Antibacterial impact of Debaromyces hansenii and Saccharomyces cerevisiae mycocons on Staphylococcus aureus and Escherichia coli in freshly prepared yoghurt

Manal, M. Abdullah 1,2*, Hend Ahmed Elbarbary1, Hamdi A. Mohamed1, Marionette, Z. Nassif2
1 Food Hygiene and Control Department, Faculty of Veterinary Medicine, Benha University, Egypt
2 Animal Health Research Institute – Benha Lab., ARC, Egypt.

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ABSTRACT
Recently, consumers have been looking for natural alternatives to chemical preservatives because of their concerns about the toxicity of these chemicals and the presence of antimicrobial-resistant pathogens found in food. So, this research sought to investigate the antibacterial effect of mycocins of Debaromyces hansenii (D. H) and Saccharomyces cerevisiae (S.C) at different concentrations (200 ppm and 400 ppm) on Staphylococcus aureus (Staph. aureus ) and Escherichia coli (E. coli) in yoghurt samples. Results revealed a decrease in coli counts. The T6 yoghurt group (400 ppm of D.H cell-free extract + 400 ppm of S.C cell-free extract + 10^8 cfu/ml E. coli strain) had the most significant results, as it recorded at zero time of storage 4.9 ± 0.02 log10 cfu/g and its count recorded <10 at the 3rd, 6th, 9th, 12th, 15th, and 18th days of storage compared with the T7 yoghurt group (control positive) (10^9 cfu/ml of E. coli strain). Conversely, the results for S. aureus decreased during the storage period. Whereas G6 (400 ppm of D.H cell-free extract + 400 ppm of S. C cell-free extract + 10^9 cfu/ml Staph. aureus strain) had the most significant results as it recorded at zero time of storage 4.1±0.03 log10 cfu/g and recorded at 3rd day of storage with mean value 1.3±0.01 log10 cfu/g and S. aureus counts were <10 at 6th, 9th, 12th, 15th, and 18th day of cold storage in comparison with G7 yoghurt group (control positive) (10^9 cfu/ml Staph. aureus strain). In conclusion, DH and SC cell-free extracts at a concentration of 400 ppm could inhibit Staph. aureus and E. coli growth during the yoghurt cooling storage period.

1. INTRODUCTION
Yoghurt’s flavor and ease of preparation have made it incredibly popular for years. It is regarded as a primary source of good fats, proteins, calcium, phosphorus, and potassium, in addition to large amounts of vitamins. A tendency to further enrich the product with specific probiotics, prebiotics, and minerals has emerged as knowledge about probiotics and yoghurt production technology has grown (Khablenko et al., 2022). Probiotics are live microorganisms that, when consumed in certain amounts, benefit the host’s health. The food matrix of traditional fermented foods is orchestrated by beneficial microorganisms, which have been validated for their functional properties, including providing consumers with probiotics (Marco et al., 2021).

When it comes to fermented dairy products made from domesticated animals, yeasts are less commonly used as starter cultures than lactic acid bacteria. Yeasts have primarily enzymatic (mainly amylolytic) functions in food fermentation, including leavening, baking, producing alcohol, and producing metabolites (Maicas, 2020). Debaromyces hansenii (D.H) is the most reported yeast species from fermented dairy foods and beverages, followed by Saccharomyces cerevisiae, which is the only commercialized probiotic yeast (Fu et al. 2020).

