Biological activity and physicochemical quality of different types of kombucha yoghurt VS traditional yoghurt during storage

Ayah, B. Abdel-Salam¹ and Gehan, F. Galal ²

¹Department of Food Hygiene & Control, Faculty of Veterinary Medicine, Cairo University, Egypt.
²Department of Microbiology, Faculty of Agriculture, Ain shams University, Egypt.

*Correspondence: setelkol2003@yahoo.com Accepted: 12 Jun 2018 Published online: 30 Sep 2018

Fermented dairy products hold an important role in functional foods field which has a great interest in human nutrition. The metabolic activity of kombucha in milk and obtaining a new yoghurt type is the subject of this research. Kombucha Fermented Solutions (KFSs) were firstly produced from six different types of tea (Bergamot Tea, Camomile Tea, Green Tea, Cardamom Tea, Moringa Tea and Black Tea) and then inoculated in milk with the traditional yoghurt starter culture and incubated until yoghurt coagulum formation. Control group without KFSs was prepared. Chemical composition (total solids, Ash, Protein and fat content) for all types of produced yoghurt was tested. Sensory evaluations, as well as acidity percent of produced yoghurt for all groups, were measured during the storage period. Biological activity of kombucha yoghurt was examined by inoculating yoghurt with a pathogenic microorganism (S. aureus) and tracking its viability in the product during storage. Data for all measures were statistically analyzed. Obtained results for chemical composition showed that TS% of treatments B & C are significantly different (less) than that of the control, Fat% of treatments B, D & F are significantly different (less) than that of the control, while Ash% of treatment E is significantly different (more) than that of the control. There were significant differences in acidity percent between groups. For sensory quality, all types of produced kombucha yoghurt were accepted overall and during storage and not significantly differ from the control group. Tracking the viability of inoculated S. aureus in the kombucha yoghurt in our study during storage showed great percentage reduction from day one especially in kombucha yoghurt with Camomile & Cardamom tea. This antibacterial effect appeared against S. aureus organism may be attributed to kombucha metabolites which have a significant effect against pathogenic microorganisms. These results encourage the availability of using kombucha yoghurt as a commercial product with high hygienic and chemical quality in addition to its acceptability for the consumer.

Keywords: kombucha yoghurt, physicochemical quality, S. aureus, traditional yoghurt.

INTRODUCTION

Fermented milk is a dairy product processed by lactic fermentation resulting in acidification (pH of about 4.6) and coagulation of milk casein (Birollo et al., 2002). Yoghurt is one of the most popular fermented dairy products widely consumed all over the world because of its organoleptic and nutritional properties (Loveday et al., 2013). Yoghurt is defined as a coagulated milk product achieved mainly by lactic acid fermentation of milk via Lactobacillus bulgaricus and Streptococcus thermophilus with or without additions of whole or skim milk powder (Codex, 2003). Type and ratio of microorganisms in starter
culture contribute to different physico-chemical and sensory characteristics of fermented dairy products. Manufacturing of desirable products is dependent on many factors, such as acidity, production of aroma compounds, textural characteristics, sensory attributes, nutritional value and therapeutic properties (Bonczar, et al., 2002). Quality of yoghurt is influenced by conversion of milk components and metabolic activities of starter culture during the gelation process (Tamime and Robinson, 2004).

Starter cultures used in yoghurt manufacturing has different purposes such as biopreservation of the product and bacteriocins production as potential food preservatives thus prolongs shelf-life of the product and enhanced its safety; improvement of sensory properties and enhancement of human health (Tamime, 2006). In recent researches, a symbiotic association of yeasts and acetic acid bacteria, known as kombucha, has been applied for milk fermentation with the aim to enlarge variety of the fermented dairy products as a functional food. Research in the field of functional foods is of great interest in human nutrition. Fermented dairy products certainly hold an important role in this field (Milanović et al., 2008; Malbaša et al., 2009; Iličić et al., 2011 and Dajana et al., 2014).

Kombucha tea is called “tea fungus” composed of fermented different types of acetic acid bacteria and yeasts in symbiotic association with two portions, a floating cellulose pellicle layer and the sour liquid broth. Kombucha tea is a popular beverage across the world for its refreshing taste and beneficial effects on human health. Kombucha tea beneficial effects are accredited to its ability to produce different acids (gluconic acid, glucuronic acid, and lactic acid), vitamins, amino acids and micronutrients during fermentation (Jayabalan et al, 2007). Kombucha tea is known to show a remarkable antimicrobial activity against a broad range of microorganisms due to the probability of antibiotic production. Many scientific studies have been done on this subject and the Kombucha broth has demonstrated inhibitory activity against many pathogenic microorganisms of both Gram positive and Gram negative origin (Dufresne and Farnworth, 2000). Kombucha is usually cultivated on a sweetened black and green tea. Also, it can be cultivated on some other types of tea or a dark beer, wine, whey and lactose (Lončar et al., 2006).

