ISOLATED NON-STARTER LACTIC ACID BACTERIA USED TO IMPROVE KAREISH CHEESE QUALITY

SHADY EL-GHAISH

Kafrelsheikh University, Faculty of Agriculture, Department of Dairy Sciences

ABSTRACT

Lactobacillus casei and *Streptococcus thermophilus* were highly proteolytic activity that collected from Egypt. *Streptococcus thermophilus* is produced metallo-proteases while *Lactobacillus casei* is produced metallo-, serine- and cysteine-proteases. The proteases of these isolates are classified as P_{III} type, which proteolyses α_{S1}-, β- and γ-caseins with optimal pH and temperature of 6.5-7.2 and 37 - 42°C, respectively. Unsalted Kareish cheese is manufactured by traditional starter [*Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris* (Treatment A) as a control and with good technological characteristics strains [*Lactobacillus casei* (Treatment B) and *Streptococcus thermophilus* (Treatment C)] that mixed with traditional starter.

After 21 days of cold storage (5±2°C), treatment B had the highest acidity (2.49 %) and total solids (36.34 %). The protein content gradually decreased during storage while there was a gradual increase in cheese soluble nitrogen content in all cheese treatments. Treatments B and C suppressed bacterial and fungal growth in cheese during all storage period as compared to control (Treatment A). The sensory evaluation of cheeses showed the best acceptability of treatment (C) as compared to other cheese treatments (B and A) at the end of storage period with total score of 92.0.

Keywords: Kareish cheese, LAB, proteolytic activity, keeping quality

INTRODUCTION

Different kinds of milk (cow, buffalo, sheep, goat and camel) are consumed or used in Egypt as a whole, half or fat-free for producing different types of dairy products (Ayad *et al.,* 2004). Egyptian dairy products are generally produced in artisan conditions from raw milks without using industrial starter cultures. Nowadays, the Egyptian Organization for Standards and Quality Control has recommended that various dairy products must be produced from pasteurized milk. This recommendation aims to produce higher quality and safer dairy products.

Therefore, there is a great demand for new starter cultures to be used in Egyptian dairy products (El-Soda *et al.,* 2003; El-Ghaish *et al.,* 2011).

The main traditional dairy products in Egypt are Ras (hard cheese), Domiati (soft cheese), Kareish (soft cheese – acid coagulant), and Zabady and Laban Rayeb (fermented milk) (Abou-Donia, 2002). Kareish cheese is considered the most important type of cheese in Egypt for consumers with limited incomes, because of its price is low. It is also a recommended diet for some patients because of its low calorie and salt content (Fahmy, 1960). Several varieties of traditional fermented milk products such as Kareish, Zabady and Laban Rayeb made in Egypt, mostly prepared by peasant/fellah women using traditional knowledge of fermentation are considered as a way for bio preservation of milk for storage and longer consumption (Desouky, 2014).

Food preservation plays a vital role in Egyptian life and in food industry. However, using low temperature for preservation is the most efficient. Food preservatives are employed principally to prevent the spoilage of foods during the storage and the distribution, retailing and the use by the consumer. In this way they help to ensure that desired shelf-lives are met, that food products are convenient to store at home and that economic losses are minimized (Russell and Gould, 1991).

Many chemical components were used as preservatives such as hydrogen peroxide, sodium or potassium sorbate, formalin, sodium nitrate, phenolic compounds, etc. These preservatives are very cheap and stable but some of them are toxic and carcinogenic. Therefore, today much more attention is focused on using natural preservatives such as spices, herbs, propolis and lactic acid bacteria (LAB).

LAB are used as commercial starter cultures according to their production and technological characteristics. They are applied and recommended in dairy industry for their properties such as acidification activity, proteolytic activities, bacteriocin production, resistance to bacteriophages and production of exopolysaccharides (Salminen *et al.,* 2004). The ability of LAB to produce extracellular proteinases is very important. These proteinases catalyze the initial steps in the hydrolysis of milk proteins, providing the cell with the amino acids that are essential for growth of LAB **(**Fira *et al.,* 2001**)**. The structural components of the proteolytic systems of LAB can be divided into three groups on the basis of their function: (i) proteinases that cleave caseins to peptides, (ii) peptidases that degrade peptides, and (iii) transport systems that translocate the cleavage products across the cytoplasmic membrane **(**Kunji *et al.,* 1996).

Proteolysis is considered one of the most important biochemical processes involved in manufacturing of many fermented dairy products. Proteolytic/peptidolytic enzymes of LAB are involved in formation of organoleptic properties of the final milk products. The

casein molecules of milk are of particular interest because they are known to harbor bioactive peptides that are latent until released by proteolysis (Pihlanto and Korhonen, 2003). Lactic fermentation of milk is important for cheese production and a lot of mesophilic and thermophilic cultures are used for this purpose. Among these cultures, *Lactococcus lactis* subsp. lactis, *Lactococcus lactis* subsp. *cremoris*, *Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Lactobacillus helveticus*, and *Lactobacillus casei* have been well described (Caplice and Fitzgerald, 1999; Xie, *et al.,* 2011).

Our objectives were to isolate and identify LAB from some traditional Egyptian dairy products based on their technological characteristics. Selected strains were used as starter or co-cultures in Kareish cheese production. Cheese storage/shelf-life quality and sensory properties during cold storage were evaluated.

MATERIAL AND METHODS

Samples collection

Sixty samples of raw milk (buffalo, cow, goat, and sheep) and fifteen samples of fermented homemade traditional dairy products (Zabady and Laban Rayeb) were collected from the local markets of the Egyptian cities of Alexandria, Kafr El-Sheikh, and El-Mehala. All samples were collected in sterile cups and kept under cooling at (5±2°C) till analysis.

Isolation, purification and pre-identification of LAB cultures

LAB were isolated and pre-identified by morphological and physiological tests as described by El-Ghaish et al. (2010). For raw milk, Zabady and Laban Rayeb, 10 ml of each sample was pipetted aseptically into 90 ml of Ringer solution and mixed thoroughly. Serial dilutions (10^{-1} to 10^{-8}) were made and 1 ml of the appropriate dilutions was pour plated onto M17 and MRS agar. All plates were incubated at 37°C for 48 h and colonies from M17 and MRS agar plates were selected according to their shape and color from plates with colony numbers between 30 and 300. They were examined microscopically for Gram staining. Catalase activity was determined by placing drops of 3% H_2O_2 (w/v) on cultures. The cultures were classified as Gram positive, catalase-negative rods and Gram positive, catalase-negative cocci. For purification, the cultures were streaked on suitable media and the purified strains were reconstituted in sterile skim milk (12.5%, w/v) supplemented with 30% (w/v) glycerol and stored at -20°C.

Bacterial proteolytic activity on ultra-high temperature (UHT) skim milk

The isolates were reactivated twice by inoculating 50 µl of the previously prepared pre-culture into 950 µl of Délisse UHT skim milk and incubated at 37°C overnight. The last mixture was diluted 1/10

(v/v) in sample buffer and heated at 100°C for 3 min then analyzed for its proteolytic activity by Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) as described by El-Ghaish et al. (2010).