Debaromyces hansenii is a haploid, halo-tolerant species, and it can be grown in a medium supplemented with up to 25% sodium chloride (Marquina et al. 2001). Furthermore, it can endure low water activity levels and various pH ranges from 3 to 10 (Capace and Romano, 2009). According to reports, D. H produces lethal toxins known as glycol-proteins, or powerful and active toxic proteins. These antimicrobial compounds may be crucial in preventing the growth of various yeast genera (Buzzini and Martini, 2001). Escherichia coli (E. coli) organisms are the most common contaminants of raw and processed milk, such as yoghurt, and they are a reliable indicator of fecal contamination of water and food such as milk and dairy products, constituting a public health hazard (Virpari et al. 2013). Because of the ongoing source of contamination, E. coli is resistant to production environments. The presence of E. coli in dairy products is thought to serve as a barometer for the cleanliness of the production facility. Children who are exposed to it can die from severe diarrhea, as reported in developing nations (Metz et al. 2020). Being an opportunistic pathogen, Staphylococcus aureus (S. aureus) can cause anything from minor skin infections to serious invasive infections and food poisoning. A serious threat to public health is the rise in antibiotic resistance in S. aureus isolates. Using substances made by probiotics may be able to solve this problem (Saidi et al., 2019).

Correspondence to: drmanalmahrous645@gmail.com
**Staphylococcus aureus** produces a variety of exotoxins, including hemolysins and enterotoxins (Berube and Wardenburg, 2013). However, *S. aureus* enterotoxin C (SEC) could not be produced in sufficient quantities to cause food poisoning at temperatures between 10 and 25 °C (Valihrah et al., 2013). The foodborne pathogen *S. aureus* forms biofilms on food and surfaces that come into contact with food (Farha et al., 2020). Therefore, preventive measures like proper manufacturing and hygiene practices and consumer safety awareness should be applied to decrease the risks associated with dairy products (Owusu-Kwarteng et al. 2020). So, the current research sought to study the antibacterial impact of *Saccharomyces cerevisiae* and *Debaryomyces Hansenii* mycocins on *E. coli* and *S. aureus* in yoghurt.

2. MATERIALS AND METHODS

2.1 Activation of *Debaryomyces Hansenii* and *Saccharomyces cerevisiae* strains

*Debaryomyces Hansenii* (D. H.) and *Saccharomyces cerevisiae* (S.C.), reference strains were obtained from Cairo MIRCEN, Faculty of Agriculture, Ain Shams University, Cairo, Egypt. The strains were activated in yeast extract peptone dextrose broth (YPD) (5 g yeast extract, 5 g peptone, 10 g dextrose, and 1 L distilled water) at 25 °C for 2-3 days. Three subcultures were conducted to activate the strains until they obtained the concentration of 1.4x10^11 cfu/ml and 2.3x10^10 cfu/ml, respectively. (Golubev, et al. 2002; Gori, et al. 2012).

2.2 Extraction of *Debaryomyces Hansenii* and *Saccharomyces cerevisiae*

After 3 days of incubation of activated D. H and S.C. with a concentration of 1.4x10^13 and 2.3x10^10 cfu/ml, respectively in YPD broth at 25 °C, yeast cells were separated by centrifugation at 3000 xg for 30 min at 4°C. To obtain cell-free extracts for D. H. and S. C. respectively (Golubev et al. 2002), the supernatant was filtered through a Seitz filter with a pore size of 0.45 μm, then stored in a refrigerator until it was needed for the antifungal assay.

2.3 Determination of antimicrobial activity

2.3.1 Pathogenic microorganism and culture condition

The reference pathogenic strains [*E. coli* (ATCC® 25922)] and [*S. aureus* (ATCC® 6538)] were obtained from the Animal Health Research Institute, Dokki, Cairo. The strains were activated on Tryptic soya broth (TSB, Himedia) at 37°C for 24 hours then the strains were cultured for 24 hours at 42°C. Transfer yoghurt starter cultures (1:1) to sterile 11% reconstituted skim milk powder, the activated strains were incubated for 24 hours at 42°C. The active starter cultures were stored in the refrigerator till be used within 24 hours (Badawi et al. 2004).