Standardization of yoghurt quality is not easy because it could be produced in many forms, with different manufacturing methods, using many ingredients in addition to consumer preferences. This situation makes yoghurt an interesting, challenging area to work in. This study aimed to investigate the possibility of application of non-conventional starter (kombucha cultivated on six different tea types: (Bergamot Tea, Camomile Tea, Green Tea, Cardamom Tea, Moringa Tea and Black Tea) in combination with traditional yoghurt starter culture for milk fermentation and yoghurt production. Then investigate the physicochemical quality, acid production and biological activity of Kombucha yoghurt as compared with traditional yoghurt during storage at 4°C for 14 days.

MATERIALS AND METHODS

Preparation of Kombucha fermented solutions (KFSs): acc. to (Reiss, 1994)

KFSs were produced from six different types of tea (Bergamot Tea, Camomile Tea, Green Tea, Cardamom Tea, Moringa Tea and Black Tea). After boiling 1L of tap water for 15 min, four grams of each substrate (Bergamot Tea, Camomile Tea, Green Tea, Cardamom Tea, Moringa Tea, and Black Tea) were added and infused for 15 min, then removed by filtration. 70 g/L of commercial sucrose was dissolved into the filtered infusion before it has cooled. After cooling to room temperature, pour the solution into a glass jar has been previously sterilized at 121°C for 20 min., these solutions were inoculated with 10% of the fermentation broth from the previous fermentation of tea obtained under the same conditions. The glass jars were covered with a clean piece of cloth and fixed with rubber bands. The fermentation was carried out at room temperature (25°C) for 10 days. The harvesting was done after 2, 4, 6, 8 and 10 days of the fermentation period. Kombucha tea was centrifuged at 10.000 rpm for 15 min and the supernatant was used as KFSs.

Yoghurt production

Used milk

Normal clean buffalo’s milk was obtained from the Department of dairy production, Faculty of Agriculture, Cairo University.

Yoghurt starter culture

Lyophilized culture for direct vat set (DVS) type Lactobacillus delbrueckii sub sp. bulgaricus and Strptococcus salivarius sub sp. thermophilus (YC-280) was used. The culture was kindly obtained from Chr. Hansen Laboratories,
Copenhagen, Denmark. The fermented culture was used according to the manufacturer's description.

**Yoghurt manufacturing step**

Yoghurt was prepared in the laboratory using the procedure described by The Egyptian Standard (1990). Samples were produced by addition of 90 mL of kombucha inoculum and 0.1g/L of starter cultures in 900 mL of pasteurized milk. Fermentation was continued until pH=4.5 were reached. Then samples were cooled to 4°C and stored in refrigerator. Depending on the used Kombucha, different groups were produced. The samples were labeled as Group A, B, C, D, E and F for Bergamot Tea, Camomile Tea, Green Tea, Cardamom Tea, Moringa Tea and Black Tea, respectively. G group is a control group using sterile distilled water instead of kombucha.

**Experiment tests**

**Viability Test**

Reference strain of *Staphylococcus aureus* (25923) S.A., was used. The organism was kindly obtained from ATCC (American type culture collection). The organism was inoculated in Brain Heart Infusion Broth for 18 hours to yield a final concentration of 10⁸ log cfu/ml as determined by spreading technique on Baired Barker agar medium.

In samples prepared for this test, milk was inoculated with nearly 10⁸ cfu/ml *Staphylococcus aureus in the seven groups (A, B, C, D, E, F & G) for measuring the antibacterial effect of different types of Kombucha. *Staphylococcus aureus* count was done on Baird-Parker medium at zero time (immediately after coagulum formation and before storage) and then periodically every 48 hours till the end of storage period (14 days). Typical colonies of *S. aureus* were counted and recorded.

**Sensory evaluation**

Three replicates of the seven groups of yoghurt were prepared for sensory evaluation of the final product after one day of storage and periodically during storage period to evaluate the effect of kombucha on organoleptic quality of yoghurt as compared with the control group. The evaluation every time was done by three panelists (belong to the department of food hygiene and control, faculty of veterinary medicine, Cairo University) following the score card of yoghurt according to Nelson and Trout, 1981.

**Chemical examination**

Chemical quality for all types of produced yoghurt was tested in triplicates after Production: Total solids, Ash, Protein and Fat were measured according to AOAC, 2003.

**Measurement of acidity percent**

Acidity percent for all samples were measured day after day during 15 days of storage for assess on changes in pH, measuring the effect of different types of kombucha on acidity and make a correlation between acidity and *S. aureus* growth control. Acidity percent determined according to APHA, 2004.

**Statistical analysis**

All data were presented as the mean ± standard error (SE). The Shapiro–Wilk test was utilized for normality analysis of the variables. A Levene’s test (Levene, 1960) was conducted to check the homogeneity of variances between ponds. Analysis of variance (ANOVA) was used for data with a normal distribution, and if there were significant differences, the Tukey's Studentized Range (HSD) test was used for multiple comparisons post hoc analysis. Dunnett’s test was used to compare the control with the experimental groups. Otherwise, the non-parametric Kruskal–Wallis test was used for data not normally distributed, and Wilcoxon signed ranks test was used to calculate the difference between samples in cases showing differences with the Kruskal–Wallis test.

