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Impact of *Debaryomyces hansenii* and *Saccharomyces cerevisiae* cell free extracts on yoghurt quality

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ABSTRACT

Beneficial yeast extracts mainly of *Debaryomyces* and *Saccharomyces* species, have been considered as one of the natural biological fermenters of food products. These yeast metabolites, specially their mycocins can be used to prolong storage time of yoghurt. This study was undertaken to assess the effect of mycocin (*Debaryomyces hansenii* (DH) and/or *Saccharomyces cerevisiae* (SC) cell free extracts) (CFEs) on yoghurt quality. Yoghurt samples examined for coagulation time, titratable acidity, sensory and microbiological quality at appropriate intervals until the appearance of spoilage (texture changes). The pre-heated skimmed milk was mixed with starter culture and then it was divided into 10 groups, each inoculated by different inoculum and a certain concentration of (DH) and/or (SC) CFEs (100, 200 and 400 ppm) and incubated at 42°C till curd formation, then refrigerated at 4±1°C and examined every 3 day till appearance signs of spoilage. The obtained results revealed a significant elongated coagulation time with the mycocin concentration (400 ppm). During the storage period, titratable acidity steadily rose in all groups. In addition, a significant improvement in the sensory quality parameters represented by flavor, appearance and texture characters appeared with the treated groups with mycocin concentration (100 ppm) compared with the other groups. Regarding to the total fungal count, treated groups with mycocin (400 ppm) showed the most significant inhibitory effect on fungal growth. In conclusion, DH and SC mycocins at concentration of 100 ppm could significantly extend shelf-life of treated yoghurt samples with the highest flavor and texture scores in comparing to the control and the other treated groups.

Introduction

The use of probiotics and recognition of their role in preventing disease and enhancing human health by fortifying the immune system, enhancing feed digestibility, and lowering metabolic disorders has emerged as one of the most promising nutritional research areas in recent years (Zielińska and Kołożyn-Krajewska, 2018).

Dairy products that have undergone fermentation are frequently utilized as the main source of probiotics for customers across the world. The fermentation of milk has also yielded a variety of probiotic microorganisms (James and Wang, 2019). One of the most significant dairy products with beneficial bacteria and one that is often consumed is yoghurt. It has been hypothesized that probiotic organisms can develop more quickly and survive longer in storage if a probiotic yeast is grown with the yoghurt microbiota and added to commercial yoghurt (Staniszewski and Kordowska-Wiater, 2021).

Beneficial yeasts are considered one of the most potent biocontrol agents due to their biology and nontoxic properties (Zohri and Marwa, 2018).

Numerous yeast species have been found to create exoproteins (mycocins), which may be able to suppress a variety of harmful bacteria. In 1963, *Saccharomyces cerevisiae* was the first organism in which the synthesis of killer toxin was identified. Since then, more than 100 yeast species from more than 20 genera have been shown to be active producers of killer toxin. Additionally, it has been shown that *Debaryomyces hansenii* produces potent and deadly poisonous proteins or glycoproteins (Al-Qaysi *et al*., 2017).

The suppression of beta-glucan production or beta-glucan hydrolysis in the cell walls of sensitive bacteria is one of the possible antimicrobial modes of action of mycocins (Muccilli and Restuccia, 2015). By fermenting lactose to create fragrance components and by exhibiting significant proteolytic and lipolytic activities, yeasts contribute to the improvement of sensory quality and taste development in dairy products (Ferreira and Viljoen, 2003).

Debaryomyces hansenii and *Saccharomyces cerevisiae*, which are found in dairy products, are two of the yeasts that are most commonly investigated (Atanassova *et al*., 2016; Bertuzzi *et al*., 2017). So, determining how *Saccharomyces cerevisiae* and *Debaryomyces hansenii* mycocin extract affect yoghurt quality and shelf life was the goal of the current investigation.

Materials and methods

Activation of probiotic strains

Both *Saccharomyces cerevisiae* and *Debaryomyces hansenii* were acquired from Cairo MIRCEN, Faculty of Agriculture, Ain Shams University, Cairo, Egypt. The strains were activated in YPD (5g yeast extract, 5g peptone, 10g dextrose, and 1L distilled water) for 48 hours at 25°C, and then three subcultures were carried out to activate the strain until it reached concentrations of 1.4×10^{11} and 2.3×10^{10} CFU/ml, respectively (Gori *et al*., 2012).

Preparation of cell-free extract

Yeast cells were separated by centrifugation at 3000xg for 30 min at 4°C after activated DH and SC with concentrations of 1.4×10^{11} and 2.3×10^{10} CFU/ml in YDP broth were incubated for 3 days at 25°C, respectively. A 0.45 m pore size filter was used to separate the supernatant to

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produce DH cell-free extract (DHE) and SC cell-free extract (SCE) (Golubev *et al*., 2002).

