The Antimicrobial Activity of Chitosan and Its Application on Kariesh Cheese Shelf Life

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ABSTRACT

Chitosan is a natural and non-toxic polysaccharide with many biological applications, largely known for its activity against a wide range of microorganisms, particularly as an antimicrobial agent. in the present study, antimicrobial properties of two kind of commercial chitosan (High (CS_H) and low (CS_L) molecular weight Chitosan) were evaluated, against *Staphylococcus aureus, Escherichia coli, Lactobacillus delbruekii spp. bulgaricus , and Lactococcus lactis spp. Cremoris* (LAB) as model bacteria besides isolated yeast culture from kariesh cheese surface in vitro was carried out using standard method (broth dilution method). The antimicrobial activities of chitosan were explored by calculation of the Minimum Inhibitory Concentration (MIC) for the tested culture in media supplemented with various concentrations lying between 0.0004% (4 µg/ml) and 0.02% (200 µg/ml). The results revealed that *E.coli* and *S. aureus* were more sensitive in CS_L (0.001, 0.0008% respectively) than in CS_H 0.006%. While the MIC of LAB strains (*Lb. bulgaricus* and *Lc. cremoris*) were higher than the previous strains (0.01% and 0.02%). Adding CS_L to the milk used in Kariesh cheese manufacturing by 0.03 and 0.05% level of CS_L which previously inoculated with *E. coli, S. aureus* and yeast showed a significant decreased in the log CFU/g of *E. coli, S. aureus* and yeast in cheese which slightly decreased during storage period at 4°C for 10 days compared with other treatment without chitosan. On other hand addition of chitosan led to keep survival of LAB during cheese making and storage at 4°C. It could be concluded that the chitosan concentration dependence antibacterial activity with variation against several pathogenic bacterial strains which indicates their possibility to be used as antimicrobial agents in dairy products.

kayword : chitosan , The antimicrobial activity , Kariesh cheese , MIC

INTRODUCTION

Chitosan is the second most abundant natural polymer after cellulose in the world; primarily composed of glucosamine and N-acetyl glucosamine residues with a 1, 4-β-linkage. Chitosan is considered as most promising materials for future applications on account of its excellent biodegradability, biocompatibility, antimicrobial activity, non-toxicity, and its economic advantages Ahmed, *et al.,* (2014).

The growing consumer demand for foods without chemical preservative has focused efforts in the discovery of new natural additives. Chitosan is one of the new generation food additives and has been accepted as potential foods preservation of natural origin (Devlieghere *et al.,* 2004). In this context, chitosan and its derivatives has been explored for its antimicrobial activity against clinical pathogen. Even though studies on antimicrobial activity of chitosan for food preservation are being done extensively, such reported investigation is considerably less for clinical applications.

Chitosan is a natural non-toxic biopolymer, which included to the GRAS (Generally Recognized as Safe) category by the FDA, is known to possess numerous technological and physiological properties useful in foods. In addition to its lack of toxicity and allergen city, its biocompatibility and bioactivity make it a very attractive substance for diverse application in food sing fields (Chien *et al.,* 2007; Kim *et al.,* 2007).

Chitosan has three types of reactive functional groups, an amino group as well as both primary and secondary hydroxyl groups at the C-2, C-3 and C-6 positions, respectively (Furusaki *et al*., 1996). Chemical modifications of these groups have provided numerous useful materials in different fields of application (Kurita, 1986).

Commercially, chitosan is found in a variety of sources such as crabs, shrimp, lobster etc., and usually is sold in powder or as flakes form. The main parameters which defines solubility and physic-chemical properties this polymer are the molecular weight and the degree of deacetylation.

Chitosan has gained significant attention and has been evaluated for numerous applications in the medical, food, agriculture and chemical industries. The applications of Chitosan to use as antimicrobial material for food have been widely reported in literatures. For example; dairy products (Evdokimov *et al.,* 2015) and (El. Diasty *et al.,* 2012). Much attention also has been focused on the safety and efficiency of chitosan from animal origin as a natural antimicrobial. Chitosan has several advantages over other types of natural

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antimicrobials, such as higher antimicrobial activity, broader spectrum of activity, higher killing rate and lower toxicity towards mammalian cells.

Despite that several different mechanisms for microbial inhibition by Chitosan have been proposed, the exact mechanism is still unknown. The most accepted one is the interaction of the positively charged Chitosan with the negatively charged residues at the cell surface of many yeast and bacteria such as *E.coli* and *S. aureus*, causing extensive cell surface alterations and altering cell permeability. This interaction would probably result in the leakage of intracellular substances (such as electrolytes, UV-absorbing material, proteins, amino acids, glucose, and lactate dehydrogenase) and thereby inhibiting the normal metabolism of microorganisms and finally leading to the death of these cells (Lim and Hudson, 2003).

Kariesh 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 may have some undesirable micro-organismes as its production of raw milk, which and because of the combination of the environmental conditions during storage reduce considerably its quality.

The objectives of this study were to determine the Minimal inhibitory concentration of chitosan on undesirable microbes including some pathogens and to examine the effect of adding it on the growth progressive of these cultures in Kariesh cheese during storage.

MATERIALS AND METHODS

Materials

1-Fresh Cow's milk was obtained from the herd Mutubis farm, Kafr El-Sheikh, Egypt.

2-Cultures; *Escherichia coli ATCC 23355*, *Staphylococcus aureus CIP 53154*, isolated yeast culture from Kariesh cheese surface, *Lactococcus lactis* subsp. *cremoris DK11* was obtained from Chr. Hansen's laboratory (Horsholm, Denmark) and *Lactobacillus delbrueckii ssp. bulgaricus LB4* was obtained from Succo srl via Manzoni29/a 22071 Cadorago Company, Italy.

