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A MIXED CULTURE OF *PROPIONIBACTERIUM THOENII* P-127, *LACTOBACILLUS RHAMNOSUS* AND *LACTOBACILLUS PLANTARUM* AS PROTECTIVE CULTURES IN KAREISH CHEESE

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Forty samples of Kareish cheese were collected from Cairo and Giza markets and examined for incidence of yeasts. All examined samples contained yeasts in considerable numbers (log 5.6 cfu/g). The most predominant species isolated were *Saccharomyces cerevisiae* (46.67%) followed by *Candida* species (30%).

The antiyeast activity of *Lactobacillus rhamnosus* B-445 and *Lactobacillus plantarum* DSA 20174 was tested alone or in combination with *Propionibacterium thoenii* P-127. A combination between *P. thoenii* and both lactobacilli was found to be the most active against tested yeasts. This led to the development of two protective cultures containing *P. thoenii* P-127 and *L. rhamnosus* B-445 or *L. plantarum* DSA 20174. These protective cultures were used to manufacture Kareish cheese to determine their antiyeast activities and their effect on the composition, sensory and textural characteristics of the cheese. The protective cultures showed inhibitory activities against yeasts in Kareish cheese at refrigerator temperatures (4°C) without an influence on the quality of the food. In conclusion, yeasts growth can be minimized using a protective culture containing *P. thoenii* P-127 and *L. rhamnosus* or *L. plantarum* without undesirable effects on sensory properties. The potential of the tested protective cultures to inhibit yeast growth on Kareish cheese (soft cheese) was a new finding in this research.

INTRODUCTION

Reports on the occurrence of yeasts in cheeses date back to the early part of this century, but it is still not widely appreciated that yeasts can be an important component of many, if not all, cheese varieties [Ferreira & Viljoen, 2003]. High numbers of yeasts are frequently observed in cheeses. They can either cause spoilage or effect desirable biochemical changes. Spoilage of cheeses caused by yeasts happens, and occurs as visible growth of yeast colonies on the surface of cheese, as unpleasant smell or taste, as changes in colour and texture or as deformation of the packets containing the cheese [Effat, 2000]. Their occurrence may be attributed to the yeast's ability to grow at low temperatures, the assimilation of organic acids like succinic, lactic and citric acid, their proteolytic and lipolytic activities, resistance against high salt concentration, low α_w and resistance to cleaning compounds and sanitizers [Martin *et al.*, 2007]. Furthermore, yeasts have the ability to tolerate low pH and water activity values [Ferreira & Viljoen, 2003].

Recently, there has been significant commercial interest in using lactic acid bacteria and propionibacteria as natural food preservatives to enhance food safety and stability as the antimicrobial systems possessed by these bacteria offer potential for effective natural preservation methods [Effat, 2000]. Selected strains of these bacterial genera can be applied as "protective cultures" with an *in situ* production of antimetabolities. Such an example on the market is Bio Profit (Danisco Niebüll GmbH, Germany), a co-culture of *Lactobacillus rhamnosus* and *Propionibacterium freudenreichii* subsp. *shermanii* JS (DSM 7067) that is suggested for growth suppression of yeasts and moulds [Schwenninger & Meile, 2004]. In contrast, Microgard™ (Wesman Foods Inc. OR, USA) is a commercially available milk product fermented by *P. freudenreichii* subsp. *shermanii* and pasteurized after fermentation. The product promises effectiveness against most Gram-negative bacteria, some yeasts and moulds [Daeschel, 1993]. Finally, purified inhibitory metabolites lacking the producer culture are used as biopreservatives. Protective cultures may be incorporated into different food products, for example cheese.

Kareish cheese is a soft cheese which is most popular in Egypt and Arabian countries. It is an acid coagulated fresh cheese, made from skim milk with soft composition, white curd and slightly salty [Francois *et al.,* 2004]. Manufacture of such a product is made at home from naturally fermented skim milk by lactic acid bacteria. Then it is ready to be consumed as fresh-cheese. It is mainly manufactured by small holders and sold at local markets. Thus, the product is exposed to contamination with several types of microorganisms, especially with yeasts.

The first aim of this study was to identify the yeast species present in Kareish cheese. The second aim was to develop protective cultures by the use of propionibacteria and lactobacilli for biopreservation of cheese with yeasts as targets.

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MATERIALS AND METHODS

Sample collection

Forty random samples of Kareish cheese were purchased from the street vendors at Cairo and Giza markets. The samples were transported to the laboratory under refrigeration and analysed on arrival.

Microbiological analysis

All samples were examined for total bacterial count (TBC) and lactic acid bacteria (LAB) according to the American Public Health Association [APHA, 1992]. The yeasts were enumerated on a yeast extract-glucose-chloramphenicol-agar (YGCA, Merck) according to Lopandic *et al.* [2006]. The colony forming units (cfu) for yeasts were determined after incubation for 5 days at 25°C. From the primary cultures three colonies with identical morphological appearance were selected for further purification. The purified yeast isolates were grown on YGCA medium and kept at 4°C until they were identified.

Identification of yeast isolates

Yeast isolates were identified by conducting the full range of morphological, sporulation, biochemical and physiological tests as described in Kreger-Van Rij [1984] and Barnett *et al.* [1983]. In addition, each isolate was examined in ATP-32C strips (Bio Merieux, Marcy-L'Eloile, France).

Screening method for anti-yeast activities

Cultures

The strains of yeasts used in this study were isolated from Kareish cheese. We selected one strain belonging to *Saccharomyces cervisiae* and another one belong to genus *Candida*. A strain of *Propionibacterium thoenii* P-127 was provided by the Department of Food Technology, Propionibacteria Culture Collection, Iowa State University. *Lactobacillus rhamnosus* B-445 was obtained from the Northern Regional Research Laboratory, Illinois, USA (NRRL). *Lactobacillus plantarum* DSA 20174 was supplied by Cairo Mircen, Faculty of Agriculture, Ain Shams University. A commercial culture of *Lactococcus lactis* subsp. *lactis* (Chr. Hansen's Lab., Hoersholm, Denmark) was used as starter for cheese making.

