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Research Article
Using of Dairy Propionibacteria as Bio-preservative in Kareish Cheese

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Abstract
Moulds and yeasts are a major cause of fermented foods spoilage, such as kareish cheese with the low pH being selective for them and can produce off-flavors, sticky texture and cheese becomes softer. Seventy two samples of kareish cheese were collected, examined for incidence of moulds and yeasts. Positive samples were non-conforming, contained 4-5 $\log_{10}$ CFU g^{-1} moulds and yeasts. Ten species of moulds and nine species of yeasts were detected in collected samples. All propionibacteria strains showed antagonistic effect against moulds and yeasts isolates using agar well diffusion technique. Cheese made with *Propionibacterium thoenii* 119 and *L. lactis* subsp. *lactis* (LPT) contained the highest folate, vitamin B12, diacetyl and acetaldehyde followed by cheese made with *Propionibacterium jensenii* 118 and *L. lactis* subsp. *lactis* (LPJ) then made with *Propionibacterium acidicpropionici* 117 and *L. lactis* subsp. *lactis* (LPA). The LPT cheese achieved the greatest of yield and sensory evaluation. The populations of *Aspergillus flavus* and *Candida albicans* decreased by (1.41, 1.24 and 1.2) and (1.49, 1.28 and 1.1) log cycle in kareish cheese made with LPT, LPJ and LPA after 30 days, respectively. Experimental cheeses were higher adhesiveness, cohesiveness, gumminess, hardness and springiness than control and contained the lowest counts of moulds and yeasts.

Key words: Propionibacteria, bio-preservatives, kareish cheese, moulds and yeasts, antimicrobial

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

More than a quarter of sold dairy products are cheeses, kareish cheese is a soft cheese commonly made and consumed in Egypt. This cheese is an excellent source of protein, amino acids, calcium, phosphorus, vitamins and many micronutrients. It has low fat and salt, so doctors advise patients to eat it.

Nevertheless, high numbers of moulds and yeasts frequently can grow best on/in kareish cheese, producing off-flavors described as fermented, yeasty, gassy and moldy appearance and sticky texture as well as cheese becomes softer, so the shelf-life of this type of cheese is short. Their occurrence may be attributed to the mould and 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 (Alvarez-Martin *et al*., 2007). Elbagory *et al*. (2014) confirmed that 35 kareish cheese samples were positive for moulds and yeasts and they isolated *A. fumigatus*, *A. terreus*, *Penicillium* spp. and *Fonsecaea* spp., *Candida albicans*, *Rhodotorula* spp. and *Candida famata*. Moulds and yeasts were detected in kareish cheese in range 27-35$\times 10^2$ CFU g^{-1} (Metwalli, 2011). About 90% of kareish cheese samples were contaminated with mould and only *Rhizopus* spp. was identified (El-Kest *et al*., 2015). An average of yeasts 5.6 $\log_{10}$ was detected in kareish cheese they were *S. cerevisiae*, *K. lactis* and *Candida* sp. (El-Shafei *et al*., 2008). Many species of fungi were isolated from cheese types (Abou Ayana *et al*., 2014).

Chemical preservatives and artificial additives causing severe harmful to consumers, scientists make unremitting efforts to find out and selection of natural compounds for use as safe alternatives to chemical preservatives. Newly, using lactic acid bacteria and propionibacteria as bio-preservatives (Dabiza, 2006) to enhance food safety and stability as the antimicrobial systems possessed by these bacteria offer potential for effective natural preservation methods. 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 yeast and moulds (Daeschel, 1993). Bio profit contains viable cells of *P. freudenreichii* subsp. *shermanii* strain JS and co-culture of *Lactobacillus rhamnosus* and is effective for inhibiting yeasts growth in dairy products, *Bacillus* spp. in sourdough bread (Schwenninger and Meile, 2004). Dairy propionibacteria have a long history of safe use in human diet and animal feed. *P. freudenreichii* is widespread consumed in Swiss cheese in which they are present in concentrations close to 10^9 CFU g^{-1}. Propionibacteria can synthesis vitamin B2, B7 (biotin), B9 and B12 and vitamin K (Burgess *et al*., 2009) beside homo and heteropolysaccharides (Dobruchowska *et al*., 2007) and produce propionic, acetic and succinic acid.

Therefore, this study aimed to isolate, identify and determine moulds and yeasts in kareish cheese samples, study the inhibitory effect of some propionibacteria strains against spoilage isolates and using them as bio-preservatives in kareish cheese.

MATERIALS AND METHODS

Sampling: Seventy two of kareish cheese samples were collected from milk shops, retail farmers, street vendors and supermarkets (18 each) from Dakahlia governorate. All samples were transported in an ice-box to laboratory and immediately analyzed on arrival to determine, isolate and identify of moulds and yeasts.

