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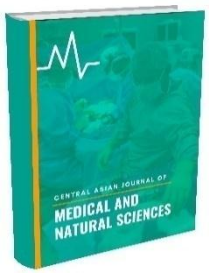

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INVESTIGATION OF THE EFFECT OF CHITOSAN NANOPARTICALS ON MDR BACILLUS CEREUS ISOLATED FROM PASTEURIZED MILK

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Abstract: The current study focused on extending the storage life of pasteurized milk by taking advantage of nanoparticles of the biopolymer known as chitosan, where the nanoparticles were synthesized based on a green technology, meaning that it does not have any negative harm to the synthetic material. The laboratory-synthesized nanoparticles were characterized by a size ranging from 100 to 140 nanometers when examined immediately after the anthesis and the size when examined two weeks after the anesthesia, the size was 115-220 nm, using a transmission electron microscope (TEM) of the type Philips CM10. By studying the inhibitory action of the nanoparticles in the Muller-Hinton agar culture medium, it was found that the synthesized particles have the ability to combat the pathogenic Bacillus cereus bacteria that form blackboards when using different concentrations (0. 25 - 0. 5 - 1%), where it was found that the best diameter of the contrast corona was (14-15 mm at the two concentrations (0. 5) (1) sequentially When testing the biological validity of pasteurized milk at a temperature of 5 semen for a period of -1-15-30 days of storage and after adding nano-chitosan using the three aforementioned concentrations, it was found that the number of B. cereus It was varied as it reached on the first day when using the three concentrations 10 FU/ml 1 x, but on the fifteenth day the numbers were as follows 10 CFU/ml 2 x 10 -3 x 10 - 5 x and according to the concentrations (0. 25) - 0. 5 - 1% On the thirtieth day, the bacterial numbers decreased in pasteurized milk fortified with nanoparticles of chitosan, and the result was as follows (10 CFU/mL (10) - 10 - 2 x relative to the three concentrations, respectively). This result was an indication of the effectiveness of nanoparticles of chitosan in preventing cell growth. vegetation and the formation of new spores compared with

the number of Bacillus cereus, which is approximately 107 CFU/ml x when pasteurized milk is stored for 15 days at a temperature of 5°C. It indicates contamination of milk and its products. It has been observed that increasing the concentration of added nano-chitosan increases the antimicrobial activity.

Key words: Nanoparticles, caritosan, pasteurized milk.

Introduction

Technological and cognitive development in the twenty-first century helped produce modern sciences, one of which was nanotechnology, which is one of the many technologies that have begun to take a large part in recent periods, and this technology has proven its importance in various fields such as medicine, agriculture, food, the environment, and many other applications. in other fields. Nanotechnology involves the study and synthesis of materials in dimensions ranging from(1 to 100) nanometers, where these nanostructured materials are characterized by different characteristics than their natural sizes, that is, before nanosynthesis processes, by increasing the surface area relative to the size (1). In order to produce nanomaterials, there is a set of methods used worldwide, some of which include thermal decomposition, chemical evaporation deposition, laser pyrolysis, and others. Many of these methods are still considered economically costly and take a long time. (2, 3, 4). As a result of the extreme importance of nanotechnology, it has become a challenge for many scientists in terms of how to apply it to food, as recent studies focused on the functional aspects of nanoparticles and tried to employ them in raising the nutritional value and preserving and safety of food. One of the first nanomaterials used in the field of food was chitosan, which is a derivative of chitin and is a type of polymer derived from the cellular components of living organisms (animals, plants, microorganisms, which are included in the structure of living cell walls, especially crustaceans and fungi (5).

Interest has increased in the recent period in chitosan, as it is one of the renewable polymeric materials that has been widely spread in various applications, for example in the food industry and in the pharmaceutical and medical industries, and there are many other uses for chitosan in various fields (6, 7). We also find in a previous study that confirmed that When NATO technology was applied to chitosan, the resulting chitosan improved its physical and chemical properties, such as increasing its water solubility, antimicrobial activity, and other sensory properties that are of importance in the food industry and preservation (8). The research aims to study the possibility of particle synthesis Nanoparticles of chitosan and its employment as a preservative for milk or its products through its inhibition of many microorganisms. It was found that the most technical problems facing the milk industry and its products is the shelf life of raw milk, as it was found that during the transportation of raw milk in refrigerated tanks at less than 7 ° C may occur Contamination with a high percentage of pathological microorganisms that make up blackboards, specifically those called psychrotrophic microbes (9), where damage to milk can occur at any manufacturing stage in the event that it is not kept in appropriate temperatures even after it has been subjected to the (pasteurization) process), it was found that storing pasteurized milk in the refrigerator for a period exceeding fourteen days encourages the growth of bacteria that form vegetative cells, which are capable of growing at temperatures less than 7 degrees Celsius, and which resist the pasteurization process, the most famous of which is the bacterial strain Bacillus (10). for a

period exceeding fourteen days encourages the growth of bacteria that form vegetative cells, which are capable of growing at temperatures less than 7 degrees Celsius, and which resist the pasteurization process, the most famous of which is the bacterial strain Bacillus (10).