Assay for anti-yeast activity

A preliminary screening for anti-yeast activities was done using a modified agar spot assay according to Schwenninger & Meile [2004]. Therefore, the test culture was spot inoculated onto an agar plate and incubated anaerobically at 37°C. Each plate was then overlaid with tempered (50°C) malt extract soft agar (Difco) containing about 10^4 cells/mL of indicator yeast. The inhibition areas around the test colonies were recorded after 48 h at 25°C. All assays were performed in duplicate, and the results presented are the means of duplicate trials.

Preparation of protective cultures

Protective cultures were produced by growing *P. thoenii* P-127, *L. rhamnosus* B-445, *L. plantarum* DSA 20174 strains separately and in combination in supplemented whey permeate (SWP) [Suomalainen & Mäkinen, 1999; Schwenninger & Meile, 2004]. After incubation for 72 h at 32°C, the cells were harvested by centrifugation and washed twice in 0.85% NaCl. Finally, they were 10 to 100 fold concentrated in sterilized skim milk (Difco).

Manufacture of Kareish cheese

Buffalo's milk was defatted and pasteurized at 80°C for 15 s and cooled to 32°C. The chemical composition of milk is given in Table 1. Milk was divided into three main portions. The first portion was applied for evaluating chemical and sensory properties. The other two portions were inoculated with *S. cerevisiae* and *Candida* spp. suspensions, respectively, to give initial count of 10^3 cfu/mL. Then each portion was divided into four equal portions. Starters were added as follows: (1) *Lac. lactis* subsp. *lactis* (2% v/v), served as a control; (2) *Lac. lactis* subsp. *lactis* (1% v/v) and *P. thoenii* P-127 (1% v/v) (Treatment I); (3) *Lac. lactis* subsp. *lactis* (1% v/v) and 1% of protective culture consisting of *P. thoenii* P-127 and *L. plantarum* DSA 20174 (Treatment II); and (4) *Lac. lactis* subsp. *lactis* and 1% of protective culture consisting of *P. thoenii* P-127 and *L. rhamnosus* B-445 (Treatment III).

The cheese was manufactured according to the procedure described by Effat *et al.* [2001]. Resultant cheeses were packed into plastic bags, and stored at 4°C for 30 days. The experiments were carried out in triplicate.

Samples of each cheese were taken, zero time, 5, 10, 15 and 30 days after manufacture for composition, rheological, microbiological analysis and sensory evaluation. Data were reported as the average of three independent trials.

Chemical analysis

Total and soluble nitrogen were determined with the Kjeldahl method [IDF, 1993]. Total solids, fat content, moisture, titratable acidity and lactose content were measured according to AOAC [1995]. The pH was determined with a digital pH meter (Hanna AT 4817). Acetaldehyde and diacetyl content of all cheeses were measured using a Shimadzu (240 UV-vis) spectrophotometer (Japan) as described by Lees & Jago [1970].

Texture analysis

Cheese samples were prepared for examination according to El-Zeny [1991]. Instron Universal Testing Machine 4202 (Instron Co., Canton, OH) equipped with 50N (5 kg) load cell was used. A plunger with a flat plate 305 cm in diameter was attached to the cross-head. The speed of the cross-head was set at 22.5 cm/sec in both upward and downward

TABLE 1. Gross chemical composition of milk used for making Kareish cheese.

Constituents	Buffalo's milk (%)
Total solids	10.5
Fat	0.15
Total protein	4.5
Acidity	0.18
pH	6.6
Lactose	4.9

directions. The record chart speed was 1.25 cm/min. The strain level of the plunger to the sample was set at one inch (75% deformation). On-tenth scale load range of 5 kg was used and two consecutive bites were taken. The texture evaluation was done in triplicate on each batch of cheese. The chart was used to calculate the area under the response plot. Area 1 and 2 (cm^2) represent the area under the curves formed during the first and second compression cycle and the work done during compression. The height of the first peck during the first bite represented the extent of hardness (force), while the distance of the sample under compression during the second bite represented springiness (cm). Cohesiveness was derived from the ratio of Area 2/Area 1, whereas gumminess was equal to hardness x cohesiveness. Chewiness was equal to gumminess x springiness. All texture measurements were done at room temperature (22 ± 2°C).

Microbial analysis

Two grams of a cheese sample were homogenized aseptically in a stomacher with 18 mL of warm (45°C) sterile 2% tri-sodium citrate solution. Further decimal dilutions were carried out as required for microbial assays in 9-mL sterile peptone water and subsequently plated in duplicate onto selective media. Populations of *L. rhamnosus* B-445 were enumerated on MRS supplemented with 0.005% (w/v) of vancomycin (MRSV) [Suomalainen & Mäkinen, 1999]. Plates were incubated anaerobically at 37°C for 3 days. *L. plantarum* DSA 20174 was enumerated using MRS with Maltose and Bromocresol Purple after 48 h of anaerobic incubation (Anaerocult C, Merck) at 37°C [Kapitula *et al.,* 2007]. *Propionibacterium thoenii* P-127 was counted according to El-Kholy *et al.* [2006] on APT agar medium (Merek, Germany). Plates were incubated at 30°C for 3-4 days under anaerobic conditions. The population of yeasts was estimated on YGCA and plates were incubated aerobically for 96 h at 28°C.

Sensory evaluation

Samples of Kareish cheese were cut into approximately 5 x 5 cm pieces and placed on white plates. Samples were tempered at ambient temperature (20 ± 2°C) and then presented to the panelists in a random order. Water was provided for mouth washing between samples. The cheeses were evaluated organoleptically after zero, 5, 10, 15 and 30 days of ripening in Dairy Science Department, National Research Center by ten members of laboratory staff familiar with Kareish cheese. Panelists evaluated cheese for appearance (10 points), body and texture (40 points) and flavour (50 points). Scores were obtained for the three sensory attributes.