Pearson's correlation coefficient (r) values were calculated for correlations between *S. aureus* reduction percent and acidity percent in the seven groups of yoghurt during storage period (14 days at 4°C). In order to evaluate the changes in physical properties occurred in all yoghurt groups during storage, repeated measures multivariate analysis of variance (MANOVA) test was conducted to test effect of yoghurt group on sensory properties. Univariate tests were performed to determine yoghurt group effect on individual sensory characteristic overtime. All statistics were calculated to the 95% confidence level. Statistical significance was set at *p* < 0.05. Analyses were performed with SAS® version 9.4 statistical software (SAS Institute Inc., Cary, NC, USA).

**RESULTS**

Table (1) showed the Reduction percentage in the count of *S. aureus* in the seven groups of yoghurt during storage period (14 days at 4°C).
**Table (1): Reduction percentage in the count of* S. aureus *in the seven groups of yoghurt during storage period (14 days at 4°C).**

<table>
<thead>
<tr>
<th>Group</th>
<th>Storage days</th>
<th>1</th>
<th>3</th>
<th>5</th>
<th>7</th>
<th>9</th>
<th>11</th>
<th>13</th>
<th>15</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td></td>
<td>23.53 ± 7.53</td>
<td>88.97 ± 0.52</td>
<td><strong>91.37</strong> a ± 0.63</td>
<td>96.77 a ± 0.59</td>
<td>96.95 a ± 0.28</td>
<td>97.90 a ± 0.37</td>
<td>99.61 a ± 0.03</td>
<td>99.91 a ± 0.00</td>
</tr>
<tr>
<td>B</td>
<td></td>
<td>92.55 a ± 0.52</td>
<td>92.51 b ± 0.81</td>
<td>95.39 ab ± 0.40</td>
<td>98.42 a ± 0.15</td>
<td>98.51 a ± 0.10</td>
<td>99.49 a ± 0.03</td>
<td>99.87 a ± 0.01</td>
<td>99.95 a ± 0.00</td>
</tr>
<tr>
<td>C</td>
<td></td>
<td>76.39 b ± 3.09</td>
<td>95.56 ab ± 0.62</td>
<td>98.53 a ± 0.14</td>
<td>99.82 a ± 0.04</td>
<td>99.82 a ± 0.02</td>
<td>99.89 a ± 0.01</td>
<td>99.91 a ± 0.01</td>
<td>99.92 a ± 0.01</td>
</tr>
<tr>
<td>D</td>
<td></td>
<td>77.51 b ± 1.57</td>
<td><strong>99.40</strong> a ± 0.08</td>
<td>99.65 a ± 0.06</td>
<td>99.82 a ± 0.02</td>
<td>99.97 a ± 0.01</td>
<td>99.98 a ± 0.00</td>
<td>99.99 a ± 0.00</td>
<td>99.99 a ± 0.00</td>
</tr>
<tr>
<td>E</td>
<td></td>
<td>48.52 d ± 4.55</td>
<td>94.73 ab ± 0.37</td>
<td>96.78 ab ± 0.57</td>
<td>97.36 a ± 0.47</td>
<td>99.10 a ± 0.15</td>
<td>99.76 a ± 0.05</td>
<td>99.94 a ± 0.01</td>
<td>99.96 a ± 0.01</td>
</tr>
<tr>
<td>F</td>
<td></td>
<td>71.70 e ± 0.22</td>
<td><strong>94.84</strong> ab ± 0.97</td>
<td>95.11 ab ± 0.67</td>
<td>98.93 a ± 0.11</td>
<td>99.00 a ± 0.04</td>
<td>99.39 a ± 0.04</td>
<td>98.39 a ± 1.23</td>
<td>99.69 a ± 0.00</td>
</tr>
<tr>
<td>G</td>
<td></td>
<td>88.67 a ± 0.51</td>
<td>89.94 c ± 0.45</td>
<td>96.51 ab ± 0.55</td>
<td>98.19 a ± 0.08</td>
<td>98.70 a ± 0.03</td>
<td>98.71 a ± 0.03</td>
<td>99.57 a ± 0.01</td>
<td>99.69 a ± 0.04</td>
</tr>
</tbody>
</table>

*Results are mean value of three trials.*

* Means were tested by Tukey’s Studentized Range (HSD) test; entries indicated by the same letter in a column do not differ at *P* < 0.05.

