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# EFFECT OF USING CHITOSAN NANOPARTICALS ON YOGHURT MANUFACTURE

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ABSTRACT

The current study was designed to prepare functional yoghurt through using three forms of chitosan in addition to control sampel on its quality charachteristics during cold storage for 21 days. The first treatment was adding the particles in its commercial form, the second in the form of nano particles with the same concentration as the commercial form, while the third was added nano particles at half of the concentration in the commercial form. Treatments were coded CHC, CHNPs(5mg/100ml milk) and CHNPs (2.5mg) respectively. All treatments Were analysed for physiochemical, microbiological, Antioxidant scavenging activity by DPPH method and organoleptic properties. Results indicated that The pH values increased in all fortified yoghurts especially CHNPs(5mg/100ml milk) (4.50) compared with control. No clear differences in protein and fat (%) of fortified yoghurt with commercial and nano sized Chitosan. Syneresis was reduced in CHNPs treatments as compared to control samples. According to the obtained results, the control group's antioxidant scavenging activity was shown to be lower. The results were reported that antioxidant radical scavenging activity was found to be lower in the control. However, adding CHC and CHNPs increased the DPPH radical scavenging activity more than control. LAB in yoghurt were significantly decreased with increasing the concentrations of nano particales CHNPs (5 mg/100 ml milk), However, coliform bacteria were not found in any treatments during cold storage. The rephrase and the conclusion can be referred to availability of using CHNPs at (2.5mg/100ml milk) to make functional yoghurt without an adverse effect on its quality. during production of yoghurt without having a negative impact on its organoleptic qualities.

## INTRODUCTION

Nanotechnology is a new scientific approach that has recently been studied as a food additive for enhancing its quality characteristics. Nanotechnology is a scientific approach that focuses on the properties of materials, chemicals, optics, and mechanical systems at the nanoscale. The word "nano" is derived from the Greek word "warf," which means "less than 100 nm." **(Mallmann *et al*., 2015)**. Chitosan is a complex polymer made of glucosamine and N-acetyl glucosamine that is produced *via* alkaline deacetylation of chitin and partial deacetylation of acetyl glucosamine. Important biological characteristics of chitin include biodegradability, biocompatibility, and bioactivity. Furthermore, it is a polycationic polymer, which is an important chemical characteristic since it contains functional groups with active amino and hydroxyl atoms **(Nam and Shin., 2017).** Chitosan, the primary chitin derivative, is a linear aminopolysaccharide mainly consisting of repeating units of -(14)2-amino-2-deoxy-D-

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glucose (D-glucosamine)**.** Previous studies have found that chitosan has hypoglycemic properties **(Lee *et al.*, 2007).** Chitosan polymer has attracted the interest of chemists, physicists, and engineers because of its biocompatibility, processability, and excellent film forming ability. These properties increased the attention to the development of a wide range of compositions containing chitosan nanomaterials, nanofibers, nanoclay, colloids, composites, and other products, either alone or in combination with natural chemical **(Rao *et al.*, 2019).** Nano-powdered chitosan has a wide range of biological activities, including blood cholesterol lowering and anti-diabetic activity, for these reasons it can be said that the resultant product is function yoghurt. The influence of nano chitosan added Maribo cheese on its physicochemical characteristics and sensory analysis after 6 months of ripening on the physiochemical and sensory properties of Maribo cheese during 6 months of ripening was followed. The results indicated that adding chitosan had no effect on cheese moisture and fat contents while its ash content increased while its protein content had lower values **(Kim *et al.*, 2014).** Chitosan can be used to extend the shelf life of perishable mozzarella cheese. The use of chitosan lactic acid solution in starter culture inhibits the growth of spoilage microflora for up to 10 days during cold storage **( Al-Altieri, *et al.*, 2005).** The use of Chitosan in dairy products is limited due to its low solubility. However, the use of nanosized chitosan could aid in better solubility and bioavailability. Another type of chitosan, chitosan nanoparticles (Ch-NPs), can be used effectively in cholesterol-reduced yoghurt, and lowering of lactic acid bacteria counts of CH-NPS-added yoghurt is observed when stored at 4°C for 20 days, the aim of this work was increasing yoghurt shelf life **(Nagpal *et al.*, 2019)**.

# MATERIALS AND METHODS

## Materials

Buffalo's milk was purchased from a private farm in North Sinai Governorate, Egypt and had 6% fat, 4% protein, and 18.21% T.S. Yoghurt culture (*Streptococcus thermophillus*, *Lactobacillus delbrueckii* ssp. bulgaricus) was obtained from DANISCO, Rue de clemencieres-BP 32, Sassenage, Denemark. Nano and commercial materials of Chitosan were obtained from Nakaa nanotechnology network (NNN), Egypt. All chemicals used in this study were of analytical grade and supplied by BDH and Sigma chemical companies.

## Methods

## Manufacture of Yoghurt Fortified With Nano and Commercial Chitosan

Fresh buffalo milk was heated to 90°C for 3 minutes before being cooled to 42°C and inoculated with 3% yoghurt starter. After that, the inoculated milk was devided to the following four portions:

Chitosan was added to milk in 3 treatments. The first treatment, it was added the particles in its commercial form, the second in the form of nano particles with the same concentration as the commercial form, while the third was added nano particles at half of the concentration of the commercial form.