Statistical analysis

Data obtained from this study were analysed statistically according to procedures outlined by Snedecor & Cochran [1982].

RESULTS AND DISCUSSION

Microbiological quality of Kareish cheese

Table 2 reveals that the average of TBC was 7.73 $\log_{10}$ cfu/g. These results are in agreement with those obtained by Kaldes [1997]. However, El-Ghaish [2004] reported lower total viable counts in Kareish cheese samples, collected from different markets in Egypt.

The same table shows that the average of LAB was 5.79 $\log_{10}$ cfu/g, which was less than that found by Moussa *et al.* [1984].

As shown in Table 2 yeasts were detected in all tested samples. The counts of yeasts ranged from 3.4 to 6.82 $\log_{10}$ cfu/g with an average of 5.6 $\log_{10}$ cfu/g, which is much higher than that allowed by the Egyptian Standards [2000] namely not more than 100 cfu/g. These results were less than those obtained by Kaldes [1997] and El-Ghaish [2004] who gave an average count of yeast and moulds in Kareish cheese as 2.4 x 10^7 cfu/g and 1.0 x 10^6 cfu/g, respectively. However, our results coincide with those obtained by Abou-Dawood *et al.* [2005].

The microbiological quality of Kareish cheese in this study appears insufficient of sanitation during manufacture and handling this type of cheese.

Yeast species

A total of 30 isolates from Kareish cheese were identified and these included 14 strains of *Saccharomyces cerevisiae*, 9 strains of *Candida* sp. and 7 strains of *Kluyveromyces lactis* (Table 3). As shown, a smaller number of yeast isolates (30%) was characterised only at the genus level.

Table 3 shows the response of several yeast species to properties likely to govern their growth in cheeses. The most frequent isolates were *S. cerevisiae*. The occurrence of this yeast species in Kareish cheese is probably based on the utilization of protein and fat breakdown products from other species [Viljoen *et al.*, 2003]. According to Roostita & Fleet [1996], this species is capable of good growth in cheeses of low salt concentration and must utilize some cheese components as growth substrate.

The very strong ability of *K. lactis* to assimilate and ferment lactose (Table 3) is considered to be a key property contributing to its growth in cheeses and dairy products [Roostita & Fleet, 1996]. In addition, the *K. lactis* isolates were able to utilize lactic acid and their ability to grow at 5 and 10°C.

The frequent occurrence of *Candida* sp. in Kareish cheese is correlated with their ability to utilize lactic and citric acids and milk casein and their growth at 5 and 10°C (Table 3). As already reported in the literature [Lopandic *et al.*, 2006], yeasts occur frequently in cheese because of the low pH and moisture, elevated salt concentration and low storage temperatures. Prillinger *et al.* [1999] isolated 76 strains assigned to 39 species and found that *K. lactis, S. cerevisiae, C. catenulata, D. hansenii* and *G. candidum* were predominant in dif-

TABLE 2. Microbiological quality of Kareish cheese collected from different districts.

Range of count	Total Bacterial Count (TBC)	Lactic Acid Bacteria (LAB)	Yeast
	$\log_{10}$ cfu/g		
Maximum	8.62	6.08	6.82
Minimum	6.52	4.17	3.4
Average	7.73	5.79	5.6

TABLE 3. Properties of yeast species that exhibited growth in Kareish cheese.

Yeast species	Fermentation of		Casein hydrolysis	Assimilation of		Growth at		Growth in 6% sodium chloride
	sucrose	lactose		lactic acid	citric acid	5°C	10°C	
S cerevisiae	14/14[a]	0/14	0/14	9/14	0/14	14/14	14/14	7/14 (w)
K. lactis	7/7	7/7	0/7	7/7	0/7	7/7 (w)	7/7	7/7
Candida sp.	9/9	0/9	6/9	7/9	5/9	4/9	9/9	9/9

(a) 14/14; means 14 strains tested; 14 strains positive; w: weak response.

ferent cheeses from Austria, Denmark, France, Germany and Italy. Andrighetto *et al.* [2000] isolated 48 strains from Italian cow, buffalo, goat and Greek ewe cheese and 42 of them were assigned to the species *S. cerevisiae, Kluyveromyces marxianus, K. lactis* and *C. lipolytica.* Considering these reports, our data agree with the species most frequently found in several types of cheese.

Anti-yeast activities

Lactobacillus rhamnosus and *L. plantarum* were tested for their anti-yeast activities alone or in combination with *P. thoenii* P-127. The strains were embedded in concentrations ranging from 10^5 to 10^7 cfu/mL in malt extract agar plates on which *S. cerevisiae* and *Candida* sp. were spotted yielding 10^4 cells/spot. Although *P. thoenii* P-127 showed only weak inhibitory activities using it alone, their combination with lactobacilli revealed high antagonistic values (Table 4). The highest activities were detected with *L. rhamnosus* B-445 and *L. plantarum* DSA-20174 each in combination with *P. thoenii* P-127 (Table 4). The commenalistic interactions observed between propionibacteria and lactic acid bacteria [Lind *et al.*, 2005] enables research on the synergistic antifungal properties of co-cultures. In a recent study by Schwenninger & Meile [2004], the inhibitory effect of mixed cultures of propionibacteria and lactic acid bacteria against food spoilage yeasts was evaluated. The selected strains showed only weak inhibitory activities separately, but mixed cultures were highly effective against spoilage yeasts and did not have any influence on the quality properties of the food.

In all approaches, cell numbers of 10^7 cfu/mL of the *Lactobacillus* strains and 10^7 cfu/mL of the *Propinobacterium* strain were necessary for high inhibitory activities. Generally, our results are in line with findings of Schwenninger & Meile [2004], they recognized comparably high anti-yeast properties in numbers of the *Lactobacillus casei* group as well as in some strains of *L. plantarum*. Preliminary *in vitro* tests in agar plates showed the synergistic effects of *P. thoenii* P-127 and different lactobacilli, an observation similar to that of Suomalainen & Mäkinen [1999].