Proteolytic activity on Na-caseinate fractions

A pre-culture was prepared by inoculating an aliquot of 50 µl of proteolytic isolates into 950 µl suitable media and incubating overnight at 37°C. An aliquot (~ 0.5 ml) of the activated pre-culture was applied to the top of milk-citrate agar (MCA) plates containing 4.4% skim milk powder, 0.8% Na-citrate, 0.1% yeast extract, 0.5% glucose and 1.5% (Fira *et al*. 2001). The plates were incubated for 48 h at 37°C prior to cell collection. Collected fresh cells were re-suspended in 100 mM Na-phosphate buffer (pH 7.2) and brought to final optical density (OD_{600}) of 10 just before application. One volume of the last suspension was combined with an equivalent volume of the solutions of different milk protein fractions taken as substrates for the proteolytic activity. Sodium caseinate and whey proteins were obtained at a laboratory scale by precipitation at pH 4.6. The concentration of whey proteins was 5 mg/ml, while that of Na-caseinate was 12 mg/ml. The final mixtures were incubated for 24h at 37°C. Controls were prepared by incubating equivalent protein fractions solutions for the same periods without adding cells. At the end of incubation periods, cells were pelleted by centrifugation (10 min at 8000 g) and the clear supernatant was analyzed by SDS-PAGE (12% polyacrylamide).

Molecular identification of some selected LAB isolates

DNA was extracted from the isolates according to Delley *et al*. (1990) and was used as a template for 16S rRNA gene amplification. In the reaction universal primers fD1 (5'-AGAGTTTGATCCTGGCTCAG-3') and rD1 (5'-TAAGGAGGTGATCCAGGC-3') were used (Weisburg *et al*. 1991). DNA amplifications were performed in DNA thermal cycler model (Techno, Barloworld scientific, Cambridge, UK). The reaction mixture contained: PCR buffer (20 mM-Tris-HCl, 50 mM KCl pH 8.4), 1.5 mM $MgCl_2$, 0.2 mM of each dNTPs, 1 U Taq DNA polymerase (Qiagen Gmbh, Hilden, Germany). 1 mM of each primer and 40 ng DNA in a final volume of 50 µl. PCR amplifications were performed under the following conditions: denaturation at 94°C for 5 min, 35 cycles of denaturation at 94°C for 1 min, primer annealing at 56°C for 1.15 min, and DNA extension at 72°C for 1.15 min. A final extension was added at 72°C for 5 min, Amplicons were analyzed on 1% (w/v) agarose gel with ethidium bromide (0.5 mg/ml) in 0.5x TAE (Tris acetate-EDTA) buffer for 30 min at 100 V and made visible by UV trans-illumination. DNA sequencing was carried out by MilleGen sequencing service (Labège, France).

Factors influencing proteolytic activity

Effect of pH

The cell suspensions, in 100 mM Na-phosphate buffer at different pH values (5.0, 5.4, 6.0, 6.5 & 7.2) or in 100 mM Na-phosphate buffer or Tris-HCl buffer at pH 8.2 were mixed with substrate (12 mg/ml Na-caseinate), dissolved in the same buffer (at the same pH) at a 1:1 volume ratio and incubated for 24 h at 37°C. A control was performed similarly without adding the bacterial cells. Protein hydrolysis and peptide formation were analyzed by SDS-PAGE. Extent of hydrolysis was quantified by determination of the color intensity of the protein fraction bands.

Effect of temperature

The cell suspensions (in 100 mM Na-phosphate buffer, pH 7.2) were mixed with Na-caseinate (12 mg/ml) dissolved in the same buffer at a 1:1 volume ratio and incubated for 24 h at different temperatures (20, 25, 37, 42 & 50°C). Control was prepared similarly without bacterial cells. Protein hydrolysis and peptide formation were analyzed by SDS-PAGE. Extent of hydrolysis was quantified by determination of the color intensity of the protein fraction bands.

Effect of inhibitors

Whole cells were re-suspended in 100 mM Na-phosphate buffer (pH 7.2) to an approximate OD_{600} of 10. Different inhibitors were added to the cellular suspension at a concentration of 10 mM and incubated at 37°C for 1 h before adding the substrate (12 mg/ml Na-caseinate in 100 mM Na-phosphate buffer pH 7.2) at a 1:1 volume ratio and extending the incubation period for further 5 h. The tested inhibitors were Ethylenediaminetetraacetic acid (EDTA), iodoacetic acid and PEFABLOC (diluted in 10 mM phosphate buffer (pH 7.2) to concentration equivalent of that used for other inhibitors). A sample prepared without addition of the bacterial cells served as control. Protein hydrolysis and peptide formation were analyzed by SDS-PAGE. Extent of hydrolysis was quantified by determination of the color intensity of the protein fraction bands.

Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

The samples were applied to a 12 % polyacrylamide slab gel. The migration buffer contained 50 mM Tris, 0.384 M glycine and 0.1 % SDS. After running at 10 mA on the stacking gel and 20 mA on the running gel, staining was performed with Coomassie Brilliant Blue followed by a convenient destaining. The gels were scanned with Image scanner III (GE Healthcare, USA) and the intensity of the bands was quantified by Image software (Ong *et al.,* 2006).

Application

Manufacture of Kareish cheese

Unsalted Kareish cheese was manufactured by the common procedure described by Fahmy (1960) from fresh buffalo's skim milk prepared using cream separator. The milk was pasteurized at 65°C for 30 min, cooled to 37°C and divided to three portions 5 kg each.

The first portion (A) was inoculated with 2 % traditional starter (*Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *Cremoris*, EZAL, Rhodia Food, France) and served as a control. The second portion (B) was inoculated with 1 % *Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris* [EZAL, Rhodia Food, France] and 1 % *Lactobacillus casei* (isolated strain). The third portion (C) was inoculated with 1 % *Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris* [EZAL, Rhodia Food, France] and 1 % *Streptococcus thermophilus* (isolated strain). All portions were gently mixed and incubated at 37°C until complete coagulation. The curd was ladle into hoops of suitable size and placed on a porous mat of cloth. The curd was enclosed in cloth to expel more whey and when the curd was firm, it was cut into pieces and kept at a refrigerator temperature at 5 ± 2°C and tested when fresh and after 7, 14, and 21 days of cold storage. Cheese yield was calculated on the basis of the quantity starting milk to the quantity of the obtained fresh cheese.

Cheese analysis

Chemical analysis of cheese

Cheese samples were analyzed when fresh and after 7, 14, and 21 days of storage. Sample was thoroughly mixed in a ceramic jar and used for pH, titratable acidity, moisture, total solids, protein and soluble nitrogen determination according to the method described by AOAC (2000). All measurements were prepared in triplicates.