3-Chitosan powder low molecular weight Chitosan from a shrimp shell (CS_L) and high molecular weight Chitosan (CS_H) from Crab Shells, is a linear copolymer composed of β (1 -4) -linked 2-acetamido- 2-deoxy-β-d-glucopyranose and 2-amino-2-deoxy-β- d-glucopyranose units provided by ROTH Bestellen Sie Zum Nulltarif, Germany. The molecular weight was $3.5x10^5$ for the high molecular weight Chitosan (CS_H) and $6.5x10^4$ for the low molecular weight Chitosan (CS_L). The deacetylation was 83% and 88% for the Chitosans of high and low molecular weights, respectively. Different concentrations of Chitosan solutions (10 to 25 $g \cdot L^{-1}$) were prepared in 1% acetic acid at pH 4.5.

Experimental procedures:

Cow's skim milk was pasteurization at 65±1?C for 30 min, and then cooled to 32±1?C. The treated milk inoculated with starter for Kariesh cheese, which were added in ratio 0.03% in milk at 32±1?C until curdling and considered it as a control for each treatment. The milk was divided into four groups each batch kept in Aluminum pots Fahmi (1960). Table (1) showed the groups of cheese were made;

Group 1; control treatment was made without Chitosan and tested microbes.

Table 1: Chitosan and clinic pathogen strains treatments a privation of Kariesh cheese experiments.

Groups	Tested strain	treatments	Chitosan %		
			0.0	0.03	0.05
Group 2	*E. coli*	E_0	+	-	-
		$E_{0.03}$	-	+	-
		$E_{0.05}$	-	-	+
Group 3	*S. aureus*	S_0	+	-	-
		$S_{0.03}$	-	+	
		$S_{0.05}$	-	-	+
Group 3	Yeast	Y_0	+	-	-
		$Y_{0.03}$	-	+	-
		$Y_{0.05}$	-	-	+

(+) Addition of Chitosan in the above mentioned level.
(-) Without addition of Chitosan in the above mentioned level.

The result cheeses were packed into plastic bags. All samples were stored at 4^oC and examined in the same day (zero time) and after 5 and 10 days.

Chemical analysis:

pH and titratable acidity were determined according to A.O.A.C (2003).

Microbiological analysis:

Lactic acid bacteria were determined for both *Lactobacillus delbruekii spp. bulgaricus* , *and Lactococcus lactis spp. Cremoris* on MRS plates according to the pour plate method, with overlay, after incubation at 32 °C for 2day as described by De Man *et al.* (1960). Yeast and mold were enumerated using Sabouraud agar as suggested by Harrigan and McCance (1990). *Escherichia coli* ATCC 23355 were determined on Violet red bile agar (V.R) and *Staphylococcus aureus* CIP 53154 was enumerated using Mannitol salt agar.

Determination of the Minimum Inhibitory Concentration (MIC)

The MIC of two type of commercial high and low molecular weight Chitosan were determined by standard methods (broth dilution method) according to Ramasamy *et al.,* (2013). In this method, a stock solution of 10 mg/ml (1% w/v acetic acid 1.0 % (v/v)) was prepared and Sterilized in autoclave at 121 °C/ 15 min. This was serially diluted to obtain various concentrations lying between 0.0004% (4 µg/ml) and 0.02% (400 µg/ml) 0.5 ml into sterile test tube containing 4.5 ml of MRS broth for LAB, neutral broth for *(E. coli,* yeast) and Brain heart broth for *S. aureus* supplemented with bromocresol purple as acidity indicator to the test tubes, 50 µl of test organisms previously adjusted to a concentration of 10^7 cells/ml was then introduced. A set of test tubes containing broth and inoculated with microbes without Chitosan were used as control. All the test tubes were then incubated at 37 °C for *E. coli and S. aureus,* 32°C for LAB and yeast for 24 h. The tubes were then studied for the visible signs of growth or change the indicator colour after the period of incubation. The lowest concentration of high molecular weight Chitosan and low molecular weight Chitosan that inhibited the growth of bacteria was considered as the Minimum Inhibitory Concentration.

Statistical analysis:

Data obtained from the experiments (in replicate) on the inhibitory effects of Low and high molecular weight chitosan, chemical analysis and (in duplicate) on microbial analysis were analyzed by one-way analysis of variance (ANOVA) using SPSS software (16 version) followed by Least significant difference test (LSD) and ±standard deviations. '*P*' values at <0.05 were considered for describing the significant levels.

RERSULTS AND DISCUSSION

As shown in table (2) the results revealed a variable inhibitory effect on all the tested strains. The MIC for *Escherichia coli ATCC 23355, Staphylococcus aureus CIP 53154, Lactococcus lactis* subsp. *cremoris DK11, Lactobacillus delbrueckii ssp. bulgaricus LB4* and isolated yeast culture by Low molecular weight Chitosan were , 0.001, 0.0008 , 0.02, 0.02 and 0.125%, respectively, while the corresponding MIC by high molecular weight Chitosan were 0.006, 0.006, 0.02, 0.01, and 0.15%, respectively.

According to the results CS_L showed more inhibition effect than CS_H at *Escherichia coli ATCC 23355, Staphylococcus aureus CIP 53154* and isolated yeast culture. The sensitive inhibitory for two type of chitosan were descending *Staphylococcus aureus CIP 53154, Escherichia coli ATCC 23355, Lactobacillus delbrueckii ssp. bulgaricus LB4, Lactococcus lactis* subsp. *cremoris DK11*and the isolated yeast culture.