Yoghurt manufacture

10 L of fresh, raw, mixed skimmed (fat percent 0.5%) cow and buffalo milk were combined to make yoghurt in accordance with Corrieu and Be'al (2016) instructions. The milk was then heated to 85°C for 30 minutes and immediately cooled to 45°C. The milk was mixed with 2% starter culture (*Lactobacillius bulgaricus* and *Streptococcus thermophilus*), then subdivided into 10 groups (2L of each group). The SCE and/or DHE mycocins were added by the concentrations of 100, 200 and 400 ppm as follow:
G1: 2% yoghurt starter cultures (1:1) only.
G2: 2% yoghurt starter cultures (1:1) + 100 ppm DHE.
G3: 2% yoghurt starter cultures (1:1) + 100 ppm SCE.
G4: 2% yoghurt starter cultures (1:1) + 100ppm DHE +100 ppm SCE.
G5: 2% yoghurt starter cultures (1:1) +200 ppm DHE.
G6: 2% yoghurt starter cultures (1:1) +200 ppm SCE.
G7: 2% yoghurt starter cultures (1:1) + 200 ppm DHE +200 ppm SCE.
G8: 2% yoghurt starter cultures (1:1) +400 ppm DHE.
G9: 2% yoghurt starter cultures (1:1) +400 ppm SCE.
G10: 2% yoghurt starter cultures (1:1) + 400 ppm DHE +400 ppm SCE.

Each group's yoghurt samples were combined, placed in 100 ml cups, and then incubated at 42°C until curd formation before being moved to a refrigerator at 4°C.

Yoghurt examination

The manufactured yoghurt samples were tested chemically, sensorial, and microbiologically at the zero time and every three days until spoiling signs started to develop. Three times were spent repeating the yoghurt preparation and tests.

Determination of coagulation time

The time of coagulation of yoghurt groups was calculated from entering the samples to incubator until curd formation (Li *et al*., 2022).

Calculating the titratable acidity

Hyo *et al*. (2013) stated that the direct titration technique was used to carry out the test.

Titratable acidity (T.A) could be calculated by applying the following formula:
T.A%= (R × Normality of NaOH (0.1)×90)/(Weight of sample(10 g)×1000)
R: The amount in ml of (NaOH 0.1 N) was recorded.

Sensory evaluation

Sensory evaluation was carried out according to Mehanna *et al*. (2000). The score given was 60 points for flavor, 30 points for body and texture and 10 points for appearance with an overall score of 100 points.

Microbiological examination

Preparation of serial dilutions

Using a sterilized rod, yoghurt samples were well stirred. A decimal dilution was created from a serial dilution, which was created by adding 1 ml of each well mixed yoghurt sample to 9 ml of sterile distilled water to create 0.1 dilution ISO 6887-1 (2017).

Enumeration of the viable *E. coli* was performed by culturing on tryptone-bile-glucuronic media (TBX) and incubated for 24 hours at 44°C. Typical colonies, according to ISO 16649-2 (2001), were greenish blue.

Enumeration of Staphylococcus aureus was performed by culturing on baired Parker agar plates and incubated for 24-48 hours at 37°C. According to ISO 6888-1 (2021), typical colonies were gray to jet black, with a light colored (off-white) edge, and an opaque zone with an outside halo.

Enumeration of total Psychrotrophs count

According to ISO 17410 (2019), Psychrotrophs were counted using plate count agar plates and incubated at 4–8 °C for 5 days.

Enumeration of total Coliform count

Coliforms were isolated using violet red agar plates, which were cultured for 24 hours at 37°C. According to ISO 4832 (2006), typical colonies were spherical, convex, and pink.

Determination of total yeast and mould counts

According to ISO 21527-1 (2008), these had been carried out using Sabouraud dextrose agar medium that had been treated with chloramphenicol.

Analytical statistics

Data were statistically analyzed using SPSS 16.0's analysis of variance (ANOVA). Utilizing one-way analysis of variance, statistical comparisons were done. According to P≤ 0.05, the outcomes were deemed substantially different (SPSS, 2018).

Results

Coagulation time of treated yoghurt samples

According to Table 1, the treated samples coagulation times significantly increased when compared to the control group. G10 had the longest coagulation time at 4:13±0.08 h: min, While, the G1 (control group) group had the least time, which was 3:23±0.01 h: min. The coagulation time of the tested yoghurt samples was considerably lengthened by the addition of *Debaryomyces* and *Saccharomyces* mycocins (P≤ 0.05). The increasing percentages of the coagulation time in G2, G3 and G4 compared with the control group were 0.928%, 4.33% and 8.67%, respectively; while G5 and G7 compared with the control group were 3.09%, 7.12% and 9.29%, respectively. Also, G8 and G9 were 7.12% and 9.29%, respectively.