Microbial cultures: The strains of moulds and yeasts used in this study were isolated from kareish cheese samples. *Aspergillus flavus* and *Candida albicans* were selected to study their behavior in the presence of propionibacteria strains (PB) in kareish cheese. *Propionibacterium acidipropionici* 117, *Propionibacterium jensenii* 118 and *Propionibacterium thoenni* 119 were provided by the Institute of Food Biotechnology, Olsztyn University of Agriculture and Technology, Heweliusz, Olsztyn, Poland. The *L. lactis* subsp. *lactis* (LL) was obtained from Chr. Hansen's Lab., Hoersholm, Denmark. Stock cultures of PB and LL were grown at 30°C in sodium lactate broth (NLB) and MRS broth, respectively and maintained at 4°C. Before using PB and LL were transferred three times at daily intervals in Enriched Whey Medium (EWM) and MRS broth, respectively.

Isolation of moulds and yeasts: According to Van der Walt and Yarrow (1984), 10 g of each sample was taken, diluted in 90 mL of sterile solution of 2% (w/v) sodium citrate and homogenized in a stomacher for 30 sec. For all samples, ten fold serial dilutions were prepared in a sterile solution of 2% (w/v) sodium citrate and the numbers of yeasts and moulds were determined by surface plating on yeast Potato Dextrose Agar (PDA) with 0.01% of chloramphenicol and Czapek-Dox's medium, respectively. Each sample analyzed in duplicate, incubated at 25°C for 5 days. Moulds and yeasts colonies were purified, morphologically identified in mycological laboratory-botany department, Faculty of Science, Mansoura University and mentioned on selective media.

Assay of antimicrobial activity using agar well diffusion technique: Sterilized skimmed milk (12%) was separately inoculated with *P. acidicpropionici* 117, *P. jensenii* 118, *P. thoenii* 119 and *L. lactis* subsp. *lactis* (2%), incubated at 30°C for 48 h, centrifuged at 400 rpm to get a very clear supernatant, then sterilized by passing through sterile 0.45 µm syringe filter to obtain Cell Free Filtrate (CFF). Each filtrate was divided into 3 parts; the 1st part was taken as it is (CFF), the 2nd has been treated ammonium sulphate until full saturation, the mixtures were left overnight at 4.0°C, then the precipitation of protein were occurred by centrifugation for 15 min at 10000 rpm at 4.0°C. Any precipitated protein compounds (peptides and bacteriocins) pellets were dissolved immediately in a minimum volume of 0.1 M phosphate buffer at pH 7.0 then has been re-mitigation in sterile water (PPB). The 3rd part was heated at 121°C for 15 min to destroy the protein compounds, while retaining the effect of organic acids (HFF). Amounts of 20 mL of sterile nutrient agar were poured into sterile petri dishes. After solidification, 100 µL of fresh activity of culture suspensions of each mould or yeast isolates were swabbed on the respective plates. All parts of CFF, PPB and HFF carefully added in the perfect wells in agar. The plates were kept in the fridge for 2 h then incubated for suitable time (3-5 days) at 30°C. After incubation the diameter of inhibition zones formed around each well were measured in mm and recorded.

Manufacture of kareish cheese: Buffalo's milk (The chemical composition of milk is given in Table 1) was defatted and heated at 80°C for 15 sec then cooled to 32°C. Milk was divided into 4 portions, cultures were added as follows: (1) *L. lactis* subsp. *lactis* (2% v/v), served as a control, (2) *L. lactis* subsp. *lactis* and *P. acidicpropionici* 117 (2%, 1:1 v/v) (LPA), (3) *L. lactis* subsp. *lactis* and *P. jensenii* 118 (2%, 1:1 v/v) (LPJ) and (4) *L. lactis* subsp. *lactis* and *P. thoenii* 119 (2%, 1:1 v/v) (LPT), 0.5% glucono-δ-lactone (GDL) was added to each treatment. Each portion was divided into two sub portion, A and B. All portions coded A were directly incubated at 30°C. All portions coded B inoculated with *A. flavius* and *Candida albicans* to give initial count of 10^3 CFU mL^{-1} then incubated at 30°C. Resultant cheeses were packed into plastic bags and stored at 6±1°C for 30 days. Samples of each cheese were taken, zero time, 5, 10, 15 and 30 days after manufacture for chemical composition, rheological, microbiological analysis and sensory evaluation. Data were reported as the average of three independent trials. Organoleptic evaluation was carried out according to the scheme of El-Shafei *et al.* (2008).

Table 1: Gross chemical composition of milk used for making kareish cheese

Defatted buffalo's milk	Ingredients (%)						
	pH	Acidity	Fat	Lactose	SNF	TS	Total protein
	6.7	0.18	0.2	4.8	10.3	10.5	4.6

Chemical analysis: Chemical analysis in this study were determined according to AOAC (2003). Determination of folate and vitamin B12, the cheese sample extraction procedure was carried out according to literature (Albala-Hurtado *et al.*, 1997) to determine of water-soluble vitamins in infant milk using High Performance Liquid Chromatography (HPLC) analysis. AcelatIdehyde and diacetyl content of all cheeses were measured using a Shimadzu (240 UV-VIS) spectrameter (Japan) as describes by Lees and Jago (1970).