Kathiresan and Nayak (2016), found that MIC of CS_L and CS_H for S. *aureus* and *E. coli* and stated that the Chitosan (322KD, DDA 85%) and phosphorylated Chitosan from the cuttlebone of *S. kobiensis* showed good antibacterial activity against almost all pathogenic bacteria and added that the antibacterial activity was found to be concentration dependent which was found to be 80µg/ml (0.008%) as MIC for Chitosan from *S. kobiensis* against bacterial strains such as *S. aureus*, *E. coli*. Furthermore, Chitosan from *S. lessoniana* (322KD, DDA 85%) reported the maximum inhibition against *S. aureus* at the highest concentration of 5 mg/ml and minimum inhibition against *K. pnemoniae* and *V. cholera* (Liu *et al.,* 2006). This result is agreed with the work of Jeon *et al.,* (2001) reported also the increased antibacterial activity with the increase in the concentration of Chitosan.

Table (2) demonstrated that the MIC values for *S. aureus* appeared to be lower than that measured for *E. coli,* as it was 0.0008% CS_L, while the MIC values for *E. coli* was 0.001% CS_L. The results of both bacteria are in complete agreement to several examples found in the literature such as Goy *et al.,* (2016) as they studied the antibacterial activity and showed that the Chitosan is consistently more active against the Gram-positive *S. aureus* than Gram-negative *E. coli*. Also these results were in agreement to those reported by Fujikawa and Morozumi, (2006) who studied the MIC of *S. aureus* and *E. coli*.

Several researchers reported that the low molecular weight Chitosan had more antibacterial effect than high molecular weight Chitosan for some microbial species; (Ortega-Ortiz *et al.,* 2010) also (Gerasimenko, *et al.,* 2004) reported that the antibacterial activity of CS_L on *S. aureus* was more effective than CS_H in inhibiting cell growth.

Raw milk contains several microorganisms reached to it during handling such as coliform bacteria, staphylococci, lactic acid bacteria, mold and yeast….etc. therefore this test was carried out to study the effect of adding Chitosan on the changes of microbial flora in raw milk during storage at 32°C by determine the progress of TTA% of raw milk free or with different level of CS_L.

As shown in table (3) adding CS_L by about 0.03 or 0.05% affected significantly on the progress of TTA% which found to be decreased in raw milk having CS_L either with 0.03% or 0.05%, for example, the TTA% of raw milk free of Chitosan after 20 hours of incubation, was decreased from 0.693% to 0.435% and 0.355% in the presence of 0.03% and 0.05% CS_L respectively.

Table 2. The minimal inhibition concentration of Chitosan on different microbial strains tested

	High molecular weight Chitosan (CS_H) and Low molecular weight Chitosan ((CSL)) %																							
	0.04		0.02		0.015		0.01		0.008		0.006		0.004		0.003		0.002		0.001		0.0008		0.0004	
Strain	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L
E.coli	+	+	+	+	+	+	+	+	+	+	¤	+	-	+	-	+	-	+	-	¤	-	-	-	-
S. aureus	+	+	+	+	+	+	+	+	+	+	¤	+	-	+	-	+	-	+	-	+	-	¤	-	-
Lb. bulgaricus	+	+	+	¤	+	-	¤	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lc. Cremoris	+	+	¤	¤	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

	High molecular weight Chitosan (CS_H) and Lowmolecular weight Chitosan ((CSL)) %															
	0.2		0.15		0.125		0.1		0.05		0.03		0.02		0.01	
	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L
Yeast	+	+	¤	-	-	¤	-	-	-	-	-	-	-	-	-	-

(+) inhibited for Chitosan or no growth; (-) resistant for Chitosan or growth in broth media and (¤) the minimal inhibition concentratio

Table 3. Effect of adding different levels of Chitosan on the running of Titratiable acidity in raw milk

Chitosan%	Titratiable acidity %				
	0 hr	8 hr	15 hr	20 hr	44 hr
0	0.2 ^{k} ±0.000	0.238 ^{jk} ±0.003	0.521 ^{cd} ±0.050	0.693 ^{c} ±0.064	0.885 ^{a} ±0.005
0.03	0.2 ^{k} ±0.000	0.22 ^{k} ±0.000	0.273 ^{ghi} ±0.023	0.435 ^{e} ±0.005	0.83 ^{a} ±0.030
0.05	0.203 ^{k} ±0.006	0.242 ^{hjk} ±0.010	0.265 ^{gj} ±0.005	0.355 ^{f} ±0.005	0.765 ^{b} ±0.005

[1]Mean values± standard deviations for TTA%, analyzed in replicate
$^{a,b,c,...,p}$ Means with the same superscripts are not significantly different ($P \leq 0.05$).

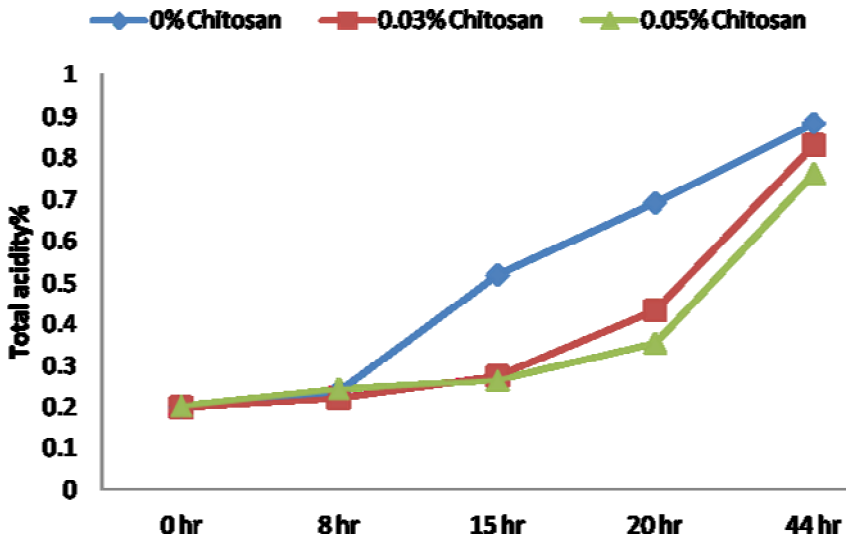

Fig. 1. Effect of adding different levels of Chitosan on the running of Titratiable acidity in raw milk

