Using of Chitosan as Antifungal Agent in Kariesh Cheese

El-Diasty, E.M.[1]; Nesreen, Z. Eleiwa[2] and Hoda, A. M. Aideia[3]

[1]Mycology Department, [2]Tanta Lab. and [3]Food Hygiene, Animal Health Research Institute, Dokki, Egypt

Abstract: In this study, the mycological quality and shelf-life of Kareish cheese treated with different concentrations of chitosan was investigated. Kareish cheese was treated with 0.5% and 1.0% chitosan solutions prepared with 1.0 % acetic acid. The samples were packed into plastic bags. All samples were stored at 4^0C and examined every 3 days until appearance of deteriorative changes. Kareish cheeses were evaluated for sensorial properties and mycological counts on days 0, 3, 6, 9, 12, 15, 18 and 21 of storage. On The chitosan treated cheese (o.5% and 1%) showed an improvement of shelf-life extended up to the 18^{th} day of storage. While in the control group of cheese, the changes of taste and texture were observed on the 6^{th} day while the changes in colour appear by the 9^{th} day. The moulds and yeasts counts ranged from 2.18 to 3.70 log cfu /g at the end of storage period in cheese samples treated with chitosan 1%, while in the control (non-treated) cheese was 3.40 log cfu /g of cheese at the 0 day of examination. This count increased during storage and reached to the high level (5.40 log cfu /g) by the end of storage period. The results indicated that the application of chitosan on the Kareish cheeses improves the mycological quality and extends the shelf-life, which could an alternative to chemical protective additives.

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Key words: Chitosan , kariesh cheese , fungi.

1. Introduction

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. Environmental conditions prevailing during storage, combined with the composition of the cheese often create possibilities for extensive development of mould on cheese surface, which reduces considerably its quality **(Reps *et al*., 2002)**.

Chitin is the second most abundant natural biopolymer after cellulose. The chemical structure of chitin is similar to that of cellulose with 2-acetamido-2-deoxy-b-d-glucose (NAG) monomers attached via β (1→ 4) linkages. Chitosan is the deacetylated (to varying degrees) form of chitin, which, unlike chitin, is soluble in acidic solutions. Application of chitinous products in foods and pharmaceuticals as well as processing aids has received considerable attention in last decades as exotic synthetic compounds are losing their appeal **(Shahidi, 1999)**.

The name `chitin' is derived from the Greek word`chiton', meaning a coat of mail **(Lower, 1984)** `, and was apparently first used by Bradconnot in 1811**(Skaugrud and Sargent, 1990)**. It is the second most abundant biopolymer on earth after cellulose and is a β (1 → 4) linked glycan, but is composed of 2-acet- amido-2-deoxy-β-D-glucose (N-acetyl glucosamine), one of the most abundant polysaccharides **(Lower, 1984)** named poly β (1→4)-2-acetamido-2-deoxy-d-glucose. Chitosan is the name used for low acetyl substituted forms of chitin and is composed primarily of glucosamine, 2- amino-2-deoxy-b-d-glucose, known as (1→4)-2-amino- 2-deoxy-(d-glucose (Fig. 1).

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)**.

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)**. Chitosan, adeacetylated derivative of chitin, is a linear copolymer composed of mainly D- glucosamine and some proportion of N-acetyl- D- glucosamine with β-1, 4- Linkage **(Rinaudo, 2006; Shahidi, 2007).**

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 allergenicity, 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).**