## Characterization of Chitosan Nanoparticles

These test were carried out at the Faculty of Nanotechnology, Sheikh Zayed, Cairo University, chitosan nanoparticles Composition class was done by raman spectroscopy which performed using Lab. RAM-HR Evolution Horiba Co. The 532 nm He–Cd edge laser line with grating 1800 (450-850 nm) and ND filter 3.2% was used to avoid burning of sample with acquisition time 10 sec, accumulations 5 without delay time and spike filter and objective was X100_VIS.

### Gross compostion analyses

Values of pH were measured by JENWAY Digital pH meter Model 3310. Titratable acidity, Total solids, Protein and Fat were determined according to **AOAC (2011)**.

**Table 1. The Experimental Treatments**

| TREATMENT | SIZE | AMOUNT/100ML |
|---|---|---|
| Control | milk sample without any additives | |
| CHC | commercial | 5mg |
| CHNPs(5mg/100ml milk) | nano | 5mg |
| CHNPs(2.5mg) | nano | 2.5mg |

## Phsyiochemical Analyses

### Dentisty

Density was determined by a gravimetric method using pycnometers (Fisherbrand, ON, Canada).

### Synersis

The degree of synersis, given as a percentage of free whey, was determined using a modest modification method developed by **Al-Kadamany *et al*. (2003).** A 10 g yoghurt sample was placed on filter paper resting on top of a funnel. After 10 minutes of draining in hoover. The remaining yoghurt was weighed, and synersis was computed as follows: The drained whey (g/100 g)/ weight of initial sample - weight of sample after filtration / weight of initial sample X 100.

### Colour

A Lab colorimeter (Colour Flex EZ's, USA) was used to measure the colour of yoghurt samples. The following colour characteristics were estimated: L* (represented as the lightness-darkness range), a* (expressed as the redness-greenness range), and b* (expressed as the yellowness-blueness range) (**Hunter, 1975)**.

### Aroma compounands of yoghurt

The acetaldehyde and diacetyl content of yoghurt samples were determined using the method given by **Lee and Jago (1969)**, with some modifications. Instead of the Conway microdiffusion cell, use one large petri dish with a cover and small one without a cover. The large dish replaced the outer compartment, while the small dish replaced the inner wall of the Conway microdiffusion cell. Then it destabilized into the largest dish's middle. The small became unstable at the centre of the large..

## Antioxidant Capacity (Raddical Scavenging) DPPH

The free radical scavenging activities of nano and commercial particles added to yoghurt were measured by the 2,2-diphenyl-1-picryl- hydrazil. **Brand-Williams *et al*. (1995)** hypothesised the (DPPH). In a nutshell, a 0.1 mM DPPH solution in ethanol was produced, and 1.0 mL of this solution was applied to 0.5 mL of samples. The absorbance was measured at 525 nm after 20 minutes. The radical scavenging activity of DPPH was calculated using the equation:

- Radical scavenging activity of DPPH (%) = [(A0- A1)/A0]* 100
- Where A0 was the absorbance in the absence of the enhanced yoghurt and A1 was the absorbance in the presence of the control.

### Toxicity test

The cytotoxicity of nano sized Chitosan of yoghurt was determined on rats (pheochromocytoma cell) with XTT according to the methods previously described by **Berridge *et al*. (2005)**. The sample was tested against pheochromocytoma cell (PC12) at concentration 5 mg/mL, for CHNPs of 1, 2, 3, 24, 48 and 72 hr.

## Microbiological Analyses

### Lactic acid bacterial count

Lactic acid bacteria (LAB) were enumerated on M17 agar medium and incubated at $37^0$ C for 3 days according to **Elliker *et al.* (1956).**

### Moluds and Yeast count

Moluds and yeast were counted on oxytetracycline glucose yeast extract agar medium as suggested by **APHA (2004).** Plates were incubated at $25^0$C for three days.

### Coliform group count

According to the **(APHA) American Public Health Association (2004)**, coliform were detected. Approving dilutions of sampels were plated on Mac Conk's agar medium and incubated at $37^0$ C for 48 hours.

### Organoleptic properties of yoghurt

A group of Faculty staff and students (90 person), Faculty of Agriculture, Arish University. Yoghurt was evaluated according to **El-Samragy and Zall (1988)**.