Determination of PB and LL: For Propionibacteria (PB) counts, NLB containing agar (15 g L^{-1}) and bromocresol purple (0.032 g L^{-1}) as an indicator with anaerobic incubation at 30°C was used. Both PB and LL grew on NLB agar. The LB formed big, white colonies after 48 h and PB formed small, yellow colonies with yellow zones after 96 h incubation (Babuchowski *et al.*, 1999), *Lac. lactis* subsp. *lactis* (LL) also was enumerated on M 17 agar at 30°C for 24 h.

Texture determination: Texture profile analysis of the kareish cheese samples were carried out by using the texture analyzer (CNS-Farnell, England). Cheese samples were cut into cubes 5 cm 3 and kept at 12°C for 1 h before analysis. The probe was TA 15 (45° and 30 mm diameter), at speed 1 mm sec^{-1} and 10 mm distance, using cycle or hold programs. Hardness, cohesiveness, springiness, gumminess and adhesiveness were calculated as described by Szczesniak and Kleyn (1963) and Bourne (1978).

Data were subjected to statistical analysis by the computer program of using the General Linear Model (GLM).

RESULTS AND DISCUSSION

Determination of moulds and yeasts: The first step in this study was collection of kareish cheese samples from 4 sources to isolate, identify and determine counts of contaminated moulds and yeasts, listed results in Table 2, illustrated that samples of retail farmers and street vendors were the worst in mold and yeast counts, all these samples were contaminated with moulds (100%), while 17 out of 18 (94.4%) retail farmers samples were contaminated with yeast, street vendor samples were completely contaminated with

Table 2: Positive kareish cheese samples for moulds and yeasts with mentioning the source and conformance with EOSQC (2005)

	Moulds			Yeasts		
Source of samples	Positive	Percentage	*Conforming or N-C	Positive	Percentage	*Conforming or N-C
Milk shops (18)	16	88.9	N-C	14	77.8	N-C
Retail farmers (18)	18	100.0	N-C	17	94.4	N-C
Street vendors (18)	18	100.0	N-C	18	100.0	N-C
Supermarkets (18)	15	83.3	N-C	13	72.2	N-C

*Standards according to EOSQC (2005), Moulds ≤10 CFU g^{-1}, Yeast ≤400 CFU g^{-1} N-C means Non-conforming

Table 3: Antimicrobial activity of dairy propionibacteria cell free against isolated moulds and yeasts from kareish cheese (the diameter of inhibition zone mm*)

	linhibition zone*								
	P. acidicpropionici 117			*P. jensenii* 118			*P. thoenii* 119		
Isolates of moulds and yeasts	CFF	HFF	PPB	CFF	HFF	PPB	CFF	HFF	PPB
Mould isolates									
Aspergillus flavus	B	B	C	A	C	B	A	B	A
Aspergillus fumigatus	B	B	C	A	C	B	A	B	A
Aspergillus nigar	A	B	C	A	C	B	A	B	A
Aspergillus terreus	B	C	D	A	C	B	A	B	B
Aspergillus ventii	A	D	D	A	C	B	A	C	B
Fonsecaea spp.	B	B	D	B	D	B	A	C	A
Geotrichum spp.	C	C	D	B	E	D	A	C	B
Cladosporium spp.	C	D	E	D	E	E	B	D	C
Nigrospora spp.	D	E	E	D	E	D	C	D	C
Penicillium spp.	B	C	D	C	E	D	A	C	B
Yeast isolates									
Candida albicans	A	B	C	B	E	D	A	C	B
Candida famata	A	B	C	B	E	D	A	C	B
Candida lipolytica	B	C	D	A	C	B	B	C	B
Candida parapasillosis	B	C	D	B	D	C	B	D	C
Candida tropicalis	C	D	E	A	C	B	B	C	C
Kluyveromyces lactis	D	E	E	C	D	C	B	C	B
Rhodotorula glabrata.	B	C	D	C	E	D	A	C	B
Saccharomyces spp.	B	C	C	C	D	C	A	C	B
Yarrowia lipolytica	C	E	D	C	D	C	B	D	C