Table 4. Effect of adding Chitosan by different level to milk used in Kariesh cheese on the survival of *E.coli* and LAB during storage period

Media	Storage period (day)	Control*	Chitosan%**		
			0	0.03	0.05
V.R.B.A	0	2.493^{h} ±0.02	5.235^{c} ±0.272	3.995^{d} ±0.11	3.864^{de} ±0.085
	5	3.156^{fj} ±0.101	5.955^{b} ±0.01	3.468^{ef} ±0.052	3.395^{fj} ±0.052
	10	4.054^{d} ±0.392	6.961^{a} ±0.035	3.252^{fj} ±0.037	3.022^{j} ±0.061
MRS	0	8.418^{a} ±0.3	6.436^{d} ±0.315	7.14^{bc} ±0.1	7.612^{b} ±0.355
	5	7.643^{b} ±0.289	5.583^{cf} ±0.088	6.544^{cd} ±0.02	6.685^{cd} ±0.062
	10	7.589^{b} ±0.202	5.54^{f} ±0.074	6.263^{d} ±0.203	6.215^{de} ±0.201

[1]Mean values of log CFU/g ± standard deviations for Kariesh cheese, analyzed in duplicate.
^{a,b,c,d,e,f,j} Means with the same superscripts are not significantly different ($P \leq 0.05$).
(*) Means standard control without adding Chitosan and *E.coli*
(**) Means this treatment inoculated with *E.coli.*

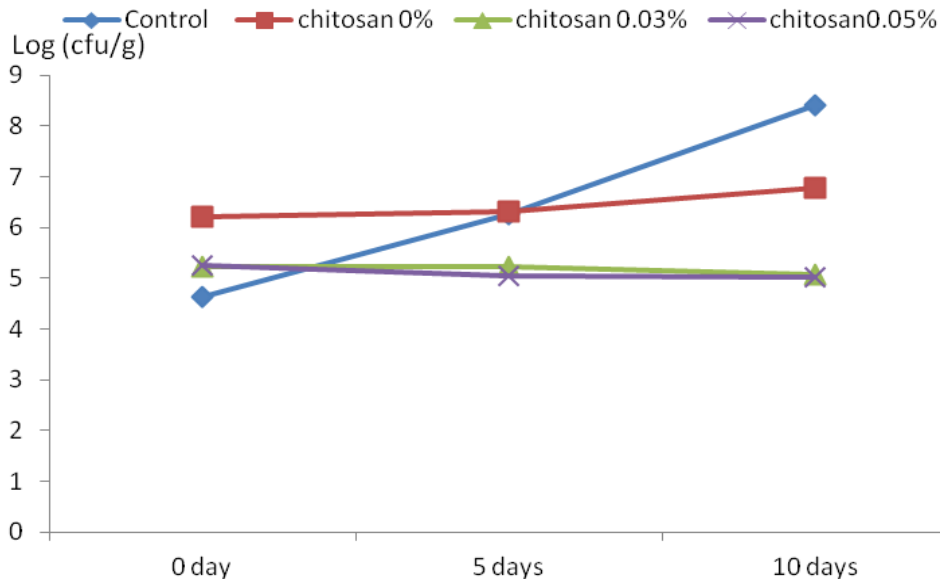

Fig. 2. Effect of adding Chitosan by different level to milk used in Kariesh cheese on the survival of E.coli during storage period

Table 5. Effect of adding Chitosan by different level to milk used in Kariesh cheese on the survival of *S. aureus* and LAB during storage period

Media	Storage period (day)	Control*	Chitosan%**		
			0	0.03	0.05
M.S.A	0	2.697^{bcd} ±0.107	3.462^{a} ±0.011	2.930^{b} ±0.047	2.733^{bc} ±0.220
	5	2.959^{b} ±0.057	3.525^{a} ±0.101	2.773^{bc} ±0.183	2.546^{cde} ±0.096
	10	3.556^{a} ±0.030	3.745^{a} ±0.032	2.442^{de} ±0.028	2.382^{e} ±0.009
MRS	0	8.418^{a} ±0.300	7.179^{c} ±0.062	7.454^{bc} ±0.365	7.978^{ab} ±0.004
	5	8.056^{ab} ±0.289	5.921^{d} ±0.505	6.467^{d} ±0.057	7.643^{bc} ±0.091
	10	8.018^{ab} ±0.202	5.004^{e} ±0.076	5.135^{e} ±0.019	7.589^{bc} 7.978^{ab}

[1]Mean values of log CFU/g ± standard deviations for Kariesh cheese, analyzed in duplicate.
[a,b,c,d,e] Means with the same superscripts are not significantly different ($P \leq 0.05$).
(*) Means standard control without adding Chitosan and S. *aureus*
(**) Means this treatment inoculated with *S. aureus.*

