

<http://www.pjbs.org>

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSI*net*

Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan



Research Article

Nanochitosan Effect on Biomolecular, Hypolipidemic in Rats and Incorporation in Functional Yogurt

¹Safaa A. Salem, ²Marwa S.M. Diab, ³Samera H. Shehata and ⁴Tarek N. Soliman

¹Department of Medicinal Food, National Organization for Drug Control and Research (NODCAR), Giza, 12553, Egypt

²Department of Molecular Drug Evaluation, National Organization for Drug Control and Research (NODCAR), Giza, 12553, Egypt

³Department of Dairy Science and Technology, Menoufia University, Shibin El-Kom, Cairo, Egypt

⁴Department of Dairy, Food Industries and Nutrition Research Division, National Research Centre El-Buhouth Street, 12622 Dokki, Cairo, Egypt

Abstract

Background and Objective: Chitosan has many functional properties and biological activities. This work aimed to prepare and characterize Chitosan Nanoparticles (CN). Then, evaluate the hypolipidemic and antioxidant effect of CN in rats. Incorporate CN in camel yogurt and evaluation of yogurt properties. **Materials and Methods:** Chitosan Nanoparticles (CN) were prepared and analyzed for the size, zeta potential and polydispersity index (PDI). Total 24 rats were divided into 4 groups, the negative control group was fed on the basal diet and the positive control group was fed on a High-Fat Diet (HFD), the group I and II were fed on the HFD+(CC) or (CN). The feeding period was 6 weeks. Prepared and Characterization stirred camel yogurt fortified by CN. **Results:** CN the size was 27.20 nm, ζ -potential+38.78. After the feeding period for CN and CC groups were a decrease in body weight, serum lipid profile and liver function in both tested groups and an increase in HDL-cholesterol and an increase in antioxidants in the CN group more than that in the CC group was observed. mRNA expression with qPCR for hepatic PPAR γ , HL, GSS and CYP2E1 genes was performed to investigate the alterations in their levels after CN treatment on the liver of rats fed with HFD. **Conclusion:** CN possesses the ability to improve the impairment of lipid metabolism as strongly associated with gene expressions related to lipogenesis and oxidative stress. Also, the addition of 2% CN to camel yogurt gave sensory acceptable and microbiological quality.

Key words: Chitosan nanoparticles, functional stirred camel yogurt, biochemical parameters, microbiological properties, viscosity, gene expression

Citation: Salem, S.A., M.S.M. Diab, S.H. Shehata and T.N. Soliman, 2021. Nanochitosan effect on biomolecular, hypolipidemic in rats and incorporation in functional yogurt. Pak. J. Biol. Sci., 24: 548-561.

Corresponding Author: Tarek Nour Soliman, Department of Dairy, Food Industries and Nutrition Research Division, National Research Centre El-Buhouth Street, 12622 Dokki, Cairo, Egypt

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Chitosan is biocompatible and biodegradable and has many various beneficial biological activities like antimicrobial, antitumor, radio-protective¹, Anti-inflammation², anti-oxidation³, antitumor⁴, Immune-stimulating effects⁵, antimicrobial and wound healing activities⁶. Chitosan also has Anti-coagulant effects⁷, anti-fungal activities⁸ and hypocholesterolemia effects⁹. Chitosan is regarded as an Anti-diabetic agent that decreases plasma glucose in diabetic rats¹⁰. Also, influences the bodyweight through its regulation of lipid and carbohydrate metabolism¹¹. Chitosan is applicable in food production because of its properties sorption properties and the ability to restore the intestinal microflora. The chitosan action mechanism on pathogenic microbial flora is associated with the integrity violation of their outer membrane composed of lipopolysaccharides, glycoproteins, phospholipids. It is shown that chitosan enhances the nonspecific resistance to adverse environmental factors. Besides, it can stimulate the growth of Bifidobacterium and beneficial intestinal flora¹. Despite the well-known benefits of utilizing chitosan in food industries, additional studies need to be done to optimize chitosan's formulations and enhance its physicochemical properties for different uses.

Currently, chitosan is using use in the dairy industry is very promising¹. Yogurt is one of the most popular fermented foods and widely consumed as a functional food in many countries for its good taste and nutritional properties and as an excellent vehicle for delivering probiotics to consumers especially when prepared stirred yogurt from Camel's milk which contains all the essential nutrients and is considered to be good nutritional sources for the human diet in many parts of the world¹² and which can use the chitosan nanoparticles for prepared functional yogurt.

Hyperlipidemia is the presence of abnormal levels of lipids in the blood and is marked by elevated cholesterol Low-Density Lipoprotein (LDL) and low or unaltered High-Density Lipoproteins (HDL) levels in the blood¹³. Hyperlipidemia is a major cause of atherosclerosis and the risk of atherosclerosis can be judged by the parameter of Atherosclerosis Index (AI)¹⁴.

This study aims to prepare chitosan nanoparticles, characterization and investigate their physicochemical properties. Also, aims to evaluate the hypolipidemic and antioxidant effect of chitosan nanoparticle in rats and Application of it in functional camel yogurt with the characterization of yogurt for physicochemical, microbiological and sensory evaluation.

MATERIALS AND METHODS

Materials: Fresh camel's milk was obtained from Barajeel, Giza, Egypt. The gross composition of raw camel's milk was 11.33% Total solids, 3.03% Protein, 4.0% Fat, 0.70% Ash, 0.51% Vitamin D, 3.6% Lactose, 0.15% Titratable acidity and 6.60 PH. Bovine sodium caseinate and Chitosan were obtained from Sigma Aldrich (St. Louis, MO, USA). Two commercial lyophilized DVS mixed bacterial starters, namely: Yo-Fast1 containing *Lactobacillus* (Lb.) *delbrueckii* ssp. *Bulgaricus* and *Streptococcus thermophiles* as a yogurt starter and ABT-5 containing *Bifidobacterium* (B). *Lactis* and *Bifidobacterium animalis* with potential probiotic properties were supplied by the Chr-Hansen company (Horsholm, Denmark). Freeze-dried bacterial starters were activated separately in sterilized (121 °C/10 min) skimmed cow's milk (0.1% fat and 10% SNF) using 0.02% (W/V) inoculums. The activated cultures were used for inoculation of the camel's milk. All chemicals were of analytical grade. The study was carried out at the Department of Dairy, National Research Centre and Department of Dairy Science and Technology, Menoufia University Shibin Elkom and animal house, NODCAR, Egypt from September, 2019-March, 2020).

Animals: Female Sprague Dawley rats weighing 140±20 g were obtained from the animal house of the National Organization for Drug Control and Research, Giza, Egypt. Animals were housed in cages under standard conditions of a 12/12 hrs dark/light cycle, temperature 25±2 °C and relative humidity. They were allowed free access to water and food and left for a week for acclimatization. The investigation complies with the guide for the care and uses laboratory animals (NODCAR/II/25/19). The experimental protocol was approved by the Institutional Ethics Committee of NODCAR, Giza, Egypt.

Preparation of chitosan nanoparticles: Chitosan nanoparticles were prepared based on the ionotropic gelation between chitosan and sodium tripolyphosphate according to Sivakami *et al.*¹⁵.

Size and morphology of chitosan nanoparticles: Chitosan nanoparticles were analyzed for their particle size and distribution using ZS/ZEN3600 Zetasizer (Malvern Instruments Ltd., UK). Scanning Electron Microscopy (JEOL JSM6300 SEM, Tokyo, Japan) was used to acquire the morphology of dried nanoparticles according to Sivakami *et al.*¹⁵.

Experimental design: Twenty four rats have randomly divided into four groups six rats for each as follow, negative control group (NF), fed on basal diet according to A.O.A.C.¹⁶, Positive control group (HF) fed on a high fat diet containing lard 10%, protein 10%, cholesterol 1.5%, bill salt 0.2% and basal diet 78.3%¹⁷.

Group I and II were fed on a high fat diet and given Commercial Chitosan (CC) or Chitosan Nanoparticle (CN) received 3 mg kg⁻¹ per day for 6 weeks, During the experimental period, body weight was recorded and the blood sample was taken from the orbital plexus of an eye of each rat after 3 weeks and at the end of the experiment. Serum was separated by centrifugation at 3000 xg for 10 min and used for serum biochemical analysis. At the end of the experimental rats were sacrificed, the liver was excised and used for biochemical assay.