* Bold means are significantly different from control (group G) at *P* < 0.05 (two-tailed Dunnett’s t-test after analysis of variance)

**Table (2): Average values of Sensory parameters of the seven groups of yoghurt during storage period (14 days at 4°C).**

<table>
<thead>
<tr>
<th>Group</th>
<th>Flavor</th>
<th>Body and texture</th>
<th>Color and appearance</th>
<th>Acidity</th>
<th>Closure</th>
<th>Overall acceptance</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>37.00 ± 0.64</td>
<td>27.17 ± 0.52</td>
<td>7.94 ± 0.26</td>
<td>7.22 ± 0.29</td>
<td>4.00 ± 0.00</td>
<td>83.33 ± 1.41</td>
</tr>
<tr>
<td>B</td>
<td>42.39 ± 0.64</td>
<td>28.72 ± 0.52</td>
<td>8.94 ± 0.26</td>
<td>8.16 ± 0.29</td>
<td>4.00 ± 0.00</td>
<td>92.22 ± 1.41</td>
</tr>
<tr>
<td>C</td>
<td>41.39 ± 0.64</td>
<td>28.44 ± 0.52</td>
<td>8.83 ± 0.26</td>
<td>8.39 ± 0.29</td>
<td>4.00 ± 0.00</td>
<td>91.06 ± 1.41</td>
</tr>
<tr>
<td>D</td>
<td>42.89 ± 0.64</td>
<td>28.33 ± 0.52</td>
<td>8.72 ± 0.26</td>
<td>8.78 ± 0.29</td>
<td>4.00 ± 0.00</td>
<td>92.72 ± 1.41</td>
</tr>
<tr>
<td>E</td>
<td>36.72 ± 0.64</td>
<td>26.94 ± 0.52</td>
<td>8.06 ± 0.26</td>
<td>7.55 ± 0.29</td>
<td>4.00 ± 0.00</td>
<td>83.28 ± 1.41</td>
</tr>
<tr>
<td>F</td>
<td>40.89 ± 0.64</td>
<td>28.22 ± 0.52</td>
<td>7.55 ± 0.26</td>
<td>7.72 ± 0.29</td>
<td>4.00 ± 0.00</td>
<td>88.39 ± 1.41</td>
</tr>
<tr>
<td>G</td>
<td>43.05 ± 0.64</td>
<td>28.83 ± 0.52</td>
<td>9.16 ± 0.26</td>
<td>8.99 ± 0.29</td>
<td>4.00 ± 0.00</td>
<td>94.05 ± 1.41</td>
</tr>
</tbody>
</table>

*Mean ± SE of sensory characteristics. Each value represents the mean of 18 value (3 panelists x 6 evaluation times)
Results in this table showed reduction percentage in the count of *S. aureus* after one day of storage with significant difference between the groups, with the highest reduction percent (92.55%) reached in group B (kombucha yoghurt with Camomile tea). After three days storage, Cardamom kombucha yoghurt shown the highest reduction percentage (99.40%) and the three groups (green, cardamom & black tea) kombucha yoghurt were significantly higher (*P* < 0.05) than the control group itself. By time of storage the reduction percentage in *S. aureus* count increased and the difference between groups decreased until 7th day of storage, there were no significant differences (*P* > 0.05) in results between groups and as compared with the control group and the reduction percent reached about 100%.

In order to evaluate the changes in physical properties occurred in all yoghurt groups during storage, repeated measures MANOVA test was conducted to test effect of yoghurt group on sensory properties. The results tabulated in table (2) showed that there was no difference among different yoghurt groups on flavor, body and texture, color and appearance, acidity and overall acceptability over time. The same closure score (4 out of 5) was assigned by all panelists to all yogurt groups at all evaluation times.

Data presented in table (3) concluded the results of analysis of sensory quality and over all acceptability of the produced yoghurt with different types of kombucha as compared with traditional yoghurt produced using yoghurt starter culture only (control group). After the first day of storage all kombucha groups were not significantly different than the control group in the flavor point except A and E groups were significantly lower. For other sensory parameters; Body and texture, color and appearance, Acidity, closure and Overall acceptance, there were no differences between the different groups including the control group. After that during the storage period and at the end of storage period after 14 days there were no differences in any of the 6 variables between groups.

**Table (3): Results of Sensory analysis after day one storage, after 14 days of storage and the storage period overall at 4°C**