2.3.2 Antimicrobial assay

The antimicrobial assay was conducted by Agar well diffusion assay (Arokiamary et al. 2012). Under the aerobic condition, 10 μl of cell-free culture supernatant (CFS) of *Debaryomyces Hansenii* and *Saccharomyces cerevisiae* with concentrations of 200 ppm and 400 ppm according to AOAC (2000), as CV = C1V1 where C= protein concentration in DHE and SCE, V is the volume to be calculated, C1 is the required concentration in the experiment (200 ppm and 400 ppm), V1 is the total volume used in the experiment. The mycocins were added separately to each well on Mueller Hinton agar (OXOID) plates that had been inocculated with 100 μl of each target bacteria. Wells (5 mm) were then cut into the plated sand. After 1 hour of refrigeration, the plates were incubated for twenty-four hours at 37 °C. We measured the diameter of the inhibition zone surrounding the wells to determine the antimicrobial activity.

2.4 Yoghurt manufacture

2.4.1 Activation of starter cultures

*Lactobacillus bulgaricus* and *Streptococcus thermophilus* (yoghurt starter cultures) were obtained from Cairo MIRCEN Faculty of Agriculture, Ain Shams University, Cairo, Egypt. Following activation on MRS broth and M17 broth, respectively, the strains were cultured for 24 hours at 42°C. Transfer yoghurt starter cultures (1:1) to sterile 11% reconstituted skim milk powder, the activated strains were incubated for 24 hours at 42°C. The active starter cultures were stored in the refrigerator till be used within 24 hours (Badawi et al. 2004).

2.4.2 Preparation of yoghurt

Yogurt was made following Corrieu and Be’al’s (2016) instructions. From Benha City supermarkets and farmers provided 8 liters of fresh, raw mixed milk from cows and buffaloes (1:1). The skimmed milk (0.5% fat) was heated to 85°C for 30 minutes and then cooled to 45°C right away. Four groups (2L of each) were created and then inoculated with two percent activated starter cultures. At the proper concentrations of 200 and 400 ppm, mycocins (SCE or DHE) were added. Yoghurt groups were ordered as follows:

- **E. coli groups:**
  - T1: 2% yoghurt starter cultures (1:1) + 200 ppm DHE + 10^6 cfu/ml *E. coli*.
  - T2: 2% yoghurt starter cultures (1:1) + 400 ppm DHE + 10^6 cfu/ml *E. coli*.
  - T3: 2% yoghurt starter cultures (1:1) + 200 ppm SCE + 10^6 cfu/ml *E. coli*.
  - T4: 2% yoghurt starter cultures (1:1) + 400 ppm SCE + 10^6 cfu/ml *E. coli*.
  - T5: 2% yoghurt starter cultures (1:1) + 200 ppm DHE + 200 ppm SCE + 10^6 cfu/ml *E. coli*.
  - T6: 2% yoghurt starter cultures (1:1) + 400 ppm DHE + 400 ppm SCE + 10^6 cfu/ml *E. coli*.
  - T7: 2% yoghurt starter cultures (1:1) + 10^8 cfu/ml *E. coli*.
  - T8: 2% yoghurt starter cultures (1:1) + 200 ppm SCE + 10^8 cfu/ml *S. aureus*.
  - G1: 2% yoghurt starter cultures (1:1) + 200 ppm DHE + 10^6 cfu/ml *S. aureus*.
  - G2: 2% yoghurt starter cultures (1:1) + 400 ppm DHE + 10^6 cfu/ml *S. aureus*.
  - G3: 2% yoghurt starter cultures (1:1) + 200 ppm SCE + 10^6 cfu/ml *S. aureus*.
  - G4: 2% yoghurt starter cultures (1:1) + 400 ppm SCE + 10^6 cfu/ml *S. aureus*.
  - G5: 2% yoghurt starter cultures (1:1) + 200 ppm DHE + 200 ppm SCE + 10^6 cfu/ml *S. aureus*.
  - G6: 2% yoghurt starter cultures (1:1) + 400 ppm DHE + 400 ppm SCE + 10^6 cfu/ml *S. aureus*.
  - G7: 2% yoghurt starter cultures (1:1) + 10^6 cfu/ml *S. aureus*.
  - G8: 2% yoghurt starter cultures (1:1)
Yoghurt samples from each group were combined, placed in 100 ml cups, and incubated at 42 °C until curd formation (pH 4.6). They were then moved to a refrigerator at 4 °C.