Serum biochemical parameters: Total lipids, Triglyceride (TG), Total Cholesterol (TC) and High-Density Lipoprotein Cholesterol (HDL.C) were measured and Low-Density Lipoprotein Cholesterol (LDL.C), Atherogenic Index (AI) was calculated by Friedewald equations according to Hellstrand *et al.*¹⁸:

$$\text{LDL.C (mmol L}^{-1}\text{)} = \text{TC} - \text{HDL.C}$$

$$\text{AI} = \frac{\text{TC} - \text{HDL.C}}{\text{HDL.C}}$$

The activities of the Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) were determined according to the method of Reitman and Frankel¹⁹, the protein was determined according to the method of Dumas²⁰. Albumin was determined according to the method of (Dumas²⁰ and globulin was calculated as follows:

$$\text{Total globulin} = \text{Total proteins} - \text{total albumins}$$

Reduced glutathione (GSH) was estimated by its reaction with dithiobis-2-nitrobenzoic acid (DTNB) according to Beutler *et al.*²¹ and catalase (CAT) activity was determined according to Aebi²².

Liver oxidant/antioxidant parameters: To measure ROS production, Nitro Blue Tetrazolium (NBT) was converted into formazan by superoxide anion²³. Total Antioxidant Capacity (TAC) was determined according to Huang *et al.*²⁴ using TAC Assay Kit Cat. No. MAK187 Sigma co.

Table 1: Oligonucleotide primer design for genes analyzed by quantitative reverse transcription PCR

Genes	Primer pairs
GAPDH	F 5'-AATGCATCCTGCA CCACCA A-3' R 5'-GATGCCATAT TCATTGT CATA-3'
PPAR γ	F 5'-TGATATCGACCAGCTGAACC-3' R 5'-GTCCTCCAGCTGTTCCGCA-3'
HL	F 5'- GAACACAGTGCAGACCATAATGCT-3' R 5'- TTCAGGTACATTTCACGAAGACTT-3'
cyp2E1	F 5'-AATGGACCTACCTGGAAGGAC-3' R 5'-CCTCTGGATCCGGCTCTCATT-3'
GSS	F 5'- CCTACATCCTCATGGAGAAGA-3' R 5'- AGAAGAGGAGTGCCAAGTACA-3'

mRNA gene expression by quantitative real-time reverse transcription PCR: Specimens (20 mg) from liver tissue of rats in the four groups were used to extract total RNA using Qiazol reagent (Qiagen, Germantown, MD, USA), then transformed to complementary DNA (cDNA) using cDNA Synthesis Kit (RevertAidTM H Minus Reverse Transcriptase, Fermentas, Thermo Fisher Scientific Inc., Canada) according to the manufacturer's protocol. For gene expression analysis, the quantitative real-time PCR was carried out in a 20 μ L reaction mixture using the QuantiFast SYBR Green fluorescence RT-PCR kit (Bioneer Inc. Korea). Target genes were analyzed by quantitative real-time reverse transcription PCR (qRT-PCR) using Applied Biosystems (Foster City, CA) 7900 Fast Real-Time PCR System. Primers were designed for qRT-PCR and sequences of the used primers are given in Table 1. A house-keeping gene, GAPDH, was used as an internal control to normalize the qRT-PCR data. The expression level of a gene in a given sample was represented as a fold change against the control group.

Preparation of plain stirred yoghurt: The stirred camel milk yogurt is manufactured using the method described by Soliman and Shehata¹², with slight modification. Manufacture yogurt had to be modified slightly as was added NaCas and difficult to see if the chitosan was dissolved fully, it was pre-dissolved and the solution was added to the milk. Raw camel milk was warmed to 40°C and fortified with 3% (w/v) sodium caseinate and sugar 10% (w/v). The mixture was blended using an electrically operated blender. Then, the addition of chitosan solution) with different concentrations (0.0, 0.1, 0.2 and 0.3%, wt. vol⁻¹) of Commercial Chitosan (CC) or Chitosan Nanoparticles (CN) were added into the milk. Then the mixture was homogenized at 1000 psi in a single-stage homogenizer Rannie homogenizer Copenhagen, Denmark. The samples were heated at 85°C for 30 min, followed by cooling to 42-43°C. The milk samples were inoculated with 3% (v v⁻¹) of mixed (1:1) Yo-Fast1 and ABT-5 activated culture

(10^8 - 10^9 CFU mL⁻¹) and inoculated at $43 \pm 1^\circ\text{C}$ for 6-8 hrs until a firm curd was obtained. Then stored at 4°C for 1 day and stirred using the mixer to prepare the stirred camel milk samples.

Physicochemical analysis: Yogurt camel milk samples were analyzed in triplicate. Physical parameters (pH, acidity) and color analysis were conducted via Hunter colorimeter (Hunter Ultra Scan VIS) according to Hunter and Harold²⁴ and chemical parameters (total solids, total protein, fat content and Ash) were determined according to the methods described by AOAC¹⁷. The total carbohydrates in milk and yogurt samples were determined as described by Krishnaveni *et al.*²⁵. Viscosity was measured according to Soliman and Shehata¹² using a Bohlin coaxial cylinder viscometer (Bohlin Instrument Inc., Sweden). Sensory evaluation, the scorecard was designed as described by Soliman and Shehata¹².

Microbiological analysis: Preparation and sterilization of the serial diluent were done as described by Richardson²⁶. Enumeration of *Streptococcus thermophiles* was carried out using a modified M-17 medium and that of *Lactobacillus bulgaricus* was done on a modified MRS medium. The plates were incubated at 37°C for 48 hrs. Pour plate techniques using a plate count agar medium incubated at 32°C for 48 hrs were used for the total bacterial count. The colonies were counted according to Marshall²⁷. The identification of purified colonies was carried out according to Barrow and Feltham²⁸.

Statistical analysis: Data are expressed as means $\pm$ SE. Comparisons across groups were performed with one-way analysis of variance (ANOVA) followed by Duncan multiple comparison tests using software of Statistical Package for the Social Sciences (SPSS Statistics version 25, USA). The presence of different superscripts over the column indicates a significant difference at $p < 0.05$.

RESULTS AND DISCUSSION

Size and zeta-potential of chitosan nanoparticles: Chitosan nanoparticles were prepared based on the ionotropic gelation interactions between positively charged amino groups NH_2 on chitosan and negative tri polyphosphate ions at the ambient room temperature to form NH^{+3} ²⁹.

The particle size, PDI and zeta potential of chitosan nanoparticles using Malvern zeta-sizer were shown in Table 2. Findings show that the minimum particle size of chitosan nanoparticles was 27.20 ± 6 nm. Also, the poly-dispersibility index was 0.602. As shown in Table 2, the chitosan nanoparticles had a zeta-potential of $+38.78 \pm 7.65$ mV.

Surface morphology: The particle morphology characterization can aid us in a better understanding of the changes in morphological or structural of materials when subjected to nano-sizing. The Scanning Electron Microscope (SEM) for commercial chitosan (A) which appears as Monolith particles the large size when the chitosan nanoparticles (B) appear as nano-beads are close (Fig. 1a, b).

Effect of chitosan nanoparticles on rat's body weight:

Gradually increasing in body weight of rats (Table 3) fed on a High Fat diet (HF group) which was significantly more than that of the negative control group which was fed on a basal diet, percentage of body weight gain increased at the end of experimental in values of 77.37 and 24.34 in the two groups, respectively. On the other hand, feeding on a diet containing commercial chitosan or chitosan nanoparticles showed a gradual decrease in body weight in both groups during and at the end of the experiment with non-significant differences between them compared to the HF group in percentage

Table 2: Size and zeta-potential of Chitosan nanoparticles

Sample	Size (nm)	PDI	Zeta-potential (mv)
CN	27.20 ± 6	0.602	$+38.78 \pm 7.65$
CC	545 ± 35	0.39	$+32.75 \pm 1.29$

CN: Chitosan nanoparticles, CC: Chitosan commercial, PDI: Poly-dispersibility index

Table 3: Effect of commercial chitosan and nanoparticle chitosan on body weight (g) after three and six weeks in rats fed on a high fat diet

Animal groups	Feeding period (weeks)				
	Initial	3 weeks	GBW (%)	6 weeks (final)	GBW (%)
NF	151.33 ± 2.11^a	168.83 ± 1.78^a	11.56	188.17 ± 2.76^a	24.34
HF	149.33 ± 2.67^a	200.00 ± 2.71^b	35.75	261.33 ± 3.74^b	77.37
GI	148.00 ± 3.65^a	195.50 ± 5.08^b	33.90	176.00 ± 1.09^a	22.50
GII	148.67 ± 7.99^a	188.16 ± 2.76^b	30.96	173.66 ± 1.62^a	18.94

Mean values with different superscript letters in the same row are significantly different at Duncan test, $p < 0.05$, NF (control negative): Rats fed on a basal diet, HF (Positive control): Rats fed on a high fat basal diet, GI: Rats fed on a commercial chitosan+high fat basal diet, GII: Rats fed on nanoparticles chitosan+high fat basal diet GBW: Gain body weight ratio

