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

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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 + 10^6 cfu/ml *E. coli* strain) had the most significant results, as it recorded at zero time of storage 4.9 ± 0.02 $\log^{10}$ 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) (10^6 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 + 10^6 cfu/ml *Staph. aureus* strain) had the most significant results as it recorded at zero time of storage 4.1±0.03 $\log_{10}$ cfu/g and recorded at 3rd day of storage with mean value 1.3±0.01 $\log_{10}$ 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) (10^6 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).

Debaryomyces 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 (Capece 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).

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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 (Valihrach *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 1.4×10^{11} cfu/ml and 2.3×10^{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 1.4×10^{11} and 2.3×10^{10} cfu/ml, respectively in YDP 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 counted on Tryptic soya agar (TSA, Himedia) after incubated at 37°C for 24 hours. The organisms were brought to a concentration of 10^6 cfu/ml through three consecutive subcultures of activation (Hassan *et al.* 2011).

2.3.2 Antimicrobial assay

The antimicrobial assay was conducted by Agar well diffusion assay (Arokiyamary *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 $CV = C_1V_1$ where C= protein concentration in DHE and SCE, V is the volume to be calculated, C_1 is the required concentration in the experiment (200 ppm and 400 ppm), V_1 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 inoculated 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) + 10^6 cfu/ml *E. coli.*
T8: 2% yoghurt starter cultures (1:1)

S. aureus groups:
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 16649-2, 2001).

2.4.3.2 Enumeration of S. aureus

Over duplicate Baired Parker agar (OXOID) plates after addition of egg yolk tellurite supplement, 0.1 ml of previously prepared serial dilutions of each sample were 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 mycocins (400 ppm) more than mycocin concentration (200 ppm). The zones of inhibition for S.C mycocin (400 ppm) against *S. aureus* and *E. coli* were 3 and 5 mm, respectively; the corresponding zones for D.H mycocin were 4 and 6 mm.

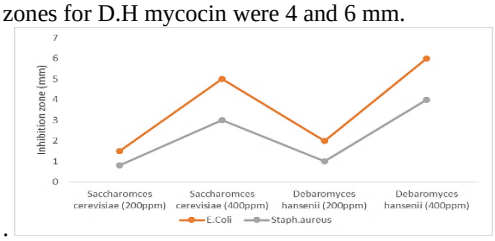

Fig.(1) Antimicrobial activities of mycocins using agar well diffusion assay

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 log_{10} cfu/g while at 6th day of the storage period recorded 1 ± 0.01 log_{10} cfu/g and less than10 on the 9th day of storage. T3 has recorded at zero day of storage 5.8 ± 0.04 log_{10} cfu/g while recorded at 6th day of storage 1.6 ± 0.01 log_{10} cfu/g and this value decreased to < 10 at 9th day of storage. T5 has recorded at zero time 5.5 ±0.03 log_{10} cfu /g while recorded at 3rd day of storage 1.6 ± 0.01 log_{10} 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 log_{10} cfu/g which declined till 12th with a mean value of 1.4 ± 0.01 log_{10} 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 mycocin extract (200 ppm) on inoculated yoghurt samples with *E. coli* counts Log_{10}.

Extract	DHE	SCE	DHE+SCE	Control +ve	Control-ve
D	T1	T3	T5	T7	T8
0	5.6^{a}±0.04	5.8^{a}±0.03	5.5^{a}±0.03	5.8^{a}±0.05	<10
3	2.6^{c}±0.02	2.8^{c}±0.02	1.6^{de}±0.01	3.9^{b}±0.03	<10
6	1^{e}±0.02	1.5^{d} 0.01	<10	2.8^{c}±0.02	<10
9	<10	<10	<10	1.9^{d}±0.01	<10
12	<10	<10	<10	1.4^{e}±0.01	1^{e}±0.01
15	<10	<10	<10	<10	1.6^{d}±0.01
18	<10	<10	<10	<10	1.9^{d}±0.01
21	s	s	s	s	2.4^{c}±0.01

DHE: Debaryomyces hansenii cell free extract. SCE: Saccharomyces cerevisiae cell free extract. D: days of storage. ^{abcde} different superscript letter within same row means significant difference ($P \leq 0.05$). S: spoiled.