Microbiological analysis of cheese

Cheese samples were analyzed when fresh and after 7, 14, and 21 days of storage (5±2°C). Samples were prepared as described by Clark *et al.,* (1978). One gram from different parts of a single piece of Kareish cheese was accurately weighed into a sterile watch glass, and then transferred to a sterile mortar jar. Nine ml of 2 % sodium citrate solution was then added and the cheese was thoroughly ground into homogenous suspension by means of a magnetic stirrer. Finally, one ml of the previous suspension was added to nine ml of a sterile saline solution, mixed well to give the 1:10 dilution and used for preparing serials of dilutions.

Total bacterial count

Total bacterial count (TBC) per gram of cheese was determined using nutrient agar (NA) medium (LAB M, Bury, England) according to Olson *et al.,* (1978). The plates were incubated at 37 ± 1°C for 48 h.

Molds and yeasts count (MYC)

Molds and yeast count was carried out on potato dextrose agar (PDA) medium according to Lück and Gavron, (1990). The plates were incubated at 24 ± 1°C for five days.

Organoleptic properties

Cheese samples were organoleptically tested for flavor, body & texture and appearance by panel constituted from members of Dairy Sciences Department, Faculty of Agriculture at Kafrelsheikh University, Food Technology Research Institute, and by some other consumers as described by Nelson and Trout, (1981). The scores of judging were 60 for flavor, 30 for body & texture and 10 for appearance.

Statistical analysis

All statistical analysis were performed using Statistical Package for Social Studies software (SPSS, 2007) at $P < 0.05$.

RESULTS AND DISCUSSION

Pre-identification of LAB

According to Gram staining and catalase activity of the isolates, LAB were classified into rods (98 LAB isolates) and cocci (262 LAB isolates), demonstrating the dominance of cocci as compared to lactobacilli. These results are in in agreement with the results of El-Soda *et al.,* (2003); El-Baradei *et al.,* (2008).

Proteolytic activity on UHT skim milk

The proteolytic activity test of the isolated bacteria using UHT skim milk showed positive results for thirty-six isolates (data not shown). These isolates were classified as rods (7 isolates) and cocci (29 isolates). Based on the rate of proteolysis, the isolates were classified into three categories, 12 isolates with high activity, 8 isolates with intermediate activity and 16 isolates with poor proteolytic activity.

Proteolytic activity on caseinate and whey milk fractions

The 36 proteolytic active isolates were further investigated in milk-citrate agar (MCA) medium as described by Fira *et al.,* (2001). All milk casein fractions were proteolyzed albeit at different degrees with 3 rods and 5 cocci isolates (data not shown). Consequently, it can be concluded that the proteases of these isolates may be classified according to Kunji *et al.,* (1996) as P_{III} type, which proteolyses α_{S1}-, β- and γ-caseins. The proteolysis process targeted mainly caseins since whey proteins were not proteolyzed.

Identification of proteolytic active isolates by amplification and sequencing of 16S rDNA

The isolates with proteolytic activity on MCA medium (3 rods and 5 cocci) were identified with 16S DNA. The rods were identified as *Lactobacillus casei* (2 isolates) and *Lactobacillus fermentum* (1 isolate). The cocci were identified as *Streptococcus thermophilus* (1

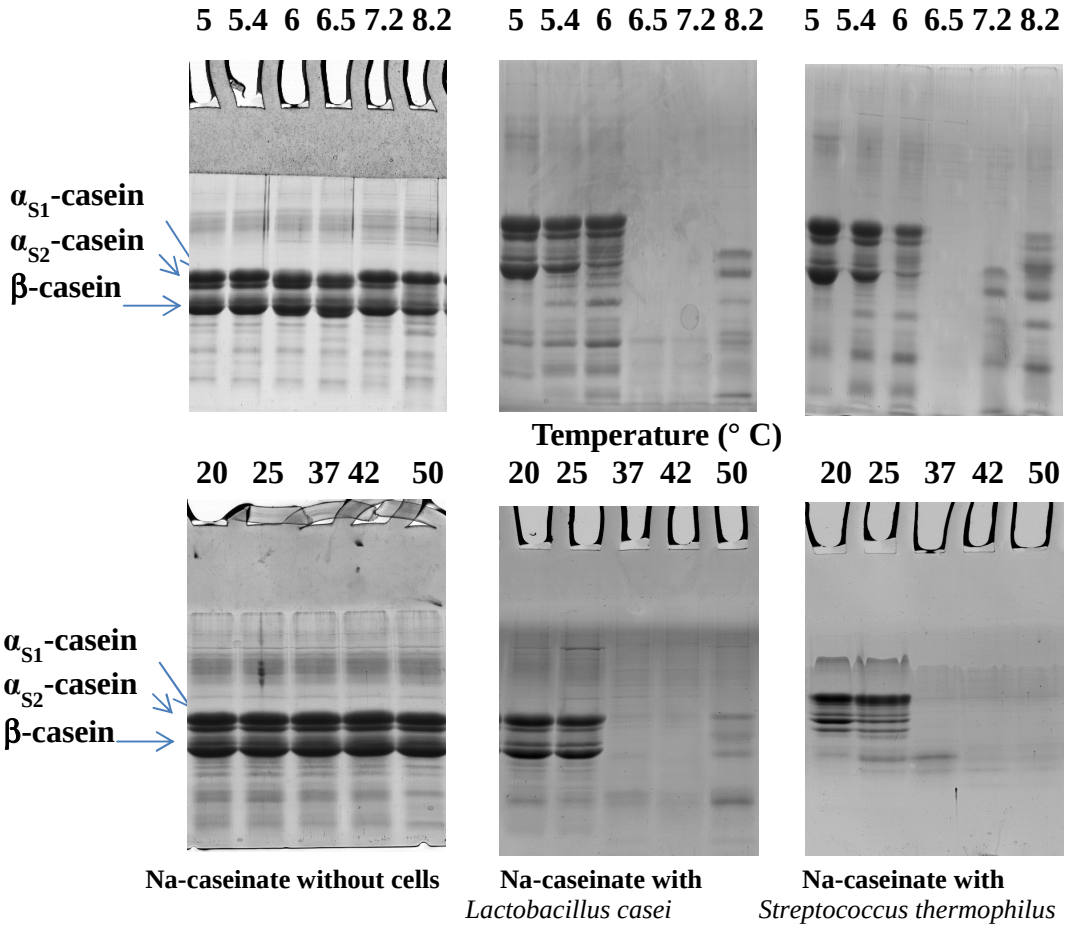
isolate) and *Enterococcus faecium* (4 isolates). *Lactobacillus casei* and *Streptococcus thermophilus* were selected for their higher proteolytic activity as compared to the other strains and used for further investigations.

Factors influencing proteolytic activity

Effect of pH

Data illustrated in Figure 1 shows the effect of pH on the proteolytic activity of the identified strains. *Streptococcus thermophilus* and *Lactobacillus casei* had no proteolytic activity at pH 5. The proteolytic activity started to be observed at pH 5.4 and reached its maximum at pH 6.5 and 7.2 for both strains, then decreased at pH 8.2. These results demonstrated that the optimal pH of the proteases secreted by these two bacterial strains was 6.5-7.2. These results are in agreement with those of Fira *et al.,* (2001); El-Ghaish, *et al.,* (2010); Sfaxi *et al.,* (2012); Ahmadova, *et al.,* (2013).