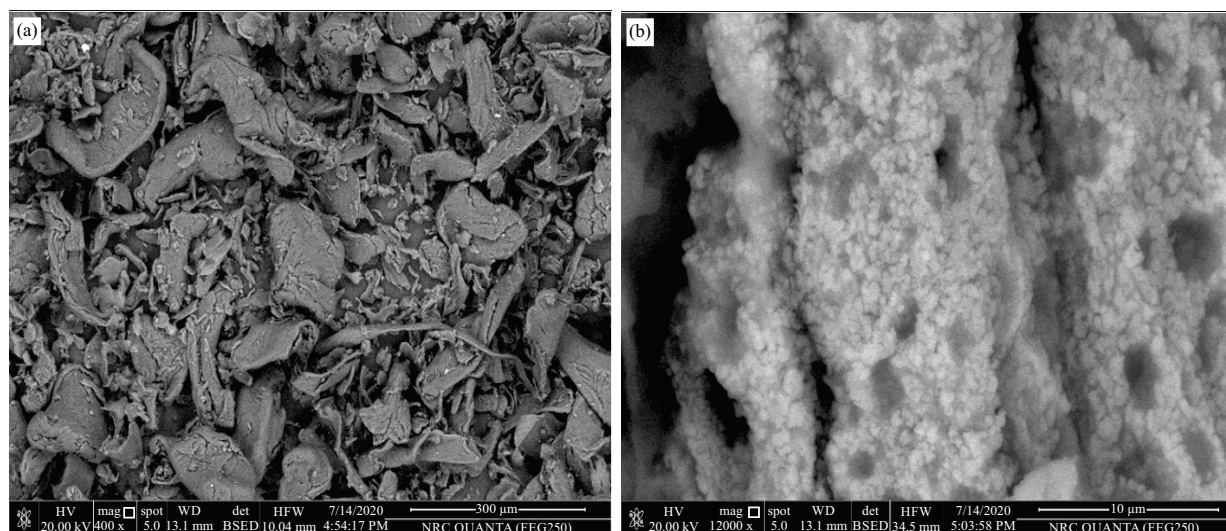


Fig. 1(a-b): Scanning Electron Microscopy (SEM) micrograph of (a) Commercial Chitosan and (b) Nanoparticles chitosan

Table 4: Effect of commercial chitosan and nanoparticles chitosan on lipid profile after three and six weeks in rats fed on the high fat diet

Parameters	Groups			
	NF	HF	GI	GII
3 weeks				
T. Lipids	240.75±14.55 ^a	633.18±9.89 ^b	511.27±19.74 ^c	389.00±5.33 ^d
Triglyceride	88.86±2.16 ^a	129.91±1.80 ^b	125.61±1.60 ^b	106.99±3.51 ^c
T. Cholesterol	92.24±1.57 ^a	186.95±3.15 ^b	140.90±0.99 ^c	126.25±1.09 ^d
HDL-cholesterol	71.37±1.32 ^a	53.86±0.52 ^b	53.14±0.34 ^b	52.57±0.62 ^b
LDL-cholesterol	21.53±1.73 ^a	133.08±3.23 ^b	87.94±0.93 ^c	73.61±1.16 ^d
6 weeks				
T. Lipids	244.80±14.55 ^a	708.14±8.64 ^b	376.18±9.98 ^c	288.96±7.15 ^d
Triglyceride	88.86±2.16 ^a	159.17±2.10 ^b	117.35±2.20 ^c	82.89±2.39 ^a
T. Cholesterol	92.24±1.60 ^a	218.73±6.48 ^b	116.41±3.30 ^c	92.16±93.81 ^a
HDL-cholesterol	71.37±1.34 ^a	38.39±0.72 ^b	71.15±1.30 ^a	73.75±0.70 ^a
LDL-cholesterol	24.15±1.74 ^a	188.17±11.49 ^b	45.43±1.30 ^c	23.63±0.70 ^a

Mean values with different superscript letters in the same row are significantly different at Duncan test, $p < 0.05$). NF (control negative): Rats fed on a basal diet, HF (Positive control): Rats fed on the high fat basal diet, GI: Rats fed on a commercial chitosan+high fat basal diet, GII: Rats fed on nanoparticles chitosan+high fat basal diet HDL: High-density lipoprotein, LDL: Low-density lipoprotein

values of 33.90, 30.96 and 35.75 in the three groups respectively, significant decreases in body weight gain in both groups compared to HF group was observed at the end of the experiment in percentage values of 22.5, 18.94 and 77.37 for GI, GII and HF group, respectively. The results showed that the best effect on B.W. was found in rats fed on chitosan nanoparticles (GII) followed by those fed on commercial chitosan (GI) these results were in agreement with the previous research³⁰. This result suggested that both found that body weight gains of groups fed on chitosan nanoparticles were lower than the groups fed on ordinary chitosan³¹. Also, it was more effective in inhibiting the increased body weights of rats. Generally, the biological activity of particles vice versa was proportional to their size. Smaller particles occupy less volume, resulting in a larger number of particles

with a greater surface area per unit mass, thus, the potential for biological interaction was increased³².

Biochemical analysis

Effect of commercial chitosan and nanoparticles on lipids profile: The effect of feeding on a basal diet supplemented with commercial chitosan or chitosan nanoparticles in the prevention of hyperlipidemia was illustrated in Table 4, gradually increases were found in lipids parameters including, total lipids, triglycerides, total cholesterol, LDL-cholesterol and decrease in HDL-cholesterol in the group fed on high fat diet (HF group) values of 708.14, 159.17, 218.73, 188.17 and 38.39 in the aforementioned parameters respectively compared to the negative control group in values of 244.8, 88.86, 92.24, 24.15 and 71.37 at the end of the experiment. The tested groups GI

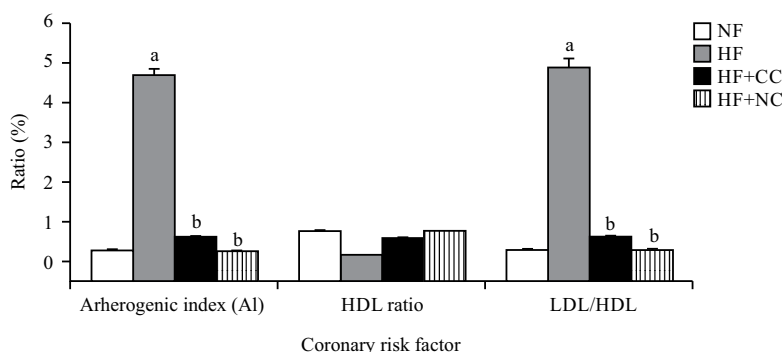


Fig. 2: Coronary risk factor

All values are expressed as mean $\pm$ SEM. "a" refers to a significant change from NF (control negative): Rats fed on a basal diet at $p < 0.05$; "b" refers to a significant change from HF (Positive control): Rats fed on the high-fat basal diet at $p < 0.05$. HF+CC: Rats fed on a commercial chitosan+high-fat basal diet, HF+NC: Rats fed on nanoparticle chitosan+high-fat basal diet

and GII which were fed on a basal diet containing commercial chitosan or chitosan nanoparticles respectively showed gradually significant decreases in all lipid parameters compared to the HF group, the highest decrease was shown in the chitosan nanoparticles group (GII) followed by commercial chitosan group (GI) in values of 288.96, 82.89, 92.16 and 23.63 for GII and in values of 376.18, 117.35, 116.41 and 45.43 in GI group in the aforementioned parameter at the end of the feeding period, which significant increase in HDL-cholesterol was found with the greatest value in GII followed by GI in values of 73.75 and 71.15 compared to HF group in value of 38.39. Chitosan may exert an effect on fecal bacterial enzyme functions. Infeed in with high cholesterol change the level of short-chain fatty acid concentrations and extend the beneficial effect to the distal colon in rats³³ also, orally administered chitosan, about 1.2 g/day per 8 weeks caused a reduction of total and LDL cholesterol³⁴.

Chitosan had fat-binding capability between positively charged amino groups and negatively charged carboxyl groups of fatty acid and bile salt³⁵, this leads to the higher activity of the LDL-receptor and thus lowers LDL-C plasma levels³⁶ and conducive to increasing excretion in feces and reducing TG, besides, to decrease of TG and TC in comparison with HF group³⁷. Also, chitosan has been shown to increase serum HDL-C, containing particle that removes excess cholesterol from tissues and delivers them to the liver for excretion³⁸.