## Statistical Analyses

Using the statistical programme SPSS 19.0, all of the study's data were expressed as means and standard deviation based on an analysis of three duplicates. According to the protocol of the study, one-way analysis of variance (ANOVA) and Duncan's multiple range tests were used to identify data with significant (P0.05) differences **(Duncan, 1955).**

# RESULTS AND DISCUSSION

## Characterization of Nanoparticles

Chitosan nanosheets were characterized by Raman spectroscopy due to its weakness crystallinity. However, Chitosan Raman shift pattern exemplify the 12 typical Raman shift peaks at 895.45, 1037.64, 1096.45, 1114.13, 1148.11, 1255.06, 1374.26, 1420.23, 1454.39, 1598.62, 34, 2880.29, and 2921.62 $cm^{-1}$, respectively. As depicted in Fig.1420.33 and 1454.49 $cm^{-1}$ denote the symmetry and asymmetry of the C-H deformation plan, respectively, whereas 895.48 and 1255.03 $cm^{-1}$ reflect the vibration of the C-H deformation plan. C-O stretch vibrations were 1037.68 and 1148.12 $cm^{-1}$, whereas C-O-C (ring) AND C-O-C (ether) vibrations were are 1096.46 and 1114.12 cm-1, respectively. C-N stretch and N-H vibration are represented by the cm-1 bands at 1374.46 and 1558.62. Last but not least, the three Raman shift peaks that are distinctive of Chitosan nanosheets are located in the Raman spectrum bands 2724, 2880.27 and 2921.60 $cm^{-1}$, respectively. These peaks correspond to the (C-H) stretching modes of (CH2) and (CH3) **(Youssef *et al*., 2021).**

## Gross Compostion

Table 2 shows the effect of commercial and nano sized Chitosan of yoghurt fortification on pH values, acidity, total solids, fat and protein(%) of yoghurt during storage. Results reported that the pH values showed adecreasing trend during storage period for all yoghurt treatmens indicating that the yoghurt quality remarkably decreased after 21 days of storage. The decrease in pH during the storage may be due to the production of lactic acid by the bacteria present in the yoghurt. The same trend for pH was reported by **El-Kholy *et al.* (2011)**. Titratable acidity: had an opposite trend to pH values. These results are in agreement with **Seo *et al*. (2009)** who observed that adding commercial and nano chitosan, 0.1 to ~0.7%, (wt/vol) into yoghurt samples reduced the titratable acidity values. Adding commercial and nano sized chitosan to yoghurt had no markable effect on total solid contents. This may be due to the small amount of added nano and commercial particles. The total solid of control was 18.29% at the end of the storage period. Moreover, the highest TS value was18.92% for CHNPs (5 mg/100 ml milk) at the end

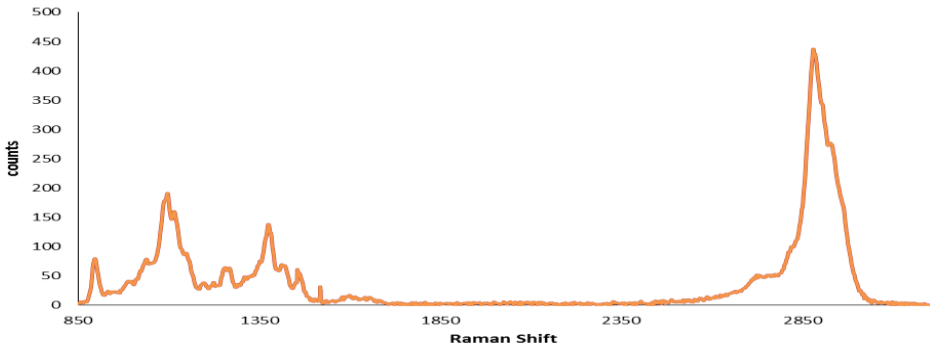


**Fig.1. The Raman shift pattern of chitosan nanosheet**

**Table 2. Gross compostion of yoghurt fortified with commercial and nano sized chitosan during storage at 4-6°C up to 21 days**