Fig. 1. SDS-PAGE profile (12 %) of Na-caseinate hydrolyzed by *Lactobacillus casei* and *Streptococcus thermophilus* at different pH values and different temperatures after 24 h incubation at pH 7.2.



Fira *et al.,* (2001) investigated the proteolytic activities of some thermophilic lactobacilli strains and found their optimal activities at pH 6.5. El-Ghaish *et al.,* (2010) described that the proteolytic activity of *Lactobacillus fermentum* IFO 3959 started to be observed at pH 5.4 and reached a maximum at pH 6.5. Also, Sfaxi *et al.,* (2012) found that the proteolytic activity of *Lactobacillus paracasei* I-N-10 started at pH 5.8 and reached its maximum in pH ranged from 6.6 to7.2. In contrast, Ahmadova *et al.,* (2013) concluded that the optimal pH value for proteinase activity of *Lactobacillus helveticus* A75 ranged from 6.6 to 7.2.

Effect of temperature

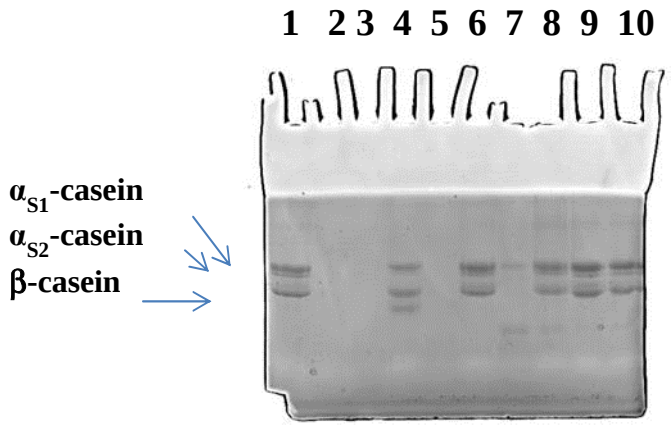
Data in Figure 1 show changes in the rate of proteolytic activity of *Lactobacillus casei* and *Streptococcus thermophilus* isolates as affected by the incubation temperature. The highest proteolytic activity was observed at 37°C until 42°C for both strains, which was stable until 50°C for *Streptococcus thermophilus* and slightly affected in *Lactobacillus casei*. On the other hand, lowering the incubation temperature under 37°C decreased the proteolytic activity for both studied strains. These results are in accordance with those obtained by Tsakalidou, *et al.,* (1999); Fira *et al.,* (2001); El-Ghaish, *et al.,* (2010); Sfaxi *et al.,* (2012); Ahmadova *et al.,* (2013). Tsakalidou *et al.,* (1999) found that the optimal temperature for □-casein hydrolysis by *Lactobacillus delbrueckii* subsp. *lactis* ACADC 178 strain was 40°C. Fira *et al.,* (2001) reported that the optimal temperatures for □-casein proteolysis by the strains *Lactobacillus acidophilus* BGRA43 and *Lactobacillus delbrueckii* BGPF1 were 45 and 40°C, respectively. El-Ghaish et. al., (2010) found that the highest proteolytic activity for *Lactobacillus fermentum* IFO 3956 was observed at 37°C-42°C but being decreased at 30°C. Sfaxi *et al.,* (2012) observed maximum casein degradation by *Lactobacillus paracasei* I-N-10 between 37 and 40°C. Ahmadova *et al.,* (2013) reported that the optimal temperature for casein hydrolysis by *Lactobacillus helveticus* A75 was at 37°C.

Effect of inhibitors

Figure 2 shows the effect of pefabloc, EDTA and iodoacetic acid inhibitors on the proteolytic activity of the investigated strains. In the presence of EDTA, the activity of proteinase totally disappeared for *Streptococcus thermophilus*; however it was stable in the presence of PEFABLOC and iodoacetic acid. From these results, we can conclude that *Streptococcus thermophilus* produces one type of proteases (metallo-proteases). On the other hand, the proteolytic activity of *Lactobacillus casei* was lost in the presence of PEFABLOC, EDTA and iodoacetic acid treatments. This means that *Lactobacillus casei* produces three types of proteases (metallo-, serine- and cysteine-

proteases). Our results are in agreement with those obtained by Pereira *et al.,* (2001); Del Papa *et al.,* (2007).

Fig. 2: SDS-PAGE profile (12 %) of Na-caseinate hydrolyzed by *Lactobacillus casei* and *Streptococcus thermophilus* after 24 h incubation at 37°C in the presence or absence of different inhibitors. Lanes 1 & 6, Na-caseinate without cells and inhibitors; lane 2, Na-caseinate with *Streptococcus thermophilus* and without inhibitors; lane 3, Na-caseinate with *Streptococcus thermophilus* and with Pefabloc; lane 4, Na-caseinate with *Streptococcus thermophilus* and with EDTA; lane 5, Na-caseinate with *Streptococcus thermophilus* and with iodoacetic acid; lane 7, Na-caseinate with *Lactobacillus casei* and without inhibitors; lane 8, Na-caseinate with *Lactobacillus casei* and with Pefabloc; lane 9, Na-caseinate with *Lactobacillus casei* and EDTA and lane 10, Na-caseinate with *Lactobacillus casei* and with iodoacetic acid.



However, they disagree with those obtained by Tsakalidou *et al.,* (1999) who found that the proteases of *Lactobacillus delbrueckii* subsp. *lactis* ACADC 178 were strongly inhibited by PMSF. However, they were slightly inhibited by EDTA in case of *Streptococcus thermophilus*. These differences were likely due to the differences in the studied strains. For *Lactobacillus casei* IM2, our result were in accordance with those of Stefanitsi*et al.,* (1995); Gilbert *et al.,* (1997); El-Ghaish *et al.,* (2010). All these studies support the presence of at least two types of proteinases at the cell surface of some lactobacilli.

3.6. Kareish cheese analysis

3.6.1. Kareish cheese yield

Table 1 shows yield of Kareish cheese patches (A, B and C) manufactured by different LAB strains as previously described. The control cheese (A) had the highest cheese yield as compared to

treatments B and C either when fresh (23.2, 20.1 and 21.5 %) or at the end of storage period (21.1, 17.9 and 19.5 %), respectively. This increase was associated with the increase in the moisture content of cheese A by 2.21 % and 1.01 % as compared to treatments B and C in fresh cheese, and 2.57 % and 0.68 % after 21 days of cold storage, respectively. Alnemr *et al.,* (2013); Awad *et al.,* (2015); Hamad, (2015) had found similar results. Alnemr *et al.,* (2013) found that the yield of Kareish cheese ranged from 18.0 to 30.7 %. Awad *et al.,* (2015) reported that the increase in Kareish cheese moisture was lead to increase in cheese yield. However, Hamad (2015) reported that Kareish cheese yield is affected by many factors such as milk composition, amount and genetic variants of casein, milk quality, milk pasteurization, coagulant type, vat design, curd firmness at cutting and manufacture parameters.

