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A Trial for Improvement of Kareish Cheese Quality by Using Chitosan Nanoparticles

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THE PRESENT study was undertaken to evaluate the addition of nano-chitosan to kareish cheese for improvement their characters, including odour, tastes, colors and mycological quality. Out of 40 samples of kareish cheese were obtainedr and mly from retail farmers and supermarkets from different district in Giza Governorate, all samples were contaminated with fungi; the mean count/g was ($1\times10^3\pm2.4$). The most common isolated mould species were *Aspergillus* spp. which isolated from (80%) meanwhile; all samples were contaminated with different yeast species. The residues of AFM_1 were detected in 42 %, and theaccurate quantitative detection was observed in immunoaffinity column method than thin layer chromatography. The results revealed that the addition or coating with nano-chitosan (0.25% and 0.5%) before or after manufacturing of kareish cheese could cause their prolonged safe preservation as the nano-chitosan have the antimicrobial potential against several bacteria and fungi. Also, the colour, odour and taste of coated or added CSNPs cheeses were more accepted and palatable good characters were observed for a longer time of storage. Hence, it is anticipated that chitosan nanoparticles have the potential of becoming a powerful and safe natural antifungal agent. Therefore, the present study may provide the possibility of the addition of CSNPs to coating kareish cheese manufacture for quality improvement and extending its shelf life.

Keywords: Milk, Kareish cheese, AFM_1, Chitosan nanoparticles, Coating, Antifungal.

Introduction

The contamination of milk and its dairy products with different species of fungi and mycotoxinscanbe occurred very widely constituting a public health hazard due to the progressive increase in the rates of consumption of these foods [1]. The fungal contamination of milk and milk products including mycotoxigenic fungi which produce mycotoxinsthat have health hazard for human. The growth of moulds is common and leads to change in colour, odour and flavour; consequently, contamination of these products leads to significant economic loss and this product become unfit for human consumption [2].

The global problem of mycotoxicosis, particularly in humid regions, including African countries are continuous of significant health hazard.Some moulds are capable of producing more than one mycotoxin, and more than one fungal species are able to produce some mycotoxins. Where as, they have dangerous effects upon consumers is ranging from acutely toxic toimmuno suppres siveorcarcinogenic

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impact to the liver [3,4]. Currently, they are hepatotoxic, nephrotoxic and immuno suppression for animals [5]. Mean while, reduced feed intake, decreased feed utilization, loss in weight gain, reduced reproductive capacity and even death may occur as a result of mycotoxicosis and leading to severe economic injuries [6]. Mycotoxins cancausevariety of shortterm as well aslong-termheal the ffects, ranging from immediate toxicres ponsetopotentiallong-termcarcinogenic and teratogenic effects [7,8].

On the other hand, milk is the most animal product exposed to mycotoxin contamination when dairy animal consumed contaminated feed with mycotoxins.The most prevalent mycotoxins in milk are aflatoxin M_1 which is a good indicator of the level of aflatoxin B_1 in the dairy feed. Cheeses as milk product cannot escape mycotoxins contamination, especially aflatoxins by its affinity to bind to milk protein casein [9]. Furthermore, the direct growths of toxigenic fungi on a rich nutrient source as milk favour the production of mycotoxinson and with in such commodities [10, 11].

Moreover, fungi are undesirable organisms which can grow at a wide range, causing spoilage, and are responsible for flavour defects, discolouration and reduced appearance of the product [12]. They were found as commensally in the gastrointestinal tract of human and animals. However, a wide variety of moulds have been involved in the infection of human, perhaps because of the intensive and prolonged use of antibiotics [13].

Up to date, nanotechnologyis employed in various fields including foodsystemssecurity,novel tools form olecular and cellularbiology, new materials for pathogendetection and protection of the environment and food industry and safety [14]. Also, the advances in the field of nanotechnology have been used in industry, medicine and the rapeutics and food packaging products [15]. Recently, chitosan nanoparticles (CSNPs) have been widely studied for the delivery of antibiotic drugs [16,17] and anti-cancer drugs, such as 5-fluorouracil, paclitaxel-doxorubicin, letrozole, and saponin[18, 19]. While, the antifungal potential of chitosan and chitosan nanoparticles (CSNPs) against *F. graminearum* colonies was detected and can be used asbiopesticides[20], growth inhibitor for *Rhizopus* sp. and *A. niger* [21].