Chitosan nanoparticles were a better cholesterol-binding capacity than commercial chitosan because nanoparticles have a small particle size and exceptionally large surface area for adsorbing organic compounds such as lipids and fatty acids³⁹. Also, it could effectively lower the plasma and liver

lipid levels in rats⁴⁰. The particle size decreases in a larger number of particle surface area per unit mass and, thus, increased potential for biological interaction⁴¹. Very high significant increases in AI and LDL.C/HDL.C ratio were found in a high fat group at the end of the experimental period in values of 4.69 and 4.89 and decrease in HDL.C/TC ratio in value of 0.17 compared to NF group in values of 0.29, 0.30 and 0.77 in the three parameters respectively at the end of the experiment as seen in Fig. 2. Tested groups that were fed on HF commercial chitosan (GI) or HF chitosan nanoparticles (GII) showed a significant decrease in AI and LDL, CL/HDL.C ratio values of 0.63 and 0.64 for GI and 0.26 and 0.31 for GII in the two parameters, respectively compared to the HF group in values of 4.69 and 4.89 in Fig. 2.

While HDL.C/TC ratio showed an increase in values of 0.60 and 0.78 for GI and GII respectively compared to the HF group in value of 0.17 at the end of the experiment.

The best effect was observed in GII in the three parameters which recovered the changes in the HF group and the values were the nearest to NF group values. GI commit in second place with less effect than those in GII. Omari-Siaw *et al.*⁴², found increases in AI which is a representative marker of atherosclerosis (plaque or lipid deposition in aorta and liver) compared to HF. The lowering of the atherogenic index by chitosan binds to negatively charged molecules such as lipids and proteins at pH above their isoelectric point, also it was shown that behaves as a fiber in the gastrointestinal tract and increases the amount of eliminated fat in the stool⁴³. The decrease in AI reduced cardiac risk and therefore revealing the good anti-hyperlipidemic activity of nanoparticle formulation and low LDL/HDL ratio is an indicator of lower risk for coronary disease⁴⁴.

Table 5: Effect of commercial chitosan and nanoparticle chitosan on function liver after three and six weeks in rats fed on high fat diets

Parameters	Groups			
	NF	HF	GI	GII
3 weeks				
AST	46.26±0.62 ^a	53.04±1.31 ^b	49.12±0.79 ^b	47.56±1.52 ^a
ALT	43.57±0.34 ^a	55.04±1.07 ^b	54.67±0.93 ^b	53.00±1.06 ^b
Total protein	7.37±0.06 ^a	7.76±0.15 ^{ab}	7.22±0.01 ^a	7.25±0.01 ^a
Albumin	3.41±0.01 ^a	3.25±0.009 ^b	3.27±0.05 ^b	3.36±0.05 ^{ab}
Globulin	3.96±0.03 ^a	4.51±0.10 ^b	3.95±0.06 ^a	3.98±0.15 ^a
6 weeks				
AST	46.09±0.52 ^a	60.20±0.65 ^b	45.62±0.48 ^a	44.56±0.37 ^a
ALT	43.51±0.41 ^a	60.97±0.54 ^b	47.16±1.38 ^c	42.95±0.729 ^a
Total protein	7.54±0.20 ^a	7.72±0.22 ^b	7.54±0.16 ^a	7.44±0.11 ^a
Albumin	3.27±0.03 ^a	3.37±0.03 ^a	3.43±0.06 ^a	3.41±0.06 ^a
Globulin	4.27±0.61 ^a	4.35±0.16 ^a	4.11±0.09 ^a	4.03±0.15 ^a

Mean values with different superscript letters in the same row are significantly different at Duncan test, $p < 0.05$, NF (control negative): Rats fed on a basal diet, HF (Positive control): Rats fed on the high fat basal diet, GI: Rats fed on a commercial chitosan+high fat basal diet, GII: Rats fed on nanoparticles chitosan+high fat basal diet, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase

Table 6: Effect of commercial chitosan and chitosan nanoparticles on oxidative stress after three and six weeks in rats fed on high fat diets

Parameters	Groups			
	NF	HF	GI	GII
3 weeks				
GSH (mmol dL ⁻¹)	44.83±0.43 ^a	32.92±1.08 ^b	36.46±0.58 ^c	38.85±1.95 ^c
CAT (IU L ⁻¹)	74.10±2.01 ^a	45.82±1.02 ^b	40.53±0.63 ^c	46.28±0.69 ^b
6 weeks				
GSH (mmol dL ⁻¹)	44.50±0.47 ^a	27.11±0.56 ^b	42.89±0.75 ^a	44.91±0.33 ^a
CAT (IU L ⁻¹)	71.99±1.67 ^a	37.18±0.67 ^b	63.56±0.83 ^c	73.96±1.29 ^a

Mean values with different superscript letters in the same row are significantly different at Duncan test, $p < 0.05$, NF (control negative): Rats fed on a basal diet, HF (Positive control): Rats fed on the high fat basal diet, GI: Rats fed on a commercial chitosan+high fat basal diet, GII: Rats fed on nanoparticles chitosan+high fat basal diet, GSH: Glutathione, CAT: Catalase

Effect of chitosan and nanoparticles on liver function: Data in Table 5 showed a significant reduction in ALT and AST activity in the two tested groups (GI and GII) compared with the HF control group, with the greatest reduction values in GII which with fed on chitosan nanoparticle in values of 44.56 and 42.95, followed by GI which was fed on commercial chitosan in values of 45.62 and 47.16, compared to HF group in values of 60.20 and 60.79, respectively De Souza Predes *et al.*⁴⁵ reported that the increased back to damages the structural integrity of the liver because they are located in the cytoplasm and are released into the circulation after hepatocyte damage. The groups feeding on a high fat diet containing commercial chitosan (GI) or chitosan nanoparticles (GII) showed significant decreases compared to HF control, non-significant changes compared to negative control was found and non-significant changes between them Liu *et al.*⁴⁶ reported that high- and low-molecular-weight chitosan supplementations, effect differences in the intestinal absorption efficiency of and their adsorption capacity of dietary lipids may outcome on the regulation of blood and liver lipid metabolism. Also, the high-dose chitosan (10 mg kg⁻¹) treatment group demonstrated the most marked improvement as indicated by

reduced hepatocellular damage and improved fibrotic liver status. On the other hand, non-significant changes were found in, total protein among normal, HF and tested groups.

Effect of chitosan and nanoparticles on serum antioxidant parameters:

Results presented in Table 6 demonstrated the effect of commercial chitosan and chitosan nanoparticles on the activity of glutathione (GSH) and catalase (CAT) content. The mean values of serum GSH level and CAT activity in the HF group were significantly lower at ($p < 0.05$) than that in the NF group in values of 27.11 and 37.18 for the two parameters respectively at the end of the feeding period. Tested groups that were fed on commercial chitosan (GI) and chitosan nanoparticles (GII) showed significant increases in both parameters in values of 42.89 and 44.91 also 63.56 and 73.96 at the end of the feeding period compared to the HF group in values of 27.11 and 37.18 in the two parameters, respectively. The best results were found in the nanoparticle chitosan group which recovered the decreases in the HF group, followed by the GI group which was fed on HF commercial chitosan. Treatment with CS preserves antioxidant enzymes, particularly the glutathione-dependent system, increases

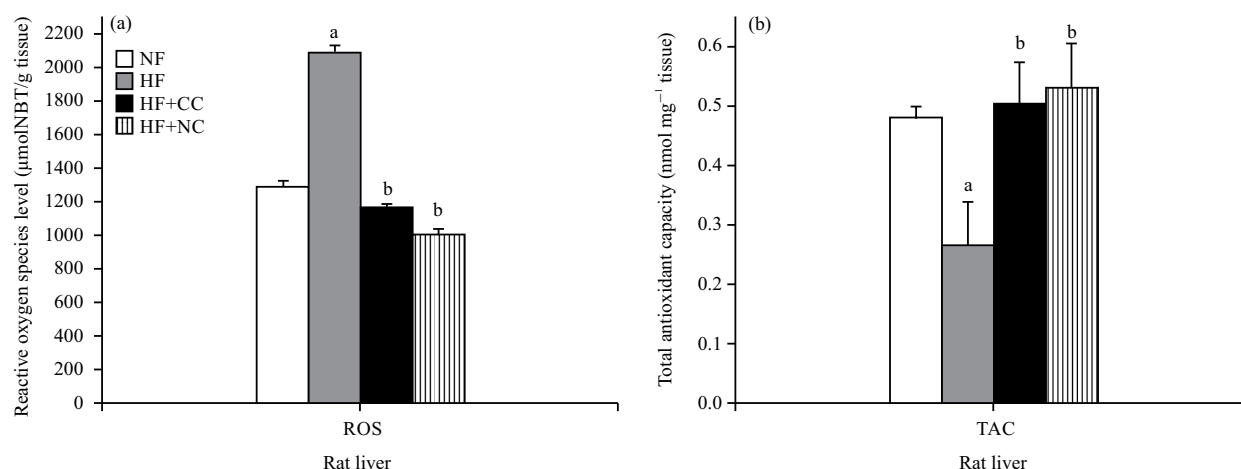


Fig. 3(a-b): Effect of commercial and nanoparticles of chitosan on ROS (a) and TAC (b) levels in rat liver