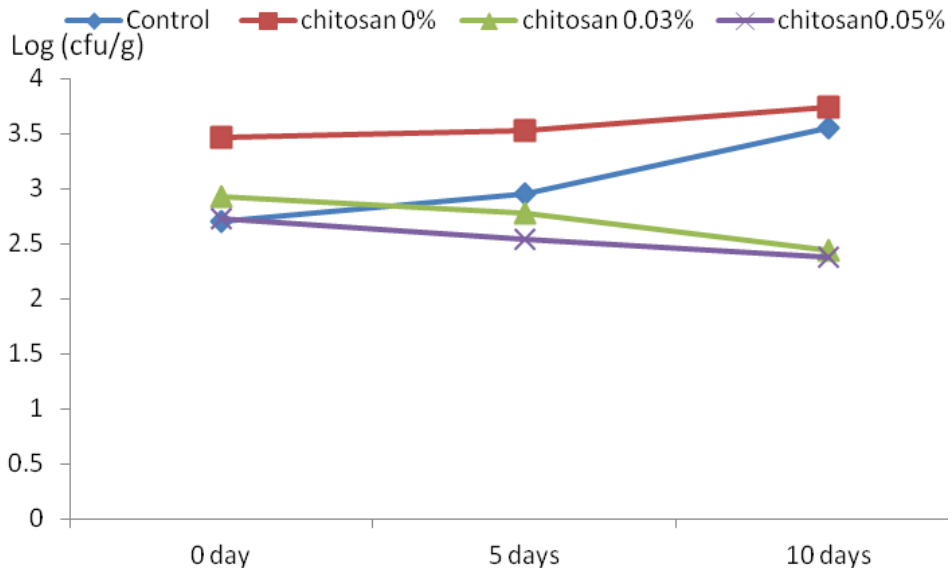

Fig. 3. Effect of adding Chitosan by different level to milk used in Kariesh cheese on the survival of *S. aureus* during storage period

Table 6. Effect of adding Chitosan by different level to milk used in Kariesh cheese on the survival of yeast and LAB during storage period

Media	Storage period (day)	Control*	Chitosan%**		
			0	0.03	0.05
Sabouraud	0	4.645^{c} ±0.212	6.221^{b} ±0.479	5.241^{c} ±0.177	5.246^{c} ±0.258
	5	6.265^{b} ±0.011	6.3175^{b} ±0.308	5.234^{c} ±0.047	5.062^{c} ±0.038
	10	6.788^{b} ±0.698	8.408^{a} ±0.151	5.074^{c} ±0.071	5.034^{c} ±0.010
MRS	0	8.418^{a} ±0.300	6.696^{e} ±0.324	6.655^{e} ±0.300	8.237^{ab} ±0.078
	5	7.589^{c} ±0.289	6.436^{ef} ±0.353	5.841^{f} ±0.089	6.693^{e} ±0.019
	10	7.643^{bc} ±0.202	6.943d^{e} ±0.087	7.267^{cd} ±0.073	7.303^{cd} ±0.099

[1]Mean values of log CFU/g ± standard deviations for Kariesh cheese, analyzed in duplicate.
^{a,b,c,d,e} Means with the same superscripts are not significantly different ($P \leq 0.05$).
(*) Means standard control without adding Chitosan and yeast
(**) Means this treatment inoculated with yeast.

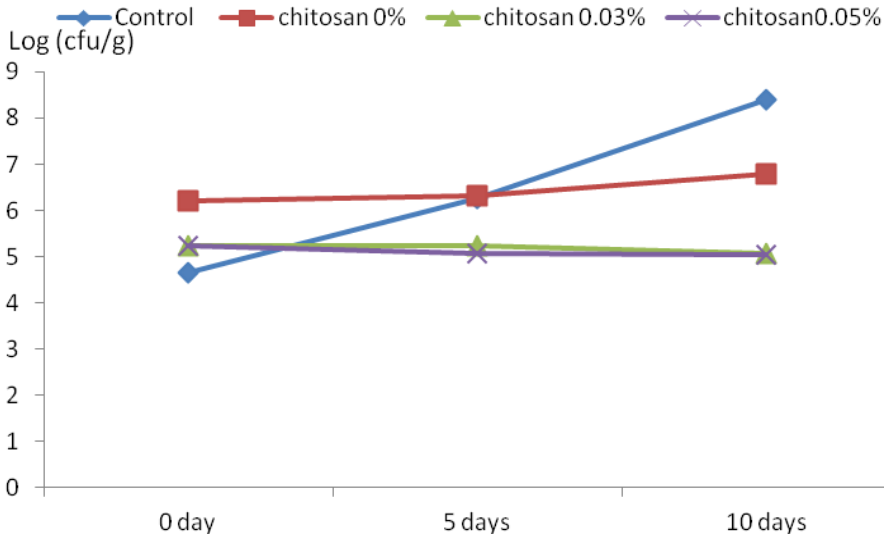

Fig. 4. Effect of adding Chitosan by different level to milk used in Kariesh cheese on the survival of yeast during storage period

This phenomenon was noticed also after the 15 and 44 hours, clearing the antibacterial effect of CS_L.

Va ´ sconez *et al,*. (2009) stated that Chitosan is nontoxic as a pharmaceutical, sensorial attributes like color, transparency, roughness or stickiness edibility, antagonistic properties against pathogenic micro-organisms, nonpolluting and low cost. For these reasons, they have attracted particular attention and have been considered in the food preservation.