2.4.3 Microbiological examination

2.4.3.1 Enumeration of E. coli viable count

After preparation of ten-fold serial dilutions, one ml of each sample’s serial dilution was added to two plates, poured with Tryptone-Bile-Glucuronic medium (TBX, Himedia). The inoculum was mixed with the medium and allowed to solidify before being incubated at 44 °C for 24 h. Typical colonies are greenish-blue colonies (ISO 16649-1, 2001).

2.4.3.2 Enumeration of S. aureus

Over duplicate Baird Parker agar (OXOID) plates after addition of egg yolk tellurite supplement, 0.1 ml of previously prepared serial dilutions of each sample was spread out, and the plates were incubated at 37 °C for 24 to 48 hours. S. aureus suspected colonies are circular, smooth, convex, gray to jet black, with light colored (off–white) margin, surrounded by an opaque zone with an outer zone (ISO 6888-1, 2018).

2.5 Statistical analysis

The data were statistically analyzed using SPSS 16.0’s analysis of variance (ANOVA) tool. Analysis of variance in one direction was used to make statistical comparisons. According to SPSS (2018), P < 0.05 indicated that the results were deemed significantly different.

3. RESULTS

The information obtained in Fig. (1) demonstrated the suppression of E. coli and S. aureus growth by S. C and D. H mycins (400 ppm) more than mycacin concentration (200 ppm). The zones of inhibition for S.C mycin (400 ppm) against S. aureus and E. coli were 3 and 5 mm, respectively; the corresponding zones for D.H mycin were 4 and 6 mm.

Fig. (1) Antimicrobial activities of mycacin using agar well diffusion assay

The data illustrated in Table (1) revealed that gradual decrease in E. coli counts in treated yoghurt samples in comparison to the control positive group. Which, T1 has recorded at zero day of 5.6 ± 0.04 log10 cfu/g while at 6th day of the storage period recorded 1 ± 0.01 log10 cfu/g and less than 10 on the 9th day of storage. T3 has recorded at zero day of storage 5.8 ± 0.04 log10 cfu/g while recorded at 6th day of storage 1.6 ± 0.01 log10 cfu/g and this value decreased to < 10 at 9th day of storage. T5 has recorded at zero time 5.5 ± 0.03 log10 cfu/g while recorded at 3rd day of storage 1.6 ± 0.01 log10 cfu/g, which decreased to < 10 at 6th, 9th, 12th and 15th day of storage period. On the other hand, the control positive group (T7) recorded at zero time 5.8 ± 0.05 log10 cfu/g which declined till 12th with a mean value of 1.4 ± 0.01 log10 cfu/g. In the control negative group (T8), the E. coli count was recorded at 2.4 ± 0.01 log10 cfu/g on the 21st day of storage, compared to a mean value of 1 ± 0.01 log10 cfu/g on the 12th day of storage.

Table (1) Effect of mycin extract (200 ppm) on inoculated yoghurt samples with E. coli counts Log10.

<table>
<thead>
<tr>
<th>Extract</th>
<th>DHE</th>
<th>SCE</th>
<th>DHE+SCE</th>
<th>Control avg</th>
<th>Control -ve</th>
</tr>
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<tbody>
<tr>
<td>0</td>
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<td>T3</td>
<td>T5</td>
<td>T7</td>
<td>T8</td>
</tr>
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</tr>
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</tr>
<tr>
<td>18</td>
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<td>&lt;10</td>
<td>&lt;10</td>
<td>1.9±0.01</td>
<td>&lt;10</td>
</tr>
</tbody>
</table>

DHE: Debaryomyces hansenii cell free extract. SCE: Saccharomyces cerevisiae cell free extract. DHE+SCE: Debaryomyces hansenii + Saccharomyces cerevisiae cell free extract. Control -ve: days of storage. a,b,c,d Different superscript letter within same row means significant difference (P ≤ 0.05). S: spoiled.