Therefore, the present study was undertaken to recover the prevalence of fungi and aflatoxins in kareish cheese and evaluation the methods of detection of aflatoxins. Also, try toexplorethe role of chitosan nanoparticles in improvement the fungal quality and the physical properties of kareish cheese.

Material and Methods

Samples

A total of 40 collective kareish cheese samples about 250 g. of each were obtained from retail farmers and supermarkets from different district in Giza Governorate. The collected samples were transferred to the laboratory in clean, dry, sterile and tightly closed wide mouth Jars, with a minimum of delay, where they were prepared for the mycological examination and aflatoxins detection.

Skimmed raw milk: Fifteen kilogram of fresh buffalo's skimmed raw milk (0.5% fat) was obtained fromadairyfarm in Giza.

Chitosannanoparticles: It was obtained from the nanotechnology lab in Agriculture Research Centre (ARC), Giza, Egypt. The average particle size was 60-70 nm.

Isolation and Identification of Fungi in Cheese Samples

Enumeration andisolationof fungi according to ICMSF[22]:

Ten grams of each sample were transferred aseptically into a sterile blender jar, to which 90 ml of 1% peptone water were added and homogenized in a sterile warring blender for 2 minutes, and tenfold serial dilutions of the homogenate were prepared [22]. One millimeter quantities of the previously prepared serial dilutions were inoculated separately into Petri dish plates and mixed with Sabouraud dextrose agar medium. The plateswere left to solidify after mixing and incubated at 25°c for 3-5 days. The counts of mould and yeast colonies were recorded. Individual suspected colonies were selected depending upon their morphological characters. The stock culture was made from each isolate and monitored on CzapeksDox, malt extract and potato dextrose agar (PDA) slopes for further identification.

Identification of isolates

The colony count and identification of mould species was carried out by observation of macroscopic and microscopic characteristics of the fungi colonies according to ISO [23]

for genus Aspergillus and other genera were identified according to Pitt and Hocking [24] while the yeast isolates were identified according to the method of Van der Walt and Yarrow[25].

Detection of Aflatoxin M1 in cheese samples

Estimation of aflatoxin M_1 by thin-layer chromatography (T.L.C.) It was proceeded according to the method described byAOAC[26].

Estimation of aflatoxin M_1 by a fluorometric immunoaffinity method

The positive samples for aflatoxin M_1 in the TLC method were measured quantitatively by the fluorometric method using specific FGisAfla test standards according to the recommended method of AOAC [27].

Experimental evaluation of the efficiency of chitosan nanoparticle in improving the characters of Kareish cheese.

Preparation of Kareish cheese:Fifteen kilograms of skimmed raw buffalo's milk were divided into 5 parts (each of 3 kg.) and every part subjected to preparation of cheese as recommended byKorish and AbdElhamid[28]. Kareishcheeses were manufactured as follows: skim milk (0. 5% fat and 8.5% SNF) was heated at 74°C for 15sec. and then rapidly cooled to 40°C. At this point yogurt starter culture was added at level of 1.5% to the base mixture for coagulation. After complete coagulation, the curd was separately transferred into gauze for wheing off in 24 hr., then cut and stored in its pasteurized salted whey (7% salt) for 24 hr. Resultant cheeses were stored at 4°C.

Cheese coating with chitosan nanoparticles [29]

Prepared kareish cheeses were divided into five parts each one was about600 gram. In the first part, no additives were used and considered as control. While in the second and third parts, the used skim milk during manufacturing of cheese was immersed in nano-chitosan at the concentration of (0.25% and 0.5%). Whereas, in the fourth and fifth parts, after manufacturing of cheese, they were oatedwithchitosannanoparticles by immersion in different concentration of (0.25% and 0.5%) up to 2 minutes. All parts were coated in sterile caped containers and stored at 4°C and 50 grams were examined every two days until the appearance of deterioration.

Mycological analyses of treated cheese [30]

The samples were analyzed for moulds and yeasts using sabouraud dextrose agar (SDA) on the first day and every 48 hrs. Ten gram sample was taken aseptically from eachcheesegroup and diluted 1:10 in sterile distilled water, and the mixture was homogenized for 2 min at 9000 rpm (Stomacher, Seward Medical, and the UK). After serial dilutions in PBS solution, aliquots of 100 µg were inoculated in selective media in duplicate by pour plate method. The plates were incubated at 25 ± 2◦c for 2-5 days, to enumerate moulds and yeasts.