All values are expressed as mean $\pm$ SEM. "a" refers to a significant change from NF (control negative): Rats fed on a basal diet at $p < 0.05$; "b" refers to a significant change from HF (Positive control): Rats fed on the high-fat basal diet at $p < 0.05$. HF+CC: Rats fed on a commercial chitosan+high-fat basal diet, HF+NC: Rats fed on nanoparticle chitosan+high-fat basal diet

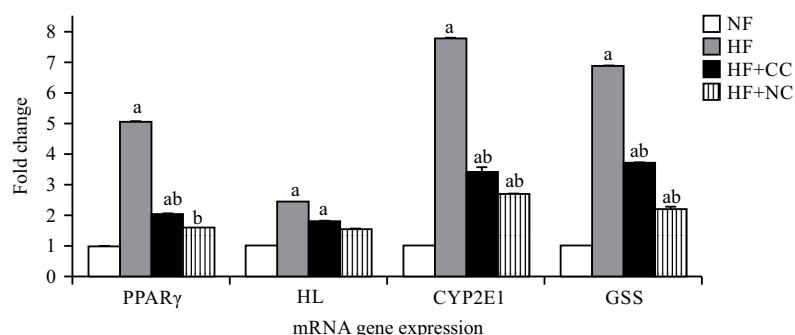


Fig. 4: mRNA gene expression by quantitative real-time reverse transcription PCR

All values are expressed as mean $\pm$ SEM. "a" refers to a significant change from NF (control negative): Rats fed on a basal diet at $p < 0.05$; "b" refers to a significant change from HF (Positive control): Rats fed on the high-fat basal diet at $p < 0.05$. HF+CC: Rats fed on a commercial chitosan+high-fat basal diet, HF+NC: Rats fed on nanoparticle chitosan+high-fat basal diet

antioxidant potential and restores redox balance. Therefore, CS decreases lipids and proteins oxidation and attenuates cellular damage⁴⁷. Generally, supplementation with antioxidants could be the protective agent against many diseases attributed to a high fat diet³². It has also been can effectively reduce the atherogenic lipoprotein profile in patients with hyperlipidemia and atherosclerosis.

Effect of commercial and nanoparticles chitosan on ROS, TAC and mRNA gene expression in rat liver: The results of hepatic ROS and TAC in female rats are shown in Fig. 3a, b. ROS level significantly increases in HF diet-fed rats than in the control group and treatment with both CC and NC can reduce this elevation significantly to normal levels (Fig. 3a). On

Contrary, TAC levels were decreased significantly in rats feed on the High fat diet than in control and both treatments increase this reduction (Fig. 3b).

Changes in gene expression were quantified by qRT-PCR analysis using liver samples of rats from all tested groups. The qRT-PCR analysis showed that the gene expression profiles were very similar to the control group results regarding the direction (up-or down-regulation) and degree of differences in expression (Fig. 4). Hepatic PPAR γ , HL, GSS and CYP2E1 expressions significantly increased in HFD rats as compared with the control rats, which were significantly decreased by chitosan and chitosan nanoparticle supplementation ($p < 0.05$). Collectively, the results have confirmed the efficiency of chitosan nanoparticles to reverse the impairments induced by HFD in female rats.

Table 7: Physicochemical properties of stirred camel milk yoghurt and fortified with CC or CN

Parameters	Control	0.1 CC	0.2 CC	0.3 CC	0.1 CN	0.2 CN	0.3 CN
Chemical characterization (g/100 g)							
Total solids	24.15±0.11	24.17±0.09	24.16±0.18	24.17±0.12	24.16±0.15	24.15±0.12	24.17±0.13
Protein	5.79±0.01	5.78±0.02	5.77±0.05	5.76±0.03	5.78±0.15	5.77±0.10	5.76±0.09
Fat	4.00±0.05	4.05±0.05	4.00±0.10	4.05±0.05	4.10±0.05	4.05±0.05	4.05±0.10
Total sugars	11.61±0.22	11.9±0.15	11.61±0.17	11.39±0.16	11.59±0.11	11.49±0.15	11.41±0.08
Ash	0.76±0.04	0.77±0.06	0.76±0.05	0.75±0.07	0.78±0.10	0.79±0.13	0.81±0.05
Physical characterization (Colour analysis)							
L*	93.65±0.05	93.25±0.36	93.10±0.04	93.08±0.01	93.70±0.04	93.76±0.09	93.77±0.04
a*	-0.58±0.01	-0.52±0.01	-0.50±0.01	-0.43±0.01	-0.59±0.01	-0.57±0.01	-0.44±0.01
b*	7.22±0.07	7.20±0.08	7.18±0.02	6.99±0.04	7.21±0.08	7.20±0.04	7.19±0.05

CN: Chitosan nanoparticles, CC: Chitosan commercial, Data presented are the means of triplicates $\pm$ SD ^{a,b}Mean in the same row followed by different superscript letters differ significantly ($p < 0.05$) L*: Value measuring black (0)/white (100); a*: Value measuring green (-)/red (+); b*: Value measuring blue (-)/yellow (+)

Up-regulation of mRNA of tested genes and an increase in ROS levels and decreases TAC activity in rats group fed on high fat diet resulted in our study in agreement with Xu *et al.*⁴⁸ in which hyperlipidemia causes an increase in the production of ROS through glucose auto-oxidation to induce oxidative stress. Chitosan has been shown to reduce the absorption of dietary fat and cholesterol and effectively improve hyperlipidemia *in vivo*³⁷. The focus of the current study was to determine whether changes in gene expression in the liver induced by a high fat diet could be attenuated or prevented by supplement with the same dose of commercial chitosan and nanoparticle chitosan. In the present study, we measured gene expression in the liver from rats that were kept on a high fat diet and commercial chitosan and nanoparticles chitosan supplement, with a focus on those genes involved in lipid homeostasis and reactive oxygen species metabolism. The results showed that HFD up-regulated these genes and chitosan treatment considerably restored mRNA levels of these genes compared with the control rat group. Observations have suggested that the expressions of hepatic PPAR γ and LH were regulated by nutritional and/or metabolic signals⁴⁹. Also, increased mitochondrial accumulation of CYP2E1 was accompanied by an increase in mitochondrial ROS production as well as increased GSS⁵⁰.

Physicochemical properties of chitosan stirred camel milk yoghurt types:

The chemical composition of stirred camel milk in the control and fortified with 0.1, 0.2 and 0.3 Commercial Chitosan (CC) or Nanoparticle Chitosan (CN) (Table 7). The findings of total solids were statistically insignificant ($p < 0.05$) for the addition of 0.0, 0.1, 0.2 and 0.3 CC of 24.15, 24.17, 24.16 and 24.17, respectively or 24.16, 24.15, 24.17 for addition 0.1, 0.2 and 0.3% of CN, respectively. Likewise, for total protein and fat ratio were statistically insignificant ($p < 0.05$) affect for additions. But, the results of ash (%) for samples fortified with 0.01, 0.2 and 0.3% CN were

statistically significant ($p < 0.05$) when increased from 0.76-0.81%. The changes of color by the effect of fortification of CC, CN with different ratios (0.1, 0.2 and 0.3%) on Values of L*, a* and b* were illustrated (Table 7). The value L* was decreased with increasing the addition of CC from 0.1-0.3% (wt vol⁻¹) was 93.25-93.08. While the addition of CN 0.1-0.3 caused an increase of 93.70-93.77, respectively. The values of a* and b* affected by additions CC or CN were finding that when increased the ratio additions from 0.1-0.3% (wt vol⁻¹) was decreased all values for a* and b*.