Table (2) had been shown that mycin concentration (400 ppm) had more significant results than mycacin concentration (200 ppm) on E. coli counts during the storage period of yoghurt samples. T2 recorded 4.4 ± 0.03 log10 cfu/g at zero-day of storage, which decreased at 3rd day of storage to record 2.1 ± 0.01 log10 cfu/g and counts were < 10 at 6th, 9th, 12th, 15th day of the storage period; while T4 recorded at zero-day 4.8 ± 0.04 log10 cfu/g and decreased to 2.5 ± 0.01 log10 cfu/g at 3rd day of storage, the counts decreased to be < 10 at 6th, 9th, 12th, 15th day of the storage period, with more attention to T6 which had the most significant results as recorded at zero time of storage 4.9 ± 0.02 log10 cfu/g and recorded < 10 at 3rd, 6th, 9th, 12th, 15th day of storage.

Table (2) Effect of mycin extract (400 ppm) on inoculated yoghurt samples with E. coli counts Log10.

<table>
<thead>
<tr>
<th>Extract</th>
<th>DHE</th>
<th>SCE</th>
<th>DHE+SCE</th>
<th>Control avg</th>
<th>Control -ve</th>
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<tbody>
<tr>
<td>0</td>
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<td>T3</td>
<td>T5</td>
<td>T7</td>
<td>T8</td>
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<tr>
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<td>4.4±0.02</td>
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<td>&lt;10</td>
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<tr>
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<td>&lt;10</td>
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<tr>
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<td>2.4±0.01</td>
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</tr>
</tbody>
</table>

DHE: Debaryomyces hansenii cell free extract. SCE: Saccharomyces cerevisiae cell free extract. DHE+SCE: Debaryomyces hansenii + Saccharomyces cerevisiae cell free extract. Control -ve: days of storage. a,b,c,d Different superscript letter within same row means significant difference (P ≤ 0.05). S: spoiled.

The data illustrated in Table (3) revealed that gradual decrease in S. aureus counts in treated yoghurt samples in comparison to the control group. G1 was recorded 5.5 ± 0.02 log10 cfu/g at zero-day, while it recorded 1.7 ± 0.01 log10 cfu/g on the 9th day of storage and then decreased to < 10 at 12th day of storage. The average value in G3 was 5.8 ± 0.04 log10 cfu/g at day zero of storage. On 9th of storage, it recorded 1.3 ± 0.01 log10 cfu/g. On 12th storage day, this value dropped to less than 10. At zero time, G5 recorded 4.8 ± 0.03 log10 cfu/g, and on 6th day of storage, it recorded 1 ± 0.01 log10 cfu/g.
log10 cfu/g. On days 9, 12, and 15 of the storage period, this value dropped to less than ten. Conversely, the positive control group (G7) recorded 5.6 ± 0.04 log10 cfu/g at zero time and decreased to 1.3 ± 0.01 log10 cfu/g at the 12th day. In the control negative group (G8), Staph. aureus counts were 1 ± 0.01 log10 cfu/g on day 12 and 2.4 ± 0.01 log10 cfu/g on day 21 of storage, respectively.

Table 3: Effect of mycocin extract (200 ppm) on inoculated yoghurt samples with S. aureus counts Log10

<table>
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<tr>
<th>Extract</th>
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<th>SCE</th>
<th>DBE+SCE</th>
<th>Control</th>
<th>Control</th>
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<tr>
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<td>G3</td>
<td>G4</td>
<td>G5</td>
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</table>

DHE: Debaryomyces hansenii cell free extract. SCE: Saccharomyces cerevisiae cell free extract. 