*Diameter of the inhibition zone, E≤8 mm, D 9-13 mm, C 14- 18 mm, B 19- 22 mm, A 22-29 mm. Maximum inhibitory zone of *L. lactis* subsp. *lactis* was nearly 2 mm (data not shown), CFF: Crude cell free filtrate, HFF: Heated cell free filtrate and PPB: Precipitated peptides and bacteriocins

yeasts. Milk shop and supermarket samples were positive for moulds at ratios 88.9 and 83.3%; the same samples included 77.8 and 72.2% contaminated samples with yeasts, respectively. It should be noted that positive samples contained 4-5 $\log_{10}$ CFU g^{-1} moulds or yeasts, thereby led to confirm that all positive sample were non-conforming because the mold and yeast counts exceeded 10 and 400 CFU g^{-1}, respectively based on EOSQC (2005). These findings surely related to reduction of the hygiene condition during the manufacturing and trading. Counts of mould in kareish cheese were 8×10^8 CFU g^{-1} (average, 3×10^4 CFU g^{-1}). So, 87% of kareish cheese samples were non-conforming due to high mycological counts exceeded 10 CFU g^{-1} mold or 400 CFU g^{-1} yeast, according to the Egyptian Standard (ES) 1008-2000 (El Sayed *et al*., 2011). Yeasts were isolated with an incidence of 52% from fresh kareish cheese samples (Hakim *et al*., 2013). Similar results were reported by Elbagory *et al*. (2014) who found that 100% kareish cheese samples were contaminated with moulds and all samples were non-conforming for EOSQC (2005). Abou Ayana *et al*. (2014) could isolate many toxigenic fungi from types of cheese.

Identification of moulds and yeasts: In Table 3 it could prove that there are 19 mould and yeast species belong to 11 fungal genera. Aspergillus and Candida were the most common genera recovered from the examined cheese samples. Aspergillus was represented by 5 mould species (*A. flavus*, *A. fumigatus*, *A. nigar*, *A. terreus* and *A. ventii*). Candida was represented by 5 yeasts species (*C. albicans*, *C. famata*, *C. lipolytica*, *C. parapasillosis* and *C. tropicalis*) too. Five other mould species (*Fonsecaea* spp., *Geotrichum* spp., *Cladosporium* spp., *Nigrospora* spp. and *Penicillium* spp.) were isolated. Four other yeast species (*Kluyveromyces*

lactis, *Rhodotorula glabrata*, *Saccharomyces* spp. and *Yarrowia lipolytica*) were isolated and identified. The enormous counts and species of moulds and yeasts associated with the low pH, the nutritional profile of kareish cheese, which are favorable for the growth of these organisms in the presence of oxygen, surface moisture, often containing lactic acid, peptides and amino acids favors rapid growth. Similar results were recorded by Elbagory *et al*. (2014). Growth of Asperigillus, Cladosporium and Penicillium species may responsible for bitterness and rancidity of cheese. This great diversity of contaminated moulds and yeasts for the cheese surely carries future connotations of spoilage or healthy risks. Chemically, *Geotrichum candidum* reduced diacetyl concentrations by 52-56% in low fat cottage cheese after 15-19 days of storage at 4-7°C (Antinone and Ledford, 1993).

Organoleptically, yeasty, fermented off-flavors, mouldy and gassy appearance and sticky texture are often detected when yeast and mould grow to 5-6 $\log_{10}$ CFU g^{-1}. Giudici *et al*. (1996) confirmed that galactose, which results from lactose hydrolysis by the lactic starter cultures was fermented by galactose-positive strains of yeasts such as *Saccharomyces cerevisiae* and *Hansenula anomala*. Lipolysis produces short-chain fatty acids that combine with ethanol to form fruity esters. Some proteolytic yeast strains produce sulfides, resulting in an egg odor. Common contaminating yeasts of cheeses include *Candida* spp., *Kluyveromyces marxianus*, *Geotrichum candidum*, *Debaryomyces hansenii* and *Pichia* spp. (Johnson, 2001).

Antagonism assay: The results in Table 3 also indicated to CFF of all tested propionibacteria strains had the strongest inhibitory effect against all isolated moulds and yeasts, PPB of *P. thoenii* 119 and *P. jensenii* 118 were higher inhibition than HFF, while HFF of *P. acidicpropionici* 117 was higher inhibition than PPB. Generally, there are not clear differences in the inhibitory effects from tested propionibacteria strains against mould and yeast isolates, these results confirmed by following -up the growth and behavior of *A. flavus* and *C. albicans* in the presence of propionibacteria cultures in kareish cheese during cold storage. In terms of efficiency, *P. thoenii* 119 was the most efficient in the inhibition all tested moulds and yeasts followed by *P. jensenii* 118 then *P. acidicpropionici* 117. Furthermore, protective cultures produce protein compounds (peptides and bacteriocins) and organic acids (e.g., acetic, propionic and succinic acids) that own anti-microbial properties. The *P. acidicpropionici* 117 has acidity inhibitory effect higher than bacteriocins unlike *P. jensenii* 118 and *P. thoenii* 119, which own higher bacteriocins effects than acidity inhibition. *Propionibacterium shermanii* and their metabolites use as a food grade biopreservative against fungi and Gram negative bacteria (Al-Zoreky *et al*., 1991). Bio profit contains viable cells of *P. freudenreichii* subsp. *shermanii* strain JS and is effective for inhibiting yeasts growth in dairy products, *Bacillus* spp. in sourdough bread (Suomalainen and Mayra-Makinen, 1999). El-Shafei *et al*. (2008) could inhibit *Sacchromyces cerevisiae* and *Candida* sp., using bacterial combinations contain lactic acid bacteria and *P. thoenii* P. 127. Bacteriocin propionicin PLG-1 and GBZ-1 produced by *P. thoenii* P-127 (Lyon *et al*., 1993), Propionicins SM1 and SM2 produced by *P. jensenii* DF1 (Miescher *et al*., 2000). Thoenicin 447 isolated from *P. thoenii* 447 (Van der Merwe *et al*., 2004). These bacteriocins are active against moulds *(Aspergillus* spp., *Apiotrichum curvatum*, *Fusarium tricinctum* and *Phialophora gregata*) and yeasts (*Saccharomyces*, *Candida* and *Scopularopsis* sp.). Lastly, propionibacteria also produce other peptides and organic acids (2-pyrrolidone-5-carboxylic acid, 3-phenyllactic acid, hydroxyphenyl lactic acid 3-phenyllactic acid) with antiviral, anti-yeasts and antifungal activities (Lind *et al*., 2007).

