiotic culture of bacteria and yeast, could be used as a non-conventional starter in producing healthy food products.

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