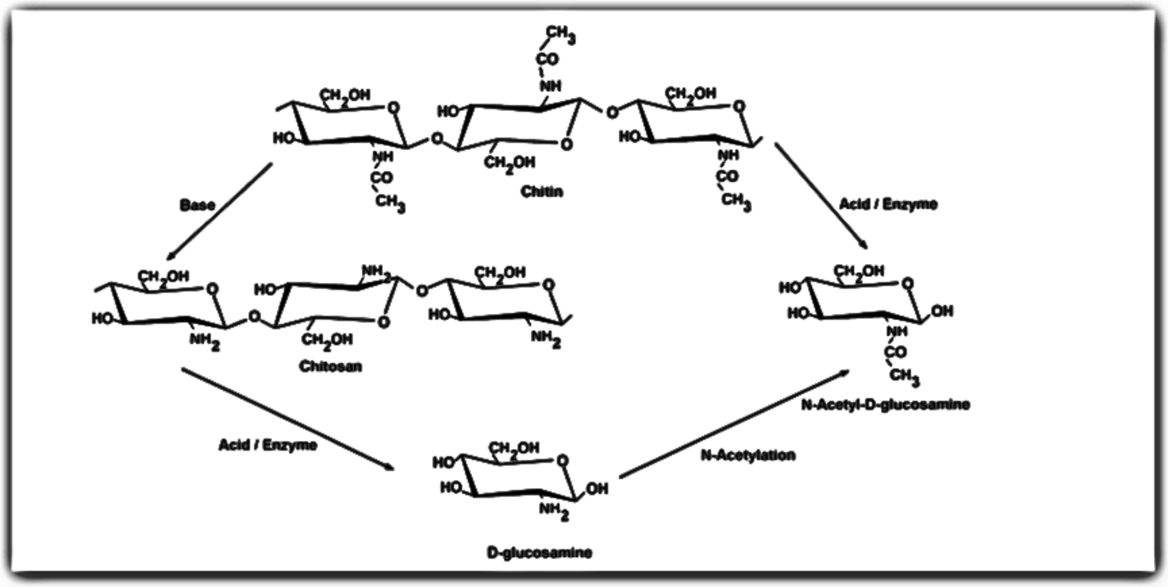

Fig.1. Preparation of Chitin derivatives from chitin.

Chitosan is antimicrobial against a wide range of target organisms. Activity varies considerable with the type of chitosan, the target organisms and the environment in which it is applied. Consequently literature reports vary somewhat and are, occasionally, contradictory. But generally speaking, yeast and moulds are most sensitive group, followed by gram-positive bacteria and finally gram-negative bacteria **(Peter, 1995)**.The possible mechanisms for chitosan´s antibacterial activity has been described by many scientists **(Papineau *et al*., 1991; Sudarshan *et al.*, 1992)**.For example, the reaction of positive charged chitosan with negative charged molecules at the bacterial cell surface may show an effect on cell permeability and a mechanism related to the binding of chitosan with bacterial DNA to inhibit RNA synthesis. The antibacterial and antifungal characteristics of chitosan and its derivatives have been effective in commercial disinfectants. Chitosan has several advantage over other types of disinfectants in that it possesses a higher antibacterial and antifungal activities, a broader spectrum of activity and a lower toxicity for mammalian cell **(Liu *et al*., 2001).**

Chitosan has gained significant attention and has been evaluated for numerous applications in the medical, food, agriculture and chemical industries. Dissolved chitosan has been used to coat grain seeds to increase the germination rate and provide resistance to plant pathogens **(Freapons, 1997),** as an antimicrobial additive (**Helander *et al.*, 2001; Zivanovic *et al.*, 2004).**For binding and recovery of proteins and metals from food processing waste water **(Pinotti *et al*., 1997)** and for preparation of wound healing sponges **(Ureno *et al*., 2001).** Due to its high molecular weight and solubility in an acidic aqueous solution, chitosan can form gels, films and fibers. However, antimicrobial and functional properties of chitosan's solutions and films depend on characteristics of chitosan molecule itself (degree of acetylation, molecular weight), other compounds in the system (type and concentration of acid, presence of proteins, lipids, ions and other food ingredients) and environmental conditions (<15% and molecular weight of 28 KDa or more have shown the strongest antimicrobial effects in aqueous solutions specially with acetic or formic acid-based films **(Begin and Van Calsteren,(1999)**.

The applications of Chitosan to use as antimicrobial material for food have been widely reported in literatures. For example, in fruit and vegetables **(Chien *et al.*, 2007;** seafood **(Tsai *et al.*, 2002; Lopez- Cabllero *et al.*, 2005)**, meat **(Sagoo *et al.*, 2002; Rao *et al.*, 2005)**, sausage **(Lin and Chao, 2001; Soultos *et al.*, 2008)** and dairy products **(Fernandez and Fox.1997)** .The addition of 0.01±0.016% chitosan to cheddar cheese whey at pH 4.5 almost completely removed the milk fat globule membrane fragments prior to ultra- filtration (**Hwang and Damodaran, 1995). Also Duan *et al.*, 2007** investigated the antimicrobial activities of chitosan-lysozyme (CL) composite films and coatings against tested microorganisms inoculated onto the surface of Mozzarella cheese .

The presence of such moulds and yeasts may cause spoilage of cheese by breaking down their components and liberating different acids and gases with subsequent changes of their odour and flavour **Pitt and Hoching (1997)**. Moreover, mould growth on cheese causes economic losses encompasses discolouration, poor appearance and off flavour.

This study was carried out to assess the feasibility of using different concentrations of chitosan in manufacture of Kareish cheese to extend the shelf-life and improved organoleptic quality

2. Material and Methods

Chitosan extracted from a shrimp shell was used (Sigma Aldrich. Low molecular weight (150,000) chitosan is 75-85 percent deacetylated. Stock solution of chitosan (1.0% w/v) was prepared in 1.0 %(v/v) acetic acid.