Effect of adding Chitosan (CS_L) by different levels (0.03% and 0.05%) to milk used in Kariesh cheese making on the survival of *E. coli*, S. *aureus* yeast and LAB during storage period

The results recorded in table (4) and fig. (2) illustrated that addition of Chitosan by 0.03% or 0.05% significantly decreased log CFU/g of *E.coli* to (3.995 and 3.864) respectively, in fresh cheese, compared to the treatment without Chitosan (E_0) which was 5.235 log CFU/g. The same phenomena was noticed after 5 and 10 days which significantly decreased log CFU /g of *E.coli* to (3.468 and 3.395) and (3.252 and 3.022) respectively, in the treatments with Chitosan $E_{0.03}$, $E_{0.05}$, while E_0 significantly increased log CFU/g of *E.coli* comparing with the fresh Kariesh cheese to (5.955) and (6.961) respectively,.

Table (5) and Fig. (3) pointed out The treatment without Chitosan which inoculated with *S. aureus* (S_0) had the highest log CFU/g; (3.462), but when Chitosan was added by 0.03% or 0.05%, it were decreased to 2.930 and 2.733 log CFU/g.

Chitosan levels affected on *S. aureus* viability during storage i.e. The populations of *S. aureus* showed a slight decrease after 5 days of storage, the log CFU/g in $S_{0.03}$ and $S_{0.05}$ were (2.773 and 2.546) respectively, and this values were lower than S_0 that slightly increased after 5 days of storage at 4°C from (3.462) to (3.525) log CFU/g, respectively. Also slightly increased of S_0 in fresh Kariesh cheese which reached to (3.745) after10 days, while the treatments $S_{0.03}$ and $S_{0.05}$ were significantly decreased to (2.442 and 2.382 log CFU/g).These results indicated that the *S. aureus* viability in the $S_{0.05}$ treatment in Kariesh cheese was 1.5 folds lower S_0.

As shown in table (6) and fig. (4), it was clear that adding Chitosan by (0.03% and 0.05%) levels to Kariesh cheese which inoculated with yeast inhibited the survival of yeast, as it significantly decreased log CFU/g of yeast to (5.241 and 5.246), compared to the treatment without Chitosan (Y_0) which was 6.221 log CFU/g.

The results in the same table showed a slightly decrease after 5 and 10 days of storage, as the values of $Y_{0.03}$ and $Y_{0.05}$ were (5.234 and 5.062 log CFU/g) after 5 days which were lower than Y_0 during the period 5 and 10 days. In the case of Y_0 it were increased to (6.3175 log CFU/g) and (8.408 log CFU/g) after 5 and 10 days of storage, respectively.

Concerning the control which free of both Chitosan and the tested cultures, the log CFU/g on M.S.A were (2.697) in fresh cheese which increased to (2.959 and 3.556), respectively, after 5 and 10 days of storage. The corresponding values on V.R.B.A were (2.493) and (3.156 and 4.054), respectively, while on Sabouraud were (4.645) and (6.265 and 6.788), respectively. Predominant primary identified cultures from M.S.A

and V.R.B.A were negative to *S. aureus* and coliform bacteria. While, those group on Sabouraud were found to be yeasts.

Altieri *et al.,* (2005) recommended that Chitosan was effective in inhibiting the growth of spoilage microorganisms such as coliform and *Pseudomonas spp* which could be advantageously used to prolong the shelf life of Mozzarella cheese.

These results were similar to those reported by others; Chitosan could inhibit the growth of *E. coli* (Liu *et al*., (2006); Tipparat & Oraphan, (2008); Zhuang, *et al.,* 2004). Also similar results observed by (Goy et *al.,* 2016) they demonstrated the growth of *E. coli* and *S. aureus*, as measured by turbidity. Both bacteria grow in a similar way but with differences in turbidity. It is recorded an exponential increasing (log phase) at first, followed by the stable stationary phase. The log phase for *E. coli* appears to be longer than that measured for *S. aureus*. These results for both bacteria are in complete agreement to several examples found in the literature (Fujikawa and Morozumi, 2006). Also similar results observed by (Simpson *et al.,* 1997) they studied the antimicrobial effect of Chitosan from raw shrimp.

Shahidi *et al.,* (1999) demonstrated antimicrobial effects of water soluble Chitosan on different bacterial species, such as *E. coli*. Numerous studies have shown the effect of Chitosan on *Escherichia coli* and other *Enterobacteriaceae* at different concentrations and under different conditions: Ouattara *et al.,* (2000) demonstrated that the inhibition growth of *Enterobacteriaceae* was increased by Chitosan. Also these results are in agreement with ;Tsai *et al.,* (1999, 2000) reported about Chitosan activity against *Escherichia coli O157*.Agreeing data were published by other authors, such as Darmadji and Izumimoto, (1994),Wang (1992),and Simpson *et al.,* (1997), they tested a range of low doses of Chitosan (i.e., from 0.0075 to 1%), obtaining a noticeable effect against *Enterobacteriaceae.*

Gerasimenko, *et al*., (2004) studied the antimicrobial activity of Chitosan on *S. aureus* and found the same trend of results.

Goy *et al.,* (2016) indicated a higher antimicrobial activity of chitosan and his derivative (N, N, N-trimethyl Chitosan (TMC)) against the Gram-positive specie *S. aureus*. For this microorganism, all tested concentrations of TMC resulted in an inhibition as close as 50% over the bacterial colonies relative to growth as measured in the control culture medium. Also Kathiresan and Nayak (2016), showed that the antibacterial activity of Chitosan against tested pathogenic strains were found to be concentration dependent. This result is also consistent with the work of Jeon, *et al*., (2001) they reported the increased antibacterial activity with the increase in the concentration of Chitosan.

Similar results were observed by (El-Diasty *et al.,* 2012) indicated that treatment of Kariesh cheese with addition of Chitosan suppressed the yeast growth and prolonged the shelf-life. From the achieved results, it is clear that the addition of Chitosan at concentration of 1% is relatively more effective than 0.5% in suppressing the moulds and yeasts growth in Kariesh cheese. The mould and yeast counts detected in the control (non-treated) cheese were 5.35 log CFU/g of fresh cheese and increased during storage and reached to the highest level by the end of storage period. Adding Chitosan resulted in reduction in yeast populations.