Table 1. Coagulation time in the treated yoghurt samples with different mycocins concentrations in refrigerating storage.

Yoghurt groups	Coagulation time	
	Time (Hours)	Progression (%)
G1	3.23±0.01^{f}	0
G2	3.26±0.01^{de}	0.93
G3	3.37±0.01^{c}	4.33
G4	3:51±0.01^{b}	8.67
G5	3:33±0.01^{cd}	3.09
G6	3:46±0.01^{b}	7.12
G7	3:53±0.01^{b}	9.29
G8	3.36±0.01^{b}	4.02
G9	3:49±0.02^{b}	8.05
G10	4:13±0.08^{a}	27.9

Data are expressed as Mean±S.E (Standard Error). Mean values in the same column followed by different superscripts (a, b, c, d, e, f) letters are significantly different (P≤ 0.05).

Yoghurt acidity profile during storage

The findings in Fig. 1, indicated the average titratable acidity in several yoghurt groups kept at a cold temperature. As the storage duration extended, the titratable acidity of yoghurt samples steadily rose in all the treated groups by varying percentages. Between the groups, there was no discernible variation in acidity. For instance, the titratable acidity of G1 yoghurt (the control group) was 0.75±0.01 on day zero and steadily climbed to 0.84±0.01 on day 15 of storage. By day zero, G2 was 0.88±0.02 and gradually climbed to 1.06±0.02 by day 33 of storage, whereas G3 was 0.91±0.01 and gradually increased to 1.08±0.02 at day 33 of storage. While G9, which recorded 0.95±0.03, had the highest percentage and increased gradually to 1.14±0.04 at the 33rd day of storage and G10 was 0.94±0.03 at day zero, and increased gradually to 1.5±0.03 at the 33rd day of storage.

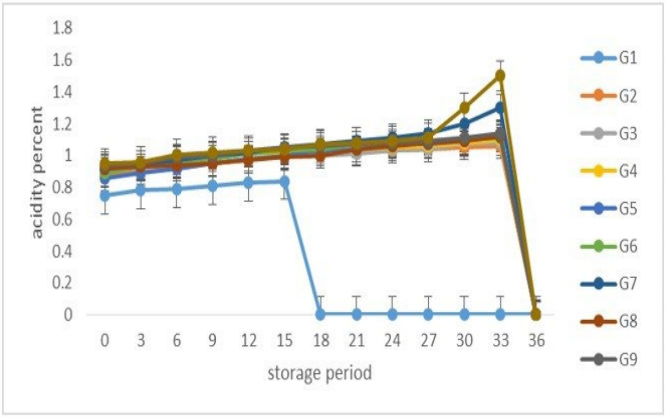

Fig. 1. Titratable acidity in treated yoghurt samples with different mycocins concentrations in cold storage (4.0±I.0° C).

Sensory characters of yoghurt during storage period

The mean flavor values for different groups of yoghurt had been shown in Fig. 2A. There was significant difference among the treated groups in relation to control group. The results of the flavor analysis indicated that the yoghurt samples with low concentration of DHE and SCE had better results than those with high concentration. For example, the flavor score in G2 and G3 was 57.9±0.2 and 57.8±0.4 at day zero and decreased gradually which recorded at the 33rd day of storage were 39.7±0.3and 40.6±0.4, but scores were still higher than other treated groups. On the other hand, G10 recorded the lowest flavor score with mean value 55.3±0.8 at zero day and decreased gradually to reach 25.6±0.6 at the 33rd day of storage time; while control group (G1) recorded 27.5 at 15th days of storage.

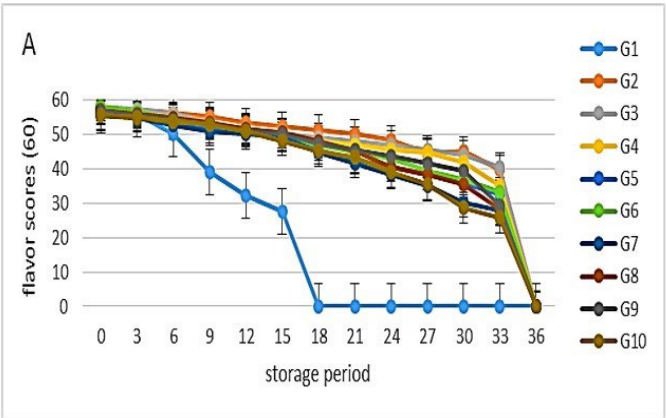

Fig. 2A. Mean values of flavor in the examined yoghurt samples

The treated samples showed a substantial improvement in body and texture ratings as compared to the control group, as shown in Fig. 2B, which showed that G2 and G3 had the highest scores at zero day with mean values of 27.7±0.3 and 27.8±0.2, respectively. These samples kept up their high scores, with G2 maintaining a mean value of 17.8±0.1 through day 33 of storage, and G3 maintaining a mean value of 18±0.03 through day 33 of refrigeration storage. At zero day, G8 and G10 had the lowest body and texture scores, with mean scores of 26.2±0.03 and 25.7±0.01, respectively. In G8 and G10, respectively, their levels dropped during storage to 14±0.6 and 13.1±0.5 at day 33.