Influence of protective cultures on the chemical composition of Kareish cheese

Table 5 records the chemical composition of Kareish cheese during storage of all treatments. The moisture content of Kareish cheese manufactured with different protective cultures gradually decreased till the end of the storage period. Higher moisture content was found in cheese manufactured with the protective culture containing *P. thoenii* P-127 and *L. plantarum* (Treat. II). This might be due to the activity of mixed strains for producing acidity [Effat *et al.*, 2001]. Also, these results indicate that *L. plantarum* mayproduce exopolysaccharides which have excellent water binding properties and moisture retention, which is vital to functionality of Kareish cheese [Francois *et al.*, 2004].

Slight differences were noticed among Kareish cheeses in titratable acidity, pH values and total protein content throughout aging (Table 5), indicating that, addition of protective cultures to cheese milk did not affect the composition of resultant cheese. These results are in agreement with those reported for different varieties of low fat cheese by others investigators [Katsiari *et al.,* 2002; Francois *et al.,* 2004] who observed that the composition of experimental low fat cheeses was not affected by various adjunct cultures in cheese making.

Ripening parameters

Data in Table 6 show that both soluble nitrogen (S.N) content and the SN/TN ratio had similar trends. No differences were found between the control and experimental cheeses at the first stages of storage indicating that the presence of protective cultures in Kareish cheese did not contribute to primary proteolysis. During storage, a gradual increase in SN and SN/TN was observed in cheese from all treatments till the end of storage. Similar trends were reported for Domiati and Tallaga cheese by Badawi & Kebary [1998].

TABLE 4. Anti-yeast activities of *P. thoenii* P. 127, *L. rhamnosus* B-445 and *L. plantarum* DSA 20174 embedded in malt extract agar.

Indicator[(a)] yeasts	*P. thoenii* P. 127	*L. rhamnosus* B-445	*L. plantarum* DSA 20174	*P. thoenii* P-127 + *L. rhamnosus* B-446	*P. thoenii* P-127 + *L. plantarum* DSA 20174
Sacchromyces cerevisiae	+	++	++	+++	+++
Candida sp.	+	++	++	+++	+++

Each strain of *Lactobacilli* and *Propionibacteria* (10^7 cfu/mL); + weak inhibition; ++ strong inhibition; +++ very strong inhibition; (a) spot-inoculation (10^4 cells/spot).

TABLE 5. Compositional parameters of Kariesh cheese manufactured with protective cultures during storage at 4°C.

Parameters	Storage period (days)	Treatments			
		Control	I	II	III
Moisture (%)	Fresh	76.75 ± 0.030	75.89 ± 0.041	77.64 ± 0.030	75.25 ± 0.030
	5	76.44 ± 0.030	75.68 ± 0.031	77.35 ± 0.031	74.52 ± 0.030
	10	75.58 ± 0.030	74.75 ± 0.031	76.96 ± 0.031	74.23 ± 0.031
	15	74.72 ± 0.030	73.80 ± 0.031	76.50 ± 0.031	73.84 ± 0.030
	30	73.80 ± 0.030	73.24 ± 0.031	75.42 ± 0.030	73.27 ± 0.031
Total acidity (%)	Fresh	0.96 ± 0.030	0.88 ± 0.030	1.04 ± 0.030	0.92 ± 0.030
	5	1.08 ± 0.036	0.94 ± 0.030	1.34 ± 0.030	1.02 ± 0.030
	10	1.10 ± 0.305	1.00 ± 0.030	1.23 ± 0.026	1.04 ± 0.030
	15	1.20 ± 0.20	1.10 ± 0.030	1.30 ± 0.030	1.06 ± 0.030
	30	1.30 ± 0.030	1.25 ± 0.030	1.42 ± 0.031	1.28 ± 0.030
pH	Fresh	5.35 ± 0.030	5.25 ± 0.030	5.20 ± 0.021	5.33 ± 0.197
	5	5.25 ± 0.030	5.17 ± 0.030	5.18 ± 0.030	5.30 ± 0.606
	10	5.16 ± 0.069	5.13 ± 0.030	5.00 ± 0.305	5.25 ± 0.030
	15	5.09 ± 0.030	5.00 ± 0.305	4.86 ± 0.030	5.18 ± 0.030
	30	5.00 ± 0.305	4.88 ± 0.030	4.60 ± 0.030	4.88 ± 0.030
Total protein (%)	Fresh	17.50 ± 0.030	17.73 ± 0.030	17.62 ± 0.030	17.60 ± 0.020
	5	17.48 ± 0.030	17.70 ± 0.030	17.58 ± 1.125	17.55 ± 0.030
	10	16.88 ± 0.030	17.23 ± 0.030	17.16 ± 0.030	16.98 ± 0.030
	15	16.51 ± 0.030	16.75 ± 0.030	16.68 ± 0.030	16.60 ± 0.030
	30	16.62 ± 0.030	16.85 ± 0.030	16.78 ± 0.040	16.74 ± 0.030

Each value is a mean of 3 replicates.

TABLE 6. Changes in ripening parameters of Kareish cheese manufactured by using some protective cultures during storage at 4°C.