Table (2) had been shown that mycocin concentration (400 ppm) had more significant results than mycocin concentration (200 ppm) on *E. coli* counts during the storage period of yoghurt samples. T2 recorded 4.4 ± 0.03 log_{10} cfu /g at zero-day of storage, which decreased at 3rd day of storage to record 2.1 ± 0.01 log_{10} 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 log_{10} cfu /g and decreased to 2.5 ± 0.01 log_{10} cfu /g at 3rd day of storage, also 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 log_{10} cfu /g and recorded <10 at 3rd ,6th, 9th , 12th , 15th , 18th day of storage.

Table (2) Effect of mycocin extract (400 ppm) on inoculated yoghurt samples with *E. coli* counts Log_{10}.

Extract	DHE	SCE	DHE+SCE	Control +ve	Control -ve
D	T2	T4	T6	T7	T8
0	4.4^{d}± 0.02	4.8^{c} ± 0.02	4.9^{b}±0.03	5.8^{a}±0.05	<10
3	2.1^{h} ±0.01	2.5^{g} ± 0.01	<10	3.9^{e}±0.03	<10
6	<10	<10	<10	2.8^{f}±0.02	<10
9	<10	<10	<10	1.9^{b}±0.01	<10
12	<10	<10	<10	1.4^{a}±0.01	1^{e}±0.01
15	<10	<10	<10	<10	1.6 ^{e}± 0.01
18	<10	<10	<10	<10	1.9 ^{h}±0.01
21	s	s	s	s	2.4^{g}±0.01

DHE: Debaryomyces hansenii cell free extract.
SCE: Saccharomyces cerevisiae cell free extract. D: days of storage. ^{abcde} different superscript letter within same row means significant difference ($P \leq 0.05$). 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 log_{10} cfu /g at zero-day, while it recorded 1.7 ± 0.01 log_{10} 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 log_{10} cfu/g at day zero of storage. On 9th of storage, it recorded 1.3 ± 0.01 log_{10} cfu/g. On 12th storage day, this value dropped to less than 10. At zero time, G5 recorded 4.8 ± 0.03 log_{10} cfu/g, and on 6th day of storage, it recorded 1 ± 0.01

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 log_{10} cfu/g at zero time and decreased to 1.3 ± 0.01 log_{10} cfu/g at the 12th day. In the control negative group (G8), *Staph. aureus* counts were 1 ± 0.01 log_{10} cfu /g on day 12 and 2.4 ± 0.01 log_{10} cfu /g on day 21 of storage, respectively.

Table (3) Effect of mycocin extract (200 ppm) on inoculated yoghurt samples with *S. aureus* counts Log_{10}

Extract	DHE	SCE	DHE+SCE	Control +ve	Control-ve
D	G2	G4	G6	G7	G8
0	4.3^{b}±0.03	4.4^{b}±0.02	4.1^{b}±0.01	5.6^{a}±0.04	<10
3	2.4^{de}±0.01	2.4^{de}±0.01	1.3^{d}±0.01	3.3^{c}±0.03	<10
6	2.1^{f} ±0.01	1.3^{g}±0.01	<10	2.6^{d}±0.01	<10
9	<10	<10	<10	2.1^{f}±0.02	<10
12	<10	<10	<10	1.3^{g}±0.01	1^{g}±0.01
15	<10	<10	<10	<10	1.6^{f}±0.01
18	<10	<10	<10	<10	1.9^{f}±0.01
21	s	s	s	s	2.4^{d}±0.01

DHE: Debaryomyces hansenii cell free extract. SCE: Saccharomyces cerevisiae cell free extract. D: days of storage. ^{abcde} different superscript letter within same row means significant difference ($P \leq 0.05$). S: spoiled.

