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Effect of Chitosan and Chitosan Nanoparticles on the Quality of Fish Fingers During Cold Storage

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ABSTRACT

Nanotechnology could be playing a key role in fish processing as well as preservation. Therefore, this study assessd the effect of using 1% shrimp and crab chitosan and their nanoparticles on the physical, chemical, microbiological, and sensory quality attributes of the catfish fingers during storage at 4°C±1 for 15 days. The main objective of this study was to assess the impact of the addition of chitosan and its nanoparticles with 1% to the formula of the catfish fingers on volatile organic compounds, fatty acid composition, and biochemical, organoleptic, and microbilogical quality of the catfish fingers stored at 4±1°C for 15 days. Chitosan or chitosan nanoparticles significantly (P<0.05) decreased myristic, palmitic, stearidonic, and rachidonic acids, while it significantly (P<0.05) increased lauric, stearic, arachidic, oleic, gadoleic, eicosapentaenoic, and docosahexaenoic acids. On the other hand, there were significant differences in volatile compounds of the catfish fingers as affected by the added chitosan and its nanoparticles, including aldehydes, alcohols, carboxylic acids, sulfur-containing compounds, aromatic compounds, and other compounds. In addition, pH value, total volatile basic nitrogen, trimethylamine, thiobarbituric acid, and total bacterial counts of stored catfish fingers at 4±1°C were significantly (P<0.05) decreased but did not exceed acceptability limit for all groups. In conclusion, chitosan and chitosan nanoparticles trials had a longer shelf-life of up to 15 days if stored at 4±1°C compared to control sample, according to obtaining quality indices results. Additionally, chitosan nanoparticles improved the quality attributes of the stored catfish fingers at 4 ±1°C.

INTRODUCTION

Nanoparticles are polymers ranging from 10 to 1000nm in size **(Kreuter, 2001)**. Nanoparticles display unparalleled chemical and physical properties due to the effects like quantum, mini, and surface size effects and the macroquantum tunnel effect **(Xu & Du, 2003)**. Nanochitosan is a bioactive and eco-friendly, natural product with excellent physicochemical and antibacterial activity characteristics prepared using the ionotropic gelation between chitosan and sodium tripolyphosphate as a controlled-release drug

carrier. Chitosan nanoparticles have inhibited bacterial growth in food because of their antimicrobial properties **(Du *et al*., 2009)**. Furthermore, using nanoparticles of chitosan-tripolyphosphates retained antioxidant activity *in vitro* using free radical scavenging and reducing power tests **(Zhang *et al*., 2008)**.

Fish finger processing technology is facing some challenges during cold storage, which does not completely prevent microbial and chemical reactions, which may lead to changes of its quality **(VidyaSagar & Srikar, 1996)**. Changes of proteins and lipid oxidation are the two major problems correlated with the freezing of fish products, which result in a rough, dry texture and an unpleasant taste **(Simeonidou *et al*., 1997)**. Numerous studies have been conducted to produce fish fingers from different fish species using different food additives **(Reddy *et al*., 1992; Zaghlool *et al*., 2023)**. Scientists have made a great effort to improve the quality of fish fingers and overcome processing, handling, and storing problems; however, very few articles have been conducted on using chitosan and its nanoparticles for the preservation of fish fingers **(Abdou *et al*., 2012; Osheba *et al*., 2013)**. Therefore, the aim of this work was to evaluate the effect of shrimp and crab chitosan and its nanoparticles at level of 1% on the physical, chemical, microbiological, and sensory quality attributes of the catfish fingers stored at 4±1°C for 15 days.

MATERIALS AND METHODS

Fresh African catfish (*Clarias gariepinus*) samples were purchased from El-Obour Market, Egypt, with an average weight of 2000-3000g and were immediately transferred in an ice box in 2h time to the Fish Processing and Technology Laboratory, Fish Research Station, National Institute of Oceanography and Fisheries. The catfish samples were carefully washed with running tap water, manually beheaded, gutted, filleted and rewashed carefully. The fillets with approximately 45% yield were kept frozen at -18±1°C up to using it. Before the day of producing the fish fingers, the frozen fillets were taken out of the freezer and kept at 4-5°C to defrost overnight. Spices, sugar, starch, salt and edible oils were purchased from local market, Cairo, Egypt. All chemicals (sodium bicarbonate, sodium polyphosphate and commercial chitosan) applied in this study were of analytical grade and purchased from Sigma-Aldrich, Germany. All other ingredients, such as onion, garlic, were purchased from reputed commercial suppliers. Shrimp and crab chitosan were used to prepare nanoparticles chitosan.