Parameters	Storage period (days)	Treatments			
		Control	I	II	III
S.N.%	Fresh	0.250 ± 0.030	0.254 ± 0.030	0.240 ± 0.031	0.240 ± 0.031
	5	0.254 ± 0.030	0.258 ± 0.030	0.246 ± 0.031	0.246 ± 0.031
	10	0.278 ± 0.030	0.284 ± 0.030	0.258 ± 0.031	0.258 ± 0.031
	15	0.280 ± 0.030	0.320 ± 0.030	0.268 ± 0.031	0.268 ± 0.031
	30	0.338 ± 0.030	0.348 ± 0.030	0.331 ± 0.031	0.331 ± 0.031
S.N. / T.N%	Fresh	9.11 ± 0.040	9.14 ± 0.030	8.73 ± 0.030	8.70 ± 0.030
	5	9.27 ± 0.381	9.31 ± 0.030	8.99 ± 0.025	8.94 ± 0.030
	10	10.49 ± 0.030	10.52 ± 0.030	9.67 ± 0.030	9.70 ± 0.030
	15	10.81 ± 0.030	12.17 ± 0.030	10.35 ± 0.030	10.30 ± 0.030
	30	12.95 ± 0.030	13.17 ± 0.030	12.70 ± 0.030	12.61 ± 0.030
Diacetyl content (mmol/100 g)	Fresh	84 ± 3.05	80 ± 3.05	88 ± 3.05	92 ± 3.05
	5	89 ± 3.05	82 ± 3.05	95 ± 3.05	105 ± 3.05
	10	94 ± 3.05	86 ± 3.05	104 ± 3.05	112 ± 3.05
	15	85 ± 3.05	75 ± 3.05	92 ± 3.05	95 ± 3.05
	30	72 ± 3.05	68 ± 3.05	84 ± 3.05	86 ± 3.05
Acetaldehyde content (mmol/100 g)	Fresh	10.3 ± 0.305	9.00 ± 0.305	11.2 ± 0.305	11.5 ± 0.305
	5	10.6 ± 0.305	10.00 ± 0.305	11.8 ± 0.305	12.2 ± 0.305
	10	8.4 ± 0.305	6.4 ± 0.305	12.00 ± 0.40	12.5 ± 0.305
	15	7.4 ± 0.305	5.2 ± 0.305	11.50 ± 0.305	12.3 ± 0.305
	30	6.2 ± 0.305	5.00 ± 0.305	10.00 ± 0.305	10.5 ± 0.305

Each value is a mean of 3 replicates.

Data presented in Table 6 show that Kareish cheese produced by a protective culture consisting of *P. thoenii* P-127 and *L. rhamnosus* B-445 had the highest acetaldehyde and diacetyl contents whether when fresh or stored. These increases may be due to both actions of aroma cultures and *L. rhamnosus* metabolize all the citrate and produce appreciable amounts of diacetyl [Wyder *et al.,* 2002]. Both acetaldehyde and diacetyl contents increased to reach maximum values after 10 days then decreased until the end of storage in all treatments. The concentration of acetaldehyde and diacetyl can differ to a great extent, depending on the medium composition, growth conditions and the specific activity of bacteria and their enzymes. In cheese, the pathway leading to acetaldehyde production is generally considered to be via lactose degradation [Salem *et al.*, 2007]. Some of the end products of citrate and pyruvate metabolism, such as diacetyl and acetaldehyde, have distinct aroma properties and contribute significantly to the quality of fermented foods [Helland *et al.*, 2004]. In general, these results coincide with those obtained by El-Nemr *et al.* [2003].

Textural properties

Several textural parameters were determined in cheese made with and without protective cultures (Table 7). It is clear that all the textural properties gradually increased till the end of the storage period. This could be mainly due to proteolysis of casein to compounds that are very soluble in water and that do not contribute to the protein network responsible for the cheese rigidity. Similar results in Edam cheese was reported by El-Batawy *et al.* [2004].

Data presented in Table 7 show that the textural properties of Kareish cheese were influenced by using the protective cultures. Values of hardness, cohesiveness, gumminess and chewiness were higher in cheese made with the protective cultures containing *P. thoenii* P-127 and *L. rhamnosus* (Treat. III) than those of other treatments and the control cheese. The higher values of textural parameters in cheese made with *P. thoenii* P-127 and *L. rhamnosus* (Treat. III) could be related to its lower moisture content [Ahmed *et al.* 2005]. Beal & Mittal [2000] suggested that high moisture weakens the protein network resulting in a less firm cheese. The high moisture content and weak protein network produce smooth cheese that coats the mouth during mastication [Ahmed *et al.*, 2005].

The differences observed in springiness and cohesiveness values may be attributed to the amount of protein matrix present and its strength. The later being dependent on factors such as moisture, salt, fat and in particular the mineral content of cheese [Innocente *et al.*, 2002; El-Batawy *et al.*, 2004].

The progressive increase in gumminess (a product of hardness and cohesiveness) and chewiness (a product of hardness and springiness) until 30 days could be attributed to the progressive increase in hardness, cohesiveness and springiness. The decrease in values of some textural parameters of the cheese manufactured with the combination of *P. thoenii* P-127 and *L. plantarum* (Treat. II) (Table 7) is in agreement with the results of previous investigators who suggested that positive results for flavour and texture development in reduced or low-fat cheeses depended strongly on the strain of adjunct culture used [El-Soda *et al.*, 2000; Zambou *et al.,* 2004]. The pliable and soft texture of the cheeses manufactured with the combination of *P. thoenii* P-127 and *L. rhamnosus* or *L. plantarum* may be due to the excellent water binding properties of metabolites produced by these lactobacilli strains which improve the hard rubbery texture of Kareish cheese.

Anti-yeast activities in Kareish cheese

The anti-yeast capacity of the protective cultures was investigated in Kareish cheese. Figure 1 shows the inhibition

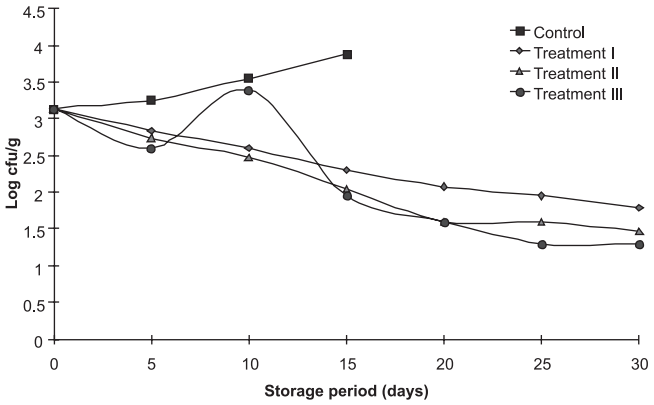
TABLE 7. Textural characteristics of Kareish cheese manufactured with and without protective cultures during storage at 4°C.