Variation of titratable acidity and pH of plain stirred yogurt and fortified with CC or CN:

Chitosan types (CC and CN) have a statistically significant effect on pH and acidity (lactic acid %) of yogurt samples ($p < 0.05$) Fig. 5a, b. Control stirred camel milk yogurt had increased its acidity than others of those significantly different from other CC and CN. It was found that when adding chitosan in its natural form (CC) affects reducing acidity much higher than if it was added in the nanoparticles of chitosan (CN). This effect is evident at 0.3% of CC form than in CN form. 0.3% CC added yogurt has the lowest acid production rate than it's in 0.3% CN and other samples fortified with CC or CN chitosan in stirred camel milk yogurt. At 2 weeks to three weeks of post-production, acidity was nearly the same trended for all treatments (Fig. 5a). It may be that chitosan forms (CC and CN) have inhibited the growth of lactic acid bacteria and reduced acid production. Lactic Acid bacteria inhibition by the interaction of anionic groups of the cell surface with the positive charge of chitosan caused the formation of an impermeable layer around the bacteria cell, which prevents the transport of essential nutrients⁵¹. They have noticed another mechanism of inhibition actions of chitosan by Aranaz *et al.*⁵². Represented in the binding of chitosan to DNA triggers inhibition of mRNA synthesis through penetration of the microbial nuclei by chitosan and interfering with the synthesis of mRNA and proteins. The fermentation

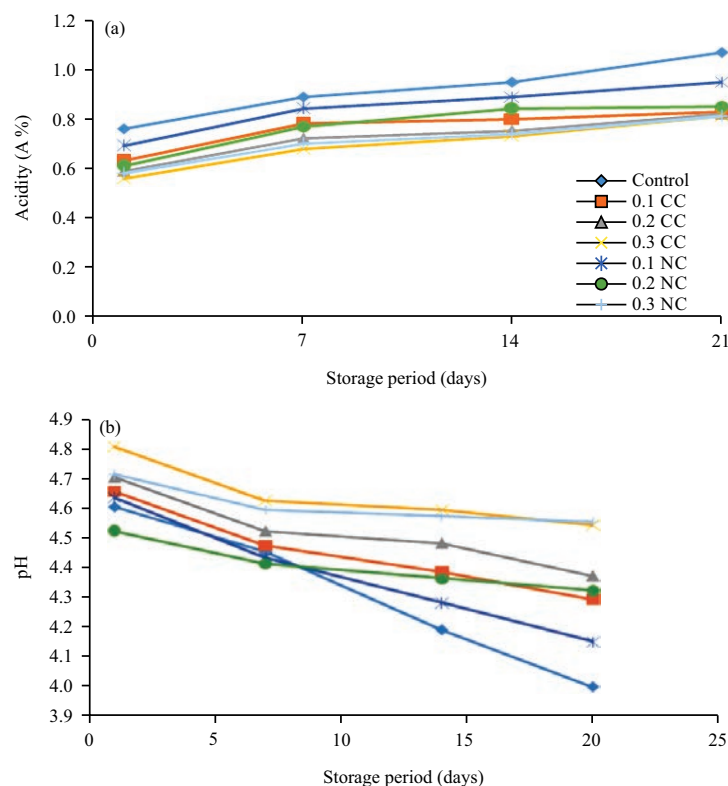


Fig. 5(a-b): Mean values and variation of (a) Titratable acidity and (b) pH of plain stirred yogurt Fortified with (0.1, 0.2 and 0.3%) of CC or CN for 20 days under 4°C refrigeration

Table 8: Changes in the survival of Lactic acid bacteria (CFU mL⁻¹) during cold storage for 21 days

Samples	Storage period (day)				
	1	5	9	14	21
Control	8.90×10^{10}	4.45×10^{10}	6.75×10^9	1.57×10^9	1.61×10^9
0.1 CC	6.55×10^{10}	5.95×10^9	2.31×10^9	1.18×10^9	9.05×10^8
0.2 CC	2.85×10^{10}	2.25×10^9	1.41×10^9	8.27×10^8	7.65×10^8
0.3 CC	1.16×10^9	7.09×10^8	4.23×10^8	2.02×10^8	1.35×10^8
0.1 CN	7.25×10^{10}	9.45×10^9	5.18×10^9	3.02×10^9	1.79×10^9
0.2 CN	6.65×10^{10}	8.95×10^9	4.45×10^9	2.35×10^9	1.08×10^9
0.3 CN	9.87×10^9	4.74×10^9	3.05×10^9	4.85×10^8	2.0×10^8

CN: Chitosan nanoparticles, CC: Chitosan Commercial

time of the camel milk yogurt samples with different ratios and forms of CC or CN chitosan not much affected when compared with control. Low pH value (pH 4.6) at the end of fermentation can be caused by the antibacterial activity of chitosan. Where chitosan forms were inversely affected by pH, with higher activity at lower pH value, these results agreed with⁵¹.

The results in Fig. 5b showed that the pH of the stirred camel milk yogurt and fortified with different ratios (0.1, 0.2 and 0.3%) of CC or CN was measured for 21 days with 7 days interval. The effect of the addition of CC or CN was significantly on the pH of stirred camel milk fortified with different forms of Chitosan ($p < 0.05$). The Plain stirred camel

milk yogurt CN. It was found that when adding chitosan in its natural form (CC) affected increasing pH much higher than if it was added in the nanoparticles of chitosan (CN). The pH of yogurt added 0.3% CC has maintained at a high pH level to the pH of plain camel milk yogurt and it was significantly different from others ($p < 0.05$).

Changes in the survival of lactic acid bacteria (cfu mL⁻¹) during cold storage for 21 days: The data of Table 8 illustrated the changes of count (*L. bulgaricus* and *Strep. thermophilus*) in stirred camel milk yogurt and fortified with CC or CN added during stored at 4°C for 21 days. The total count of lactic acid bacteria (*L. bulgaricus* and *Strep.*

Table 9: Coliform, *E. coli*, yeast and mold counts (cfu g⁻¹) of stirred camel milk yogurt samples and fortified with CC and CN during cold storage at 4°C

Table 31. Coliform, <i>Er</i> cell, yeast and mould counts (cfu g ⁻¹) obtained earlier from yogurt samples and stored with 0.2 and 0.3 wt vol ⁻¹ during cold storage at 7 °C										
Concentration of sample (% wt vol ⁻¹)	Storage period (day)								Coliform	<i>E. coli</i>
	Yeast 10 ⁻¹ dilution				Moulds 10 ⁻¹ dilution					
	1	7	14	21	1	7	14	21		
Control	Nil	Nil	Nil	Nil	Nil	1	2	4	Negative	Negative
0.1 CC	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Negative	Negative
0.2 CC	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Negative	Negative
0.3 CC	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Negative	Negative
0.1 CN	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Negative	Negative
0.2 CN	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Negative	Negative
0.3 CN	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Negative	Negative

CN: Chitosan nanoparticles, CC: Chitosan Commercial, PDI: Poly-dispersibility index

Table 10: Sensory evaluation of stirred camel's milk yogurt fortified with different ratios of CC or CN when fresh and after 21 days of storage at 5±1°C

Samples	Flavor 50		Body and texture 35		Color 15	
	Fresh	21days	Fresh	21 days	Fresh	21 days
Control	44.50±1.29	42.00±0.82	30.00±0.82	29.25±0.96	11.5±0.58	11.00±0.82
0.1 CC	44.75±1.89	42.75±0.82	31.75±0.96	30.00±0.82	10.75±1.26	10.25±0.58
0.2 CC	45.25±2.22	43.00±1.29	33.00±0.82	31.00±1.63	10.00±0.82	9.50±1.15
0.3 CC	45.25±1.96	43.50±1.50	33.20±2.06	31.25±0.96	9.75±0.50	9.25±0.86
0.1 CN	45.25±0.82	43.00±0.82	31.50±0.86	30.75±0.50	12.25±0.96	11.00±0.82
0.2 CN	47.75±2.08	44.50±2.58	34.25±0.96	32.50±1.29	13.50±0.58	11.75±0.50
0.3 CN	46.25±1.50	44.00±1.41	34.50±1.76	32.75±0.96	13.75±0.50	11.50±1.29

CN: Chitosan nanoparticles, CC: Chitosan commercial, PDI: Poly-dispersibility index

thermophilus) in control stirred camel milk yogurt was the greatest count of 8.90×10^{10} compared to yogurt fortified with different ratios from CC or CN. Moreover, increasing the concentrations of CC or CN from 0.1-0.3% (wt vol⁻¹) on the first-day storage showed a statistically significant ($p < 0.05$) decrease in the mean microbial counts from 6.55×10^{10} - 1.16×10^9 CFU mL⁻¹ and 7.25×10^{10} - 9.87×10^9 CFU mL⁻¹, respectively. All samples showed a decrease in the mean microbial counts during storage periods.

Coliform, *E. coli*, yeast and mould counts (cfu g⁻¹) of stirred camel milk yogurt samples and fortified with CC and CN during cold storage at 4°C.

Also, all the samples were not contaminated with Coliform and *Escherichia coli* and it showed by the absence of air bubble formation in the test tube. The result of Table 9 showed that there was some mould growth in control stirred camel milk yogurt while the samples fortified with CC or CN have successfully controlled the mould growth for 21 days.

The results could be explained by the fact that chitosan forms (CC or CN) affect antimicrobial⁵¹. NoH *et al.*⁵³ noted that chitosan markedly inhibited the growth of gram-positive bacteria such as *Staphylococcus aureus*, *Lactobacillus bulgaricus*, *Lactobacillus plantarum* and *Lactobacillus brevis*. It detected that chitosan has the direct intervention of fungal growth and activation of several defense processes and it has good antimicrobial activity against mould⁵⁴.