Table 4: Yield percentage, total points of sensory evaluation and changes in chemical composition of kareish cheese manufactured using some propionibacteria strains during storage at 6±1°C

		Treatments			
Parameters	Storage period (days)	Control	LPA	LPJ	LPT
SN (%)	Zero	0.250	0.253	0.241	0.245
	10	0.271	0.282	0.272	0.274
	20	0.292	0.298	0.281	0.285
	30	0.340	0.349	0.330	0.333
	Overall means	0.288^{b}	0.295^{a}	0.281^{c}	0.284^{b}
SN/TN (%)	Zero	8.98	9.11	8.85	8.91
	10	9.17	9.23	9.02	9.03
	20	11.12	11.35	10.96	10.72
	30	12.65	12.81	11.89	11.61
	Overall means	10.48^{b}	10.63^{a}	10.18^{c}	10.10^{d}
Diacetyl content	Zero	85	81	85	88
(mmol/100 g)	10	96	88	97	102
	20	83	75	88	90
	30	71	67	81	87
	Overall means	83.75^{c}	77.75^{d}	87.75^{b}	91.75^{a}
Acetaldehyde content	Zero	10.1	9.5	11.3	11.5
(mmol/100 g)	10	8.5	8.2	12.1	12.5
	20	7.1	6.5	11.2	11.8
	30	6.3	5.6	10.3	10.7
	Overall means	8^{d}	7.45^{d}	11.23^{b}	11.63^{a}
Yield (%)	Zero	19.2	22.5	22.8	23.3
	10	18.9	22.2	22.3	23.0
	20	18.3	21.7	21.8	22.6
	30	17.8	21.3	21.5	22.2
	Overall means	18.55^{d}	21.93^{b}	22.1^{c}	22.78^{a}
Total points of	Zero	79	83	85	88
sensory evaluation	10	81	85	86	89
	20	82	88	90	92
	30	84	89	92	94
	Overall means	81.5^{d}	86.25^{c}	88.25^{b}	90.75^{a}

LPA: *P. acidicpropionici* 117+*L. lactis* subsp. *lactis*, LPJ: *P. Jensenii* 118+*L. lactis* subsp. *lactis* and LPT: *P. acidicpropionici* 117+*L. lactis* subsp. *lactis*

Evaluation of cheese: Many factors involving milk composition, amount and genetic variants of casein, milk quality, milk pasteurization, coagulant type, vat design, curd firmness at cutting and manufacturing parameters influence on cheese yield. From Table 4 the results showed that the yield affected the cultures used, LPT produced the greatest yield (23.3%), which reached 22.2% at the end of storage period. This treatment followed by LPJ, LPA, then control that recorded the lowest yield. Generally, cheese yield gradually declined along storage period. Surely, these results due to the deference of cultures used particularly presence of propionibacteria, it can biosynthesis exopolysaccharides. Dobruchowska *et al*. (2007) confirmed ability of propionibacteria to biosynthesis exopolysaccharides.

Concerning ripening parameters, data in Table 4 show that both Soluble Nitrogen (SN) 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 increment in SN and SN/TN was observed in cheese from all treatments till the end of storage. Similar trends were reported for kareish cheese by El-Shafei *et al*. (2008).

Data listed in the same table show that kareish cheese produced with *P. thoenii* 119 and *L. lactis* subsp. *lactis* had the highest acetaldehyde and diacetyl contents, whether when fresh or stored. These increases may be due to both actions of aroma cultures and *P. thoenii* 119 metabolize all the citrate and produce appreciable amounts of diacetyl (Frohlich-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 coincided with those obtained by El-Shafei *et al*. (2008).