Table 1: Chemical composition and yield of Kareish cheeses manufactured with different strains of lactic acid bacteria and stored at refrigerator temperature (5 ± 2°C)

Time of storage (Days)	Cheese Treatments	Moisture %	Total solids %	Protein %	Protein / Dry mater %	SN %	Yield %
0	A	77.1 ± 0.51	22.9 ± 0.51	16.3 ± 0.04	71.4 ± 1.50	0.45 ± 0.01	23.2^{b} ± 0.23
	B	74.9 ± 0.77	25.1 ± 0.77	16.4 ± 0.03	65.4 ± 1.95	0.46± 0.01	20.1^{a} ± 0.79
	C	76.1 ± 0.26	23.9 ±0.26	16.5 ± 0.07	69.1 ± 0.87	0.48 ± 0.01	21.5^{ab} ± 0.44
7	A	73.5^{b} ± 0.54	26.5^{a} ± 0.54	16.0^{b} ± 0.07	60.4^{b} ± 1.22	0.74^{a} ± 0.01	22.3 ± 0.68
	B	69.6^{a} ± 0.34	30.4^{b} ± 0.34	15.4^{a} ± 0.05	50.7^{a} ± 0.58	0.77^{ab} ± 0.01	19.2 ± 0.67
	C	72.1^{ab} ± 1.06	27.9^{ab} ±1.06	15.4^{a} ± 0.03	55.5^{ab} ± 2.05	0.78^{b} ± 0.01	21.3 ± 1.10
14	A	70.7^{b} ± 0.96	29.3^{a} ± 0.69	15.6^{b} ± 0.04	53.2^{b} ± 1.57	0.98^{a} ± 0.01	21.5^{b} ± 0.32
	B	66.5^{a} ± 0.47	33.4^{b} ± 0.47	14.7^{a} ± 0.09	44.1^{a} ± 0.82	1.21^{b} ± 0.03	18.2^{a} ± 0.81
	C	67.9^{ab} ± 0.78	32.1^{ab} ±0.78	14.8^{a} ± 0.05	46.2^{a} ± 1.22	1.24^{b} ± 0.01	21.2^{b} ± 0.567
21	A	66.2^{b} ± 0.12	33.8^{a} ± 0.12	15.2^{b} ± 0.14	45.0^{b} ± 0.43	1.18^{a} ± 0.01	21.1^{c} ± 0.30
	B	63.7^{a} ± 0.32	36.3^{b} ± 0.32	14.0^{a} ± 0.22	38.5^{a} ± 0.41	1.71^{b} ± 0.01	17.9^{a} ± 0.40
	C	65.6^{b} ± 0.48	34.5^{a} ± 0.48	13.8^{a} ± 0.02	40.2^{a} ± 0.60	1.75^{c} ± 0.01	19.5^{b} ± 0.38

Data are mean ± SE for 3 replicates
$^{a, b \text{ and } c}$ Means with unlike superscripts are significantly different $p < 0.05$.
A: Kareish cheese made with *Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris* (Control)
B: Kareish cheese made with *Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris* and *Lactobacillus casei*
C: Kareish cheese made with *Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris* and *Streptococcus thermophilus*

Chemical analysis of Kareish cheese

Data in Table 1 shows some chemical analysis including moisture, total solids (TS), total protein (TP) and soluble nitrogen (SN) and data in Table 2 shows pH value and titratable acidity of Kareish cheese samples manufactured using different LAB strains. It is apparent from Table 1 and 2 that the acidity of treatment B was significantly related to its moisture content during storage period compared to the other cheese treatments. The highest cheese acidity 0.94 % and 2.49 %, the lowest cheese moisture 74.9 % and 63.7 % at zero time and after 21 days of cold storage, respectively. This might be

explained by the fact that the development of acidity leads to expel the whey from the curd (Effat *et al.,* 2001). Our data are different from those reported by Abd El-Tawab *et al.,* (1988) who found that the moisture content of Kareish cheese ranged from 62 % to 79 %. These differences might be attributed to the differences in the method of cheese manufacture and type of milk. Table 1 also shows that the moisture content of cheese is related to its total solids content, which gradually increased during storage period.

Cheese total protein content (TP %) shown in Table 1 was 16.3 %, 16.4 % and 16.5 % at zero time for treatments A, B and C, respectively, and decreased gradually to 15.2 %, 14.0 % and 13.8 % at the end of storage period (21 days) for cheese A, B and C, respectively. The reduction of total protein during storage period was accounted by 7.04, 14.82 and 16.22 % for cheeses A, B and C, respectively with significant differences after one week of storage. The same trend was found for the protein dry matter ratio in all cheese treatments during storage period (Table 1). Our results are in agreement with those obtained by El-Shafei *et al.,* (2008) who reported that the decrease in total protein content may be attributed to the loss of water soluble nitrogen compounds and protein degradation during storage period of Kareish cheese made with proteolytic LAB.

Data in Table 1 show also cheese soluble nitrogen (SN) content during storage period. Slight differences were found between the control cheese and other treatments after one week of storage. A gradual significant increase in SN was observed in cheese from all treatments until the end of storage, which was predominant in treatments B (1.71 %) and C (1.75 %) as compared to control (1.18 %) at the end of storage period. This increase might be attributed to the proteolysis of protein during storage. Our results are in accordance with those obtained by El-Shafei *et al.,* (2008) and Mahmoud *et al.,* (2013) who found similar trend and directly linked the increase of Kareish cheese SN to storage period.

Titratable acidity for treatments B and C slightly varied for the same period of storage when compared with treatment A (control). It increased gradually during all treatments when extending the period of storage (Table. 2). These results are in agreement with those described by Abou-Donia *et al.,* (1975) and Ayad *et al.,* (2004). Abou-Donia *et al.,* (1975) carried out a survey on the chemical composition of 26 samples of Kareish cheese collected from the local market at Alexandria city, Egypt. The obtained results (with range in parentheses) were as follows: fat, 3.37 % (1.00-11.50 %); salt, 3.94 % (0.23-7.11 %); moisture, 69.48 % (61.69-73.69 %); total protein, 19.96 % (9.70-28.34 %); soluble nitrogen, 0.22 % (0.07-0.58 %); amino nitrogen, 0.11 % (0.01-0.3 %) and titratable acidity 2.32 % (1.76-3.00

%). The variations in acidity might be attributed to the production season, type of starter, and manufacture processes.