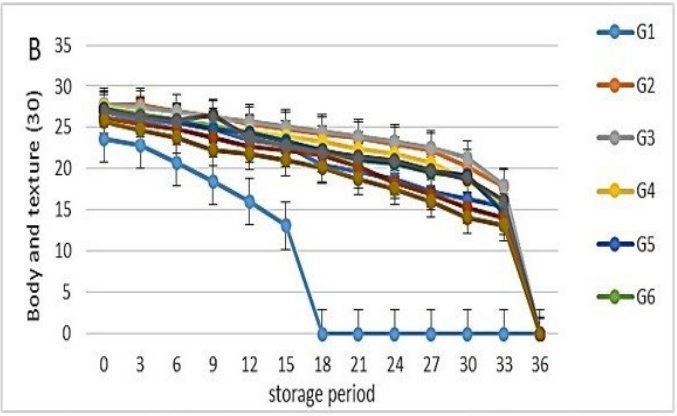

Fig. 2B. Mean values of texture in the examined yoghurt samples

Table 2. Overall sensory scores (100) for the examined yoghurt groups during their refrigeration storage (4°C).

Days	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10
0	88.5±1.6^{d}	93.6±0.1^{ab}	94.5±0.7^{a}	93.1±1^{ab}	92.3±0.8^{ab}	93.4±1.04^{ab}	91.4±0.7^{bc}	91.1±0.5^{abc}	92.4±1^{ab}	89.3±0.9^{cd}
3	87.4±0.6	93±0.5^{a}	93.2±0.8^{a}	91.1±0.7^{b}	89.1±1.2^{ab}	91.7±2^{a}	89.5±1.1^{ab}	98.6±1.1^{ab}	90.2±0.5^{ab}	87.3±1.1^{ab}
6	83±1.9^{c}	91.3±0.3^{a}	91.5±0.9^{a}	88.3±1.6^{ab}	87±1.8^{bc}	88.2±1.5^{ab}	86.2±0.9^{bc}	87.4±0.8^{ab}	88.2±0.8^{ab}	84.6±1.3^{bc}
9	69.2±1.5^{c}	89.2±0.3^{a}	87.5±2.5^{ab}	85.5±0.9^{abc}	83.9±1.4^{bc}	85.4±1.9^{abc}	83.1±1.3^{bcd}	84.5±1.9^{abc}	86.7±2.3^{abc}	82.3±0.9^{cd}
12	54.6±1.8^{d}	86.1±1.2^{a}	85.3±2.7^{ab}	82.3±1.7^{ab}	81.5±1.3^{ab}	82.8±2^{ab}	81.4±1.6^{ab}	81.1±3^{ab}	82.1±1.4^{ab}	79.4±0.7^{bc}
15	42.8±1.7^{d}	84.3±0.8^{a}	82.6±1.9^{ab}	79.9±1.4^{ab}	79.8±1.7^{ab}	79.5±1.8^{ab}	79.2±2.7^{ab}	79.2±3.1^{ab}	80±1.1^{ab}	75.6±1.4^{b}
18	S	82.6±0.7^{a}	80.6±1.5^{ab}	77.2±1.5^{abc}	72.2±2.8^{c}	75±0.2^{bc}	73.4±1^{bc}	75.9±3.6^{abc}	74±2.9^{abc}	71.2±2.3^{bc}
21	S	80.3±0.9^{a}	78.4±1.7^{ab}	75.9±0.7^{abc}	68.3±2.4^{d}	71.8±0.5^{bcd}	68.6±1.5^{d}	71.1±3.4^{bcd}	71.5±3.1^{bcd}	67.8±2.3^{cd}
24	S	77.7±0.4^{a}	76.8±1.6^{a}	72.5±2^{ab}	64.7±2.6^{c}	69±0.3^{bc}	64.3±1^{c}	64.3±3.2^{bc}	67±2.8^{abc}	62±2.7^{bc}
27	S	72.6±2.3^{ab}	74.1±1.6^{a}	70.6±2.5^{abc}	60.6±2.3^{d}	64±0.6^{cd}	59.6±1.7^{d}	60.4±3.4^{bcd}	62.9±2.9^{abcd}	56.3±2.9^{abcd}
30	S	70.9±0.6^{a}	70.7±2^{a}	65±2.4^{ab}	56.9±2.1^{ba}	59.9±0.9^{bc}	54±2.6^{c}	55.6±3.2^{bc}	58.3±2.6^{ab}	48.2±2.7^{ab}
33	S	62.6±2.5^{ab}	63.6±1.3^{a}	55.4±3.3^{abc}	52.3±1.9^{d}	53.3±2.3^{bcd}	46.4±2.6^{d}	45.9±2.2^{cd}	49.1±2^{abc}	41.8±2.9^{cd}
36	S	s	s	s	s	s	s	s	s	s

Data are expressed as Mean±S.E (Standard Error). Mean values in the same row followed by different superscripts letters (a, b, c, d) are significantly different (P≤ 0.05). S: Spoiled samples; ND: Not detected.