For sensory evaluation, the cheeses were evaluated for taste, flavour, body and texture and appearance during storage at 6°C are recorded in Table 4. According to the panelists, cheese made with LPT culture received more score points; it enhanced the flavor, body and texture of the treated

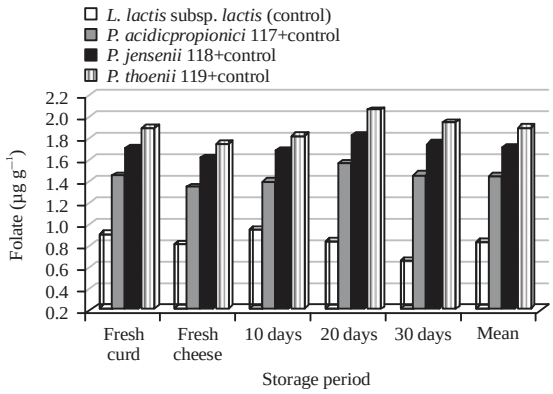

Fig. 1: Folate (µg g^{-1}) in kareish cheese made with *L. lactis* subsp. *lactis* or *L. lactis* subsp. *lactis*+Propionibacteria strains during storage periods

kareish cheese compared with the control. This trend continued along storage. The LPJ treatment came in the second place in total points of sensory evaluation then LPA treatment which was lower flavor, appearance and body and texture, hence total score against treatments. All experimental cheeses had good appearance, flavor and texture and body, the yeasty and mouldy flavor disappeared opposite the control that had some changes, it was slightly sticky, had slightly yeasty taste and lower flavor. These changes due to the high load of moulds and yeasts which can hydrolysis diacetyl (Antinone and Ledford, 1993). Enhancing of body and texture in experimental cheeses due to exopolysaccharides that are produced by propionibacteria strains.

Vitamins B9 and B12: Dairy propionibacteria have the ability to biosynthesis a package of important and necessary vitamins for the host health, such as B2 (riboflavin), B7 (biotin), B9 (folic acid) and B12 (cobalamin), vitamin K (MK-9 (4H) and its precursor DHNA with bifidogenic activity. Folate or folic acid, is an important B-group vitamin, participates in many metabolic pathways, such as DNA and RNA biosynthesis and amino acid inter-conversions. Mammalian cells cannot synthesize folate; therefore, an exogenous supply of this vitamin is necessary to prevent nutritional deficiency (LeBlanc *et al*., 2007). Among lactic acid bacteria many *Lactobacillus* spp., *Lactococcus* spp., *L. plantarum*, *L. bulgaricus*, *L. lactis*, *S. thermophilus* and *Enterococcus* spp. have the ability to produce folate so it was necessary to determine folate. Figure 1 shows the levels of folate in fresh curd and cheese after 0, 10, 20 and 30 days of storage. Propionibacteria strains were used simultaneously with *L. lactis* subsp. *lactis* that was only used in control. The results show significantly differences between the control and

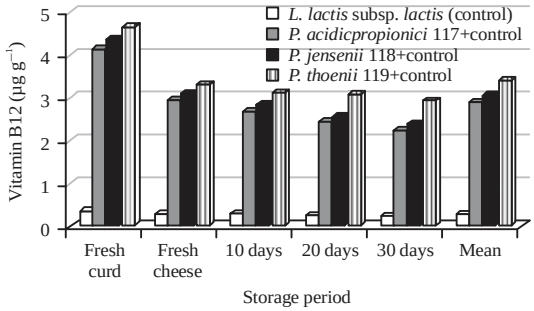

Fig. 2: Vitamin B12 (µg g^{-1}) in kareish cheese made with *L. Lactis* subsp*. lactis* or *L. lactis* subsp. *lactis*+Propionibacteria strains during storage periods

Table 5: Textural characteristics of Kareish cheese manufactured with and without protective cultures during storage at 6±1°C

Parameters	Storage period (days)	Treatments			
		Control	LPA	LPJ	LPT
Adhesiveness (g sec^{-1})	Zero	8.13	8.25	8.36	8.61
	30	9.24	10.22	11.20	12.92
	Overall means	8.69^{d}	9.24^{c}	9.78^{b}	10.77^{a}
Cohesiveness (ratio)	Zero	0.78	0.83	0.91	0.95
	30	1.02	1.11	1.32	1.45
	Overall means	0.9^{d}	0.97^{c}	1.12^{b}	1.2^{a}
Gumminess (g sec^{-1})	zero	261	275	270	275
	30	305	314	332	377
	Overall means	283^{d}	294.5^{c}	301^{b}	326^{a}
Hardness (g)	zero	200	190	195	211
	30	241	263	270	275
	Overall means	220.5^{d}	226.5^{c}	232.5^{b}	243^{a}
Springiness	zero	6.15	6.13	6.32	7.11
elasticity (mm)	30	7.29	8.20	9.50	12.50
	Overall means	6.72^{d}	7.20^{c}	7.91^{b}	9.81^{a}