The treatment of cheese with Chitosan lead to the inhibition and retardation of molds and yeasts growth and lowered the maximum growth levels in the cheese, as mentioned by (Sagoo, *et al.,* 2002), they reported a similar sensitivity to Chitosan for yeasts and molds; they concluded that the yeasts and molds counts in sausage dipped in 1.0% Chitosan were reduced approximately by 2 log CFU /g at the end of 18 storage days at 4^{o}C. Also, Duan *et al.,* (2007) mentioned yeast and mold increased to 10^{5} CFU/g in untreated Mozzarella cheese after 30 d storage. Bostan and Mahan, (2011) revealed that yeast and mold counts in sausage treated with Chitosan during cold storage were considerably lower than non-treated sausage at all sampling days.

Concerning The survival of LAB cultures in the Kariesh cheese mention previously the results in table (4, 5 and 6) revealed that adding Chitosan by different level to the milk inoculated with *E. coli, S. aureus* and yeast that used in Kariesh cheese manufacture on the survival of LAB; It is indicated that there was significant differences between the treatment without Chitosan and that inoculated with, *E. coli, S. aureus* and yeast to the treatments with Chitosan (0.03 and 0.05%) throughout the three tables. These results proved that Chitosan keep the survival of LAB, it affected on LAB viability and growth, as it increased log CFU/g compared to the treatment with Chitosan-free and inoculated by tested organisms. After the period of storage all the treatments with Chitosan and those which were Chitosan-free decreased in log CFU/g. Although all the treatments decreased during the storage, however, the reduction rate of log CFU/g to LAB during the storage was lower in the treatments with Chitosan than without Chitosan.

Altieri *et al.,* (2005) inferred that the presence of Chitosan does not affect the growth of lactic acid bacteria saving the functional dairy microbiota, and

Chitosan influenced neither cell viability and growth nor functional and technological properties. Also, Ouattara *et al.,* (2000) have verified that Chitosan had no or little effect on the numbers of lactic acid bacteria on the surface of Bologna or Pastrami after 21 d of storage at 4 or 10°C.

Effect of adding Chitosan by different level on the pH values and development of Total Titratiable Acidity in Kariesh cheese:

The results recorded in table 7, 8, and 9 demonstrated that adding Chitosan by different level had no significant differences of pH value in Kariesh cheese inoculated with *E.coli*, *S. aureus* and yeast. The treatments $CS_{0.03}$ of all tested organisms were lower than other treatments, while the treatment $CS_{0.05}$ was the highest. Throughout storage period there was a decrease in the pH values in all treatments.

The results also illustrate that adding Chitosan significantly increased the development of total titratiable acidity throughout the tables 7, 8, and 9. During the period of storage an increase was occurred to the development of total Titratiable acidity in all treatments.

Table 7. Effect of adding Chitosan by different level to Kariesh cheese inoculated with *E.coli* on the development of total Titratiable acidity and pH value during storage period

Media	Storage period (day)	Control*	Chitosan%**		
			0	0.03	0.05
pH	0	4.317^{a} ±0.049	4.240^{ab} ±0.010	4.160^{a-d} ±0.046	4.323^{a} ±0.075
	5	4.200^{abc} ±0.036	4.127^{bcd} ±0.031	4.023^{de} ±0.075	4.250^{ab} ±0.050
	10	4.127^{bcd} ±0.116	4.070^{cde} ±0.044	3.957^{e} ±0.040	4.040^{cde} ±0.026
Total acidity %	0	1.027^{e} ±0.015	1.037^{e} ±0.015	1.250^{d} ±0.050	1.367^{c} ±0.029
	5	1.077^{e} ±0.025	1.073^{e} ±0.028	1.383^{bc} ±0.029	1.510^{b} ±0.036
	10	1.273^{cd} ±0.068	1.223^{d} ±0.025	1.500^{b} ±0.100	1.650^{a} ±0.050

[1]Mean values of log CFU/g ± standard deviations for Kariesh cheese, analyzed in replicate.
^{a,b,c,d,e} Means with the same superscripts are not significantly different ($P \leq 0.05$).
(*) Means standard control without adding Chitosan and E.coli.
(**) Means this treatment inoculated with *E.coli.*

Table 8. Effect of adding Chitosan by different level to Kariesh cheese inoculated with *S. aureus* on the development of total titratable acidity and pH value during storage period

Media	Storage period (day)	Control*	Chitosan%**		
			0	0.03	0.05
pH	0	4.313^{ab} ±0.025	4.300^{ab} ±0.050	4.217^{abc} ±0.104	4.317^{a} ±0.049
	5	4.200^{abc} ±0.036	4.160^{abc} ±0.020	4.127^{abc} ±0.108	4.050^{bc} ±0.142
	10	4.127^{abc} ±0.116	4.060^{bc} ±0.017	4.017^{c} ±0.168	3.963^{c} ±0.100
Total acidity %	0	1.027^{e} ±0.015	1.043^{e} ±0.025	1.217^{cd} ±0.076	1.300^{b} ±0.050
	5	1.077^{e} ±0.025	1.120d^{e} ±0.030	1.220^{cd} ±0.010	1.417^{ab} ±0.015
	10	1.273^{c} ±0.068	1.227^{cd} ±0.015	1.443^{a} ±0.102	1.493^{a} ±0.051