Characteristics and characterization of chitosan To diagnose chitosan, there is more than one method, such as TEM FTIR, Isa et al. , 2012; Sandra et al. (2014) The purpose of employing the transmission electron microscope (TEM) technique is to characterize the external shape of the nanoparticle by studying the external appearance and size, which is represented by the use of a beam of electrons passing through the sample, which is magnified and focused by an objective lens (electrostatic). or electromagnetic), then the results of the radiation generated due to the resulting potential difference are displayed on an imaging screen (13)

As for the technique of infrared spectroscopy (FTIR), through which chitosan is diagnosed on the basis of each of the 1- chemical composition, molecular level, bonding and arrangement of components in the coherent biopolymer (14). Materials can be identified using an infrared spectrometer with a frequency often in the range of 400 - 4000 cm - (15). The FTIR technique is one of the important and easy techniques in diagnosing the presence of some functional groups in molecules, and it has many advantages as it is a fast method and is not similar to other spectroscopic methods and does not require that the sample be pure and the sample does not need to be dissolved in any solvent to perform the examination, and this technique was also used to determine The purity of the compounds and the detection of pollutants present with those particles (16)

The applications of chitosan are the presence of the positive charge in chitosan due to the presence of free amino groups attached to the carbon atom No. 2, which makes it easily soluble in organic acids such as acetic acid, and then it also contributes to making it a chelating agent that possesses biological and chemical properties that make it enter into many applications Including various food, medical and industrial

MaterIals and Methods

The materials and devices used in the research are: (HCL, Agar, Peptone, Yeast extract, Ethanol, NaCl, Tungstophsphoric acid, Tripolyphospate TPP, CH3COOH, Aceton, NaOH) and devices used she (Balance, Autoclave, Vacuum Drying Oven, vortex, Centrifuge bench type, PH meter, water bath, Heating Magnetic Stirrer, Incubator, Refrigerator, Microscope, FTIR -600 Fourier Transform, Infrared spectrophotometer, TEM, Nylon syringe 0. 22um mesh, DTD Ultrasonic Cleaner, UV/vis spectrophotometer). The culture media used are (Nutrient Broth, Nutrient Agar, Muller-Hinton Agar, Chromogenic Agar).

Where nanoscale chitosan is synthesized, 85% dehydrated chitosan with a molecular weight of 3783 kDa was purchased from (Chemical Point. Co). % volume / volume) of the acetic acid solution, mixing the reaction mixture in the magnetic mixer at a speed of 750 (rpm / min) at room temperature until the solution became clear, then the pH of the solution was adjusted to 6 by adding 0. 1 M of sodium hydroxide. Then gradually 10 mm was added of tripolyphosphates at a concentration of 0. 80 mg / (mL). Then the prepared solution was exposed to a TD Ultrasonic Cleaner 200 for 10 minutes (28) Finally the resulting solution was filtered using a nylon syringe 0. 22 um mesh and then dried in the oven at a temperature of 50 degrees Celsius to conduct Subsequent tests.

Diagnosis of nano-chitosan using the FTIR device Study the chemical composition of nano-chitosan prepared by means of the infrared spectroscopy device (FTIR), mg of the dried product was added to 100 mg of dry powder potassium bromide at a ratio of 1: 5 by means of a ceramic mortar for 10 minutes and the mixture was compressed using a special hydraulic press FTIR device at 8 bar pressure for 60 seconds (29).

Characterization of chitosan nanoparticles using TEM microscopy In order to investigate the phenotypic properties of chitosan nanoparticles, a transmission electron microscope (TEM) of the Philips CM10 type was used by suspending a dried sample with distilled water, then the suspension was dispersed for 3 minutes by means of a vortex and a drop of this suspension was taken It was left to dry on a copper grid plate at room temperature and finally the sample was stained with tungstophosphoric acid

Evaluation of the antagonistic activity of nano-chitosan. Twenty samples were collected from raw milk that was produced in daily quantities during the spring season from several farms in different cities in Babylon Governorate, for the purpose of isolating and diagnosing Gram-positive Bacillus cereus by culturing 0. 2 ml of each milk sample. Crude on sterile petri dishes containing sterilized chromogenic agar culture medium at a temperature of 121 ° C and a pressure of 15 pounds / inch for 15 minutes. After that, the cultivated bacterial colonies belonging to the Bacillus cereus strain were counted, and then four local isolates of the same strain were obtained, which were grown on three replicates to ensure their purity, and then they were transferred to a test tube containing the nutrient broth medium, and then incubated for 24 hours at a temperature 37 percent. In order to investigate the efficiency of nano-chitosan in inhibiting the bacterial isolate, 106 CFU/mL was transferred from the nutrient broth and cultivated by spreading method using L-shape on the surface of sterile Petri dishes containing sterile Muller-Hinton agar medium under the same aforementioned conditions, and then a hole was made in the middle of the medium. The culture was 6 mm in diameter and a 50ml nano-chitosan solution was placed, then the dishes were incubated at a temperature of 37 for 24 hours. The diameter of the contrast zone was estimated after the end of the incubation period (30).