Fresh buffalo skim milk was obtained from dairy shops in Giza. The milk used for preparing Kareish cheese according to the method recommended by **Fahmi (1960)**.The milk was divided into three groups, each group kept in earthenware pots. Concerning the first group, the sample was made without chitosan treatment as a control. The second and third groups were mixed with 0.5% and 1.0% of chitosan in two replications. Resultant cheeses were packed into plastic bags. All samples were stored at 4^0C and examined every 3 days until appearance of deterioration.

Organoleptic score:

All Kareish cheese were judged organoleptically when fresh and every three days of refrigerated storage for flavor(50 points), texture(40 points) and colour (10 points) according to **Nelson and Trout(1951).**

Cheese analysis:

Preparation of cheese homogenate was conducted according to **APHA (2001).**

Mould and yeast counts according to FDA (2001):

Aseptically pippet 0.1 ml of each dilution on prepared poured solidified Dichloran rose bengal agar plates and spread inoculum with sterile bent glass rod. Plate each dilution in triplicate. Inoculated plates were incubated at 25^0C for 5 days. The first examination of plates was done after 3days incubation to determine the degree of yeast growth, and if large numbers are visible, a count was made and repeated on the 5^{th} day. The yeast and mould colonies were counted.

3. Results and Discussion

Protective food additives should not cause any undesirable sensorial changes to the product. In the present, the kareish cheese samples were examined organoleptically. The panelists who carried out the sensory evaluation detected no significant differences among the treated cheese with respect to the colour, consistency, flavor and odour.

The results recorded in table (1) determine the sensory evaluation carried out on kareish cheese treated with chitosan and stored at 4 ^{0}C during 0, 3, 6, 9, 12, 15, 18 and 21days of storage. It is evident that kareish cheese containing chitosan was significantly different from control one and was more acceptable. Regarding to the control group of cheese, the changes the taste and texture were observed on the 6 th day while the changes in colour appear by the 9^{th} day.

The degree of these changes increased gradually until the twenty one day of storage. On the other hand the chitosan –treated cheese (0.5% and 1%) showed an improvement of shelf-life extended up to the 18^{th} day of storage. Similar findings havebeen reported by **Purnama and Masathoshi (1996), Youn *et al*. (1999) and Bostan and Mahan (2011)** who reported that the application of chitosan on the sausage surface by dipping extends the shelf-life.

Table (1): Organoleptic properties of examined samples:

Time of storage (days)	Flavour (50 points)			Texture (40 points)			Colour (10 points)			Total points (100)		
	Chitosan concentration			Chitosan concentration			Chitosan concentration			Chitosan concentration		
	0%*	0.5%	1%	0%*	0.5%	1%	0%*	0.5%	1%	0%*	0.5%	1%
0	47	47	47	39	39	39	10	10	10	96	96	96
3	47	47	47	39	39	39	10	10	10	96	96	96
6	44	45	47	38	38	39	10	10	10	92	93	96
9	43	44	47	38	38	39	9	10	10	90	92	96
12	40	41	47	38	38	39	9	10	10	87	89	96
15	40	41	46	37	38	39	8	9	10	85	88	95
18	39	40	45	35	37	38	7	7	10	81	84	93
21	35	38	44	33	35	37	5	5	8	73	78	89

0% *= control

Regarding to the results recorded in table (2) and figure (2), the mould and yeast counts detected in the control (non-treated) cheese was 5.35 log cfu/g of cheese at the 0 day of examination. This count increased during storage and reached to the highest level (6.96 log cfu/g) by the end of storage period. The treatment of cheese with chitosan lead to the inhibition and retardation of moulds and yeasts growth and lowered the maximum growth levels in the cheese.