<table>
<thead>
<tr>
<th>Group</th>
<th>Day</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavor</td>
<td>Day-1</td>
<td>36.67 ± 1.67</td>
<td>41.00 ± 1.00</td>
<td>40.67 ± 2.96</td>
<td>41.67 ± 0.88</td>
<td>35.00 ± 0.00</td>
<td>41.67 ± 1.67</td>
<td>44.33 ± 0.67</td>
</tr>
<tr>
<td></td>
<td>Day-14</td>
<td>36.67 ± 1.67</td>
<td>42.00 ± 1.00</td>
<td>40.67 ± 2.96</td>
<td>42.67 ± 1.45</td>
<td>37.33 ± 2.34</td>
<td>39.33 ± 2.33</td>
<td>41.33 ± 0.67</td>
</tr>
<tr>
<td></td>
<td>Overall</td>
<td>37.00 ± 0.64</td>
<td>42.39 ± 0.64</td>
<td>41.39 ± 0.64</td>
<td>42.89 ± 0.64</td>
<td>36.72 ± 0.64</td>
<td>40.89 ± 0.64</td>
<td>43.05 ± 0.64</td>
</tr>
<tr>
<td>Body And texture</td>
<td>Day-1</td>
<td>28.00 ± 1.15</td>
<td>29.00 ± 1.00</td>
<td>29.67 ± 0.33</td>
<td>29.33 ± 0.67</td>
<td>29.00 ± 1.00</td>
<td>29.67 ± 0.33</td>
<td>29.67 ± 0.33</td>
</tr>
<tr>
<td></td>
<td>Day-14</td>
<td>27.00 ± 1.00</td>
<td>28.67 ± 0.88</td>
<td>28.33 ± 1.20</td>
<td>27.67 ± 1.45</td>
<td>26.67 ± 0.88</td>
<td>28.33 ± 0.88</td>
<td>28.33 ± 0.88</td>
</tr>
<tr>
<td></td>
<td>Overall</td>
<td>27.17 ± 0.52</td>
<td>28.72 ± 0.52</td>
<td>28.44 ± 0.52</td>
<td>28.33 ± 0.52</td>
<td>26.94 ± 0.52</td>
<td>26.82 ± 0.52</td>
<td>28.83 ± 0.52</td>
</tr>
<tr>
<td>Color and appearance</td>
<td>Day-1</td>
<td>8.33 ± 0.66</td>
<td>9.33 ± 0.33</td>
<td>9.33 ± 0.33</td>
<td>8.67 ± 0.33</td>
<td>8.67 ± 0.33</td>
<td>8.00 ± 1.00</td>
<td>9.33 ± 0.33</td>
</tr>
<tr>
<td></td>
<td>Day-14</td>
<td>8.00 ± 0.58</td>
<td>8.67 ± 0.88</td>
<td>8.67 ± 0.88</td>
<td>8.33 ± 0.88</td>
<td>8.33 ± 0.67</td>
<td>7.67 ± 0.88</td>
<td>9.00 ± 0.58</td>
</tr>
<tr>
<td></td>
<td>Overall</td>
<td>7.94 ± 0.26</td>
<td>8.94 ± 0.26</td>
<td>8.83 ± 0.26</td>
<td>8.72 ± 0.26</td>
<td>8.06 ± 0.26</td>
<td>7.55 ± 0.26</td>
<td>9.16 ± 0.26</td>
</tr>
<tr>
<td>Acidity</td>
<td>Day-1</td>
<td>7.33 ± 1.33</td>
<td>8.00 ± 1.15</td>
<td>8.00 ± 1.15</td>
<td>8.93 ± 0.33</td>
<td>8.33 ± 0.88</td>
<td>7.67 ± 1.20</td>
<td>9.33 ± 0.33</td>
</tr>
<tr>
<td></td>
<td>Day-14</td>
<td>7.00 ± 0.58</td>
<td>8.00 ± 1.53</td>
<td>8.00 ± 1.53</td>
<td>8.33 ± 0.67</td>
<td>7.00 ± 1.00</td>
<td>7.67 ± 0.88</td>
<td>8.67 ± 0.67</td>
</tr>
<tr>
<td></td>
<td>Overall</td>
<td>7.22 ± 0.29</td>
<td>8.16 ± 0.29</td>
<td>8.39 ± 0.29</td>
<td>8.78 ± 0.29</td>
<td>7.55 ± 0.29</td>
<td>7.72 ± 0.29</td>
<td>8.99 ± 0.29</td>
</tr>
<tr>
<td>Closure</td>
<td>Day-1</td>
<td>4.00 ± 0.00</td>
<td>4.00 ± 0.00</td>
<td>4.00 ± 0.00</td>
<td>4.00 ± 0.00</td>
<td>4.00 ± 0.00</td>
<td>4.00 ± 0.00</td>
<td>4.00 ± 0.00</td>
</tr>
<tr>
<td></td>
<td>Day-14</td>
<td>4.00 ± 0.00</td>
<td>4.00 ± 0.00</td>
<td>4.00 ± 0.00</td>
<td>4.00 ± 0.00</td>
<td>4.00 ± 0.00</td>
<td>4.00 ± 0.00</td>
<td>4.00 ± 0.00</td>
</tr>
<tr>
<td></td>
<td>Overall</td>
<td>4.00 ± 0.00</td>
<td>4.00 ± 0.00</td>
<td>4.00 ± 0.00</td>
<td>4.00 ± 0.00</td>
<td>4.00 ± 0.00</td>
<td>4.00 ± 0.00</td>
<td>4.00 ± 0.00</td>
</tr>
<tr>
<td>Overall acceptance</td>
<td>Day-1</td>
<td>84.33 ± 3.76</td>
<td>91.33 ± 3.18</td>
<td>91.67 ± 4.36</td>
<td>93.00 ± 0.58</td>
<td>85.00 ± 2.65</td>
<td>91.00 ± 4.00</td>
<td>96.67 ± 0.33</td>
</tr>
<tr>
<td></td>
<td>Day-14</td>
<td>82.67 ± 3.18</td>
<td>91.33 ± 4.26</td>
<td>89.67 ± 6.33</td>
<td>91.00 ± 4.36</td>
<td>83.33 ± 4.06</td>
<td>87.00 ± 4.00</td>
<td>91.33 ± 1.45</td>
</tr>
<tr>
<td></td>
<td>Overall</td>
<td>83.33 ± 1.41</td>
<td>92.22 ± 1.41</td>
<td>91.06 ± 1.41</td>
<td>92.72 ± 1.41</td>
<td>83.28 ± 1.41</td>
<td>88.39 ± 1.41</td>
<td>94.05 ± 1.41</td>
</tr>
</tbody>
</table>
In table (4), the acidity percent of yoghurt expressed as lactic acid from 0 day of storage (immediately after coagulum formation) and then day by day until the end of storage period (14 days) at 4 °C were illustrated. Acidity percent after coagulum formation and after one day storage had no significant difference ($P < 0.05$) between the seven groups but after three days of storage Moringa kombucha yoghurt was significantly higher ($P < 0.05$) than other groups even the control group with acidity percent 1.15±0.08 and it showed the highest acidity percent till the end of storage period. After seven days of storage, black tea kombucha yoghurt showed significantly lower ($P < 0.05$) acidity percent than control group till the end of storage period. By the end of storage period, there were difference in the acidity percent between groups with the groups A, C & F had significant lower results ($P < 0.05$) than control group.