| Sample | Storage (day) | pH | Acidity | T.S | Fat | Protein |
|---|---|---|---|---|---|---|
| **Control** | **Fresh** | 4.66 $^{a}$ ± 0.01 | 0.87 $^{a}$ ± 0.01 | 18.21 $^{a}$ ± 0.01 | 6.00 $^{a}$ ± 0.01 | 4.10 $^{a}$ ± 0.01 |
| | **3** | 4.64 $^{a}$ ± 0.01 | 0.88 $^{a}$ ± 0.01 | 18.22 $^{a}$ ± 0.01 | 6.00 $^{a}$ ± 0.01 | 4.19 $^{a}$ ± 0.01 |
| | **7** | 4.63 $^{a}$ ± 0.01 | 0.89 $^{a}$ ± 0.01 | 18.22 $^{a}$ ± 0.01 | 6.10 $^{a}$ ± 0.01 | 4.30 $^{a}$ ± 0.01 |
| | **14** | 4.48 $^{a}$ ± 0.01 | 0.90 $^{a}$ ± 0.01 | 18.26 $^{a}$ ± 0.01 | 6.20 $^{a}$ ± 0.01 | 4.33 $^{a}$ ± 0.01 |
| | **21** | 4.42 $^{a}$ ± 0.01 | 0.92 $^{a}$ ± 0.01 | 18.29 $^{a}$ ± 0.01 | 6.30 $^{a}$ ± 0.01 | 4.46 $^{a}$ ± 0.01 |
| **CHC** | **Fresh** | 4.68 $^{a}$ ± 0.01 | 0.82 $^{a}$ ± 0.01 | 18.75 $^{a}$ ± 0.01 | 6.40 $^{a}$ ± 0.01 | 4.21 $^{a}$ ± 0.01 |
| | **3** | 4.66 $^{a}$ ± 0.01 | 0.86 $^{a}$ ± 0.01 | 18.85 $^{a}$ ± 0.01 | 6.45 $^{a}$ ± 0.01 | 4.30 $^{a}$ ± 0.01 |
| | **7** | 4.64 $^{a}$ ± 0.01 | 0.88 $^{a}$ ± 0.01 | 18.86 $^{a}$ ± 0.01 | 6.50 $^{a}$ ± 0.01 | 4.31 $^{a}$ ± 0.01 |
| | **14** | 4.52 $^{a}$ ± 0.01 | 0.89 $^{a}$ ± 0.01 | 18.86 $^{a}$ ± 0.01 | 6.50 $^{a}$ ± 0.01 | 4.36 $^{b}$ ± 0.01 |
| | **21** | 4.48 $^{a}$ ± 0.01 | 0.90 $^{a}$ ± 0.01 | 18.88 $^{a}$ ± 0.01 | 6.52 $^{b}$ ± 0.01 | 4.40 $^{c}$ ± 0.01 |
| **CHNPs (5mg/100 ml milk)** | **Fresh** | 4.70 $^{b}$ ± 0.01 | 0.81 $^{a}$ ± 0.01 | 18.76 $^{a}$ ± 0.01 | 6.50 $^{a}$ ± 0.01 | 4.20 $^{a}$ ± 0.01 |
| | **3** | 4.67 $^{b}$ ± 0.01 | 0.84 $^{a}$ ± 0.01 | 18.78 $^{a}$ ± 0.01 | 6.55 $^{a}$ ± 0.01 | 4.25 $^{a}$ ± 0.01 |
| | **7** | 4.65 $^{b}$ ± 0.01 | 0.86 $^{a}$ ± 0.01 | 18.82 $^{a}$ ± 0.01 | 6.60 $^{a}$ ± 0.01 | 4.30 $^{a}$ ± 0.01 |
| | **14** | 4.55 $^{a}$ ± 0.01 | 0.90 $^{a}$ ± 0.01 | 18.90 $^{b}$ ± 0.01 | 6.60 $^{a}$ ± 0.01 | 4.34 $^{a}$ ± 0.01 |
| | **21** | 4.50 $^{a}$ ± 0.01 | 0.92 $^{b}$ ± 0.01 | 18.92 $^{b}$ ± 0.01 | 6.70 $^{b}$ ± 0.01 | 4.38 $^{c}$ ± 0.01 |
| **CHNPs (2.5 mg)** | **Fresh** | 4.67 $^{a}$ ± 0.01 | 0.83 $^{a}$ ± 0.01 | 18.45 $^{a}$ ± 0.01 | 6.33 $^{a}$ ± 0.01 | 4.10 $^{a}$ ± 0.01 |
| | **3** | 4.64 $^{a}$ ± 0.01 | 0.87 $^{a}$ ± 0.01 | 18.55 $^{a}$ ± 0.01 | 6.35 $^{a}$ ± 0.01 | 4.15 $^{a}$ ± 0.01 |
| | **7** | 4.63 $^{a}$ ± 0.01 | 0.89 $^{a}$ ± 0.01 | 18.60 $^{a}$ ± 0.01 | 6.40 $^{a}$ ± 0.01 | 4.18 $^{a}$ ± 0.01 |
| | **14** | 4.50 $^{b}$ ± 0.01 | 0.89 $^{b}$ ± 0.01 | 18.75 $^{a}$ ± 0.01 | 6.45 $^{b}$ ± 0.01 | 4.22 $^{a}$ ± 0.01 |
| | **21** | 4.45 $^{b}$ ± 0.01 | 0.90 $^{b}$ ± 0.01 | 18.75 $^{a}$ ± 0.01 | 6.50 $^{b}$ ± 0.01 | 4.22 $^{a}$ ± 0.01 |

**a,b,c,d and e values in the same colum with the same alphabet do not differ significantly(p> 0.05).

of storage period. The same trends were reported by **El-Shibiny *et al.* (1979) and Mehnna and Gonc (1988)** who reported similar trend and attributed these changes due to the evapouration of some yoghurt water during cold storage. No clears differences were watched in protein and fat of fortified yoghurt with commercial and nano sized chitosan. The protein ranged from 4.10% to 4.21% in fresh samples. Moreover, protein and fat in all treatments increased gradually as the storage period progressed. This may be probably due to the increase of total solids. These results agree with **Zommara and Prokisch (2015).**

## Physiochemical Properties

Table 3 shows the effect of commercial and nano sized Chitosan of yoghurt fortification on % Syneresis (ml/100 g) and density (kg/m$^3$). Adding nano particles decreased the syneresis values more than the same concentration of the commercial particales. The syneresis value of all yoghurt samples increased during storage for all samples. Syneresis value was lower in Chitosan treatments than control. The lowest syneresis value was 34.9 (ml/100 g) in CHNPs(2.5mg) at the end of storage period. In general results revealed that the gradual addition of CHNPs led to gradual decrease in the syneresis of fortified yoghurt and the values of synersis in the control sample were higher than treated samples. In general data revealed that the gradual addition of CHNPs led to gradual decrease in the syneresis of fortified yoghurt and the values of syneresis in the control sample were higher than treated samples. These results are in harmony with **Soe *et al.* (2009).**

The obtained results revealed that the density was slightly higher in CHC yoghurt sample than that in the control yoghurt at the end of storage period. In addition, the values of density were slight increased with increasing storage period up to 21 days, and increase addition of NPs levels in all treatments. These results are in harmony with of **Soe *et al.* (2009)**. They reported that the syneresis of yoghurts fortified with minerals did not vary significantly when compared to the control.