Testing the biological validity of pasteurized milk after adding nano-chitosan to investigate the efficiency of laboratory-synthesized nano-chitosan in prolonging the shelf life of milk. Three ratios of chitosan nanoparticles were tested as follows (0. 25-0. 5-1. Each of the mentioned ratios was added separately to twelve three test tubes). (Replicates with a capacity of 15 ml containing raw milk taken from the same four sources that were investigated for containing Bacillus cereus bacteria. The samples were pasteurized at a temperature of 72 ° C for a period of 15 seconds, and the milk was kept for a period of 30 days at a temperature of 5 ° C. With conducting a set of microbiological tests to detect the growth of a target type of microorganisms (Bacillus cereus) on three cycles (1-1-3 days).

Results

Diagnosis of nanoscale chitosan by FTIR

Figure (3-1) shows that the FTIR examination of nanoscale chitosan gave a distinctive band at a frequency of 3440 cm.

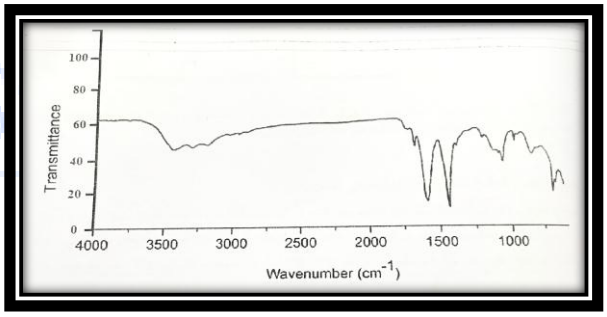

Figure (3-1) FTIR diagram of nanoscale chitosan prepared from 100 mg of chitosan in 1% (vol/vol) of acetic acid solution using an ultrasound device.

Characterization of nanoscale chitosan by TEM

From the following figure (3-2), the appearance of the nanoparticles was observed immediately after the synthesis process (a) and after two weeks of storage at room temperature (b).

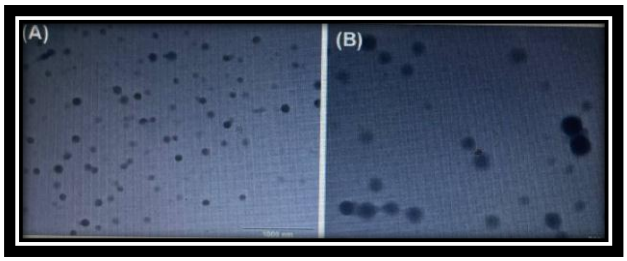

Figure (2-3) Two sections of laboratory-synthesized chitosan nanoparticles using (A) Philips CM device, examined directly after synthesis. (B) The subject examined after two weeks of storage

Estimation of the antifungal activity of nanoscale chitosan

Table (3-1) shows the antagonistic activity of laboratory-synthesized nano-chitosan against the growth of Bacillus cereus.

Table (3-1) Antifungal activity of nano-chitosan synthesized at three concentrations against

Diameter of contrast halo (mm)	Nano-chitosan concentrations %
6	0. 25
14	0. 5
15	1

Physico-chemical test of pasteurized milk after adding nano-chitosan

Table (3-2) shows that the results shown show that on the first day of examining pasteurized milk samples stored at a temperature of 5 semen after adding nano-chitosan.

Table (2-3) shows the number of Bacillus cereus cells for four samples of pasteurized milk stored at 5°C over periods that include (1-15-30 days).

B. cereus cell count (CFU/mL)

Milk storage period (days)	0%Nano -chitosan	0. 25Nano-chitosan	0. 5%Nano-chitosan	1%Nano-chitosan
1	1 x 10	1 x 10	1 x 10	1 x 10
15	6 x 10	5 x 10	3 x 10	2 x 10
30	$< 10^{8}$	2 x 10	>10	> 10

Discussion

Diagnosis of nanoscale chitosan by FTIR

the FTIR examination of nanoscale chitosan gave a distinctive band at a frequency of 3440 cm, which is due to the vibrational extension of NH and CH2OH groups, as this group appears in polymers of chitin () and chitosan because it is one of the groups that does not It is affected by the process of removing acetyl groups or decomposition processes, so it is considered as an internal standard reference to indicate the presence of this type of polymer (31) Absorption bands at frequencies 1650 - 1950 cm and 1315 cm resulted from -1 adsorption of the primary amide band 1 (CONH2), and the bond of ---- and the triple amide band ||, respectively. CH2 gave an absorption band at 1410cm and also the absorption band at 1150cm due to the stretching vibration of PO of the phosphate group of nano-chitosan, which is a discriminant band between chitosan and nano-chitosan.