Evaluation of sensory properties of treated cheese with chitosan nanoparticles [31].

Kareish cheese samples of all batches about 40 gm were judged at zero-days and every 48 hours of refrigerated storage for taste, odour and colour which were evaluation. Taste, odour and colour were investigated on 10-point scale acceptability evaluation used a 10-point scale (1 = dislike very much, 5 = neither like nor dislike and 10 = like very much). Samples were presented in randomly coded glass plates.

Statistical analysis

Data obtained were analyzed statistically for descriptive statistics (mean, maximum, minimum and standard error) using SPSS 14 (2006)[32].

Results and Discussion

Occurrence of fungi in kareish cheese

Milk products as different types of cheese are most essential foodstuffs consumed by human and considered a source of high-quality animal protein having all the essential amino acids. It is also a rich source of calcium, phosphorus and many other micronutrients. All dairy products as cheese are probably most often spoiled by mould growth and may constitute public health hazards. While, Milk and milk products can quickly become moulded by a large number of species of fungi and a group of unicellular fungal organisms (yeast) during ripening, after cutting and slicing and during storage in shops or at home[28].

Recently, the role of microbes and their toxins in food safety gained colossal attention and high research activity. Not able to the prevalence of mycotoxigenic fungi and aflatoxins in food due to its toxicity, mutagenicity, teratogenicity and carcinogenicity [33].

In the present study, all examined kareish cheese samples were contaminated with fungi; the highest mean count cfu/g was ($1X10^3$±0.4). The most common isolated mould species in fresh kareish cheese samples were *Aspergillus*

spp, which isolated from (80%). Regarding the incidence of members of *Aspergillus* species in milk and its products, *A.ochraceus, A. candidus* and *A.flavus* were recovered (45%, 45% and 20%) of kareish cheese samples. All samples were incriminated different yeast species. Several studies revealed that recovered different species of fungi from raw milk which represented six genera of moulds. The most prevalent fungi in these samples were the genus *Aspergillus* (60) with the mean of the count of ($1.6 \times 10^2 \pm 0.1$)[34].

All examined kareish cheese samples were contaminated with yeast. The most predominant species of yeast was *C.albicans* which recovered (50%), followed by *Trichosporoncutaneum* (25%), *Cr.neoformans* and *Geotrichumcandidum* (12.5% for each) and *Torulopsis* sp. isolated from (7.5%) of kareish cheese samples, respectively.

In a similar study, [35] recovered several mouldsfrom samples of cheese made from ewe milk Spain. They were identified as *Penicillium* spp. in all cheese samples besides to other species, *Aspergillus, Geotrichum, Pullularia (Aureobasidium), Mucor, Paecilomyces, Candida* and *Acremonium*. However, Diaz et al. [36] isolated 24 strains of *Penicillium* and *Aspergillus* species from 22 samples of Spanish cheese and found that one strain only of *A. flavus* was toxigenic and capable of production of aflatox in M_1.

On the other hand**,** Hassan et al. [1] recovered members of genus *Aspergillus* spp. from fresh Kareish cheese, fresh Damietta cheese and Yoghurt samples at rates of (70%, 65% and 60%) respectively. The species of *A. flavus* was recovered from 30% of Yoghurt, 25% of fresh Damietta cheese and 15% of fresh Kareish cheese, followed by *A. niger* which was recovered at the rates of (25%, 15%, and 30%) respectively.

TABLE 1. Prevalence and statistical analytical results of fungi in kareish cheese.

Fungal isolates	Incidence of Fungi		Count (cfu/g)		
	No.+ve	%	Min.	Max.	Mean ± SE
Total fungi	40	100	$1X10^2$	$5X10^7$	$1X10^3$±0.4
Aspergillus sp.	32	80	1X10	$2X10^7$	$1X10^5$±0.9
A.flavus	8	20	$2X10^3$	$1.5X10^4$	$5X10^3$±0.99
A.ochraceus	18	45	$1.0X10^2$	$3X10^3$	$1.5X10^3$ ± 0.6
A.candidus	18	45	$2X10^1$	$2.2X10^4$	$2.5X10^2$±0.45
Yeast species	40	100	$2.4X10^2$	$1X10^4$	$3.7X10^3$±0.5

TABLE 2. Identification and statistical analytical of Yeasts that Recovered from Kareish Cheese.