## Aroma Compounds of Yoghurt

The effect of using nanoparticales on acetaldehyde and diacetyel (mg/L) concentration during the storage period of different yoghurt treatments is shown in Table 4. Results indicated that on the first day the highest content of acetaldehyde was recorded for CHNPs (5 mg/100 ml milk), while the lowest acetaldehyde content in fresh yoghurt was recorded for control sample. Decreasing pH reduces acetaldehyde due to oxidation of acetaldehyde to acetate **Tamime and Robinson (1999).** The content of acetaldehyde increased in CHNPs treatments compared with the CHC treatments. Moreover, acetaldehyde content of all samples decreased during the storage.

There were signification differences among all treatments in diacetyel content. It was clear that the diacetyl contents of all treatments were increased and reached to a maximum value at the 21+ days of storage. The effect of addition CHNPs on diacetyl during the storage period of yoghurt treatments was very clear. Results indicated that the lowest value was obtained for control. While the highest value was recorded for CHNPs(5 mg/100 ml milk). Yoghurt sample contained CHNPs showed higher values of diacetyl content than yoghurt fortified with the same commercial concentration (CHC). At the same time, the treatments with less nano concentration gave results very close to the commercial treatments (CHC). Similar results were reported by **Salama *et al.* (2021).**

## Colour

Table 5 presents is the comparative statistical analysis of the three colour parameters based on the Hunter scale.

**Table 3. Physiochemical properties of yoghurt fortified with commercial and nano sized chitosan during storage at 4-6°C up to 21 days**

| Sample | Storage (day) | Synersis (ml/100g) | Density kg/m$^3$ |
|---|---|---|---|
| **Control** | Fresh | 32.8 $^{a}$ ± 0.01 | 1044.14 $^{a}$ ± 0.12 |
| | 3 | 36.7 $^{a}$ ± 0.01 | 1040.25 $^{a}$ ± 0.32 |
| | 7 | 38.5 $^{a}$ ± 0.01 | 1039.23 $^{b}$ ± 0.52 |
| | 14 | 40.2 $^{a}$ ± 0.01 | 1043.65 $^{b}$ ± 0.45 |
| | 21 | 43.1 $^{a}$ ± 0.01 | 1043.83 $^{b}$ ± 0.38 |
| **CHC** | Fresh | 30.8 $^{c}$ ± 0.01 | 1046.21 $^{a}$ ± 0.61 |
| | 3 | 32. $^{c}$ 2± 0.01 | 1048.22 $^{b}$ ± 0.42 |
| | 7 | 33.7 $^{c}$ ± 0.01 | 1048.26 $^{ab}$ ± 0.19 |
| | 14 | 36.3 $^{c}$ ± 0.01 | 1045.00 $^{c}$± 0.59 |
| | 21 | 37.2 $^{c}$ ± 0.01 | 1047.21 $^{b}$ ± 0.08 |
| **CHNPs (5 mg/100 ml milk)** | Fresh | 30.2 $^{b}$ ± 0.01 | 1047.44 $^{ab}$ ± 0.18 |
| | 3 | 30.9 $^{b}$ ± 0.01 | 1047.32 $^{bc}$ ± 0.74 |
| | 7 | 31.3 $^{b}$ ± 0.01 | 1047.60 $^{a}$ ± 0.80 |
| | 14 | 32.5 $^{a}$ ± 0.01 | 1046.19 $^{a}$ ± 0.54 |
| | 21 | 34.7 $^{bc}$ ± 0.01 | 1046.98 $^{b}$ ± 0.36 |
| **CHNPs(2.5mg)** | Fresh | 30.4 $^{a}$ ± 0.01 | 1046.14 $^{bc}$ ± 0.81 |
| | 3 | 31.6 $^{a}$ ± 0.01 | 1043.25$^{a\ b}$ ± 0.32 |
| | 7 | 32.5 $^{b}$± 0.01 | 1045.26 $^{b}$ ± 0.45 |
| | 14 | 33.6 $^{b}$ ± 0.01 | 1043.65 $^{a}$ ± 0.76 |
| | 21 | 34.9 $^{b}$ ± 0.01 | 1043.83 $^{b}$ ± 0.61 |