In addition, appearance scores of the treated yoghurt samples showed significant improved characters in comparing with control group, Fig. 2C illustrated that G2, and G3 exhibited the highest appearance scores at zero day with mean values of 8.7±0.03 and 8.8±0.02, respectively. These samples kept their high scores with a mean value of 5.1±0.01 and 5±0.01 until 33 day of refrigerated storage in G2 and G3, respectively. The lowest scores for appearance were obtained for G9 and G10 at the 33rd day with mean values of 3.4±0.02 and 3.1±0.01 respectively; while control group (G1) recorded 7.8±0.04 at zero day of storage and decreased gradually till reach 15th day with mean value 4.4±0.02.

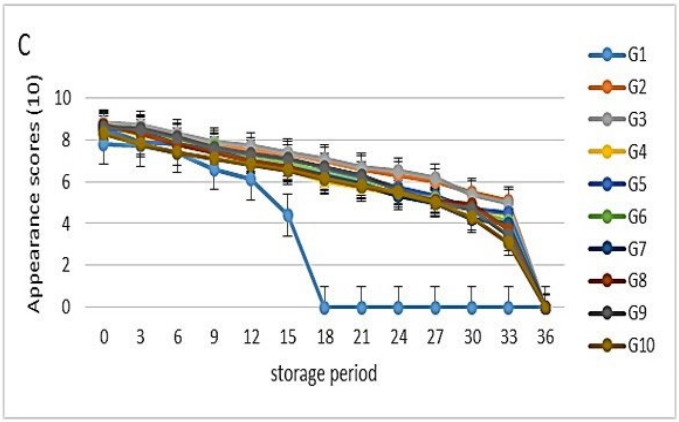

Fig. 2C. Mean values of appearance in the examined yoghurt samples.

According to the findings in Table 2, the total sensory ratings of the yoghurt samples under examination steadily declined during the course of storage time, starting out with high scores at the beginning. G2 and G3 showed the greatest overall sensory scores at zero day with mean values of 93.6±0.7 and 94.5±0.1, respectively. G1 (control group) recorded at zero 88.5±1.6 which reduced to 42.8±1.7 at 15th day of storage. These samples kept their high scores, with G2 having a mean value of 62.6±2.5 and G3 having a mean value of 63.6±1.3 through day 33rd of storage. G8 and G10 received the lowest total sensory ratings at zero day, with mean values of 91.1±0.5 and 89.3±0.9 respectively, and these values decreased during storage to 45.9±2.2 at day 33 in G8 and 41.8±2.9 at day 33 in G10. The other treated yoghurt groups had been recorded nearly similar results.

Table 3 shows how the yeast and mold counts of the yoghurt samples changed during the course of storage. From the first day of refrigerated storage until the ninth day, no samples included yeast or mould, but their presence grew as the samples were kept. Counts were discovered in G1 (control yoghurt samples) on days 12 and 15, with mean levels of 3±0.01 and 3.5±0.1 $\log_{10}$ CFU/g, respectively. At the same time, they began to emerge in the treated samples (G2, G3, G4, G5, G6, and G7) at the 15th day with mean values of 3.2±0.03, 3.4±0.2, 3.1±0.05, 3±0.06, 3.1±0.05, and 2.9±0.08 $\log_{10}$ CFU/g, respectively. From zero day until the fifteenth day of storage, yeast and mould could not be found in G8 or G9, but by the eighteenth day of storage, these yogurt samples had been recorded with 3.1±0.09 and 3.2±0.1 $\log_{10}$ CFU/g, respectively. The most significant impact on the fungal count, however, was caused by G10, which was not discovered until the 18th day of storage and was recoded at the 21st day with a mean value of 3±0.1 $\log_{10}$ CFU/g. This value rose during the course of storage as it was recorded at 3.7±0.05 $\log_{10}$ CFU/g at the 33rd day of storage.

However, the bacterial profile showed that no *E. coli*, *S. aureus*, Coliforms, and *Psychrotrophs* were found (Data not shown).

Discussion

Fungal spoilage may have a number of effects on milk and milk products, including yoghurt. One of the main factors contributing to food degradation globally is microbial infection. After milk products are spoiled by fungus, several physical problems, such as off color, loss of firmness, and loss of scent, may appear. According to Garnier *et al*. (2017), yeast and mould are known to be a significant factor in the deterioration of a variety of dairy products.