Viscosity: The viscosity values of all the samples studied increased sharply increased with different ratios addition from CC or CN. The result of Fig. 6 showed the highest viscosity value for stirred camel milk fortified with 0.6% CN, then 0.4% CN. Therefore, the viscosity improves by adding chitosan in the nano form more than adding it as a commercial form. Similar results were reported by Habtegebriel and Admassu⁵⁵ for camel's milk fortified with pectin as a stabilizer.

Sensory evaluation: The data of Table 10 showed the sensory evaluation of the control camel's yogurt and Camel yogurt fortified with 0.1, 0.2 and 0.3% CC or CN fresh and after 21 days of cold storage. The samples were sensory evaluated by 15 trained panelists from the Dairy department, National Research center, for color, body and texture and flavor. The statistical analysis of fresh yogurt samples showed a significant ($p < 0.05$) effect for adding Chitosan on all parameters of sensory. The body and Texture were affected by an increased ratio of addition CC (0.1-0.3) from 31.75-33.20, while the control of 30. Also, color and flavor take the same trend. Notably, the effect of addition chitosan nanoparticles gives the best significant ($p < 0.05$) scoring of sensory acceptability for an addition 0.2% chitosan nanoparticle for all parameters flavour, body and texture and color of 47.75, 34.25 and 13.50, respectively. The same trend for all samples was found with 21 days of cold storage as those obtained from fresh samples.

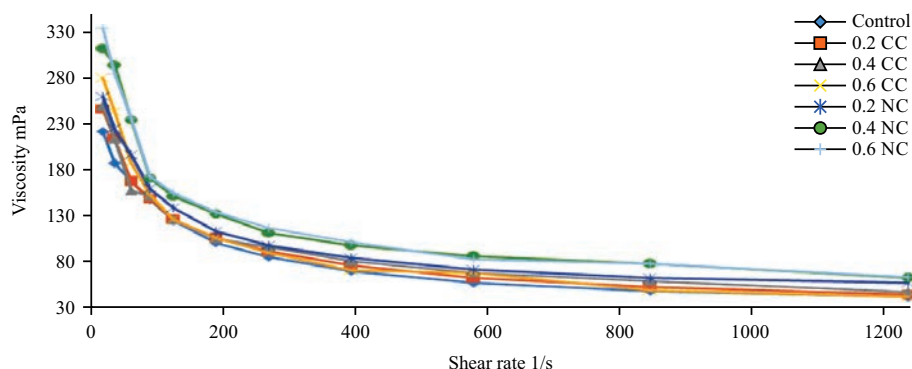


Fig. 6: Viscosity of stirred camel's milk fortified with different ratios of CC or CN at 5°C

CONCLUSION

The findings of the current study support the argument that HFD induces hyperlipidemia. Furthermore, in the present analyses of the rat liver mRNA, increased PPAR γ , HL, GSS and CYP2E1 expressions indicated that high fat diet-induced lipid metabolism disorder and oxidative damage. Chitosan nanoparticles supplementation significantly suppresses high fat-diet-induced damage. The results expand the current understanding of hyperlipidemia induced by HFD and provide a potential interventional strategy by supplementing chitosan. Besides, we used CN in the manufacture of yogurt, which had the effect of improving the acceptability of all parameters of the functional stirred camel yogurt. Can applicable Chitosan nanoparticles in the production of yogurt and the best ratio of addition 0.2% CN, because of her functional properties and more economical than commercial chitosan. Where, these effects improving the viscosity and appearance and also activate the starter bacteria, prevent the emergence of fungi and yeasts and improves the sensory and technological properties of the functional stirred camel yogurt.

SIGNIFICANCE STATEMENT

The present study found the chitosan nanoparticle supplementation significantly suppresses high fat-diet-induced damage. Additionally, the results are to expand the current understanding of hyperlipidemia induced by HFD and provide a potential interventional strategy by supplementing chitosan. Also, can applicable Chitosan nanoparticles in the production of yogurt and the best ratio from chitosan-nanoparticle addition is 0.2%, because of her functional properties and more economically than commercial chitosan. Furthermore, chitosan nanoparticles affect improving viscosity and appearance and also activate the starter bacteria, prevent

the emergence of fungi and yeasts and improve the sensory acceptability and technological properties of the functional stirred camel yogurt. Hence, it is recommended to use chitosan nanoparticles in functional dairy products.

REFERENCES

1. Evdokimov, I., L. Alieva, V. Varlamov, V. Kurchenko, V. Haritonov and T. Butkevich, 2015. Usage of chitosan in dairy products production. *Foods Raw Mater.*, 3: 29-39.
2. Meng, Z.Q., Z.H. Tang, Y.X. Yan, C.R. Guo and L. Cao *et al.*, 2014. Study on the anti-gout activity of chlorogenic acid: Improvement on hyperuricemia and gouty inflammation. *Am. J. Chin. Med.*, 42: 1471-1483.
3. Anraku, M., M. Tanaka, A. Hiraga, K. Nagumo and T. Imafuku *et al.*, 2014. Effects of chitosan on oxidative stress and related factors in hemodialysis patients. *Carbohydr. Polym.*, 112: 152-157.
4. Kumar, S. and J. Koh, 2012. Physiochemical, optical and biological activity of chitosan-chromone derivative for biomedical applications. *Int. J. Molec. Sci.*, 13: 6102-6116.
5. Jeon, Y.J. and S.K. Kim, 2002. Antitumor activity of chitosan oligosaccharides produced in ultrafiltration membrane reactor system. *J. Microbiol. Biotechnol.*, 12: 503-507.
6. Arpa, M.D., A. Yoltaş, E.O. Tarlan, C.Ş. Şenyüz, H. Sipahi, A. Aydın and N.Ü. Okur, 2020. New therapeutic system based on hydrogels for vaginal candidiasis management: Formulation-characterization and *in vitro* evaluation based on vaginal irritation and direct contact test. *Pharm. Dev. Technol.*, 25: 1238-1248.
7. Hu, W., M. Liu, X. Yang, C. Zhang and H. Zhou *et al.*, 2019. Modification of chitosan grafted with collagen peptide by enzyme crosslinking. *Carbohydr. Polym.*, 206: 468-475.
8. Qin, Y., R. Xing, S. Liu, K. Li and X. Meng *et al.*, 2012. Novel thiosemicarbazone chitosan derivatives: Preparation, characterization and antifungal activity. *Carbohydr. Polym.*, 87: 2664-2670.