Types of Yeast	Prevalence of yeast isolates		
	No. of +ve sample (40)	%	Count (cfu/g) Mean ± SE
Yeast species	40	100	$3.7X10^3$±0.5
Candida albicans	20	50	$1.0X10^2$±0.5
Trichosporoncutaneum	10	25	$1.5X10^1$±0.2
Cr. Neoformans	5	12.5	$3.0X10^2$±0.5
Geotrichumcandidum	5	12.5	$2.1X10^2$±0.5
Torulopsis	3	7.5	$1.1X10^2$±0.1
Total	43		

TABLE 3. Comparison between immune-affinity column and thin layer chromatography methods in detection of aflatoxins in kareish cheese.

Method of aflatoxin M_1 detection	Incidence of aflatoxin M_1 in samples		Levels of detected aflatoxins M_1 (PPb)(ug/kg)		
	No. of +ve	%	Min.	Max.	Mean ± SE
Immune-affinity column	16	42%	2.4	5.9	0.414.2±
Thin-layer chromatography	16	42%	1.1	2.5	1.7±0.11

*Food and Drug Administration (FDA) guidelines on the acceptable levels of aflatoxins in milk which has an action level of 0.5 ppb of aflatoxins.

Prevalence of aflatoxins inkareish cheese

In general, aflatoxins are known to be mutagenic, teratogenic, carcinogenic and immune suppressive in animals and possibly in man. This situation requires efficient analytical methods which are sensitive, quantitative and reliable to minimize the danger of these toxins through detection of the lowest quantities of Aflatoxin M_1 in food.

In the present study, the samples of kareish cheese were examined for detection of AFM1 by thin-layer chromatography and immunoaffinity column methods. The residues of AFM_1 in 42 % were detected by both methods, but the sensitivity for higher quantitative detection was observed in immunoaffinity column than thin layer chromatography where the detection levelranges was higher in fluorometric method (2.4 ppb-5.9 ppb) with the mean levels of (4.2 ppb±0.41). While, in case of thin-layer chromatographic method, the detected levels ranged from (1.1ppb - 2.5 ppb), with mean levels of (1.7ppb±0.11). Hence, the fluorometricimmunoaffinity column method ismore effectivelyusedfor the detection of AFs in food than thin layer chromatography.

So, we concluded that the fluorometric method is more efficient than thin layer chromatography in detection of mycotoxins as aflatoxins.

Aflatoxin M_1contamination of milk is considered as indicator of the level of aflatoxins B_1 and B_2 in feed,consequently cheese as milk product are able to be contaminated by their metabolic products M_1 and M_2, these findings are supported by many investigators [1,11,37, 38]. Several research studies detected aflatoxins B_1 and M_1 [39, 40] and aflatoxin M_1only in the examined cheese samples [41-44].

The high levels of AFM_1 in kareishcheese in the present work could be attributed to the high moisture content of cheese and caused the growth of moulds which may assist the formation of mycotoxins in cheese [45]. Also, kareish cheese showed the highest level of aflatoxins contamination which may be attributed to the bad handling and long duration of storage.

Herein, the detection of aflatoxins in food by immunoaffinity column method is more accurate,sensitiveand short time consuming than other old chromatographic methods. Hence in our study, the majority of kareish cheese samples had the aflatoxins levels more than 0.5 ppb and detected in high rate (42%), and this product was rejected and considered unfit for human consumption. Similar findings were detected by several authors [1,11,46] who identified the ability of the fluorometric method to detectthe lower levels of mycotoxins which not detected by the chromatographic method. Some researchers [47,48] established regulatory working guidelines on the acceptable levels of aflatoxins in milk which has an action level of 0.5 ppb of aflatoxins.

Hassan et al. [1] detected AFM_1 residues in fresh Kareish cheese over the permissible limits (0.5 ppb). Similarly, Ali, [11] detected AFs in milk and its products, the mean values of total aflatoxin residues (µg/kg) in fresh kareish cheese samples were (3.30 ± 2.21). The effects of aflatoxins in animal and human health variedac cording to the age, nutritional status, and amount of aflatoxins, frequency and composition of the diet [49]. The significant effects of Aflatoxins were mutagenic, carcinogenic, and teratogenic [50].

Therefore, numerous methods had been proposed to remove or inactivate microbial contamination in different types of food.

Influence of addition of Chitosan Nanoparticles to Kareish cheese during or after their preparations on survival of moulds and yeast count.