The use of biopreservation in dairy products is of significance. Shelf life is extended and/or safety of food products is enhanced by using natural or bio-controlled by different ways of biological fermentation with beneficial yeasts like (*Debaryomyces* and *Saccharomyces*) and/or their metabolites, especially their mycocins. They also play a significant role in the taste development of fermented foods and are widely recognized for their antagonistic activity versus pathogenic bacteria and fungus. The competition for resources and the release of antimicrobial substances like "mycocins" or toxins that kill off fungi are linked to these activities (Hatoum *et al*., 2012).

Milk coagulates as a result of milk casein precipitating in acidic conditions at a pH of about 4.6 (Lucey, 2016).

Referring to the obtained results in Table 1, significant increase in the coagulation time was observed in the higher concentration of SC and DH extracts when compared with the control group. Obviously, G10 had the longest coagulation time (4h and 13 min) with increase% 27.9% while, control group (G1) recorded (3h and 23min). According to Heller (2001), variations in the lactic acid bacteria's (LAB) capacity to develop in fermenting milk may be the cause of variations in the coagulation time, which may be affected by the addition of SCE and DHE. The delayed pace of acid production caused by the mycocins inhibitory impact on the yoghurt starter cultures' capacity to create acid may be the cause of this rise.

Yeast and LAB would compete with one another in the same environment in addition to coexisting microbes (Chen *et al*., 2015). According to Yue *et al*. (2022), *Saccharomyces cerevisiae* YE4 cell free supernatant hindered LAB's development by inhibiting its physiology, metabolism, and synthesis of acid. This effect was caused by yeast metabolites.

The obtained results agreed with those obtained by Eissa *et al*. (2015) who recorded increasing in coagulation time in yoghurt samples inoculated by DHE with mean value 4:00±0.04 h: m in comparing with control 3.37±0.04 h: m. *Saccharomyces cerevisiae* YE4 CFS reduced the growth, physiology, and metabolism of LAB strains, including the generation of acid and the development of biofilms, as demonstrated by Xu *et al*. (2019).

Table 3. Yeast and mold counts (log10 cfu/g) in the control and treated yoghurt samples with different mycocins concentrations in refrigerated storage.

	0	3	6	9	12	15	18	21	24	27	30	33	36
G1	$<10^2$	$<10^2$	$<10^2$	$<10^2$	3±0.01^{c}	3.5±0.1^{bc}	s	s	s	s	s	s	s
G2	$<10^2$	$<10^2$	$<10^2$	$<10^2$	$<10^2$	3.2±0.03^{b}	3.3±0.1^{b}	3.4±0.09^{b}	3.7±0.05^{a}	3.8±0.02^{a}	3.9±0.04^{a}	4.1±0.03^{a}	s
G3	$<10^2$	$<10^2$	$<10^2$	$<10^2$	$<10^2$	3.4±0.2^{b}	3.5±0.1^{b}	3.6±0.07^{b}	3.8±0.02^{a}	3.9±0.03^{a}	4±0.04^{a}	4.3±0.04^{a}	s
G4	$<10^2$	$<10^2$	$<10^2$	$<10^2$	$<10^2$	3.1±0.05^{a}	3.3±0.09^{ab}	3.5±0.1^{ab}	3.7±0.09^{a}	3.8±0.02^{a}	3.9±0.02^{a}	4±0.01^{a}	s
G5	$<10^2$	$<10^2$	$<10^2$	$<10^2$	$<10^2$	3±0.06^{a}	3.6±0.07^{ab}	3.6±0.08^{ab}	3.7±0.1^{a}	3.7±0.05^{a}	3.8±0.05^{a}	3.9±0.03^{a}	s
G6	$<10^2$	$<10^2$	$<10^2$	$<10^2$	$<10^2$	3.1±0.05^{a}	3.5±0.1^{ab}	3.6±0.07^{ab}	3.8±0.07^{a}	3.8±0.09^{a}	3.9±0.04^{a}	4.1±0.02^{a}	s
G7	$<10^2$	$<10^2$	$<10^2$	$<10^2$	$<10^2$	2.9±0.08^{a}	3.2±0.08^{a}	3.3±0.1^{a}	3.5±0.1^{a}	3.7±0.03^{a}	3.7±0.09^{a}	3.8±0.02^{a}	s
G8	$<10^2$	$<10^2$	$<10^2$	$<10^2$	$<10^2$	$<10^2$	3.1±0.09^{a}	3.2±0.07^{a}	3.3±0.08^{a}	3.5±0.2^{a}	3.7±0.06^{a}	3.9±0.01^{a}	s
G9	$<10^2$	$<10^2$	$<10^2$	$<10^2$	$<10^2$	$<10^2$	3.2±0.1^{a}	3.4±0.09^{ab}	3.6±0.2^{a}	3.7±0.07^{a}	3.8±0.06^{a}	4±0.08^{a}	s
G10	$<10^2$	$<10^2$	$<10^2$	$<10^2$	$<10^2$	$<10^2$	$<10^2$	3±0.1^{a}	3.1±0.09^{a}	3.2±0.08^{a}	3.5±0.05^{a}	3.7±.0.05^{a}	s

Data are expressed as Mean±S.E (Standard Error). Mean values in the same row followed by different superscripts letters (a, b, c, d) are significantly different (P≤ 0.05). D: storage days; S: Spoiled samples; ND: Not detected.