LPA: *P. acidicpropionici* 117+*L. lactis* subsp. *lactis*, LPJ: *P. Jensenii* 118+*L. lactis* subsp. *lactis* and LPT: *P. acidicpropionici* 117+*L. lactis* subsp. *lactis*

treatments. *L. lactis* subsp. *lactis* (control) produced a limited quantity of folate compared to the treatments. The LPT (control+*P. thoenii* 119) produced the greatest level of folate followed by LPJ (*P. jensenii* 118+control) then LPA (*P. acidicpropionici* 117+control) generally, folate slightly increased to reach the maximum level on the 20th day of storage at 6±1°C then decreased. Hugenholtz *et al*. (2002) found some propionibacteria strains as productive as *Streptococcus thermophiles*. Folate decreased in experimental fresh cheeses against fresh curds, fresh cheeses recorded the lowest folate levels. Furthermore, folate decreased on the 30th day but still more than in fresh cheese. These results nearly similar obtained results by Holasova *et al*. (2004) for fermented milks, Ibrahim *et al*. (2014) confirmed that some lactic acid bacteria isolated from Egyptian dairy products able to synthesize levels of folate. These increases of folate during storage are associated with the growth of propionibacteria.

Vitamin B12 or cobalamin is an essential nutrient for the human body that plays a key role in the normal functioning of the brain and nervous system, the formation of blood and also the metabolism of every cell, especially affecting DNA synthesis and regulation, fatty acid synthesis and energy production. Its deficiency leads to a serious physiological disorder called pernicious anemia. Many microorganisms such as bacteria able to produce vitamin B12 by fermentative processes. Propionibacteria produce vitamin B12 intracellularly and excrete mainly propionic acid and acetic acid extracellularly. In this study, remarkable differences in vitamin B12 production among the different starters used to make kareish cheeses are presented in Fig. 2. Production of vitamin B12 by single *L. lactis* subsp. *lactis* (C) or accompanied with *P. acidicpropionici* 117 (LPA), *P. jensenii* 118 (LPJ) and *P. thoenii* 119 (LPT) was determined. The LPT treatment was significantly higher in vitamin B12 production compared to control, LPA or LPA. Fresh curds contained the greatest vitamin B12 against fresh or stored cheeses. Sharp decline was noticed in vitamin B12 in fresh cheeses followed by a gradual increase till the 10th day of storage period. The quantities of vitamin B12 declined again on the 20th day and continued to decrease until the end of storage period. These results related to the moisture content of the curds and the growth of bacteria, Vitamin B12 is water- soluble so loses in the syneresis of whey. Furthermore, genetic differences of strains in ability to produce vitamin B12. Sharaf *et al*. (2014) obtained similar findings using encapsulated *Propionibacterium shermanii* or free cells.

Textural measurements: It was necessary to determine the textural parameters in cheese made with and without propionibacteria cultures (Table 5). Textural profile was determined at fresh and after 30 days. 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 findings in kareish cheese was reported by El-Shafei *et al*. (2008). All the textural properties of kareish cheese were influenced by using the protective cultures. Cheese made of protective cultures containing *P. acidicpropionici* 117, *P. jensenii* 118 or *P. thoenii* 119 were higher adhesiveness (g sec^{-1}), cohesiveness (ratio), gumminess (g sec^{-1}), hardness (g) and springiness elasticity (mm) than control which recorded the lowest texture profile parameters. The LPT treatment was the highest in textural parameters followed by LPJ, LPA. These results could be related to exopolysaccharides synthesis by protective cultures.

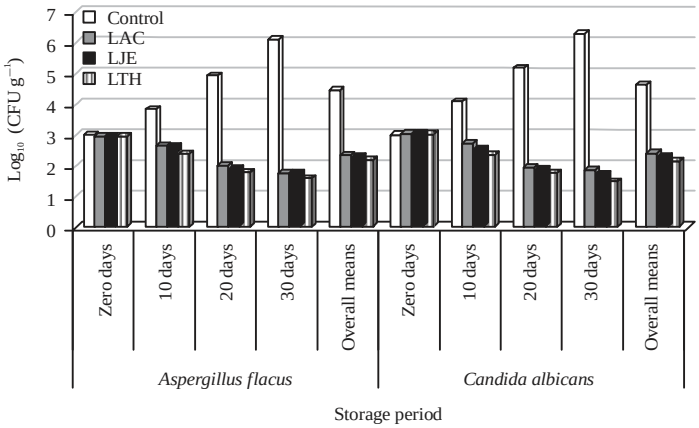

Fig. 3: Behavior of *A. flavus* and *C. albicans* in kareish cheese in the presence of propionibacteria cultures

Table 6: Changes of propionibacteria strains populations, *L. lactis* subsp. *lactis*, moulds and yeasts in kareish cheese during storage at 6±1°C