Parameters	Storage period (days)	Treatments			
		Control	I	II	III
Hardness (N)	Fresh	1.378 ± 0.030	1.205 ± 0.030	1.124 ± 0.030	1.398 ± 0.030
	15	5.842 ± 0.030	4.949 ± 0.025	4.493 ± 0.030	8.538 ± 0.030
	30	7.504 ± 0.030	9.751 ± 0.037	7.953 ± 0.030	16.491 ± 0.030
Cohesiveness	Fresh	42.203 ± 0.030	8.48 ± 0.030	24.63 ± 0.036	76.72 ± 0.030
	15	53.975 ± 0.030	17.717 ± 0.30	35.365 ± 0.030	90.605 ± 0.030
	30	54.77 ± 0.030	77.01 ± 0.025	65.41 ± 0.030	80.34 ± 0.030
Springiness (Elasticity, mm)	Fresh	12.330 ± 0.036	6.214 ± 0.030	11.81 ± 0.030	13.16 ± 0.030
	15	13.860 ± 0.030	7.708 ± 0.030	14.05 ± 0.036	17.370 ± 0.030
	30	14.31 ± 0.030	14.88 ± 0.030	14.98 ± 0.030	14.27 ± 0.030
Gumminess (N)	Fresh	58.16 ± 0.030	10.22 ± 0.030	27.68 ± 0.030	107.26 ± 0.031
	15	321.164 ± 0.030	87.686 ± 0.030	158.895 ± 0.030	773.587 ± 0.032
	30	403.48 ± 0.030	750.91 ± 0.031	520.23 ± 0.031	1324.95 ± 0.030
Chewiness (Nmm)	Fresh	717.06 ± 0.030	63.51 ± 0.030	326.90 ± 0.030	1411.54 ± 0.030
	15	5093.66 ± 0.032	675.02 ± 0.030	2232.47 ± 0.032	13437.2 ± 0.307
	30	5370.31 ± 0.032	11248.63 ± 0.032	7741.03 ± 0.032	18907.10 ± 0.204

Each value is a mean of 3 replicates.

of tested yeasts in Kareish cheese treated with protective cultures. The results indicate that the protective cultures were able to totally inhibit the growth of tested yeasts during storage for 30 days at 4°C. The use of the protective cultures led to a reduced viability of both tested yeasts. The reduction in the *S. cerevisiae* culture was nearly similar to the culture of *Candida* sp. The populations of *S. cerevisiae* decreased by 1.84, 1.67 and 1.36 log cycle in Kareish cheese containing protective cultures consisting of *L. rhamnosus* B-445 and *L. plantarum* DSA 20174 each in combination with *P. thoenii* P-127 and *P. thoenii* P-127 alone, respectively, after 30 days (Figure 1-a), whereas

(a) On the growth of *Sacchromyces cervisiae*



(b) On the growth of *Candida* sp.

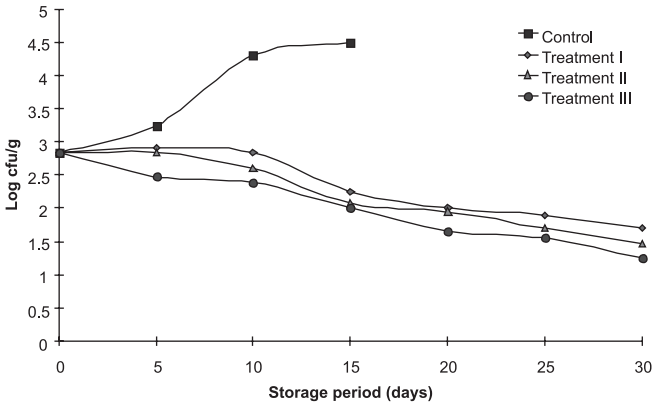


FIGURE 1. Effect of protective cultures on the growth of *S. cervisiae* and *Candida* sp. in Kareish cheese at 4°C.

Candida sp. decreased by 1.58, 1.37 and 1.14 log cycle during the same time (Figure 1-b). Therefore, strains of *S. cerevisiae* and *Candida* sp. did not differ in their sensitivity to the tested protective cultures. These findings clearly demonstrate that a complex synergistic action may take place originating from a minimal metabolism of the protective cultures during the storage period. Generally, our results confirmed those of Suomalainen & Mäkinen [1999] and Schwenninger & Meile [2004]. On the other hand, the yeast counts increased sharply in control samples and spoiled after 15 days of storage. Visual observation done on the growth of yeasts indicates that visible yeast growth occurred in control cheese after 15 days. Control cheese became softer in texture and developed a less attractive odour at the end of the storage period.

In all three cheese treatments in the present study, the numbers of *P. thoenii* P-127 remained high during the storage period and never declined to values below 10^9 cfu/g cheese (Table 8). Enumeration of *L. plantarum* DSA 20174 and *L. rhamnosus* B-445 in Kareish cheese during storage of the two treatments (II and III) indicated their enhanced survival after 5 days of storage. A decrease (less than 1 log unit) in both lactobacilli counts was observed, initiated within 15 days after storage at 4°C. These findings confirmed the data of Schwenninger & Meile [2004]. They reported that the cell number of the protective cultures neither increased nor decreased during storage of yoghurts.

Sensory evaluation

The cheeses were evaluated for taste, flavour, consistency and appearance. Data pertaining to the overall evaluation and preference of cheese during storage at 4°C are depicted in Figure 2. According to the panelists, cheese manufactured with a protective culture consisting of *L. rhamnosus* B-445 and *P. thoenii* P-127 received more score points. As shown, the use of this protective culture enhanced the flavour and improved body and texture of the treated Kareish cheese if compared with the control. At the five time points examined, the cheese containing *L. rhamnosus* B-445 and *P. thoenii* P-127 as a protective culture exhibited the highest score and improved flavour compared with control and other treatments. It was characterised by good nature flavour, typical smooth body and texture without off-flavours throughout storage. It was followed by cheese containing *L. plantarum* DSA 20174 and *P. thoenii* P-127 as a protective culture. This

TABLE 8. Changes of *P. thoenii, L. plantarum* and *L. rhamnosus* populations in Kareish cheese samples during storage at 4°C.