Characterization of nanoscale chitosan by TEM

the appearance of the nanoparticles was observed immediately after the synthesis process (a) and after two weeks of storage at room temperature (b), as the newly prepared appearance gave a spherical shape with a few with an average diameter ranging from 900 - 1800 nm. Synthesized and tested nato-chitosan, after two weeks of storage, the appearance of the nanoparticles became larger due to swelling resulting from osmosis in the medium containing triple phosphates, and these swollen particles fragmented to become weak branches with an average diameter ranging between 115-220 nm, and the mixture retained the

transparent appearance during a period Storage No, the synthesized nanoparticles did not grow to a size that leads to the occurrence of turbidity in the mixture.

Estimation of the antifungal activity of nanoscale chitosan

the antagonistic activity of laboratory-synthesized nano-chitosan against the growth of Bacillus cereus ,It was found that the effect of nano-chitosan was clear, as the diameter of the anti-halo against the growth of these bacteria was 6 mm, at a concentration of 0. 25%, while it reached 14 mm at a concentration of 0. 5%. When using a concentration of 1%, the diameter of the anti-halo was 15 mm. Nano-chitosan seems to affect the antagonism of Bacillus cereus to varying degrees, depending on its concentration. These results agree to some extent with what many researchers have found that have studied the effect of increasing the concentration of chitosan, as it leads to an increase in its effect as an inhibitor of the growth of microorganisms (32).

Physico-chemical test of pasteurized milk after adding nano-chitosan

the results shown show that on the first day of examining pasteurized milk samples stored at a temperature of 5 semen after adding nano-chitosan, the number of x 10 B. cereus bacteria was 1 CFU/mL in the three different concentrations (0. 25 - 0. 5-1) compared to the number of cells for the same bacteria in pasteurized milk free of nano-chitosan x 10 CFU/mL 1. As for the samples that were examined on the fifteenth day of storage, it was found that the number of cells increased in significant proportions and was as follows (10 x 10 - 5 x 3- 10 CFU/mL (2 x and according to the aforementioned concentrations sequentially due to the growth of some spores that are resistant to pasteurization, but these numbers still do not affect the microbiological quality of milk due to the antagonistic property possessed by chitosan, which contributed to limiting the increase in the numbers of vegetative bacteria compared to milk Pasteurized without nanoparticles, where the number of cells x 10 CFU / mL 6, which is considered an indication of the inefficiency of heat treatment alone in the elimination of pathological plaques of bacteria, that is, milk contamination and spoilage on the fifteenth day of refrigerated storage On the 30th day of refrigerated storage at 5 °C, the bacterial numbers in the pasteurized milk fortified with nanoparticles of chitosan were as follows (10 CFU/mL (10 - 10 - 2 x) with respect to the aforementioned concentrations of nanoparticles of chitosan, sequentially, an indication of the effectiveness of chitosan particles in preventing cell growth vegetative and the formation of new spores and benefit from the nutrients in milk, noting the significant increase in the number of vegetative cells of B cereus in pasteurized milk free of nano-chitosan, which amounted to > 108 CFU / mL. With the number of cells from B. cereus, according to sources (34, 33, 34) which indicate that the permissible limits for the microbiological quality of chilled raw milk should not exceed 105 CFU / mL x 7. 5.

and it was observed that by increasing the concentration of nano-chitosan added, the antagonistic activity Microbiology also increases, and concentrations (0. 5-1) gave similar results in eliminating bacteria at rates higher than the concentration of 0. 25%. As a result, it is recommended to adopt a concentration of 0. 5% for its effectiveness as well as for the economic cost.

Conclusions

The conclusions were as follows:

1. The infrared spectrometer (FTIR) used to investigate the nano-chitosan synthesized between the presence of P = 0 phosphate groups at the absorption beam 1150 cm.
2. It has been shown that it is possible to synthesize nanoscale chitosan based on green chemistry, especially after it was confirmed by a transmission electron microscope with a size ranging from -180 - 2200 nm, at a maximum limit of.
3. The synthetic nano-chitosan eliminated the

pathogenic bacteria Bacillus cereus and its spores at a high rate.

4. It was found that the best percentage of nano-chitosan added to milk during pasteurization was 0. 5% to extend the storage life at a temperature of 5 semen and eliminate Bacillus cereus.

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