The moulds and yeasts counts ranged from the beginning 2.18 to 3.70 log cfu /g at the end of storage period in cheese samples treated with chitosan 1%, while in samples treated with chitosan 0.5% the count ranged from 2.30 - 4.36 log cfu /g. 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 kareish cheese. **Sagoo *et al.* (2002)** reported a similar sensitivity to chitosan for yeasts and moulds. They concluded that the yeasts and moulds counts in sausage dipped in 1.0% chitosan were reduced approximately by 2 log cfu /g at the end of 18 storage days at 4^0C. Also, **Duan *et al.* (2007)** mentioned Mold and yeast increased to 10^5 CFU/g in untreated Mozzarella cheese after 30 d storage. Growth of mold was completely inhibited in cheese packaged with CL films, while 0.24- to 1.90- and 0.06- to 0.50-log reductions in mold populations were observed in cheese packaged with CL-laminated films and coatings, respectively. All CL packaging applications resulted in 0.01- to 0.64-log reduction in yeast populations. **Nour *et al.* (2011)** reported that application of pure chitosan films reduced microbial counts on minced meat slices from 1 to 4 log. **Bostan and Mahan (2011)** revealed that yeast and mould counts in sausage treated with chitosan during cold storage were considerably lower than non-treated sausage at all sampling days. They mentioned that 1.0% concentration of chitosan was relatively more effective than 0.25% and 0.5% in suppressing the yeasts and moulds growth in sausage. Several mechanisms for antifungal action of chitosan have been proposed. For example , it has been suggested that chitosan may inhibits microbial growth by acting as a chelating agent rendering metals , trace elements or essential nutrients unavailable for the moulds and yeasts at normal rates. The growth rates of fungal hyphae have been shown to be sensitive to all factors which influence intracellular calcium ions, including variations in extracellular calcium concentrations and the presence of calcium transport inhibitors (**Jackson and Heath, 1993**). Therefore, it is conceivable that chitosan limits the growth of filamentous fungi indirectly by making calcium and other essential minerals and nutrients inaccessible. Several authors have proposed that the antimicrobial action of chitosan against filamentous fungi could be explained by a more direct disturbance of membrane performance (**Leuba and Stossel, 1986 and Muzzarelli, 1996).**

Table (2): Changes of moulds and yeast count (log CFU/g) of Kareish cheese with added chitosan during storage at 4^0C.

Storage time(day)	Moulds and Yeast count (log CFU/g)		
	0% conc.*	0.5% conc.	1.0% conc.
0	3.40	2.30	2.18
3	3.49	3.04	2.23
6	3.59	3.18	2.30
9	3.61	3.30	2.36
12	3.70	3.40	2.48
15	4.90	3.48	2.54
18	5.04	4.08	3.30
21	5.40	4.36	3.70

0% conc.*= control

Conclusion

The results obtained in this study indicate that treatment of Kareish cheese by addition of chitosan inhibits the mould and yeast growth and extends the shelf-life. Chitosan concentration of 0.5 % was sufficient in respect to the slowing down of mould and yeast growth, but higher concentration 1.0 % was needed for inhibition yeast and mould growth. We concluded that chitosan can be used as an alternative natural preservative in the Kareish cheese.

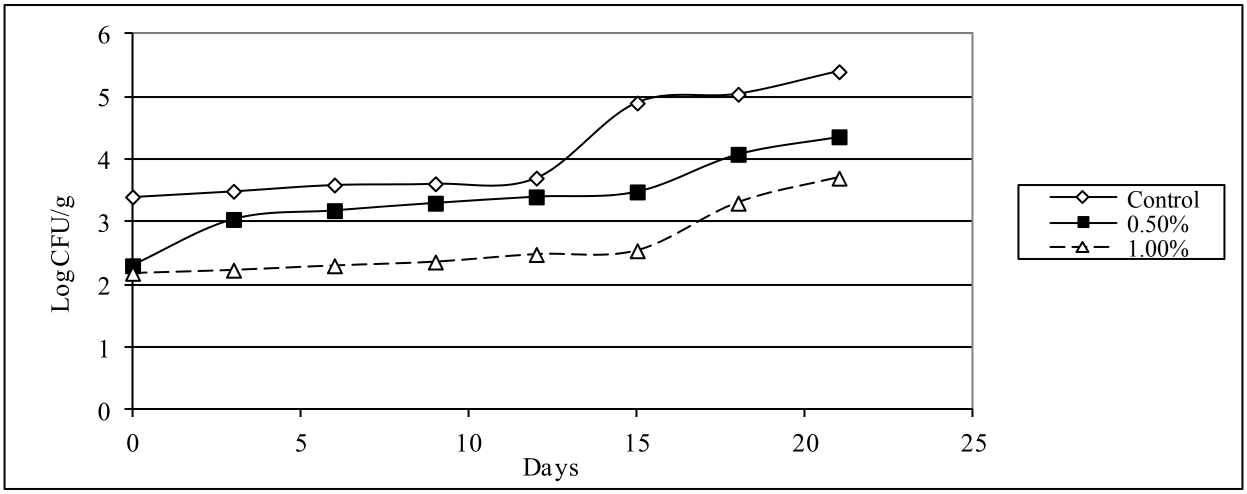

Fig. (2): Growth of mould and yeast in Kareish cheese stored at 4^0Cfor 21 days.

Corresponding author
El-Diasty, E.M.
Mycology Department, Animal Health Research Institute, Dokki, Egypt

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