**Figure (1): Chemical composition of the seven groups of yoghurt after one day storage.**

Means indicated by (*) within each variable are significantly different than control (group G) at $P<0.05$. 

**Means indicated by (*) within each variable are significantly different than control (group G) at $P<0.05$.**
Table (4): Titratable acidity percent of the seven groups of yoghurt from zero days and during storage period (14 days at 4°C).

<table>
<thead>
<tr>
<th>Group</th>
<th>Storage days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>A</td>
<td>0.86±0.01</td>
</tr>
<tr>
<td>B</td>
<td>0.88±0.01</td>
</tr>
<tr>
<td>C</td>
<td>0.85±0.01</td>
</tr>
<tr>
<td>D</td>
<td>0.90±0.02</td>
</tr>
<tr>
<td>E</td>
<td>0.89±0.01</td>
</tr>
<tr>
<td>F</td>
<td>0.85±0.01</td>
</tr>
<tr>
<td>G</td>
<td>0.93±0.02</td>
</tr>
</tbody>
</table>

* Results are mean value of three trials ± SEM.* Average values with different alphabetical superscripts within column are significantly different at P<0.05. * Bold means are significantly different from control (group G) at P<0.05 (two-tailed Dunnett's t-test after analysis of variance).
DISCUSSION

Biological activity

Occurrence and survival of food borne pathogens for up to several days in fermented dairy products, such as yoghurt, illustrates the potential health risks on consumer health. The presence of these pathogens in yoghurt may be attributed to low quality of raw milk or post-processing contamination. For that, there is a need for investigation of the survival period of these pathogens in yoghurt before the finished product reaches the consumer (Dineen et al., 1998). The survival of different pathogens in fermented dairy products is attributed to several factors: fermentation conditions, type of fermentative microorganisms, acid type, acid concentration, acid tolerance and type of pathogen (Pitt et al., 2000). In fermented food as a general and yoghurt specifically, acidity could be considered as the main defense line against food borne pathogens; that could be easily improved as shown in fig. (2) through finding out the correlation between acidity percent and \( S. aureus \) reduction percentage in all produced yoghurt groups. This correlation is conventional but is not enough to lower the incidence of pathogens below its critical limits, so that acidity should be supported with other antibacterial substances. Due to the increasing interest of consumers for ‘healthy’ food, it is necessary to introduce some functional ingredients in the food (Lazarides, 2009). Recently many additives are added during yoghurt manufacture as functional ingredients to increase the final product quality and safety such as probiotic bacteria, bacteriocins, ethanol, natural plant oils and kombucha.

Kombucha is a well-known symbiotic association of yeast (Saccharomyces Ludwigia, Saccharomyces cerevisiae, Saccharomyces bisporus, Torulopsis sp. and Zigosacharomyces sp) and acetic acid bacteria (Acetobacter xylinum and Gluconobacter oxydans). The metabolic activity of black tea sweetened with sucrose creates a pleasant, slightly sour sparkling beverage, which contains many important nutritional and pharmacological components with positive influence on human health (Mirjana and Katarina, 2012).