Methods

Fish fingers preparation

Catfish fingers were prepared according to the method of **Talab *et al.* (2022)** with some modifications. The fingers formulation consisted of 81.83% minced fish, 1.23% salt, 0.08% onion powder, 0.08% garlic powder, 0.16% ground coriander seed, 0.08% black pepper powder, 0.16% monosodium glutamate, 4.09% starch, 4.09% vegetable oil, and 8.18% ice water. The catfish fillets were divided into seven groups, then minced separately by a meat grinder with a 5mm-hole plate, weighted, and then added to other

ingredient. All ingredients were thoroughly mixed by a kitchen blender and weighed. The catfish fingers were then shaped and formed, packed, wrapped with polyethylene sheets, and stored at 4±1°C for 15 days. Chitosan solution was prepared by dissolving 20g of chitosan in 4973.8mL of distilled water with 6.25g of acetic acid under a mechanical stirring for 15min. After which, agitated was used on a constant agitation at 37°C for 24h **(Qi *et al.*, 2004)**. Seven groups of catfish fingers were prepared as follows: T1 was the control sample of the catfish fingers which do not contain chitosan; and the other samples contained chitosan as follows: T2 sample of 1% commercial chitosan, T3 sample of 1% commercial chitosan nanoparticles, T4 sample of 1% shrimp chitosan, T5 sample of 1% shrimp chitosan nanoparticles, T6 sample of 1% crab chitosan, and T7 sample of 1% crab chitosan nanoparticles. Deep-frying was carried out using a preheated sunflower oil at 160°C for 5–6min, and the fried samples were used only for the sensory evaluation.

Analysis

The moisture and ash contents of fresh sample were determined by drying at 105°C up till constant weight (about 16h), then ash content was determined in dried sample at 500°C according to the guidelines of **AOAC (2000)**. The protein content was determined using Kjldahl method as described in **AOAC (2000)**. Crude fat was determined following Bligh and Dyer method **(Bligh & Dyer, 1959)**. The pH value and Trimethyl amine nitrogen (TMA-N), were determined according to **AOAC (2002)**. Total volatile basic nitrogen (TVB-N) and thiobarbituic acid (TBA) were determined according to **Pearson (1991)**. Total bacterial count was estimated as stated by **Downes and Ito (2001)**. Sensory evaluation was carried out according to **Fey and Regenstein (1982)**.

Fatty acids composition

The analysis of fatty acids composition was carried out in the National Research Center, Giza, Egypt. Cold extraction of fat and the identification of fatty acids using gas chromatography, flame ionization detector, Supelco™ SP-2380 capillary column, HP 6890 were conducted according to the method described by **Zahran and Tawfeuk (2019)**.

Volatile compounds

Volatile compounds were carried out in the National Research Center, Giza, Egypt using GLC-MSS. Volatile compounds of catfish fingers were analyzed according to **Centonze *et al.* (2019)** using a solid-phase microextraction (SPME), (Agilent 8890 GC System), coupled to a mass spectrometer (Agilent 5977B GC/MSD). All the obtained results were expressed as a mean value of three replicates ± SD (Microsoft Office Excel, 2010).

Statistical analysis

Statistical analysis was conducted using triplicate samples and represented as mean ±SD. Statistical analysis was performed using SAS program (Statistical Analytical Systems, Cary, NC).

RESULTS and DISCUSSION

Chemical composition of catfish fingers

Chemical composition of different catfish fingers samples are shown in Table (1). The obtained results showed that the moisture content of all the fortified catfish finger did not get affected compared to control sample, where they ranged between 55.58 – 57.18%. The same trend was also noticed in gross chemical contents, where protein, lipid and ash ranged between 18.00-18.90%, 21.91-19.29%, and 2.65-2.20%, respectively. This result could be due to using a small amount (1%) of shrimp chitosan or 1% crab chitosn. Consequently, an addition of a small amount of chitosan has no effects on the chemical composition of the product. Moreover, it could be noticed that using the same percentage of nano particle size of shrimp chitosan or crab chitosan has no effect on the chemical composition of catfish finger. This result agreed with the fact that there is no change in chemical composition of any compound if converted to nano particle size. Similar results were reported by **Cakli *et al.* (2005)**, **Talab (2014)**, **Inanli and Amin (2022)**, **Talab *et al.* (2022)** and **Zaghlool *et al.* (2023)**.