Titratable acidity (TA), which measures the overall acidity of milk and is frequently used to gauge how milk is fresh, TA is a more accurate indication of subtle variations in milk acidity (Burke *et al*., 2018). Since it impacts the shelf life and acceptability of dairy products, the variation in titratable acidity (TA) is the key element (Mehdizadeh *et al*., 2019).

The titratable acidity steadily rose in all of the groups over the storage duration, according to what was seen in Fig. 1, with no significant differences among treatment groups. These outcomes could be explained by the metabolites made by yeast that encourage the growth of starter culture bacteria, resulting in high microbial metabolic activity with lactose consumption and the production of lactic acid and other organic acids during the storage of yoghurt samples (Vahedi *et al*., 2008; Fadahunsi and Olubodun, 2021).

The current findings were in agreement with Niamah (2017), who noted that yoghurt manufactured with starter culture including *Saccharomyces* boulardii had a slower pH decline than the control sample of yoghurt, with PH values of 3.77, 3.65, and 3.55 for 1% yeast, 2% yeast, and 3% yeast at the 21st day of storage, respectively, and Eissa *et al*. (2015) who demonstrated that the average values of titratable acidity with DHE varied from 0.81±0.01 at zero day up to 1.50±0.04 at day 34 of storage. The pH of *L. hordei* and *S. cerevisiae* co-cultures rose to about 3.49, according to Kang *et al*. (2021), as opposed to 3.28 for *L. hordei* monoculture.

Consumer approval is ultimately influenced by sensory qualities (taste, texture, appearance) (Karagul-Yuceer and Drake, 2013). Yoghurt sensory qualities cannot be changed by probiotics (Antunes *et al*., 2005).

In the current study, the addition of *Debaryomyces* and *Saccharomyces* extracts alone and with low concentration (100 ppm) had better results than other higher concentration on the flavor of the treated yoghurt samples as G2 and G3 with the records of 39.7±0.3and 40.6±0.4 at 33rd day of storage, respectively (Fig 2 A) . The enhanced proteolytic and lipolytic activities of SC and DH filtrate, which may have a significant impact on the flavor, may be responsible for the enhanced flavors of the treated yoghurt samples (Aquilanti *et al*., 2007; Andrade *et al*., 2010). These findings were very comparable to those of Eissa *et al*. (2015) who observed a mean value of taste scores from the start of yoghurt sample production with a mean value of 53.85±0.09 to the end of storage at 40.66±0.33.

The quality and popularity of yoghurt are strongly influenced by its body and texture. According to Riener *et al*. (2010), the yoghurt curd's characteristics should be smooth when being consumed and have a low propensity to separate into serum and water when being stored. According to Hassan *et al*. (2003), a variety of factors, including the starter culture, incubation temperature, processing conditions (heat treatment and homogenization), and the compositional characteristics of the milk base, influence the body and texture of yoghurt.

In the current study, the treated groups with low concentrations of mycocins had better body and texture scores than those with higher concentration and also the control group during storage period. For example, G2 and G3 had higher scores with mean values of 17.8±0.1 and 18±0.03 at 33rd storage day, respectively; while lowest score was obtained for G10 with mean value 13.1±0.5 at the 33rd day of storage, moreover, the control group spoiled at the 18th day of storage time as shown in Fig 2B. These results were almost identical to those of Eissa *et al*. (2015) who found that the mean body and texture scores at the end of storage for yoghurt samples inoculated with DHE were 23.29±0.19 at day 34th.

Regarding to yoghurt appearance, Fig 2C shows that G2 and G3 were obtained the highest scores with mean values of 5.1±0.01 and 5±0.01 respectively, while the lowest score was obtained for G10 with mean value 3.1±0.01 at storage time the 33rd day. The obtained results were nearly similar to Eissa *et al*. (2015) who recorded that mean value of appearance scores at zero time of storage 7.77±0.06, while it recorded 6.1±0.04 at 34th day of yoghurt samples inoculated with DHE.

In the same context, G2 and G3 received the highest overall sensory scores, with mean values of 62.6 and 63.6 at the 33rd days of storage, respectively, while G8, G9, and G10 received the lowest overall sensory scores, with mean values of 45.9, 49.5, and 41.8 at the 33rd days of storage, respectively (Table 2). These findings corroborated those of Eissa *et al*. (2015), who found that the mean sensory overall scores of the DHE-inoculated yoghurt samples under examination decreased from 88.8 at the beginning of storage to 70.18 at the end of storage.