Table 4 had shown that 400 ppm of mycocin had more significant results than mycocin concentration of (200 ppm) on S. aureus growth during storage period of yoghurt samples. G2 was recorded 4.3 ± 0.03 log10 cfu/g at zero day of storage, which decreased at 3rd day of storage to record 2.4 ± 0.01 log10 cfu/g and then counts were <10 at 6th, 9th, 12th, 15th, 18th day of storage period. In the same context, G4 recorded 4.4 ± 0.03 log10 cfu/g at zero day and declined to 1.3 ± 0.01 log10 cfu/g at 6th day of storage. Also the counts in G4 decreased to be <10 at 9th, 12th, 15th, 18th day of storage period. On the other hand G6 had the most significant results as it recorded at zero time of storage 4.1 ± 0.03 log10 cfu/g and recorded at 3rd day of storage with 1.3 ± 0.01 log10 cfu/g and S. aureus counts were <10 at 6th, 9th, 12th, 15th, and 18th day of cold storage.

Table 4: Effect of mycocin extract (400 ppm) on inoculated yoghurt samples with S. aureus counts Log10

<table>
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<tr>
<th>Extract</th>
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<th>SCE</th>
<th>DBE+SCE</th>
<th>Control</th>
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<tr>
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DHE: Debaryomyces hansenii cell free extract. SCE: Saccharomyces cerevisiae cell free extract. 