Table (4) had been 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 log_{10} cfu /g at zero day of storage, which decreased at 3rd day of storage to record 2.4 ± 0.01 log_{10} 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 log_{10} cfu /g at zero day and declined to 1.3 ± 0.01 log_{10} 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 log_{10} cfu /g and recorded at 3rd day of storage with 1.3 ± 0.01 log_{10} 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 Log_{10}

Extract	DHE	SCE	DHE+SCE	Control +ve	Control-ve
D	G1	G3	G5	G7	G8
0	5.5^{a}±0.02	5.8^{a}±0.04	4.8^{b}±0.03	5.6^{a}±0.04	<10
3	3.6^{cd}±0.02	3.6^{cd}±0.02	2.7^{e}±0.02	3.3^{c}±0.03	<10
6	2.3^{e}±0.01	2^{e}±0.01	1^{h}±0.01	2.6^{e}±0.01	<10
9	1.7^{g}±0.01	1.3^{h}±0.01	<10	2.1^{e}±0.02	<10
12	<10	<10	<10	1.3^{h}±0.01	1^{h}±0.01
15	<10	<10	<10	<10	1.6^{g}±0.01
18	<10	<10	<10	<10	1.9^{f}±0.01
21	s	s	s	s	2.4^{e}±0.01

DHE: Debaryomyces hansenii cell free extract. SCE: Saccharomyces cerevisiae cell free extract. D: days of storage. ^{abcde} different superscript letter within same row means significant difference ($P \leq 0.05$). S: spoiled.

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 *Debayomyces 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. hansenii* 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*. Fakruddin *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 log_{10} cfu/g while it recorded at the 3rd day of storage of 1.6 ±0.01 log_{10} 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 log_{10} 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 4.82 log_{10} cfu/mL to 3.30 log_{10} 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 Az-12* extract in a modified culture medium (YPDA), the number of viable E. coli cells dropped significantly, from 5 log_{10} cfu/ml to 3.4 log_{10} cfu/ml. These results disagreed with Karagözlü *et al.* (2007), who detected that the count of *E. coli O157:H7* increased when kefir (including *S.C* mycocin) was inoculated at 10^3 cfu/ml, which increased during the fermentation period at 23 ± 1°C, whereas *E. coli* grew from 3.22 ± 0.04 log_{10} cfu/ml at zero time to 6.78 ± 0.99 log_{10} cfu/ml at 24 h.

The antibacterial effect of mycocins (200 ppm) on *S. aureus*, as shown in Table 3, 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 log_{10} cfu/g, while it was recorded at the 6th day of

storage of 1±0.01 log_{10} 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 log_{10} cfu/g and recorded at 3rd day of storage with a mean value of 1.3±0.01 log_{10} 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 log_{10} cfu/mL to 0.30 log_{10} cfu/mL. Saidi *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 (YPDA) of *S.C* Az-12 extract and *S. aureus*, there was a decrease in the number of viable *S. aureus* cells from 5 log_{10} cfu/ml to 4.1 log_{10} 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 6th 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 *Debayomyces hansenii* and *Saccharomyces 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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6. REFERENCES