Recently, the nanomaterials are widely used in diversified sectors, including food systems. The Nano-sizedparticles have higher potential than their conventional sources, and thus they reduce the quantity required [51, 52]. They have more significant growth-promoting, immunomodulatory, antibacterial effects than conventional counterparts. The chitosan nanoparticle (CSNPs) have the amine groups change dunder weak acid conditionin to the– $NH3^+$ anion, which caninteract with the cell wall of bacteria and hinder the growth of the microorganism [53].

In the present study, the addition of CSNPs before or after manufacturing of kareish cheesecausedprolonged safe preservation of cheese as the CSNPs have the antimicrobial potential against several bacteria and fungi. Where the addition and coating of cheesewith CSNPs prevent the contamination and growth of yeasts for more extended period than the control groups which not coated with chitosannanoparticles (Table 4, Fig.1). When the added concentrations of CSNPs to kareish cheese increased the prolonged inhibition of fungal contamination and

colony count was decreased in comparison with the control cheese. Currently, the more antifungal potential was observed incase of addition of CSNPs after manufacturing of kareish cheese than in case of addition during ripening and preparation of kareish cheese. This is maybe due to the contamination may occur after addition of CSNPs during preparation, but the addition after preparation overcome mostfungal contamination. In general, both methods addition of CSNPs are efficient in the preservation and safe storage of kareish cheese for longer of time (Table 4, Fig.1).

TABLE 4. Influence of total fungi count (log cfu/g) after addition of Chitosan Nanoparticles to Kareish cheese during or after their preparations.

Prevalence of fungi in cheese (log cfu/g) before& after treatments with gradual concentrations of chitosan nanoparticles					Time of treatment(day)
0.5%		0.25%		0.0%	Time after treatments (days)
3rd gp***	2nd gp**	3rd gp ***	2nd gp **	1st gp*	
1.85	2.10	2.00	2.25	2.50	0
1.70	2.00	2.50	2.20	3.10	2
1.50	2.00	2.30	2.10	3.20	4
1.35	1.90	2.00	2.00	3.60	6
2.10	1.95	2.50	3.35	3.90	8
2.00	2.45	2.60	3.70	4.10	10
2.50	2.90	3.00	4.30	4.50	12
2.70	3.10	3.30	4.40	4.70	14
2.95	3.34	3.60	4.60	4.90	16
3.14	3.54	3.8	4.79	5.15	18
3.20	3.67	4.00	4.90	5.38	20

1st gp *= Control group, non-treated with CSNPs– 2nd gp **Second group (cheese treated during its preparation)-3rd gp ***Third group (cheese treated after preparation).

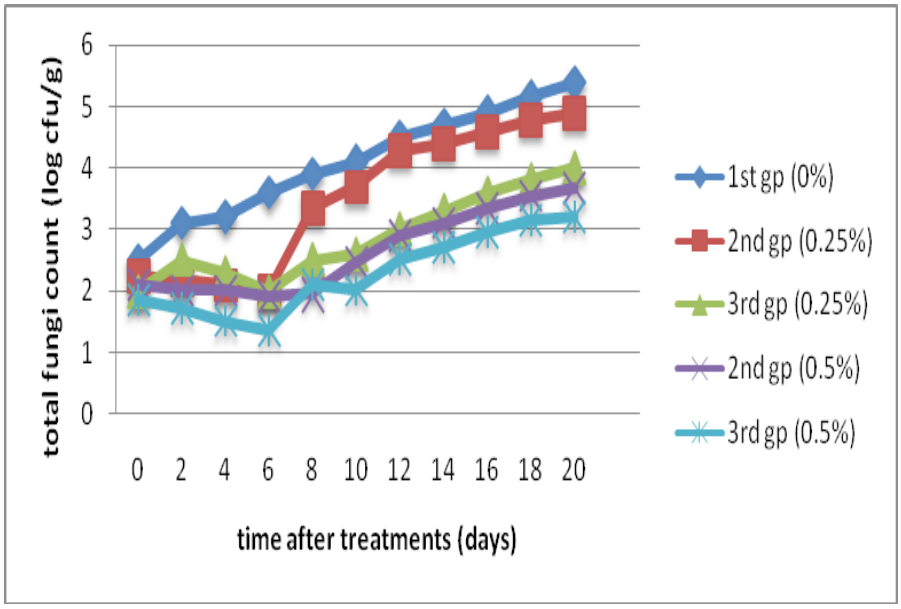

Fig. 1. Influence of total fungi count (log cfu/g) after addition of chitosan nanoparticles to Kareish cheese during & after their preparations.