[1]Mean values± standard deviations for Kariesh cheese, analyzed in duplicate.
^{a,b,c,d,e} Means with the same superscripts are not significantly different ($P \leq 0.05$).
(*) Means standard control without adding Chitosan and *S. aureus*
(**) Means this treatment inoculated with *S. aureus.*

Table 9. Effect of adding Chitosan by different level to Kariesh cheese inoculated with yeast on the development of total titratable acidity and pH value during storage period

Media	Storage period (day)	Control*	Chitosan%**		
			0	0.03	0.05
pH	0	4.260^{ab} ±0.085	4.247^{ab} ±0.050	4.223^{abc} ±0.075	4.317^{a} ±0.049
	5	4.200^{a-d} ±0.036	4.143^{a-e} ±0.049	4.033^{cde} ±0.081	4.063^{a-e} ±0.152
	10	4.127^{a-e} ±0.116	4.150^{a-e} ±0.070	3.997^{e} ±0.091	4.007^{de} ±0.090
Total acidity %	0	1.027^{d} ±0.015	1.083^{d} ±0.029	1.250^{c} ±0.076	1.283^{bc} ±0.050
	5	1.077^{d} ±0.025	1.083^{d} ±0.076	1.290^{bc} ±0.036	1.443^{ab} ±0.040
	10	1.273^{c} ±0.068	1.267^{c} ±0.090	1.360^{bc} ±0.036	1.583^{a} ±0.065

[1]Mean values± standard deviations for Kariesh cheese, analyzed in duplicate.
[a,b,c,d,e] Means with the same superscripts are not significantly different ($P \leq 0.05$).
(*) Means standard control without adding Chitosan and yeast
(**) Means this treatment inoculated with yeast.

These results are in accordance with those reported by (Evdokimov *et al.,* (2015) they studied the Chitosan-protein complexes formation, that the pH interaction was studied. The pH factor is very important for the implementation of many of intermolecular interactions, as it affects the ionization of certain functional groups of polymer compounds.

These studies is in complete agreement to several examples found in the literature (El-Sisi *et al.,* 2015) as they reported that there were changes in total titratiable acidity increased significantly ($P < 0.05$) with the extension of the ripening period. It is evident that acid content is one of the most important factors in determining Ras cheese flavor.

In conclusion, the present investigation revealed that the Chitosan (CS_L) inhibits the growth of all the tested undesirable micro-organisms in low MIC than CS_H which indicates that the Chitosan may contain a broad range of antibacterial activity. Additionally the present study brings out the possibility of using the peel shrimp (CS_L) as promising source for natural antimicrobial agent. The use of Chitosan can significantly inhibited pathogenic and some undesirable micro-organisms and increase the shelf life of dairy products.

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الملخص العربي

القدرة التثبيطية الميكروبية للكيتوزان وتطبيقها على إطالة مدة حفظ الجبن القريش

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يعتبر الكيتوزان منتج طبيعى من عديدات التسكر غير سام وله عدة تطبيقات بيولوجية والمعروفة بنشاطها التثبيطى لمجموعة واسعة من الكائنات الحية الدقيقة وبالتالى فهو يستخدم كعامل مضاد للميكروبات وقد استخدم فى هذه الدراسة نوعين من الكيتوزان عالى ومنخفض الوزن الجزيئى ضد مجموعة من الميكروبات الممرضة والمتلفة للمنتجات اللبنية بالإضافة الى بكتريا حامض اللاكتيك والتى شملت

Escherichia coli ATCC 23355, Staphylococcus aureus CIP 53154, Lactococcus lactis subsp. cremoris DK1,Lactobacillus delbrueckii ssp. Bulgaricus LB4.

بالإضافة الى سلالة خميرة عزلت من على سطح الجبن القريش. وقد قدر التأثير التثبيطى للكيتوزان عن طريق تقدير التركيز الأدنى للتثبيط للسلالات المختبرة لمستويات مختلفة من الكيتوزان ما بين ٠.٠٠٠٠٤% (٤ ميكروجرام|مل) و٠.٠٠٢%(٢٠٠ ميكروجرام|مل).

وقد أظهرت النتائج أن أقل تركيز مثبط من الكيتوزان منخفض الوزن الجزيئى لكلا من *E. coli, S. aureus* كان ٠.٠٠٠١% و ٠.٠٠٠٠٨% على التوالى بالمقارنة بالكيتوزان عالى الوزن الجزيئى والذى كان فى حدود ٠.٠٠٠٦% لكليهما، بينما كان التركيز الأدنى المثبط لسلالات بكتريا حامض اللاكتيك المختبرة

Lc. Cremoris, Lb. bulgaricus ٠.٠٠١% و ٠.٠٠٢% على التوالى مما يوضح أن الميكروبات الممرضة كانت أكثر حساسية للكيتوزان من بكتريا حامض اللاكتيك.

إشتمل الجزء الثانى على دراسة تأثير إضافة الكيتوزان منخفض الوزن الجزيئى للبن المستخدم فى صناعة الجبن القريش بتركيز ٠.٠٣% و ٠.٠٥% الذى سبق تلقيحه بكلا من *E. coli*أو *S. aureus* أوالخميرة. وقد أدت الإضافة الى نقص معنوى فى log CFU/g للـــ *E. coli, S. aureus* و الخميرة فى الجبن الناتج مع تناقص طفيف خلال فترة التخزين على ٤°م لمدة ١٠ أيام بالمقارنة بمعاملات الجبن الذى لم يضاف له كيتوزان.

ويمكن تلخيص النتائج فى أن الكيتوزان له تأثير تثبيطى لعديد من الميكروبات ذا العلاقة بصناعة الألبان وبذلك يمكن إستخدامة كمضاد ميكروبى لإطالة مدة حفظ المنتجات اللبنية.