Table 1. Gross chemical composition of different catfish fingers samples (on fresh weight basis)

Trials	Moisture (%)	Protein (%)	Lipid (%)	Ash (%)	Energy (Kcal/100g)
T1	56.48±0.2	18.35±0.1	19.84±0.1	2.53±0.1	257.31±1
T2	56.47± 0.1	18.28±0.1	20.84±0.2	2.42±0.1	266.03±1
T3	55.79± 0.2	18.25±0.1	21.30±0.2	2.37±0.1	270.05±1
T4	55.06±0.1	18.33±0.1	21.91±0.2	2.65±0.1	275.90±1
T5	57.70±0.1	18.12±0.2	19.29±0.1	2.20±0.1	251.37±1
T6	57.18±0.2	18.09±0.1	19.67±0.1	2.40±0.1	254.67±1
T7	55.58±0.1	18.90±0.1	20.46±0.2	2.33±0.1	265.25±1

T1: control sample (without adding chitosan); **T2:** chitosan-based catfish fingers prepared with 1% commercial chitosan; **T3:** chitosan-based catfish fingers prepared with 1% commercial chitosan nanoparticles; **T4:** chitosan-based catfish fingers prepared with 1% shrimp chitosan; **T5:** chitosan-based catfish fingers prepared with 1% shrimp chitosan nanoparticles; **T6:** chitosan-based catfish fingers prepared with 1% crab chitosan; **T7:** with 1% crab chitosan nanoparticles. Each values represent average of three replicate samples ± SD.

Fatty acid composition of fish fingers

Fatty acids composition of different catfish fingers samples are shown in Table (2). The results showed that the main saturated fatty acid in control catfish fingers (T1) was palmitic acid (26.82%), followed by stearic acid (5.10%). In case of using catfish fingers chitosan-based prepared with 1% commercial chitosan (T2), chitosan-based catfish fingers prepared with 1% commercial chitosan nanoparticles; T4: chitosan-based catfish fingers prepared with 1% shrimp chitosan; T5: chitosan-based catfish fingers

prepared with 1% shrimp chitosan nanoparticles; T6: chitosan-based catfish fingers prepared with 1% crab chitosan; T7: with 1% crab chitosan nanoparticles.

Table 2. Fatty acids composition of different catfish fingers

Fatty acids (% of total fatty acids)	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**
Saturated fatty acids (SFA)							
Lauric acid (C12:0)	nd*	nd	0.11	nd	nd	nd	nd
Myristic acid (C14:0)	2.94	1.49	1.51	1.28	0.96	1.55	1.22
Palmitic acid (C16:0)	26.82	24.23	24.13	22.18	23.01	25.82	25.14
Stearic acid (C18:0)	5.10	5.47	5.42	6.25	6.20	5.47	5.40
Arachidic acid (C20:0)	nd	0.87	1.77	1.65	1.35	1.97	nd
Unsaturated fatty acids (USFA)							
Palmitoleic acid (C16:1), n9	5.74	4.33	4.33	5.11	5.00	4.33	4.21
Oleic acid (C18:1n9c)	25.82	31.25	30.25	30.19	31.46	29.80	32.53
Linoleic acid (C18:2n6c)	14.56	14.10	14.10	14.88	15.31	14.10	14.48
α- Linolenic acid (C18:3n3)	2.42	1.75	1.75	2.53	2.28	1.75	1.49
Stearidonic acid (C18:4) n3	1.49	1.39	1.39	1.43	1.12	1.15	1.11
Gadoleic acid (C20:1)	nd	0.15	0.87	0.93	0.59	0.82	nd
Arachidonic acid (C20:4)	9.12	1.97	0.15	2.11	1.83	nd	1.72
Eicosapentaenoic acid (C20:5)	1.63	3.88	4.81	3.17	2.95	3.81	3.73
Docosahexaenoic acid (C22:6)	4.38	9.12	9.42	8.29	8.35	9.44	9.04
Total (%)							
Saturated fatty acids	65.16	67.94	67.07	68.64	68.89	65.2	68.31
Unsaturated fatty acids	34.86	32.06	32.94	31.36	31.52	34.81	31.76

nd: not detected*;* **T1:** control sample (without adding chitosan); **T2:** chitosan-based catfish fingers prepared with 1% commercial chitosan; **T3:** chitosan-based catfish fingers prepared with 1% commercial chitosan nanoparticles; **T4:** chitosan-based catfish fingers prepared with 1% shrimp chitosan; **T5:** chitosan-based catfish fingers prepared with 1% shrimp chitosan nanoparticles; **T6:** chitosan-based catfish fingers prepared with 1% crab chitosan; **T7:** with 1% crab chitosan nanoparticles. Each values represent average of three replicate samples ± SD.

This result could be due to using small amount (1%) of shrimp chitosan (or 1% crab chitosn. There were small difference in palmitic acid for catfish fingers chitosan-based prepared with 1% commercial chitosan (T2), chitosan-based catfish fingers prepared with 1% commercial chitosan nanoparticles; T4: chitosan-based catfish fingers prepared with 1% shrimp chitosan; T5: chitosan-based catfish fingers prepared with 1% shrimp chitosan nanoparticles; T6: chitosan-based catfish fingers prepared with 1% crab chitosan; T7: with 1% crab chitosan nanoparticles.