Due to the unfavorable alterations that cause off-flavors, off color, and inferior product quality, the presence of yeast and moulds in milk and dairy products is undesirable even in low concentrations (Abdel hameed, 2011).

It has been noted that antifungal action of yeast metabolites appears to be higher in lower pH ranges with reference to yeast and mold counts (Rouse *et al*., 2008; Borrelli and Trono, 2017). This may show the antifungal effects of DHE and SCE in yoghurt conditions that are acidic. Yoghurt shouldn't include more than 10 CFU/g of mold and 400 CFU/g of yeast, according to the Egyptian Organization for Standardization and Quality Control EOSQ (2005). G8 and G9 findings were within acceptable ranges until the 18th day of storage, whereas G10 values were within acceptable ranges through the 21 day of storage (Table, 2).

It is regarded as an addition to DHE and SCE, which have inhibitory activities resulting from DNA damage or inhibition of DNA replication, attacking the cell membranes of target organisms, the formation of pores, changes in pH of the medium as a result of the production of organic and volatile acids, high concentrations of ethanol, and hydrogen peroxide (Suzuki *et al*., 2001; Liu *et al*., 2015). These acids pass through the target species' membranes in their hydrophobic, dissociated state, which lowers the cytoplasmic pH and results in cell death, loss of potassium ions, and energy loss in the form of ATP (Dalié *et al*., 2010).

In contrast to Eissa *et al*. (2015), who found lower yeast and mold counts in yogurt samples inoculated with DHE, with mean values of 1.00±0.00 $\log_{10}$ CFU/g at the seventh day of storage and 2.33±0.52 $\log_{10}$ CFU/g at the 34th day of storage. These results came in agreement with the recorded results of detected yeast and mold after 9 days of refrigeration storage of yoghurt samples and increased gradually until end of shelf life (14 days). According to Abdel-Kareem *et al*. (2018), an 83% decrease in *Aspergillus flavus* was seen in the crude supernatant of *Saccharomyces cerevisiae* EBF101. These findings demonstrated *Saccharomyces cerevisiae*'s capacity to create high temperatures-stable secondary metabolites that are antifungal against aflatoxigenic fungus; and Lowes *et al*. (2000) who recorded that the 83 AU/ml HMK mycocin reduced the yeast count in yoghurt samples from 10^4 by day 2 to mean value 10 CFU/g, while the higher concentration (167 AU/ml) reduced it to below the level of detection by day 1 at 20°C by using an agar well diffusion experiment, Al-Qaysi *et al*. (2017) investigated the antagonistic activity of *D. hansenii* against 4 fungal pathogenic strains (*T. rubrum, T. concentricum, A. alternata*, and *C. lunata*) in vitro using an agar well diffusion assay. The findings demonstrated that *D. hansenii* supernatant strongly suppressed the development of all pathogens examined, with zones of inhibition for *T. rubrum*, *A. alternata*, *T. concentrcum*, and *C. lunata* being 43, 47, 46, and 35 mm, respectively.

However, yoghurt bacterial analysis indicated that coliform, psychrotrophs, *E. coli* and *S. aureus* could not be found. (Data not shown) These results were consistent with EOSQ (2005), which stated that yoghurt should be free from any pathogenic bacteria which is strongly associated with proper heat treatment of milk, high sanitary standards of processing, and also influence cell free extract applied. Additionally, Al-Kadamany *et al*. (2003) were unable to find any coliform bacteria in labneh samples.

Moreover, Al-Qaysi *et al*. (2017) found that *Debaryomyces hansenii*'s toxin-killing has an inhibitory efficacy at 25°C against *E. coli* and *S. aureus* with inhibition zones 36 mm and 35 mm respectively. Moreover, Rajkowska *et al*. (2012) and Saidi *et al*. (2019) studied how *S. cerevisiae* S3 supernatant extract accompanied by incubation with yeast cells and recorded a significant decrease in the number of *S. aureus*/g of the tested yoghurt sample.

Referring to the Egyptian standards, yoghurt samples are supposed to be free of *E. coli*, according to EOSQ (2005), but NFSA (2021) says that *E. coli* shouldn't be more than 2 ×102 CFU/g of yoghurt.

Conclusion

The addition of SCE and DHE has a significant improvement on the physio-chemical and microbiological quality of yoghurt, especially with the concentration of 100 ppm, which gave the yoghurt favorable characteristics, including the best scores for flavor, body, texture, and appearance up to the 33rd day of storage period as well as inhibiting the fungal growth and strongly recommended to be used in the yoghurt production.

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Conflict of interest

The authors declare that they have no conflict of interest.

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