**a,b,c,d and e values in the same colum with the same alphabet do not differ significantly(p> 0.05).

**Table 4. Acetaldehyde and diacetyle values (mg/L) of fortified yoghurt with commercial and nano sized chitosan during storage at 4-6°C. up to 21 days**

| Treatment | Storage(day) | | | | | Mean |
|---|---|---|---|---|---|---|
| | Fresh | 3 | 7 | 14 | 21 | |
| **Acetaldehyde values (mg/L)** | | | | | | |
| **Control** | 21.02 | 20.00 | 18.00 | 17.03 | 16.00 | 18.41±0.028$^{a}$ |
| **CHC** | 33.00 | 30.00 | 25.33 | 23.00 | 21.00 | 26.46±0.032$^{b}$ |
| **CHNPs (5mg/ 100ml milk)** | 40.00 | 35.10 | 29.00 | 26.50 | 23.00 | 30.72±0.042$^{d}$ |
| **CHNPs(2.5mg)** | 30.95 | 25.20 | 24.06 | 20.09 | 18.20 | 23.70±0.029$^{a}$ |
| **Diacetyle values (mg/L)** | | | | | | |
| **Control** | 2.51 | 2.98 | 3.32 | 4.00 | 5.21 | 3.60±0.029$^{a}$ |
| **CHC** | 4.37 | 4.50 | 7.63 | 9.64 | 10.13 | 7.25±0.038$^{b}$ |
| **CHNPs (5 mg/100 ml milk)** | 5.37 | 5.45 | 8.63 | 9.50 | 10.13 | 7.81±0.040$^{d}$ |
| **CHNPs (2.5mg)** | 3.21 | 3.29 | 4.00 | 5.02 | 6.00 | 4.30±0.034$^{b}$ |

**Table 5. Colour of fortified yoghurt with commercial and nano chitosan during storage at 4-6°C up to 21 days**

| Treatment | Storage (day) | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | | | 3 | | | 7 | | | 14 | | | 21 | | |
| | L | A | B | L | A | B | L | A | B | L | a | B | L | A | b |
| **Control** | 88.96 | 3.04 | 5.81 | 88.92 | 3.05 | 6.01 | 88.88 | 3.07 | 6.14 | 87.80 | 3.20. | 6.84 | 87.80 | 3.27 | 7.06 |
| **CHC** | 88.70 | 2.65 | 6.48 | 88.56 | 2.74 | 6.52 | 88.61 | 2.79 | 6.70 | 85.21 | 2.86 | 7.13 | 86.58 | 2.94 | 7.43 |
| **CHNPs (5mg/100ml milk)** | 86.00 | 2.71 | 6.47 | 85.21 | 2.73 | 6.40 | 86.30 | 3.18 | 6.43 | 80.57 | 3.02 | 6.49 | 84.11 | 3.08 | 6.53 |
| **CHNPs(2.5mg)** | 88.86 | 2.81 | 6.29 | 87.40 | 3.05 | 6.33 | 88.82 | 3.08 | 6.40 | 89.11 | 3.20 | 6.42 | 86.58 | 3.23 | 6.46 |

Because the additional particles were white, there were no discernible differences between the fresh samples fortified with CHC and CHNPs and the control for the L, a*, and b* parameters. The L and b* parameters significantly dropped during storage, however all of the samples' a* parameters significantly raised. Minerals present during storage encourage the oxidation of lipids in yoghurt, reducing brightness and affecting parameters a* and b*. These outcomes support by **Ramirez-Sucre and Vélez-Ruiz (2013)**.

## Antioxidant Scavenging Activity by DPPH Method

The antioxidan scavenging activity (DPPH) of different yoghurt treatments at the 7$^{th}$ day of storage at 4-6°C is shown in Table 6. Adding CHC and CHNPs increased the antioxidan scavenging activity than control**.** However, it was discovered that the antioxidan scavenging activity was higher in CHNPs (5mg/100ml milk) 50.8% than CHC 40.5%. Generally Nano particales has greater antioxidant activity because of it's longer surface area and functional group exposure, resulting in increased antiradical scavenging activity (**Kitts *et al.*, 2000; Park *et al*., 2007; Ahn *et al.*, 2013).**

### Totoxicity effect

Table 7 shows the totoxicity of yoghurt fortified with nano and commercial chitosan. In the examined rat's pheochromocytoma (PC12) cells, no cytotoxicity was seen at 1, 2, 3, 24, 48, or 72 hours at a concenteration of 5 mg/100 mL for CHNPs. Yoghurt fortified with CHNPs did not enter the cells, according to the results of an XTT test for the examination of cytotoxicity. Instead, it appeared to be sitting on the cell's surface. At any tested of concenteration. CHNPs showed maximal contact and entry in cells without a harmful effect. Therefor, CHNPs are considered safe because they did not induce any noticeable toxicity, even over extended periods of time, and we may conclude that they do not cause any measurable cytotoxicity. Because of milk protein can bind nanoparticles which difficult to release **El-Sayed *et al.* (2015).** As a result, no harmful activity was noticed whey protein has a high propensity for binding to tive and other hydrophobic compounds. Eight binding sites in whey protein allow minerals to CHNPs to their compact structure, which may account for their potent ability to bind to the additional nanoparticles **(El-Saadony *et al.* 2021).** Similarly with a study further indicated significant differences in cytotoxicity profiles in consideration of cell type or the presence of serum matter for nanoparticle toxicology studies **(Caro *et al.*, 2019)**.