Table 2: pH values and titratable acidity of Kareish cheeses manufactured with different strains of lactic acid bacteria and stored at refrigerator temperature (5 ± 2°C)

Time of storage (Days)	Cheese Treatments	pH	Acidity %
0	**A**	4.48 ± 0.25	0.90 ± 0.04
	B	4.15 ± 0.11	0.94 ± 0.04
	C	4.29 ± 0.11	0.92 ± 0.07
7	**A**	4.26 ± 0.05	1.48 ± 0.01
	B	4.15 ± 0.07	1.57 ± 0.04
	C	4.16 ± 0.05	1.53 ± 0.03
14	**A**	3.77 ± 0.10	1.96 ± 0.09
	B	3.65 ± 0.41	2.03 ± 0.03
	C	3.70 ± 0.09	1.99 ± 0.02
21	**A**	3.74 ± 0.04	2.36 ± 0.12
	B	3.62 ± 0.06	2.49 ± 0.06
	C	3.65 ± 0.30	2.41 ± 0.02

Data are mean ± SE for 3 replicates
A: Kareish cheese made with *Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris* (Control)
B: Kareish cheese made with *Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris* and *Lactobacilluss casei*
C: Kareish cheese made with *Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris* and *Streptococcus thermophilus*

Ayad *et al.,* (2004) found that the majority of lactococci strains showed a medium acidification but lactobacilli and streptococci strains had faster rate of acidification. The fast acidifying strains are good candidates in milk fermentation process as starter organisms. Table 2 shows opposite trend of pH values with cheese acidity. These results remain in agreement with those given by Abou-Donia *et al.,* (1975) who reported that the pH value of different Kareish cheese samples varied from 3.80 to 5.00.

Microbiological analysis of the manufactured cheese

Total Bacterial Count (TBC)

TBC in fresh and cold stored cheeses are shown in Table 3. No significant differences were found in fresh cheeses (15.2 x 10^4, 14.2 x 10^4 and 14.3 x 10^4, CFU/gram cheese for treatments A, B, and C, respectively. The increase of the storage period led to gradual TBC increase in all cheese treatments, however the increasing rate was less in treatments B and C as compared to control (treatment A). The control cheese (A) had higher TBC as compared to other treatments at the end of storage period (44.7 x 10^4, 35.0 x 10^4 and 32.4 x 10^4 CFU/gram cheese for treatments A, B, and C, respectively). The reduction in TBC for treatments B and C might be attributed to the proteolysis in protein that may produce antimicrobial active peptides or

producing antimicrobial components such as bacteriocin and H_2O_2 by some bacterial strain (s).

Table 3: Microbiological composition of Kareish cheeses manufactured with different strains of lactic acid bacteria and stored at refrigerator temperature (5 ± 2°C)

Time of storage (Days)	Cheese Treatments	Total Bacterial		Fungi and Yeasts	
		Colony Forming Unit	Log10	Colony Forming Unit	Log10
0	A	15.2×10^4	5.18 ± 0.02	1.05×10^2	2.02 ± 0.01
	B	14.2×10^4	5.16 ± 0.00	1.02×10^2	2.00 ± 0.02
	C	14.3×10^4	5.16 ± 0.00	0.95×10^2	1.98 ± 0.01
7	A	27.6×10^4	5.44^b ± 0.00	1.79×10^2	2.25 ± 0.04
	B	22.0×10^4	5.34^a ± 0.01	1.50×10^2	2.18 ± 0.01
	C	21.5×10^4	5.33^a ± 0.02	1.48×10^2	2.17 ± 0.01
14	A	38.1×10^4	5.50^b ± 0.01	4.45×10^2	2.65^b ± 0.02
	B	25.6×10^4	5.40^a ± 0.00	2.03×10^2	2.31^a ± 0.01
	C	23.9×10^4	5.38^a ± 0.01	1.81×10^2	2.26^a ± 0.03
21	A	44.7×10^4	5.65^c ± 0.01	6.07×10^2	2.78^c ± 0.02
	B	35.0×10^4	5.54^b ± 0.00	2.55×10^2	2.41^b ± 0.00
	C	32.4×10^4	5.51^a ± 0.00	2.35×10^2	2.37^a ± 0.01

Data are mean ± SE for 3 replicates

[a, b and c] Means with unlike superscripts are significantly different $p < 0.05$.

A: Kareish cheese made with *Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris* (Control)

B: Kareish cheese made with *Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris* and *Lactobacillus casei*

C: Kareish cheese made with *Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris* and *Streptococcus thermophilus*

Molds and Yeasts Count (MYC)

Table 3 shows cheese molds and yeasts counts (MYC) during storage period. Counts of MYC gradually increased with increasing storage period with no significant differences up to 2 weeks of storage. There was a gradual increase in all cheese treatments during storage with higher count in control cheese (A) than in the other cheese treatments (B and C). The MYC increased from 1.05×10^2 at zero time to 6.07×10^2 CFU/ gram cheese after 21 days of cold storage for control. Compared to control cheese, using of *Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *cremoris* and *Lactobacillus casei* in treatment B, and *Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *cremoris* and *Streptococcus thermophilus* in treatment C significantly reduced the count of molds and yeasts in cheeses throughout storage period. Increments in fungi and yeasts counts in treatment B was accounted by 1.02×10^2 at zero time and 2.55×10^2 CFU/ gram cheese after 21 days of storage. Same trend was found in treatment C that had 0.95×10^2 at zero time and 2.35×10^2 CFU/ gram cheese after 21 days of storage. The reduction in molds and yeasts counts for treatments B and C might be due to the proteolytic activity of the used strains that may produce antimicrobial active peptides or antimicrobial components such as bacteriocin and H_2O_2. Our data are

in agreement with those of Kaldes, (1997) who found an average count of molds and yeasts of 2.4 x 10^7 CFU/gram fresh Kareish cheese. These results might be related to *Streptococcus thermophilus* TA061 or *Lactobacillus helveticus* LH110 showing anti-fungal effects in cultured medium reported by (Ayana and Ibrahim, 2015) or to the results of Schwenniger and Meile, (2004) who reported that *Lactobacillus casei* had high anti-yeast properties.

Organoleptic properties

Organoleptically, the use of proteolytic LAB strains (*Streptococcus thermophilus* and *Lactobacillus casei*) with Kareish cheese starter improved the acceptability of Kareish cheese. Compared to control cheese (A), treatment C obtained the highest sensory points, followed by treatment B, either when fresh or during storage period (Table 4).