4. DISCUSSION

Probiotics, which are naturally occurring substances made by other microorganisms, may be able to help prevent and manage infections. According to reports, probiotic yeasts have antagonistic properties against other microorganisms, which is one of their important roles (Hatoum et al. 2012). The antimicrobial activity of mycocin produced by Debaryomyces hansenii and Saccharomyces cerevisiae was determined using two reference strains of pathogenic bacteria. The data in Fig. 1 showed no significant difference between the two extracts, as mycocins with a concentration of 400 ppm had shown more moderate inhibition against E. coli and S. aureus than mycocins with a concentration of 200 ppm. These results agreed with Helmy et al. (2019), who illustrated the antibacterial activity of S.C mycocin isolated from sweetened kareish cheese against S. aureus and E. coli using an agar-well diffusion assay, whereas the inhibition zone was 1.2 cm for S. aureus and 0.3 cm for E. coli strains. While the results of the current study were less than the results recorded by Al-Qaysi et al. (2017), who showed that the toxin killing activity of D. hanseni using an agar well diffusion assay at 25 °C was demonstrated by the largest inhibition zones of 36 mm and 35 mm for E. coli and S. aureus, respectively, Younis et al. (2017) recorded that S.C exhibited moderate antimicrobial activity against E. coli (12 mm) and low antimicrobial activity against S. aureus (8 mm). Abd Elatif et al. (2016) found that both S.C and D.H inhibited the growth of both S. aureus and E. coli strains recovered from yoghurt samples by using an agar-well diffusion assay. Srinivas et al. (2017) investigated the antagonistic activity of S. cerevisiae OBS2 against several bacterial pathogens, including S. aureus, using an agar-well diffusion test. The results showed high inhibitory activity against S. aureus. Lima et al. (2017) reported that strains of S.C had powerful antibacterial efficacy against S. aureus. Fakrudin et al. (2017) investigated the antagonistic activity of the supernatant of the yeast S.C (IFST062013) against S. aureus using the agar-well diffusion method. The results indicated that the supernatant possessed a strong antimicrobial effect against S. aureus. The antimicrobial effect of mycocin (200 ppm) against E. coli in yoghurt samples has been illustrated in Table 1. It showed that T5 has more significant results in comparison to other treated yoghurt samples and the control group, as it has recorded a mean value at zero time of 5.5 ± 0.03 log10 cfu/g while it recorded at the 3rd day of storage of 1.6 ± 0.01 log10 cfu/g. This value decreased to <10 on the 6th, 9th, 12th, and 15th days of the storage period. More attention was paid to T6 (400 ppm), which had the most significant results as it had been recorded at zero time of storage with a mean value of 4.9±0.02 log10 cfu/g and recorded <10 at the 3rd, 6th, 9th, 12th, 15th, and 18th days of cold storage, as shown in Table 2. These results came in harmony with Kovanc and Yapoco (2019) observation that E. coli counts dropped from 4.8 log10 cfu/mL to 3.3 log10 cfu/mL at the end of the kefir fermentation process. According to Latif et al. (2023), following the second day of co-cultivation with S.C Acl-12 extract in a modified culture medium (YPDA), the number of viable E. coli cells dropped significantly, from 5 log10 cfu/mL to 3.4 log10 cfu/mL. These results disagreed with Karagözü et al. (2007), who detected that the count of E. coli O157:H7 increased when kefir (including S.C mycocin) was inoculated at 103 cfu/mL, which increased during the fermentation period at 23 ± 1°C, whereas E. coli grew from 3.22 ± 0.04 log10 cfu/mL at zero time to 6.78 ± 0.99 log10 cfu/mL at 24 h. The antibacterial effect of mycocins (200 ppm) on S. aureus, as shown in Table 2, revealed a gradual decrease in S. aureus counts in treated yoghurt samples in comparison to the control group. In which, G5 was recorded as having a mean value at zero time of 4.8 ± 0.03 log10 cfu/g, while it was recorded at the 6th day of
storage of 1.40.01 log10 cfu/g. This value decreased to <10 on the 9th, 12th, and 15th days of the storage period. On the other hand, G6 (400 ppm) had the most significant results as it had been recorded at zero time of storage with a mean value of 4.1±0.01 log10 cfu/g and recorded at 3rd day of storage with a mean value of 1.3±0.01 log10 cfu/g, and S. aureus counts were <10 at the 6th, 9th, 12th, 15th and 18th days of cold storage, as shown in Table 4. These results came in agreement with Kovanc and Yapoco (2019), who recorded that at the end of the fermentation process of kefir, the S. aureus number dropped from 4.78 log10 cfu/mL to 0.30 log10 cfu/mL. Saïd et al. (2019) recorded that the highest concentration (2048 µg/ml) of supernatant extract of D.H using a culture medium resulted in a 69% reduction in the S. aureus strain. Latif et al. (2023) showed that after 32 hours of co-cultivation in a modified culture medium (YPD) of S.C Az-12 extract and S. aureus, there was a decrease in the number of viable S. aureus cells from 5 log10 cfu/ml to 4.1 log10 cfu/ml.

The current results may be attributed to the ability of yeasts to reduce the growth of harmful bacteria. This antibacterial activity is associated with the synthesis of peptides, organic acids, and diacetyl, growth-coupled ion exchange that alters the pH medium, tolerance to high ethanol concentrations, or the production of a volatile thermo-labile toxic extract called mycocin (killer toxin) (Fadahunsi and Olubodun, 2021), which interacts with the components of cell walls to inhibit the synthesis of β-glucan. As an alternative, it could hydrolyze the β-glucan in the target cell wall or prevent tRNA cleavage and DNA synthesis. Ultimately, the development of channels in the cytoplasmic membrane and the inhibition of calcium uptake result in ion leakage (Marquina et al., 2002).

5. CONCLUSIONS

Spoilage microorganisms cause changes in the primary characteristics and properties of yoghurt causing serious health problems. So, the current study concluded that 400 ppm of SCE and DHE separately have a significant inhibitory effect against S. aureus at the 9th day of storage. However, E. coli inhibited the growth of S. aureus on the 9th day of storage, whereas a mixture of both extracts at the same concentration inhibited the growth of E. coli on the 3rd day of storage of yoghurt samples. It is advisable to apply Debaryomyces Hansenii and Saccharomycyes cerevisiae as natural antimicrobials for promoting the shelf life and quality of yoghurt.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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