## Microbiological Evaluation

The counts of LAB were clearly decreased as the fortification with CHNPs and storage period progressed.Table 8 In fresh yoghurt, the control sample exhibited the highest rate of 8.15 $10^{10}$cfu. Because of the antibacterial activity of CHNPs, the LAB in yoghurt dropped dramatically with

**Table 6. The antioxidant scavenging activity of fortified yoghurt with commercial and nano chitosan at 4-6°C at the 7 day of storage**

| Treatment | DPPH (%) |
|---|---|
| **Control** | 29.5±0.7[a] |
| **CHC** | 40.5±0.8[b] |
| **CHNPs(5mg/100ml milk)** | 50.8±0.5[c] |
| **CHNPs(2.5mg)** | 38.5±0.4[bc] |

**Table 7. The totoxicity of yoghurt fortified with nano and commercial sized of chitosan at the 7th day of cold storage**

| Treatment | Rat's pheochromocytoma PC12 cells |
|---|---|
| **Control** | 22.0±2.3[b] |
| **CHC** | 21.6±3.8a |
| **CHNPs(5mg/100ml milk)** | 21.4±4.7a |
| **CHNPs(2.5mg)** | 21.2±3.5a |

increasing concentrations of nanoparticales (CHNPs (5 mg/100 ml milk)). The addition of CHC and CHNPs to yoghurt has a significant effect on the lactic acid bacterial count after yoghurt formation and after 21 days of cold storage ($p > 0.05$). This result is consistent with the findings of **Sawant *et al.* (2015).**

During storage, the concentration of lactic acid bacteria decreased. The count of lactic acid bacteria ranged from $10^7$ cfu/ gram till the end of the shelf-life, which was recorded on all days of storage. This suggests that the starting culture in fermented dairy products is stable. Coliform and Moulds and Yeast were both absent in all treatments when fresh or during storage at 4-6°C for up to 21 days, indicating that the set yoghurt manufacturing was in good and clean condition.

## Organoleptic Properties of Yoghurt

Table 9 shows the influence of fortified yoghurt with commercial and nano-sized chitosan on the organoleptic properties of yoghurt during storage at 4-6°C for up to 21 days. The flavour scores for all treatments decreased gradually throughout cold storage. However, the highest flavour score at fresh yoghurt was 47 for CHNPs (5 mg/100 ml milk). Furthermore, till the end of the storage period, the lowest flavour scores 38 was found for yoghurt treated with CHC. Body and texture scores for all treatments gradually decreased over the storage period up to 21 days. However, the highest scores at the end of the storage period was 28 for CHNPs (5 mg/100 ml milk). The appearance scores for all treatments significantly declined over the storage time. However, the greatest score at the conclusion of storage period was 12 for CHNPs (5 mg/100 ml milk). Total acceptance scores for all treatments declined gradually over the storage period. The greatest score was observed at the end of the storage period of up to 21 days for CHNPs (5mg/100ml milk). Similar results were observed by **Seo *et al.* (2009)**. The using of CHNPs increased the sensory properties of yoghurt, so its lowest particles size resulted in occurrences of homogeneity and general acceptance of yoghurt when compared to the control and the commercial particles.

**Table 8. Lactic acid bacteria count (cfu/ml) of fortified yoghurt with commerciall and nano sized Chitosan during storage at 4-6°C. up to 21 days**

| Treatment | Storage (day) | | | | |
|---|---|---|---|---|---|
| | Fresh | 3 | 7 | 14 | 21 |
| **Control** | $8.15\times10^{10c}\pm0.01$ | $1.50\times10^{10c}\pm0.01$ | $4.50\times10^{9b}\pm0.01$ | $1.70\times10^{9b}\pm0.01$ | $1.75\times10^{9b}\pm0.01$ |
| **CHC** | $1.08\times10^{10a}\pm0.01$ | $6.55\times10^{8a}\pm0.01$ | $3.20\times10^{8b}\pm0.01$ | $1.40\times10^{8b}\pm0.01$ | $2.60\times10^{8b}\pm0.01$ |
| **CHNPs (5 mg/ 100 ml milk)** | $1.85\times10^{10a}\pm0.01$ | $1.52\times10^{9a}\pm0.01$ | $8.20\times10^{8a}\pm0.01$ | $4.89\times10^{8c}\pm0.01$ | $4.65\times10^{8c}\pm0.01$ |
| **CHNPs (2.5mg)** | $2.49\times10^{10b}\pm0.01$ | $2.17\times10^{9b}\pm0.01$ | $1.90\times10^{8c}\pm0.01$ | $8.90\times10^{8a}\pm0.01$ | $8.65\times10^{8c}\pm0.01$ |