		Counts of microorganisms (Log_{10} CFU g^{-1})					
		Propionibacteria					
Treatments	Store period (days)	*P. acidicpropionici* 117	*P. jensenii* 118	*P. thoenii* 119	*L. lactis* subsp. *lactis*.	Moulds	Yeasts
Control	Zero	-	-	-	10.34	-	-
	10	-	-	-	10.47	0.59	1.39
	20	-	-	-	10.33	0.96	2.25
	30	-	-	-	10.12	1.27	2.32
LPA	Zero	10.21	-	-	8.81	-	-
	10	1029	-	-	8.92	-	-
	20	10.08	-	-	8.65	0.97	1.32
	30	9.85	-	-	8.41	1.11	1.63
LPJ	Zero	-	10.31	-	8.87	-	-
	10	-	10.43	-	8.91	-	-
	20	-	10.29	-	8.59	-	1.42
	30	-	9.97	-	8.38	0.90	1.88
LPT	Zero	-	-	10.29	8.75	-	-
	10	-	-	10.39	8.86	-	-
	20	-	-	10.24	8.56	-	1.17
	30	-	-	9.95	8.33	0.84	1.34

LPA: *P. acidicpropionici* 117+*L. lactis* subsp. *lactis*, LPJ: *P. Jensenii* 118+*L. lactis* subsp. *lactis* and LPT: *P. acidicpropionici* 117+*L. lactis* subsp. *lactis*

The differences observed in springiness and cohesiveness values may be attributed to the amount of protein matrix present and its strength. These results in agreement Abou Ayana and Ibrahim (2015).

Microbial properties of kareish cheese: In Table 6 LPA, LPJ and LPT had an inhibitory effect against moulds and yeasts have been shown. In control, moulds andyeasts could growth on the 10th day and rapidly increased along storage to reach the maximum populations among all treatments (1.27 and 2.32 log_{10} CFU g^{-1}), respectively. In the presence of propionibacteria strains, LPT restricted and prohibited the growth of these organisms till 20 days, only 1.17, 1.42 and 1.32 log_{10} CFU g^{-1} of yeasts were detected after 20 days in LPT, LPJ and LPA, respectively. Moulds appeared in LPA after 20 days but were detected after 30 days in LPJ and LPT. At the end of storage period moulds and yeasts recorded (0.84 and1.34), (0.9 and 1.88) and (1.11and1.63) log_{10} CFU g^{-1} in LPT, LPJ and LPA respectively. Nevertheless, all experimental cheeses were conformance with EOSQC (2005). Off-flavor, yeasty and moldy appearance disappeared. Thus, it is possible to prolong the shelf life of kareish cheese to month in refrigerator. On the other hand, propionibacteria strains slightly increased then decreased on the 20th day to reach 9.85, 9.97 and 9.95 log_{10} CFU g^{-1} for *P. acidicpropionici* 117, *P. jensenii* 118 and *P. thoenii* 119 at the end of cold storage, respectively. The *L. lactis* subsp. *lactis* took the same trend.

Antifungal activity in kareish cheese: The most isolated moulds and yeasts from collected samples were Aspergillus and Candida, therefore, *Aspergillus flavus and Candida albicans* were selected to further study. Data presented in Fig. 3 clearly showed the inhibition of both tested microorganisms in experimental kareish cheese made with propionibacteria cultures. The protective cultures (Propionibacteria) were able to totally inhibit the growth of *A. flavus* and *C. albicans* during storage for 30 days at 6±1°C. In the control (without propionibacteria strains), the growth of *C. albicans* was faster than *A. flavus*; it was 5.2 log CFU g^{-1} on 20th day. The LPJ and LPT treatments inhabited *C. albicans* more than *A. flavus* whilst LPA treatment inhabited *A. flavus* more than C. *albicans*. Generally, *P. thoenii* 119 could inhabit *A. flavus* and *C. albicans* more than *P. jensenii* 118 and *P. acidicpropionici* 117. The populations of *A. flavus* and *C. albicans* decreased by (1.41, 1.24 and 1.2) and (1.49, 1.28 and 1.1) log cycle in kareish cheese made with LPT, LPJ and LPA, respectively, after 30 days. Therefore, isolates of *A. flavus* and *C. albicans* did not clearly differ in their sensitivity to the tested protective cultures. These results reflect the antagonism effects of propionibacteria cultures against the mould and yeast, this antagonism resulting due to biosynthesis compounds of propionibacteria cultures that revealed by the results recorded in Table 3. The results of this research point was confirmed the results of Schwenninger and Meile (2004) and El-Shafei *et al*. (2008). The visible fungal growth occurred in the control cheese after 10 days, it became softer in texture and developed a less attractive odor, unacceptable and rejected at the end of the storage period.

CONCLUSION

Finally, the moulds and yeasts represent a nuisance to producers and consumers of soft cheese particularly manufactured by fermentation as kareish cheese. Using protective cultures contain propionibacteria strains such as *P. thoenii* 119, *P. jensenii* 118 and *P. acidicpropionici* 117 can be limiting the growth of moulds and yeasts allowing prolonging the validity of this type of cheese up to a month with improving sensory evaluation, microbial, rheological properties and increase the content of some of the necessary vitamins.

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