Time of storage (days)	Treatment I *P. thoenii*	Treatment II		Treatment III	
		L. plantarum	*P. thoenii*	*L. rhamnosus*	*P. thoenii*
	Log cfu/g				
0	10.25	9.81	10.27	9.84	10.16
5	10.3	10.27	10.38	10.10	10.36
10	10.32	10.29	10.39	10.13	10.39
15	10.36	10.25	10.38	10.10	10.33
20	10.25	10.09	10.29	10.07	10.30
25	10.0	10.06	10.23	10.01	10.27
30	10.0	9.97	10.17	9.95	10.17

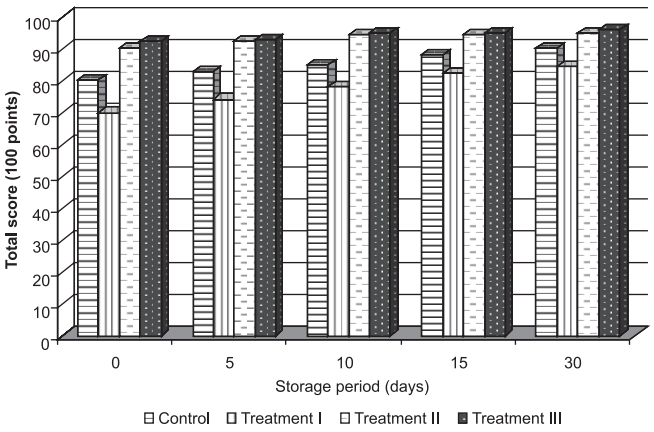


FIGURE 2. Organoleptic properties of Kareish cheese containing protective cultures.

could be attributed to the fact that propionibacteria produce vitamins and some growth factors which stimulate the starter cultures [El-Kholy *et al.*, 2006]. These results coincide with those obtained by El-Kholy [2000] and El-Kholy *et al.* [2006]. Moreover, the improvement of texture in these treatments might be observed due to the production of exopolysaccharides. For both propionibacteria and lactobacilli the production of exopolysaccharides was described and reviewed by Cerning [1995]. An *in-situ* production of exopolysaccharides in Kareish cheese improving stability might thus be a positive side-effect of suitable protective cultures potentiating novel applications.

CONCLUSION

In conclusion, the presence of yeasts in Kareish cheese represents a major concern for cheese manufacturers. The results indicate that the yeast growth can be minimized using a protective cultures consisting of *P. thoenii* P-127 and *L. rhamnosus* or *L. plantarum* without undesirable effects on sensory properties. These peculiars can have a positive impact as a "natural" and gentle way to preserve food, with a view to improving the quality, hygiene and safety of food.

REFERENCES

1. Abou-Dawood A.I., Taha S.H., Mohamed M.A., Chemical and microbiological quality of raw milk, soft and hard cheeses collected from some districts at Giza government. Egypt. J. Dairy Sci., 2005, 33, 201–214.
2. Ahmed N.H., El-Soda M., Hassan A.N., Frank J., Improving the textural properties of an acid-coagulated (Karish) cheese using exopolysaccharide producing cultures. LWT-Food Sci. Technol., 2005, 38, 843–847.
3. Andrighetto C., Psomas E., Tzanetakis N., Suzzi G., Lombardi R., Randomly amplified polymorphic DNA (RAPD) PCR for the identification of yeasts isolated from dairy products. Lett. Appl. Microbiol., 2000, 30, 5–9.
4. AOAC, Association of Official Analytical Chemists. Official Methods of Analysis. 1995.Vol. II, 16th Ed., Arlington, VA, USA.
5. APHA, American Public Health Association, Standard Methods for the Examination of Dairy Products. 1992, Amer. Publ. Halth Assoc. Inc. 12th Ed., New York.
6. Badawi R.M., Kebary K.M.K., Influence of fat replacers on the quality of low fat Tallaga cheese. 1998, *in*: Proc. 7th Egypt. Conf. Dairy Sci. and Technol., 1998, pp. 347–361.
7. Barnett J.A., Payne R.W., Yarrow D., Yeasts: Characteristics and identification. 1983, Cambridge University Press, pp. 679–695.
8. Beal P., Mittal G.S., Vibration and compression responses of Cheddar cheese at different fat content and age. Milchwissenschaft, 2000, 55, 139–142.
9. Cerning J., Production of exopolysaccharides by lactic acid bacteria and propionibacteria. Lait, 1995, 75, 462–472.
10. Daeschel M.A., Applications and interactions of bacteriocins from lactic acid bacteria in foods and beverages. 1993, *in*: Bacteriocins of Lactic Acid Bacteria (eds. D.G. Hoover, L.R. Steenson). Academic Press, Inc. San Diego, New York, Boston, London, Sydney, Tokyo, Toronto, pp. 62–91.
11. Effat B.A., Antifungal substances from some lactic acid bacteria and propionibacteria for use as food preservatives. J. Agric. Sci. Mansoura Univ., 2000, 25, 6291–6304.
12. Effat B.A., Salem M.M.E., Kawther El-Shafei, Effect of using different starters on quality of Kareish cheese. Egypt. J. Food Sci., 2001, 29, 95–108.
13. Egyptian Standards for Kareish and Domiati Cheese, Egyptian Organization for Standards and Quality Controls. ES:1008/2000, 2000.
14. El-Batawy M.A., Metry W.A., Zedan M.A., El-Tawel H.S.M., Influence of salting method on the quality of Edam cheese. 2004, *in*: Proc. the 9th Egyptian Conf. for Dairy Sci. & Tech., 9–11 Oct. Cairo, Egypt, pp. 493–508.
15. El-Ghaish S.N.R., Chemical and microbiological studies to improve Kareish cheese quality. 2004, M.Sc. Thesis, Faculty of Agriculture, Kafr El-Sheikh, Tanta Univ., Egypt,
16. El-Kholy W.I., Effat B.A., Tawfik N.F., Sharaf O.M., Improving soft cheese quality using growth stimulator for *Bifidobacterium bifidum*. Deuttsche Lebensmittel-Rundschau, 2006, 102, 101–109.
17. El-Kholy W.I., The role of propionic acid bacteria metabolites in some diary products. 2000, Ph.D. Thesis, Faculty of Agriculture, Ain Shams University, Cairo.
18. El-Nemr T.M., Awad S.A., Ali A.H., Increasing of probiotic and therapeutic action in Kareish cheese using tolue balsam extract. Egypt. J. Food Sci., 2003, 31, 213–226.
19. El-Soda M., Madkor S.A., Tong P.S., Adjuncts cultures: Recent developments and potential significance to the cheese industry. J. Dairy Sci., 2000, 83, 609–619.
20. El-Zeny H.M., Rennet coagulation of salted milk and rheology of soft white cheese produced from it. 1991, Ph.D. Thesis, Michigan State Univ., East Lansing, 48824 MI, USA.
21. Ferreira A.S., Viljoen B.C., Yeasts as adjunct starters in matured Cheddar cheese. Int. J. Food Microbiol., 2003, 86, 131–140.
22. Francois Z.N., Ahmed N., Félicité M.T., El-Soda M., Effect of ropy and capsular exopolysaccharides producing strain of *Lactobacillus plantarum* 162 RM on characteristics and functionality of fermented milk and soft Kareish type cheese. African J. Biotechnol., 2004, 3, 512–518.
23. Helland M.H., Wicklund T., Narvhus J.A., Growth and metabolism of selected strains of probiotic bacteria in milk- and water-based cereal puddings. Int. Dairy J., 2004, 14, 957–965.