*Control group, Non-treated with nano chitosan - **Second group (cheese treated during its preparation) - ***Third group (cheese treated after preparation)

In a similar study, the CSNP shave been used to improve the anti-bacterial effects [54]. Other research, detected that bio-nano composite of fish gelatin and chitosan nanoparticle have a good candidate for food packaging applications to the extent the shelf life of foods and products [55]. In addition, another research reported that CSNP sexhibit higher antibacterial activity than chitosan solution [56]. Furthermore, El-Diasty et al. [2] revealed that the moulds and yeasts counts in case of treatment of kareishcheese with 1% chitosan were lower than control untreated cheese in which the fungal count increased during storage and reached to the high level by increase of storage period.

Evaluation of sensory properties of treated cheese with chitosan nanoparticles.

Some species of moulds are used in manufacturing and ripening of certain varieties of cheese even though other moulds are responsible for certain flavour defects and surface discolouration of cheese. Furthermore, some of these fungi produce toxic metabolites (mycotoxins) which may penetrate the cheese and polishing its surface might not sufficiently remove them and affect the health of consumers [1, 57].

In the present study, the colours, taste and odour of kareisk cheese coated or added with CSNPs were improved more than 20 days and more accepted by the consumer, and palatable good characters were observed (Tables, 5, 6 and 7). Where, it is showed that the acceptability score evaluation was higher in treated cheese with CSNPs, mainly if coating occurred after the preparation of cheese and as the concentration of CSNPs increased up to 0.5 %, the prolonged preservation and acceptability increased more than 20 days. While in the control group of untreated kareish cheese, the sensory properties (colour, taste and odour) were changed and loss its acceptability by consumers. Nearly similar,[2] detected that the treated cheesewith chitosan (0.5% and 1%) showed an improvement of shelf-life extende dup to the 18th day of storage while in the control group of cheese, the changes of taste and texture were observed on the 6th day while the variations in colour appear by the 9th day.

From the obtained results, it becomes apparent that the addition of CSNPs to the Kareish cheese improves their preservation from fungal contamination changed their sensoryproperties to prolong their shelf-life. Moreover, CSNP scoating after manufacture is more efficient in the preservation of kareish cheese than its addition to the cheese during production.

In spite of the different benefits of the application of nanotechnology in food products, the mainfocus, especially regarding milk and products coating. Onthe other hand, lackof knowledgea bout the impact of the senano material sonhuman healthisa significant obstaclefornano technology application, especially in food. Hence, more development of specific methods and models fore valuation of the potential toxicity insomenano structure materials isneeded.

Therefore, extensive researches are still needed to support the effectiveness and safety of nano technology, avoid inganyharmto the environment orhuman beings and animals' health.

TABLE 5. Sensory colour property of Kareish cheese before and afteraddition of chitosan nanoparticles.

Time after treatments (days)	Changes in the colour of kareish cheese before and after treatments with gradual concentrations of Nano-chitosan				
	0.0%	0.25%		0.5%	
Colour	1stgp *	2ndgp **	3rdgp ***	2ndgp **	3rdgp ***
0	10	10	10	10	10
2	10	10	10	10	10
4	10	10	10	10	10
6	5	10	10	10	10
8	5	10	10	10	10
10	1	10	10	10	10
12	1	5	10	10	10
14	1	5	10	10	10
16	1	5	5	5	10
18	1	1	5	5	5
20	1	1	1	5	5

1stgp *Control group, Non-treated with nano chitosan - 2ndgp ** (skim raw milk treated during its preparation) - 3rdgp *** (cheese treated after preparation). The overall acceptability score evaluation was a 10-point scale (1 = dislike very much, 5 = neither like nor dislike and 10 = like very much).

TABLE 6. Sensory odour property of Kareish cheese before and after addition of chitosan nanoparticles.

Time after treatments (days)	Changes in the odour of kareish cheese before and after treatments with gradual concentrations of Nano-chitosan				
	0.0%	0.25%		0.5%	
Odour	1st gp *	2nd gp **	3rd gp ***	2nd gp **	3rd gp ***
0	10	10	10	10	10
2	10	10	10	10	10
4	10	10	10	10	10
6	10	10	10	10	10
8	5	10	10	10	10
10	1	10	10	10	10
12	1	5	10	10	10
14	1	5	10	10	10
16	1	5	10	`10	10
18	1	1	5	5	10
20	1	1	5	5	10

1st gp * Control, Non-treated with nano chitosan -2nd gp ** (skim raw milk treated during its preparation) - 3rd gp *** (cheese treated after preparation).The overall acceptability score evaluation was a 10-point scale (1 = dislike very much, 5 = neither like nor dislike and 10 = like very much).