1. Abdelatif, S.S., Elsayed, S.M., Bahout, A.A. and Bayoumi, A.M., 2016. Studies on beneficial yeasts isolated from some Egyptian dairy products. Zagazig Veterinary Journal 441:75-84.
2. Al-Qaysi, S. A. S., Al-Haideri, H., Thabit, Z. A., Al-Kubaisy, W. H. A. and Ibrahim, J. A., 2017. Production, Characterization, and Antimicrobial Activity of Mycocin Produced by Debaryomyces hansenii DSMZ70238. International Journal of Microbiology. https://doi.org/ 10.1155/2017/2605382
3. AOAC, 2000: Dairy products. In: Official methods of analysis. Association of official and Analytical Chemists, Inc., Gaithersburg, USA.
4. Arokiyamary, A. and Sivakumar, P.K., 2012. Antibacterial spectrum, and mode of action of bacteriocin produced by Lactobacillus sp. isolated from traditional dairy products. International Journal of Pharm Tech Research 4 1:315-320.
5. Badawi, R.M. and El-Sonbaty, A.H., 2004. Viability of Staphylococcus aureus and *Escherichia coli* in zabady made with Bidifidobacteria. Egyptian Journal of Dairy Science 25: 217-233.
6. Berube, B.J. and Bubeck Wardenburg, J., 2013. Staphylococcus aureus alpha-toxin: nearly a century of intrigue. Toxins 5 6: 1140-1166.
7. Buzzini, P. and Martini, A., 2001. Large-scale screening of selected *Candida maltosa, Debaryomyces hansenii* and *Pichia anomala killer* toxin activity against pathogenic yeasts. Sabouradia 39 6: 479–482.
8. Capece, A. and Romano, P. l., 2009. Pecorino di Filiano' cheese as a selective habitat for the yeast species, *Debaryomyces hansenii.* International Journal of Food Microbiology 1322-3:180–184.
9. Corrieu, G., and Be´al, C., 2016. Yogurt: The Product and its Manufacture. The Encyclopedia of Food and Health 5, 617-624.
10. Fadahunsi, I.F. and Olubodun, S., 2021. Antagonistic pattern of yeast species against some selected food-borne pathogens. Bulletin of the National Research Centre 45 1: 1-19.
11. Fakruddin, M., Hossain, M.N. and Ahmed, M.M., 2017. Antimicrobial and antioxidant activities of Saccharomyces cerevisiae IFST062013, a potential probiotic. BMC Complementary and Alternative Medicine. 17: 1-11.
12. Farha, A.K., Yang, Q.Q., Kim, G., Zhang, D., Mavumengwana, V., Habimana, O., Li, H.B., Corke, H. and Gan, R.Y., 2020. Inhibition of multidrug-resistant foodborne *Staphylococcus aureus* biofilms by a natural terpenoid +-nootkatone and related molecular mechanism. Food Control 112: 107-154.
13. Fu, W., Xue, W., Liu, C., Tian, Y., Zhang, K. and Zhu, Z., 2020. Screening of lactic acid bacteria and yeasts from sourdough as starter cultures for reduced allergenicity wheat products Foods 9 6: 751. https://doi.org/10.3390/foods9060751
14. Golubv, W.I., Pfeiffer, I. and Golubev, E., 2002. Mycocin production in *trichosporon pullulans* population colonizing tree exudates in the spring. FEMS Microbiology Ecology, 40 2: 151-157.
15. Gori, K., Sorensen, L. M., Peterson, M. A., Jesperen, L. and Arneborg, N., 2012. Debaryomyces hansenii strains differ in their production of flavor compounds in a cheese-surface model. Microbiology Open 12: 161-168.
16. Hassan, P.A.H., Dobias, J. and Long, M., 2011. Antimicrobial activity of metabolites of various strains of Lactobacillus acidophilus. Proceeding of the International Conference on Advanced Science. Engineering and Information Technology. Malaysia.
17. Hatoum, R., Labrie, S. and Fliss, I., 2012. Antimicrobial and probiotic properties of yeasts: from fundamental to novel applications. Front Microbiology 3: 421-433.
18. Helmy, E.A., Soliman, S.A., Abdel-Ghany, T.M. and Ganash, M., 2019. Evaluation of potentially probiotic attributes of certain dairy yeast isolated from buffalo sweetened Karish cheese. Heliyon 5 5: 01649-01658. [https://doi.org/10.1016/j.heliyon.2019.e01649].
19. ISO, 2001. International Organization for Standardization No.16649-2. Microbiology of food and

animal feeding stuffs — Horizontal method for the enumeration of glucuronidase-positive Escherichia coli - Part 2: Colony-count technique at 44 °C using 5-bromo-4-chloro-3-indolyl-D-glucuronide.