Table 4: Sensory evaluation of Kareish cheeses manufactured with different strains of lactic acid bacteria and stored at refrigerator temperature (5 ± 2°C)

Time of storage (Days)	Cheese treatments	Apparence 10	Body & Texture 30	Flavour 60	Total 100
0	A	$7.4^{a} \pm 0.22$	$25.3^{a} \pm 0.78$	$52.1^{a} \pm 2.08$	$84.8^{a} \pm 2.68$
	B	$8.5^{b} \pm 0.22$	$26.4^{ab} \pm 0.43$	$53.6^{ab} \pm 0.64$	$88.5^{ab} \pm 2.22$
	C	$8.1^{ab} \pm 0.33$	$27.3^{b} \pm 0.30$	$56.8^{b} \pm 0.44$	$92.2^{b} \pm 0.87$
7	A	$7.3^{a} \pm 0.21$	$25.8^{a} \pm 0.44$	$52.1^{a} \pm 1.35$	$85.2^{a} \pm 1.07$
	B	$9.1^{c} \pm 0.18$	$26.6^{a} \pm 0.40$	$55.9^{b} \pm 0.38$	$91.6^{b} \pm 1.61$
	C	$8.2^{b} \pm 0.29$	$28.7^{b} \pm 0.30$	$56.8^{b} \pm 0.94$	$93.7^{b} \pm 1.34$
14	A	$8.2^{a} \pm 0.25$	$26.4^{a} \pm 0.64$	$52.8^{a} \pm 1.47$	$87.4^{a} \pm 1.78$
	B	$9.3^{b} \pm 0.26$	$27.2^{a} \pm 0.42$	$55.9^{ab} \pm 0.38$	$92.4^{a} \pm 0.90$
	C	$8.2^{a} \pm 0.29$	$28.8^{b} \pm 0.20$	$57.2^{b} \pm 0.44$	$94.2^{b} \pm 0.69$
21	A	$6.8^{a} \pm 0.33$	25.5 ± 0.73	$49.5^{a} \pm 0.64$	$81.8^{a} \pm 2.22$
	B	$8.0^{b} \pm 0.30$	25.7 ± 0.54	$52.4^{a} \pm 1.65$	$86.1^{a} \pm 0.90$
	C	$8.0^{b} \pm 0.30$	27.2 ± 0.36	$56.8^{b} \pm 0.44$	$92.0^{b} \pm 0.39$

Data are mean ± SE for 3 replicates

[a, b and c] Means with unlike superscripts are significantly different $p < 0.05$.

A: Kareish cheese made with *Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris* (Control)

B: Kareish cheese made with *Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris* and *Lactobacillus casei* IM2

C: Kareish cheese made with *Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris* and *Streptococcus thermophilus* IM2

These results might be attributed to the proteolysis of protein that introduces some flavor enhancing components.

CONCLUSION

The use of isolated *Streptococcus thermophilus* and *Lactobacillus casei* as starter cultures in Kareish cheese production improved its quality and sensory acceptability. Consequently these starters can be recommended as a natural microflora accompanying other LAB starters in Kareish cheese industry.

REFERENCES

Abd El-Tawab, G., El-Din, A. G., El-Demerdash, O., Zedan, M., 1988. Chemical composition and nutritive value of Kareish cheese from buffalo skim-milk. Egypt. J. Dairy Sci. 16, 65-70.

Abou-Donia, S. A., 2002. Recent development in Ras cheese research: a review. Egypt. J. Dairy Sci. 30, 155-166.

Abou-Donia, S. A., Sirry, I., Abd El-Rahman, S. M., 1975. Chemical and microbiological studies on Kareish cheese. Alex. J. Agri. Res. 23, 234-247.

Ahmadova, A., El-Ghaish, S., Choiset, Y., Rabesona, H., Drouet, M., Chobert, J-M., Haertlé, T., 2013. Modification of IgE binding to β- and αs_1-caseins by proteolytic activity of *Lactobacillus helveticus* A75. J. Food Biochem. 37, 491-500.

Alnemr, T. M., Abd El-Razek, A. M., Hasan, H. M. A., Massoud, M. I., 2013. Improving of Kareish cheese by using enhanced technological texturizing inulin. Alex. J. Agri. Res. 58, 173-181.

A.O.A.C., 2000. Association of Official Agricultural Chemists. Official Methods of Analysis, 17th ed. Washington, DC: USA.

Awad, R. A., Salama, W. M., Ragb, W. A., 2015. Enhancing yield and acceptability of Kareish cheese made of reformulated milk. Ann. Agri. Sci. 60, 87-93.

Ayad, E. H. E., Nashat, S., El-Sadek, N., Metwaly, H., El-Soda, M., 2004. Selection of wild lactic acid bacteria isolated from traditional Egyptian dairy products according to production and technological criteria. Food Microbiol. 21, 715–725.

Ayana, I. A. A., Ibrahim, A. E., 2015. Attributes of low-fat yogurt and Kareish cheese made using exopolysaccharides producing lactic acid bacteria. Am. J. Food Technol. 10, 48-57.

Caplice, E., Fitzgerald, G. F., 1999. Food fermentations: role of microorganisms in food production and preservation. Int. J. Food Microbiol. 50, 131-149.

Clark, W. S., Brazis, Jr. A. R., Fowler, J. L., Johns, C. K., Nelson, F. E., 1978. Standard plate count method. Ch. 5 In: Standard Methods for the Examination of Dairy Products. Edited by E. H. Marth, 14th, American Public Health Association, DC, U.S.A.

Del Papa, M. F., Hancock, L. E., Thomas, V. C., Perego, M., 2007. Full activation of *Enterococcus faecalis* gelatinase by a C-terminal proteolytic cleavage. J. Bacteriol. 189, 8835–8843.

Delley, M., Mollet, B., Hottinger, H., 1990. DNA probe for *Lactobacillus delbrueckii.* Appl. Enviro. Microb. 56, 1967-1970.

Desouky, M. M., 2014. Oxidative storage stability of traditional butter oil produced from spontaneous fermentation of goats' milk. Egypt. J. Dairy Sci. 42, 71-80.

Effat, B. A., Salem, M. M. E., El-Shafei, K., 2001. Effect of using different starters on quality of Kareish cheese. Egypt. J. Food Sci. 29, 95-108.

El-Baradei, G., Delacroix-Buchet, A., Ogier, J.C., 2008. Bacterial biodiversity of traditional Zabady fermented milk. Int. J. Food Microbiol. 121, 295-301.

El-Ghaish, S., Dalgalarrondo, M., Choiset, Y., Sitohy, M., Ivanova, I., Haertlé, T., Chobert, J.-M., 2010. Characterization of a new isolate of *Lactobacillus fermentum* IFO 3956 from Egyptian Ras cheese with proteolytic activity. Eur. Food Res. Technol. 230, 635-643.

El-Ghaish, S., Rebesona, H., Choiset, Y., Sitohy, M., Haertlé, T., Chobert, J.-M., 2011. Proteolysis of *Lactobacillus fermentum* IFO 3956 from Egyptian milk products decrease α_{s1}-casein immune-reactivity. J. Dairy Res. 230, 635-643.

El-Shafei, K., Abd El-Gawad, M. A. M., Dabiza, N., Sharaf, O. M., Effat, B. A., 2008. A mixed culture of *Probionibacterium thoenii* P-127, *Lactobacillus rhamnosus* and *Lactobacillus plantarum* as protective cultures in kareish cheese. Pol. J. Food Nutr. Sci. 58, 433-441.

El-Soda, M., El-Ziney, M., Awad, S., Osman, G., Omran, N., Gamal, G., Ezzat, N., El-Shafei, H., 2003. A culture collection of lactic acid bacteria isolated from raw milk and traditional Egyptian dairy products. Egypt. J. Dairy Sci. 31, 23-41.

Fahmy, A. H., 1960. Manufacture of Kareish cheese. Ph.D. Thesis, University of Reading, England.