9. Kerch, G., 2015. The potential of chitosan and its derivatives in prevention and treatment of age-related diseases. *Mar. Drugs*, 13: 2158-2182.
10. Kim, J.N., I.Y. Chang, H.I. Kim and S.P. Yoon, 2009. Long-term effects of chitosan oligosaccharide in streptozotocin-induced diabetic rats. *Islets*, 1: 111-116.
11. Xiao, D., W. Ren, P. Bin, S. Chen and J. Yin *et al.*, 2016. Chitosan lowers body weight through intestinal microbiota and reduces IL-17 expression via mTOR signalling. *J. Funct. Foods*, 22: 166-176.
12. Soliman, T.N. and S.H. Shehata, 2019. Characteristics of fermented camel's milk fortified with kiwi or avocado fruits. *Acta Sci. Pol. Technol. Aliment.*, 1: 53-63.
13. Nelson, R.H., 2013. Hyperlipidemia as a risk factor for cardiovascular disease. *Primary Care: Clin. Office Pract.*, 40: 195-211.
14. Li, J., C.X. Chen and Y.H. Shen, 2011. Effects of total glucosides from paeony (*Paeonia lactiflora* Pall) roots on experimental atherosclerosis in rats. *J. Ethnopharmacol.*, 135: 469-475.
15. Sivakami, M.S., T. Gomathi, J. Venkatesan, H.S. Jeong, S.K. Kim and P.N. Sudha, 2013. Preparation and characterization of nano chitosan for treatment wastewaters. *Int. J. Biol. Macromolec.*, 57: 204-212.
16. Atkinson, M.A. and Furth, S. L., 2016. Anemia in children with chronic kidney disease. In: *Official Methods of Analysis of AOAC International 20th(Ed.) Current Through Revision 2*. A.O.A.C., AOAC International USA 3172.
17. Wang, Z., F. Zhang, Y. Yan, Z. Zhang, L. Wang and C. Qin, 2019. Lipid-lowering activities of chitosan and its quaternary ammonium salt for the hyperlipidemia rats induced by high-fat diets. *Int. J. Biol. Macromol.*, 132: 922-928.
18. Hellstrand, S., E. Sonestedt, U. Ericson, B. Gullberg, E. Wirfält, B. Hedblad and M. Orho-Melander, 2012. Intake levels of dietary long-chain PUFAs modify the association between genetic variation in FADS and LDL-C. *J. Lipid Res.*, 53: 1183-1189.
19. Reitman, S. and S. Frankel, 1957. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *Am. J. Clin. Pathol.*, 28: 56-63.
20. Doumas, B.T., W.A. Watson and H.G. Biggs, 1971. Albumin standards and the measurement of serum albumin with bromocresol green. *Clin. Chim. Acta*, 31: 87-96.
21. Beutler, E., 1963. Improved method for the determination of blood glutathione. *J. lab. clin. Med.*, 61: 882-888.
22. Aebi, H., 1984. Catalase *in vitro*. *Meth. Enzymol.*, 105: 121-126.
23. Vrablic, A.S., C.D. Albright, C.N. Craciunescu, R.I. Salganik and S.H. Zeisel, 2001. Altered mitochondrial function and overgeneration of reactive oxygen species precede the induction of apoptosis by 1 O octadecyl 2 methyl rac glycerol 3 phosphocholine in p53 defective hepatocytes. *FASEB J.*, 15: 1739-1744.
24. Krishnaveni, S., T. Balasubramanian and S. Sadasivam, 1984. Sugar distribution in sweet stalk sorghum. *Food Chem.*, 15: 229-232.
25. Hunter, R.S. and D.L. MacAdam, 1976. The measurement of appearance. *Physics Today*, Vol. 29. 10.1063/1.3024412.
26. Richardson, G.H., 1985. Standard methods for examination of dairy products. 15th Edn., Vol. 15. American Public Health Association, Washington, D.C., ISBN: 9780875531328 Pages: 412.
27. Marshall, R.T., 1992. Standard Methods for the Examination of Dairy Products. 16th Edn., American Public Health Association (APHA), Washington, DC, USA, ISBN-13: 9780875532080, Pages: 546.
28. Barrow, G.I. and R.K. Feltham, 2004. Cowan and Steel's Manual for the Identification of Medical Bacteria. 3rd Edn., Cambridge University Press, England, ISBN: 9780521543286, Pages: 331.
29. Lee, S.T., F.L. Mi, Y.J. Shen and S.S. Shyu, 2001. Equilibrium and kinetic studies of copper(II) ion uptake by chitosan-tripolyphosphate chelating resin. *Polymer*, 42: 1879-1892.
30. Zhang, H.L., Y. Tao, J. Guo, Y.M. Hu and Z.Q. Su, 2011. Hypolipidemic effects of chitosan nanoparticles in hyperlipidemia rats induced by high fat diet. *Int. Immunopharmacol.*, 11: 457-461.
31. Zhang, J., J. Liu, L. Li and W. Xia, 2008. Dietary chitosan improves hypercholesterolemia in rats fed high-fat diets. *Nut. Res.*, 28: 383-390.
32. Xia, T., N. Li and A.E. Nel, 2009. Potential health impact of nanoparticles. *Annu. Rev. Public Health*, 30: 137-150.
33. Han, X.Y., W.L. Du, C.L. Fan and Z.R. Xu, 2010. Changes in composition a metabolism of caecal microbiota in rats fed diets supplemented with copper loaded chitosan nanoparticles. *J. Anim. Physiol. Anim. Nutr.*, 94: e138-e144.
34. Bokura, H. and S. Kobayashi, 2003. Chitosan decreases total cholesterol in women: a randomized, double-blind, placebo-controlled trial. *Eur. J. Clin. Nutr.*, 57: 721-725.
35. Zhang, J., W. Xia, P. Liu, Q. Cheng, T. Tahi, W. Gu and B. Li, 2010. Chitosan modification and pharmaceutical/biomedical applications. *Mar. Drugs*, 8: 1962-1987.
36. Gu, H.M. and D.W. Zhang, 2015. Hypercholesterolemia, low density lipoprotein receptor and proprotein convertase subtilisin/kexin-type 9. *J. Biomed. Res.*, 29: 356-361.
37. Zhang, J., W. Zhang, B. Mamadoubay and W. Xia, 2012. A comparative study on hypolipidemic activities of high and low molecular weight chitosan in rats. *Int. J. Biol. Macromol.*, 51: 504-508.
38. Cao, P., H. Pan, T. Xiao, T. Zhou, J. Guo and Z. Su, 2015. Advances in the Study of the antiatherogenic function and novel therapies for HDL. *Int. J. Mol. Sci.*, 16: 17245-17272.
39. Jin, Q., H. Yu, X. Wang, K. Li and P. Li, 2017. Effect of the molecular weight of water-soluble chitosan on its fat-/cholesterol-binding capacities and inhibitory activities to pancreatic lipase. *Peer J.*, Vol. 5. 10.7717/peerj.3279.

40. Pan, H., Q. Yang, G. Huang, C. Ding and P. Cao *et al*, 2016. Hypolipidemic effects of chitosan and its derivatives in hyperlipidemic rats induced by a high-fat diet. Food Nut. Res., Vol. 60. 10.3402/fnr.v60.31137.
41. Jeevanandam, J., A. Barhoum, Y.S. Chan, A. Dufresne and M.K. Danquah, 2018. Review on nanoparticles and nanostructured materials: history, sources, toxicity and regulations. Beilstein J. Nanotechnol., 9: 1050-1074.
42. Omari-Siaw, E., Q. Wang, C. Sun, Z. Gu and Y. Zhu *et al*, 2016. Tissue distribution and enhanced *in vivo* anti-hyperlipidemic-antioxidant effects of perillaldehyde-loaded liposomal nanoformulation against Poloxamer 407-induced hyperlipidemia. Int. J. Pharm., 513: 68-77.
43. Capuano, E., 2017. The behavior of dietary fiber in the gastrointestinal tract determines its physiological effect. Crit. Rev. Food Sci. Nut., 57: 3543-3564.
44. Rawal, T., N. Mishra, A. Jha, A. Bhatt, R.K. Tyagi, S. Panchal and S. Butani, 2018. Chitosan nanoparticles of gamma-oryzanol: Formulation, optimization and *in vivo* evaluation of anti-hyperlipidemic activity. AAPS PharmSciTech, 19: 1894-1907.
45. De Souza Predes, F., M.A. da Silva Diamante, M.A. Foglio, C.A. Camargo and H. Aoyama *et al*, 2014. Hepatoprotective effect of *Arctium lappa* root extract on cadmium toxicity in adult Wistar rats. Biol. Trace Element Res., 160: 250-257.
46. Liu, S.H., C.Y. Chiu, C.M. Shi and M.T. Chiang, 2018. Functional comparison of high and low molecular weight chitosan on lipid metabolism and signals in high-fat diet-fed rats. Mar. Drugs, Vol. 16. 10.3390/md16080251.
47. Luo, Y. and Q. Wang, 2013. Recent advances of chitosan and its derivatives for novel applications in food science. J. Food Proc. Beverages, 1: 1-13.
48. Xu, Y., B. Shi, S. Yan, T. Li, Y. Guo and J. Li, 2013. Effects of chitosan on body weight gain, growth hormone and intestinal morphology in weaned pigs. Asian-Australas. J. Anim. Sci., 26: 1484-1489.
49. Zhou, Y. and L. Rui, 2010. Major urinary protein regulation of chemical communication and nutrient metabolism. Vitamins Horm., 83: 151-163.
50. Raza, H., S.K. Prabu, M.A. Robin and N.G. Avadhani, 2004. Elevated mitochondrial cytochrome P450 2E1 and glutathione S-transferase A4-4 in streptozotocin-induced diabetic rats: Tissue-specific variations and roles in oxidative stress. Diabetes, 53: 185-194.
51. Rajapaksha, D.S.W. and K.A.H.T. Kodithuwakku, 2014. Evaluation of chitosan for its inhibitory activity on post-acidification of set yoghurt under cold storage for 20 days. J. Chitin Chitosan Sci., 2: 16-20.
52. Aranaz, I., M. Mengibar, R. Harris, I. Panos and B. Miralles *et al*, 2009. Functional characterization of chitin and chitosan. Curr. Chem. Biol., 3: 203-230.
53. No, H.K., N.Y. Park, S.H. Lee and S.P. Meyers, 2002. Antibacterial activity of chitosans and chitosan oligomers with different molecular weights. Int. J. Food Microbiol., 74: 65-72.
54. Tikhonov, V.E., E.A. Stepnova, V.G. Babak, I.A. Yamskov and J. Palma-Guerrero *et al*, 2006. Bactericidal and antifungal activities of a low molecular weight chitosan and its *N*-2(3)-(dodec-2-enyl)succinoyl/-derivatives. Carbohydr. Polym., 64: 66-72.
55. Habtegebriel, H. and S. Admassu, 2016. Optimization of the processing conditions of stirred yoghurt from camel milk using linear programming technique. Optimization, 53: 33-38.