24. IDF, International Dairy Federation, Milk determination of nitrogen content. International Dairy Federation Standard 2B, 1993, p. 163.
25. Innocente N., Pittia P., Stefanuto O., Corradini C., Correlation among instrumental texture, chemical composition and presence of characteristic holes in a semi-hard cheese. Milchwissenschaft, 2002, 57, 204–208.
26. Kaldes Y.Th., Microbiological examination of soft cheeses manufactured in Minia city. Assiut Vet. Med. J., 1997, 38, 39–46.
27. Kapitula M.M., Klebukowska L., Kornacki K., Influence of inulin and potentially probiotic *Lactobacillus plantarum* strain on microbiological quality and sensory properties of soft cheese. Pol. J. Food Nutr. Sci., 2007, 57, 143–146.
28. Katsiari M.C., Voutsimas L.P., Kondyli E., Improvement of sensory quality of low fat kefalograviera-type cheese with commercial adjunct cultures. Int. Dairy J., 2002, 12, 757–764.
29. Kreger-van Rij (ed.). The Yeasts – A Taxonomic Study.1984, Elsevier Science Publisher, Amsterdam, pp. 105–465.
30. Lees G.J., Jago G.R., The estimation of diacetyl in the presence of other carbonyl compounds. J. Dairy Res., 1970, 37, 129–132.
31. Lind H., Jonsson H., Schnürer J., Antifungal effect of dairy propionibacteria – contribution of organic acids. Int. J. Food Microbiol., 2005, 98, 157–165.
32. Lopandic K., Zelger S., Bánszky L.K., Eliskases-Lechner F., Prillinger H., Identification of yeasts associated with milk products using traditional and molecular techniques. Foods Microbiol., 2006, 23, 341–350.
33. Martin P.A., Florez A.B., López-Diaz T.M., Mayo B., Phenotypic and molecular identification of yeast species associated with Spanish blue-veined Cabrales cheese. Int. Dairy J., 2007, 17, 961–967.
34. Moussa A.M., Zein G.N., Nofel A., Gomaa E.A., Studies on Kareish cheese in the local markets of Monoufia. II. Bacteriological properties. Egypt. J. Dairy Sci., 1984, 12, 117–122.
35. Prillinger H., Molnar O., Lechner F.E., Lopandic K., Phenotypic and genotypic identification of yeasts from cheese. Antonie van Leeuwenhoek, 1999, 75, 267–283.
36. Roostita R., Fleet G.H., The occurrence and growth of yeasts in Camembert and blue veined cheeses. Int. J. Food Microbiol., 1996, 28, 393–404.
37. Salem M.M.E., Abd El-Gawad M.A.M., Hassan F.A.M., Effat B.A., The use of synbiotics for production of functional low fat Labneh. Pol. J. Food Nutr. Sci., 2007, 57, 151–159.
38. Schwenninger S.M., Meile L., A. mixed culture of *Propionibacterium jensenii* and *Lactobacillus paracasei* subsp. *paracasei* inhibits food spoilage yeasts. System. Appl. Microbiol., 2004, 27, 229–237.
39. Snedecor G.W., Cochran W.G., Statistical Methods. 1982, 7th ed. Iowa State Univ. Press, Ames, Iowa, USA.
40. Suomalainen T.H., Mäkinen A.M., Propionic acid bacteria as protective cultures in fermented milks and breads. Lait, 1999, 79, 165–171.
41. Viljoen B.C., Knox A.M., De Jager P.H., Hattingh A.L., Development of yeast populations during processing and ripening of blue veined cheese. Food Technol. Biotechnol., 2003, 41, 291–297.
42. Wyder M.T.F., Bachmann H.P., Casey M.G., Interaction between propionibacteria and starter/non starter lactic acid bacteria in Swiss-type cheese. Lait, 2002, 82, 1–15.
43. Zambou N.F., Nour A.E., Félicite M.T., El-Soda M., Effect of ropy and capsular exopolysaccharides producing strain of *Lactobacillus plantarum* 162 RM on characteristics and functionality of fermented milk and soft Kareish type cheese. African J. Biotechnol., 2004, 3, 512–518.

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