Fira, D., Kojic, M., Banina, A., Spasojevic, I., Strahinic, I.,Topisirovic, L., 2001. Characterization of cell envelope-associated proteinases of thermophilic lactobacilli. J. Appl. Microbiol. 90, 123–130.

Gilbert, C., Blanc, B., Frot-Coutez, J., Portalier, R., Atlan, D., 1997. Comparison of cell surface proteinase activities within the *Lactobacillus* genus. J. Dairy Res. 64, 561-571.

Hamad, M. N. F., 2015. Effect of adding glucono-δ-lactone, different starters, rennet on the chemical composition, yield and economic study of Kareish cheese. Int. J. Food Sci. Nut. Eng. 5, 130-140.

Kaldes, T. TH., 1997. Microbiological examination of soft cheeses manufactured in Minia city. Assiut Vet. Med. J. 38, 39-46.

Kunji, E. R., Mierau, I., Hagting, A., Poolman, B., Konings, W. N., 1996. The proteolytic systems of lactic acid bacteria. Anton. Leeuw. 70, 187-221.

Lück, H., Gavron, H., 1990. Quality control in the dairy industry. Ch. 7 In: Dairy Microbiology (the microbiology of milk products). Edited by R. K. Robinson, (1990). 2nd ed., Vol. 2, Elsevier Science Publishers, Ltd, U.K.

Mahmoud, S. F., El-Halmouch, Y., Montaser, M. M., 2013. Effect of probiotic bacteria on Kareish cheese production. Life Sci. J. 10, 1279-1284.

Nelson, A. T., Trout, G. M., 1981. Judging Dairy Products 4th ed. AVT Publishing Co. INC, Wesport. Connecticut, USA.

Olson, N. F., Anderson, R. F., Sellars, R., 1978. Microbiological methods for cheese and other cultured products. Ch. 12 In: Standard Methods for the Examination of Dairy Products. Edited by E. H. Marth, 14th, American Public Health Association, DC, U.S.A.

Ong, L., Henriksson, A., Shah, N. P., 2006. Development of probiotic cheddar cheese containing *Lactobacillus acidophilus*, *Lactobacillus casei, Lactobacillus paracasei* and *Bifidobacterium* spp. and the influence of these bacteria on proteolytic patterns and production of organic acid. Int. Dairy J. 16, 446-456.

Pereira, C. I., Barreto Crespo, M. T., San Romão, M. V., 2001. Evidence for proteolytic activity and biogenic amines production in *Lactobacillus curvatus* and *Lactobacillus homohiochii*. Int. J. Food Microbiol. 68, 211–216.

Pihlanto, A., Korhonen, H., 2003. Bioactive peptides and proteins. In Advances in Food and Nutrition Research, edited by S.L. Taylor. Elsevier Inc., San Diego, CA. pp. 175-276.

Russell, N. J., Gould, G. W., 1991. Food Preservatives. 1st ed., Blackie and Son Ltd., AVI., New York, USA.

Salminen, S., von Wright, A., Ouwehand, A., 2004. Lactic Acid Bacteria: Microbiological and Functional Aspects, 3rd Revised and Expanded Edition Edited by New York, Marcel Dekker.

Schwenniger, S. M., Meile, L., 2004. A mixed culture of *Propionobacterium jensenii* and *Lactobacillus paracasei* subsp. *paracasei* inhibits food spoilage yeasts. Syst. Appl. Microbiol. 27, 229-237.

Sfaxi, H. I., El-Ghaish, S., Ahmadova, A., Rabesona, H., Haertlé, T., Chobert, J-M., 2012. Characterization of new strain *Lactobacillus paracasei* I-N-10 with proteolytic activity: Potential role in decrease in □-casein immuno-reactivity. Eur. Food Res. Technol. 235, 447-455.

Statistical Package for Social Studies software (SPSS, 2007). Version 16.

Stefanitsi, D., Sakellaris, G., Garel, J.R., 1995. Presence of two proteinases associated with the cell wall of *Lactobacillus bulgaricus*. FEMS Microbiol. Lett. 128, 53–58.

Tsakalidou, E., Anastasiou, R., Vandenberghe, I., van Beeumen, J., Kalantzopoulos, G., 1999. Cell-wall-bound proteinase of *Lactobacillus delbrueckii* subsp. *lactis* ACA-DC 178: Characterization and specificity for □-casein. Appl. Environ. Microb. 65, 2035-2040.

Weisburg, W. G., Barns, S. M., Pelletier, D. A., Lane, D.J., 1991. 16S ribosomal DNA amplification for phylogenetic analysis. J. Bacteriol. 173, 697-703.

Xie, Y., An, H., Hao, Y., Qin, Q., Huang, Y., Luo, Y., Zhang, L., 2011. Characterization of an anti-*Listeria* bacteriocin produced by *Lactobacillus plantarum* LB-B1 isolated from koumiss, a

traditionally fermented dairy product from China. Food Control. 22, 1027-1031.

الملخص العربي

استخدام بكتيريا حامض اللاكتيك فى تحسين جودة الجبن القريش

شادى الغايش
***قسم الألبان ـ كلية الزراعة ـ جامعة كفرالشيخ.**

بكتيريا الـ *Lactobacillus casei* و *Streptococcus thermophilus* ذات نشاط بروتينى عالى. بكتيريا *Streptococcus thermophilus* تنتج بروتينيز معدنى بينما بكتيريا *Lactobacillus casei* تنتج العديد من انواع البروتينيز (معدنى ـ سيرين ـ سيستئين). البرتينيز الناتج من السلالات المعزولة من نوع P_{III} حيث يكون له القدرة على تحليل كل شقوق الكازين. أفضل اس ايدروجينى للتحلل البروتينى يتراوح ما بين 6,5 الى 7,2 وأفضل درجة حرارة تتراوح ما بين 37 الى 42 درجة مئوية. تم تصنيع ثلاث معاملات 1ـ المعاملة الأولى (باستخدام بادئ الجبن القريش فقط وهو *Lactococcus lactis* subsp. *lactis* و *Lactococcus lactis* subsp. *cremoris*) معاملة A 2ـ المعاملة الثانية (باستخدام بادئ الجبن القريش فقط وهو *Lactococcus lactis* subsp. *lactis* و *Lactococcus lactis* subsp. *cremoris*) مع بكتيريا *Lactobacillus casei* معاملة B 3ـ المعاملة الثالثة (باستخدام بادئ الجبن القريش فقط وهو *Lactococcus lactis* subsp. *lactis* و *Lactococcus lactis* subsp. *cremoris*) مع بكتيريا *Streptococcus thermophilus* معاملة C. بعد 21 يوما من التخزين البارد (5 ± 2 درجة مئوية) كانت المعاملة B أعلى حموضة (2.49٪) وجوامد صلبة كلية (36.34٪). ينخفض محتوى البروتين تدريجيا أثناء التخزين بينما كانت هناك زيادة تدريجية في محتوى النيتروجين الذائب في جميع المعاملات. التقييم الحسى للمعاملة B و C حصلت على اعلى الدرجات مقارنة بالكنترول.