Kombucha has positive effect on tonsillitis, headaches, atherosclerosis, rheumatism and digestive difficulties. Prophylactic and therapeutic properties of kombucha are the result of metabolic activity with the production of: acetic acid, ethanol, glycerol and other metabolites. Kombucha tea has demonstrated the ability to inhibit the growth of pathogens such as Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus cereus and Salmonella typhimurium (Hartman et al., 2000; Sreeramulu et...
Tracking the viability of inoculated *S. aureus* in the laboratory manufactured yoghurt in our study during the storage period (14 days) showed great reduction percentage in the count. After one day of storage at 4°C acidity in all groups increased up to 0.9 % and more with no significant difference \((P < 0.05)\) between the seven groups including the control one (table 4). On the other hand, reduction percentage in the count of *S. aureus* after one day of storage showed significant difference between the groups as seen in table (1), with the highest reduction percent (92.55\%) reached in group B (kombucha yoghurt with Camomile tea). These results indicate that the antibacterial effect appeared against *S. aureus* organism is not only caused by increasing acidity but it may be also attributed to other kombucha metabolites which have significant effect against pathogenic microorganisms. After three days storage, Cardamom kombucha yoghurt shown the highest reduction percentage (99.40) and the three groups (green, cardamom & black tea) kombucha yoghurt were significantly higher \((P < 0.05)\) than the control group itself, that increase the hypothesis of production of antibacterial metabolites which act symbiotically with the acidity against *S. aureus* organism. By time of storage the reduction percentage in *S. aureus* count increased and the difference between groups decreased until 7\textsuperscript{th} day of storage, there were no significant differences \((P < 0.05)\) in results between groups and as compared with the control group. Data presented in fig. (2) and table (1) summarized that, with storage the acidity percentage increased with subsequent reduction in *S. aureus* count, but this reduction in count could be enhanced from day one of storage by using kombucha as fortification during yoghurt manufacture.

**Sensory evaluation**

Product quality and consumer satisfaction are the main points to increase the sales of various types of yoghurt products (Debbie et al., 1991). In all branches of food and dairy industry (yogurt production in particular), there is a tendency towards a constant improvement of product quality. Quality assessment encompasses specifications, sampling, testing procedures and recording or reporting. Specifications are typically set by the manufacturer but the consumer is the final judge of quality.

Yogurt is one of the most popular fermented dairy products widely consumed all over the word because of its organoleptic and nutritional properties are much sought after by consumers (Loveday et al., 2013). The commercial yoghurt starter culture (*Lactobacillus bulgaricus* and *Streptococcus thermophiles*) acidify milk and produce specific yoghurt flavor and aroma. The acetaldehyde formed during lactic acid fermentation is the main constituent of the specific yogurt flavor (Sahan et al. 2008). As a result of milk fermentation by kombucha, new refreshing fermented dairy product with mild sour taste is obtained. Sensory analysis of the product shows its quality characteristics and consumer satisfaction (Malbaša et al., 2009).

Color, taste and aroma are the important factors which determine the acceptance or rejection of the food article. Sensory evaluation of the different yoghurt groups were conducted by specialist panelists according to score card.

In order to evaluate the changes in physical properties occurred in all yoghurt groups during storage, repeated measures MANOVA test was conducted to test effect of yoghurt group on sensory properties. The results showed that there was no difference among different yoghurt groups on flavor, body and texture, color and appearance, acidity, and overall acceptability over time, \(F(120, 280) = .39, p = 1.00, \eta^2 = .14\). Univariate tests also indicated there was no effect of yoghurt group on individual sensory characteristic overtime, \(F(30, 70) = .45, p = .99, \eta^2 = .16\) for flavor, \(F(30, 70) = .29, p = 1.00, \eta^2 = .11\) for body and texture, \(F(30, 70) = .29, p = 1.00, \eta^2 = .11\) for color and appearance, \(F(30, 70) = .19, p = 1.00, \eta^2 = .07\) for acidity, and \(F(30, 70) = .18, p = 1.00, \eta^2 = .07\) for overall acceptability. The same closure score (4 out of 5) was assigned by all panelists to all yogurt groups at all evaluation times. Thus, no effect of group on closure (table 2).

Sensory quality and over all acceptability of the produced yoghurt with different types of kombucha as compared with traditional yoghurt produced using yoghurt starter culture only was evaluated from day one after production and alongside the storage period for 14 days at 4°C and analyzed using ANOVA test. Data presented in table (3) concluded the results of analysis after the first day of storage, at the end of storage period and the storage period overall and it showed that all kombucha groups were not significantly different than the control group in the flavor point, while A and E groups were significantly lower. For other sensory parameters
(Body and texture, color and appearance, Acidity, closure and Overall acceptance) there were no differences between the different groups including the control group. After that during the storage period and by the end of it after 14 days there were no differences in any of the 6 variables between groups.

Milanović, et al., 2012 concluded that textural characteristics of black tea kombucha yoghurt samples were enhanced significantly after storage and these results indicated positive influence of black tea ingredients on samples gel during storage. Makvandi et al., 2016 noted that raising the kombucha concentration caused reduction of overall acceptability score in samples (p<0.01); and during storage, this score decreased (p<0.01) a little in comparison with that in control sample.