20. ISO, 2018. International Organization for Standardization No. 6888-1. Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of coagulase- positive staphylococci *Staphylococcus aureus* and other species – Part 1: Technique using Baired Parker agar medium.
21. Khablenko A, Danylenko S, Yalovenko O, Duhan O, Potemskaia O. 2022. Potential of using Saccharomyces boulardii to produce fermented milk products. Food Science and Technology. 16,1:12-24. https://doi.org/10.15673/fst.v16i1.2290
22. Karagözlü, N., Karagözlü, C. and Ergönül, B., 2007. Survival characteristics of *E .coli* O157:H7, *S. typhimurium* and *S. aureus* during kefir fermentation. Czech Journal of Food Science 254: 202–207.
23. Kovanc, M. and Yapoco, E., 2019. Survival of *Escherichia coli* O157:H7 and *Staphylococcus aureus* during the fermentation and storage of kefir. Food Science and Technology 39 1: 225-230. [https://doi.org/10.1590/fst.39517].
24. Latif, A. S., Saparbekova, A. A., Akhmedova, Z. R., Kaldybekova, G. and Daugaliyeva, S. T., 2023. Probiotic yeast *Saccharomyces cerevisiae* Az-12 isolated from pomegranate juice presented inhibitory effects against pathogenic bacteria. Brazilian Journal of Biology. 83, 271997-2711016. https://doi.org/10.1590/1519-6984.271997
25. Lima, M.D.S.F., Souza, K.M.S., Albuquerque, W.W.C., Teixeira, J.A.C., Cavalcanti, M.T.H. and Porto, A.L.F., 2017. Saccharomyces cerevisiae from Brazilian kefir-fermented milk: An in vitro evaluation of probiotic properties. Microbial Pathogenesis 110: 670-677. DOI: 10.1016/j.micpath.2017.05.010
26. Maicas, S., 2020. The role of yeasts in fermentation processes. Microorganisms, 8 8: 1142. [https://doi.org/10.3390/microorganisms8081142].
27. Marco, M.L., Sanders, M.E., Gänzle, M., Arrieta, M.C., Cotter,P.D., De Vuyst, L. *et al.*, 2021. The International Scientific Association for Probiotics and Prebiotics ISAPP consensus statement on fermented foods. Nature Reviews Gastroenterology and Hepatology 183: 196–208.
28. Marquina, D., Barroso, J., Santos, A. and Peinado, J. M., 2001. Production and characteristics of *Debaryomyces hansenii* killer toxin. Microbiological Research 156 4: 387–391.
29. Marquina, D., Santos, A. and Peinado, J. M., 2002. Biology of killer yeasts. *International. Microbiology* 5 2:65–71. https://doi.org/ 10.1007/s10123-002-0066-z.
30. Metz, M., Sheehan, J. and Feng, P.C., 2020. Use of indicator bacteria for monitoring sanitary quality of raw milk cheeses –A literature review. Food Microbiology 85: 103283-103295.
31. Owusu-Kwarteng, J., Akabanda, F., Agyei, D., Jespersen, L., 2020. Microbial Safety of Milk Production and Fermented Dairy Products in Africa. Microorganisms 85:752. [https://doi.org/ 10.3390/microorganisms8050752].
32. Saidi, N., Owlia, P., Marashi, S.M.A. and Saderi, H. 2019. Inhibitory effect of probiotic yeast *Saccharomyces cerevisiae* on biofilm formation and expression of α-hemolysin and enterotoxin A genes of Staphylococcus aureus. Iranian Journal of Microbiology 11 3:246-254.
33. SPSS, 2018. IBM Corp. Released 2018. IBM SPSS Statistics for Windows, Version 26.0. Armonk, NY, IBM Corp.
34. Srinivas, B., Rani, G.S., Kumar, B.K., Chandrasekhar, B., Krishna, K.V., Devi, T.A., *et al*., 2017. Evaluating the probiotic and therapeutic potentials of *Saccharomyces cerevisiae* strain OBS2 isolated from fermented nectar of toddy palm. AMB Express, 71: 1-14. doi: 10.1186/s13568-016-0301-1.
35. Valihrach, L., Alibayov, B. and Demnerova, K., 2013. Production of Staphylococcal enterotoxin C in milk. International dairy Journal 302: 103-107.
36. Virpari, P. k., nayak, J. B., Brahmbhatt, M.N. and Thaker, H.C., 2013. Study on isolatin, molecular detection of virulence gene and antibiotic sensetivity pattern of *E.coli* isolated from milk and milk products. Veterinary world 68: 541-545.
37. Younis, G., Awad, A., Dawod, R.E. and Yousef, N.E., 2017. Antimicrobial activity of yeasts against some pathogenic bacteria. Veterinary World 108: 979-983. http://doi.org/10.14202/vetworld.2017.979-983.