TABLE 7. Sensory taste property of Kareish cheese before and afteraddition of chitosan nanoparticles.

Time after treatment(days)	Changes in the taste of kareish cheese before and after treatments with gradual concentrations of Nano-chitosan				
	0.0%	0.25%		0.5%	
Taste	1st gp *	2nd gp **	3rd gp ***	2nd gp **	3rd gp ***
0	10	10	10	10	10
2	10	10	10	10	10
4	10	10	10	10	10
6	5	10	10	10	10
8	1	7	10	10	10
10	1	7	10	10	10
12	1	5	10	10	10
14	1	5	10	10	10
16	1	5	8	5	10
18	1	1	5	5	10
20	1	1	5	5	10

1st gp *Control, non-treated with nano chitosan -2nd gp ** (skim raw milk treated during its preparation) - 3rd gp *** (cheese treated after preparation). The overall acceptability score evaluation was a 10-point scale (1 = dislike very much, 5 = neither like nor dislike and 10 = like very much).

Conclusion and Recommendations

Fungal contaminated milk products are widely distributed in nature and may lead to food poisoning which renders them unfit for consumption causing high economic losses, or they may be incriminated in mycotoxicosis to the consumers.

Under favourable environmental conditions (temperature, relative humidity, water activity, suitable medium and enough time), moulds can grow well and produce their mycotoxins particularly aflatoxins, which are considered to be one of the most potent threats to human beings.

Thus, special care should be taken with manufacturing and storage practices of milk products. This could be achieved by proper handling of milk product, good manufacturing and storage. Also, chitosan nanoparticle is an effective natural antifungal preservative against yeasts and moulds at very low concentrations.

Cheese treated with chitosan nanoparticles could extend the shelf-life of during refrigeration storage period which is desired by manufacturers and consumers.

Although specialattention is required for the toxicology of nanomaterials before nano technology application inthe food system, hence further studie sareurgently needed to addres stoxicologicalrisks of the application of nano technology in food technology to support the effectiveness and safety of nanotechnology, avoid inganyharmto the environment orhuman beings and animals' health.

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محاولة لتحسين جودة الجبن القريش بإستخدام جسيمات الشيتوزان النانوية

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قد أجريت هذه الدراسة لتقييم إضافة نانو الشيتوزان إلى الجبن القريش لتحسين مواصفاتها ، من ناحية الرائحة والطعم واللون والجودة الميكروبية. تم تجميع ٤٠ عينة من الجبن القريش من البائعين في الأسواق ومحلات السوبر ماركت من احياء مختلفة في محافظة الجيزة , كانت جميع العينات ملوثة بالفطريات. كان أعلى متوسط للعد (١ × ١٠^{٣}± ٢,٤) لكل جرام و أكثر الأنواع شيوعا هو فطر الأسبرجيللس تم عزله بنسبه ٨٠٪ و جميع العينات كانت ملوثة بأنواع مختلفة من الخمائر. و قد كان ٤٢٪ من العينات بها سموم الافلاتوكسين م١ وتم الكشف عن بقايا السموم بطريقه العمود المناعية التي كانت أفضل من طريقة الكروماتوجرافي. و لقد اتضح لنا ان اضافة او تغطية الجبن القريش قبل او بعد التصنيع بالنانو شيتوزان بنسبه (٠,٢٥٪ و ٠,٥٪) تسببت في الحفظ الآمن لفترة طويلة حيث ان النانو شيتوزان له القدرة المضادة للميكروبات ضد الكثير من الفطريات. و أيضا الخصائص الطبيعية للجبن المغلفة او المضاف اليها نانوشيتوزان من حيث اللون و الرائحة و الطعم كانت مقبولة و كذلك ادت الي إطالة مدة صلاحية الجبن القريش دون التأثير علي خواصها الفيزيائية و بالتالي فان هذه الدراسة توضح إمكانية تغليف الجبن القريش بالنانوشيتوزان حيث انها طبيعيه و قويه وآمنه و مضاد للفطريات لتحسين الجودة و زيادة مدة الصلاحية.