Chemical Composition

Milk nutrients are partially modified during fermentation process for yoghurt manufacture. Mainly lactose and galactose are decrease; also there is a significant reduction in vitamin B12 and pantothenic acid. By a general viewpoint, yoghurts contain more abundant nutrients in comparison with the original milk, excluding lactose (Baglio, 2014).

The possibility of using kombucha as non-conventional starter culture is newly introduced to dairy industry (Malbaša et al., 2009). Dajana et al., 2014 examined the difference in composition between milk and kombucha yoghurt and obtained results revealed that dry matter, milk fat, total proteins and ash of samples produced at 37°C and 42°C have not been significantly changed during fermentation process, and also, differences are not significant between samples produced at different temperatures. Milanović et al., 2012 produced different kombucha yoghurt and examined its chemical composition and found that Samples produced with kombucha cultivated on thyme tea had slightly higher level of dry matter and ash than samples produced with kombucha cultivated on black tea. All samples had similar level of milk fat and proteins content.

In our study, by comparing the chemical composition (total solids, fat, protein and Ash) of six types of kombucha yoghurt with traditional yoghurt (control group) after one day storage at 4°C, data illustrated in fig. (1) showed that total solids percent was significantly lower (P < 0.05) in camomile and green tea kombucha yoghurt than other groups including the control group. Also camomile and cardamom kombucha yoghurt were significantly lower (P < 0.05) than control group in fat percent. On the other hand, Ash percent was significantly higher (P < 0.05) in moringa kombucha yoghurt than other groups including the control group, while there were no significant difference (P < 0.05) between all groups for the fat percent. These results are in accordance with results obtained by Milanović, et al., 2012 and Dajana et al., 2014.

Acidity percentage

Yoghurt is considered ready to eat depending on lactic acid level. Acidity is measured by taking a sample of yoghurt and titrating it with alkaline (sodium hydroxide) in the presence of color indicator. A value of at least 0.9% acidity is the minimum standard for yoghurt manufacture. The usual fermentative pathway in the yoghurt manufacture is coincident with the common homolactic fermentation. LAB utilizes carbohydrates and produce organic acids as lactic acid or acetic acid, with an increase of acidity and a decrease in pH of yoghurt (Luquet, 1990 and Baglio, 2014). Sokolinska et al., (2004) indicated that the pH values of milk under processing, from the time it was inoculated with bacterial cultures to the time the yoghurt was manufactured, decreased from 6.70 to 4.34. The majority of food borne contaminants, either pathogenic or non-pathogenic is sensitive to the resulting low pH (Maganusson and Schnurer, 2001).

Duraković et al., 2008 and Milanović, et al., 2012 indicated that kombucha inoculums cultivated on different tea types could be used in combination with yoghurt starter culture in fermented dairy products technology and they concluded that fermentation of milk with kombucha and yoghurt starter lasts from 3.5 to 4.5 h and increasing the incubation temperature caused the decreasing of the fermentation time.

Data presented in table (4) showed the acidity percent in yoghurt expressed as lactic acid from 0 day of storage (immediately after coagulum formation) and then day by day until the end of storage period (14 days) at 4 °C. Acidity percent after coagulum formation and after one day storage had no significant difference(P < 0.05) between the seven groups but after three days of storage Moringa kombucha yoghurt was significantly higher (P < 0.05) than other groups even the control group with acidity percent 1.15±0.08 and it showed the highest acidity percent till the end of storage period. After seven days of storage, black tea kombucha yoghurt showed significantly lower (P < 0.05) acidity percent than control group till the end of storage period and by the end of it after 14 days there were no differences between the different groups including the control group. After that during the storage period and by the end of it after 14 days there were no differences in any of the 6 variables between groups.

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period. By the end of storage period, there were difference in the acidity percent between groups with the groups A, C & F had significant lower results \((P < 0.05)\) than control group. Acidity percent in all groups at the end of storage period didn’t increase than the legal requirements of the Egyptian standards (ES. 2005)

**CONCLUSION**

Obtained results in this study revealed that kombucha inoculums cultivated on different tea types could be used in combination with traditional yoghurt starter culture as one of the functional ingredients which could be used in fermented dairy products technology to improve sensory characters, technological parameters and the hygienic quality of the finished products; with subsequent increase in product shelf-life, consumer satisfaction and public health hazards control. Chemical composition of yoghurt fortified with different kombucha tea inoculum is nearly similar to traditional yoghurt and Ash content could increase. Also, it was guaranteed that different sensory parameters of kombucha yoghurt aren’t affected during storage at 4°C for 14 days.

**CONFLICT OF INTEREST**

The authors declared that present study was performed in absence of any conflict of interest.

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**AUTHOR CONTRIBUTIONS**

ABA designed and performed the experiments, analyzed the data collected and also wrote the manuscript. GFG prepared the Kombucha solutions (KFS), helped in data collection and reviewed the manuscript. All authors read and approved the final version.

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