**Table 9. Sensory characteristies of fortified yoghurt with commercial and nano chitosan during storage at 4-6°C up to 21 days**

| Parameter | Flavours 50 | | | | Body and Texture 35 | | | | Appearance 15 | | | | Total acceptance 100 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Storage (day) Treatment | 0 | 7 | 14 | 21 | 0 | 7 | 14 | 21 | 0 | 7 | 14 | 21 | 0 | 7 | 14 | 21 |
| **Control** | $46^{a}\pm0.1$ | $43^{a}\pm0.1$ | $42^{a}\pm0.1$ | $40^{b}\pm0.1$ | $31^{b}\pm0.1$ | $30^{b}\pm0.1$ | $28^{b}\pm0.1$ | $27^{b}\pm0.1$ | $13^{b}\pm0.1$ | $12^{b}\pm0.1$ | $12^{b}\pm0.1$ | $11^{b}\pm0.1$ | $90^{a}\pm0.1$ | $85^{b}\pm0.1$ | $82^{b}\pm0.1$ | $78^{b}\pm0.1$ |
| **CHC** | $43^{ab}\pm0.1$ | $42^{ab}\pm0.1$ | $40^{ab}\pm0.1$ | $38^{a}\pm0.1$ | $32^{b}\pm0.1$ | $31^{b}\pm0.1$ | $29^{b}\pm0.1$ | $26^{b}\pm0.1$ | $13\pm0.1$ | $12\pm0.1$ | $12\pm0.1$ | $11\pm0.1$ | $88^{a}\pm0.1$ | $85^{a}\pm0.1$ | $81^{a}\pm0.1$ | $76^{b}\pm0.1$ |
| **CHNPs (5 mg/100ml milk)** | $47^{b}\pm0.1$ | $44^{b}\pm0.1$ | $42^{b}\pm0.1$ | $41^{c}\pm0.1$ | $34^{c}\pm0.1$ | $32^{c}\pm0.1$ | $30^{c}\pm0.1$ | $28^{c}\pm0.1$ | $14^{c}\pm0.1$ | $13^{c}\pm0.1$ | $13^{c}\pm0.1$ | $12^{c}\pm0.1$ | $95^{b}\pm0.1$ | $89^{b}\pm0.1$ | $85^{b}\pm0.1$ | $81^{b}\pm0.1$ |
| **CHNPs (2.5mg)** | $46^{b}\pm0.1$ | $43^{b}\pm0.1$ | $42^{b}\pm0.1$ | $39^{b}\pm0.1$ | $33^{c}\pm0.1$ | $32^{c}\pm0.1$ | $29^{c}\pm0.1$ | $27^{c}\pm0.1$ | $13^{c}\pm0.1$ | $12^{c}\pm0.1$ | $12^{c}\pm0.1$ | $11^{c}\pm0.1$ | $92^{b}\pm0.1$ | $87^{b}\pm0.1$ | $83^{b}\pm0.1$ | $77^{c}\pm0.1$ |

## Conclusion

The results found that the addition of nano chitosan at (5 mg/100 ml milk) improved the sensory properties of yoghurt. So, its small size led to the events of homogeneity and general acceptance of yoghurt compared to control and commercial particles. Also, CHNPs possessed better antioxidant capability than CHC.

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## الملخص العربي

# تأثيـــر اســـتخـــدام جـــزيئـــــات الشيتوزان النانـــــــويـــة فـــــي صنـــاعـــة اليوجهورت

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صممت الدراسة الحالية للتحقق من استخدام ثلاثة أشكال من الشيتوزان في تصنيع اليوجهورت أثناء التخزين عند 4-6 درجات مئوية حتى 21 يومًا على النحو التالي: المعاملة الأولى كانت إضافة الجزيئات في شكلها التجاري، والثانية في شكل من جسيمات النانو بنفس تركيز الشكل التجاري، بينما أضيفت جسيمات النانو بنصف التركيز في الشكل التجاري. تم ترميز المعاملات CHC ، CHNPs (5mg/100ml milk) و CHNPs (2.5mg) على التوالي. تخضع جميع المعالجات للتقييم الفيزيوكيميائي والميكروبيولوجي و DPPH والتقييم الحسي. أشارت النتائج إلى أن قيم الأس الهيدروجيني تزداد في جميع أنواع اليوجهورت المدعم وخاصةCHNPs (5mg/100ml milk ) (4.50) مقارنة مع مجموعة المقارنة. لا توجد فروق واضحة في نسبة البروتين والدهون في اليوجهورت المدعم بالشيتوزان التجاري والنانو الحجم. تم تقليل التآزر في معاملات CHNPs مقارنة بمجموعة المقارنة وفقًا للبيانات، تبين أن نشاط الشقوق الحرة لـ DPPH من مجموعة المقارنة كان أقل. اظهرت النتائج أن نشاط الشقوق الحرة لـ DPPH كان أقل في مجموعة المقارنة. وتبين أن نشاط الشقوق الحرة لـ DPPHكان أقل في مجموعة المقارنة. ومع ذلك، فإن إضافة CHC و CHNPsزاد من نشاط الشقوق الحرة لـ DPPH أكثر من التحكم. انخفض LAB في اللبن بشكل كبير مع زيادة تركيزات جزيئات النانو 5مجم/100ملجم، ومع ذلك، لم يتم العثور على بكتيريا القولون في أي معاملات أثناء تخزينها. أشارت النتائج إلى أن اضافة CHNPs (2.5 ملغ) قد يكون ضروريًا أثناء إنتاج اليوجهورت دون أن يكون له تأثير سلبي على صفاته الحسية.

**الكلمات الإسترشادية:** الشيتوزان، جسيمات النانو، اليوجهورت.

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