The main unsaturated fatty acids in control catfish fingers were 25.82% oleic acid followed with 14.56% linoleic acid, 9.12% arachidonic acid, 5.74% palmitoleic acid, 4.38% docosahexaenoic acid, 2.94% myristic acid, 2.42% α- linolenic acid, 1.63% eicosapentaenoic acid, and 1.49% stearidonic acid. Catfish fingers were rich in palmitic, followed by oleic and linoleic acids. The control fish fingers had 65.16% saturated fatty

acids and 34.86% unsaturated fatty acids. It was observed that the content of myristic, palmitic, stearidonic, and arachidonic acids were decreased, while the content of lauric, stearic, arachidic, oleic, gadoleic, eicosapentaenoic, docosahexaenoic acids increased by adding chitosan or its nanoparticles to the formula of catfish fingers. The most prevalent fatty acids in both control and treated fish fingers were palmitic, oleic, linoleic, respectively (Table 2).

These results agree with **Tokur *et al.* (2006),** who reported that the dominant fatty acids in carp fingers produced from washed mince mirror was found to be linoleic acid (54.7%) and oleic acid (25.0%). On the other hand, Oleic acid levels of the catfish fingers increased significantly ($P \leq 0.05$) in the treated groups with chitosan and its different nano-particles. Significant differences ($P \leq 0.05$) were observed in the percentage of most FAs between the control catfish fingers and catfish fingers. **Tokur *et al.* (2006)** attributed the higher amount of linoleic acid to pre-frying treatment of carp fingers that led to absorption of the frying oil. Similar results were reported by **Talab *et al.* (2023)** in fish burgers processed using commercial shrimp and crab chitosan and chitosan nanoparticles.

Volatile flavor compounds of fish fingers

Volatile flavor compounds of different fish fingers samples were determined and recorded in Table (3). A total of 42 volatile compounds were identified in different samples. The obtained volatile compounds were mainly aldehydes, alcohols, carboxylic acids, carboxylic acids, sulfur compounds and aromatic compounds. The catfish finger samples contained higher number of volatile compounds reaching 11 in aromatic compounds, followed by 7 aldehydes, 6 alcohols, 5 carboxylic acids, and 2 sulfur-containing compounds. The volatile compounds of different catfish fingers samples were affected slightly when using chitosan or its nano-particles.

It could be noticed in Fig. (1) that the aromatic compounds represent the main volatile compounds in different catfish fingers samples, followed by alcohols and aldehydes. The control sample had higher level of cuminaldehyde (14.08), followed by eugenol (9.94), methyl n-hydroxybenzene carboximidoate (6.35), palmitic acid (5.81), and oleic acid (5.46). On the other hand, there were slight differences in volatile compounds in samples as affected by adding chitosan and its nanoparticles, including aldehydes, alcohols, carboxylic acids, sulfur-containing compounds, aromatic compounds and other compounds.

Similar results were reported by **Talab *et al.* (2023)** for fish burgers formulated with the nanoparticles of commercial shrimp and crab chitosan. These results could be caused by protein decomposition producing peptides and amino acids during storage **(Wang *et al.*, 2020; Agboola *et al.*, 2021)**. Following additional reactions of decarboxylation and deamination, it released an unpleasant odor **(Hoque *et al.*, 2018)**.

Table 3. Volatile flavor compounds of different catfish fingers samples

Compounds	T1	T2	T3	T4	T5	T6	T7
Aldehydes (7)							
Nonanal	0.58	nd*	0.96	0.66	0.46	nd	nd
α-Terpinen-7-al	1.47	3.00	1.57	1.62	1.36	1.17	2.04
γ-Terpinen-7-al	2.16	3.87	2.41	2.50	1.98	1.64	3.04
1-Pentanol	Nd	nd	nd	0.44	0.83	0.28	nd
3-p-Menthen-7-al	Nd	nd	nd	nd	nd	0.38	nd
Cuminaldehyde	14.08	23.28	15.43	16.03	13.17	11.47	18.79
Benzaldehyde	nd	1.44	nd	nd	nd	nd	nd
Alcohols (6)							
Eucalyptol	3.99	2.78	4.59	5.80	6.28	6.82	3.12
Linalool	3.78	5.60	5.57	6.73	6.50	6.32	4.78
α-Terpineol	0.52	1.02	0.63	0.66	0.59	0.57	0.71
Camphol	0.61	nd	nd	0.81	0.79	0.83	nd
Eugenol	9.94	34.25	10.40	8.60	7.90	6.58	14.32
Methyleugenol	0.68	2.13	0.62	0.53	0.54	0.43	1.17
Carboxylic acids (5)							
Allantoic acid	nd	nd	nd	nd	0.72	0.88	nd
Palmitic acid	5.81	3.07	3.95	1.3	1.34	1.15	4.64
Linoleic acid	1.13	nd	nd	nd	nd	nd	nd
Oleic acid	5.46	nd	1.19	nd	nd	nd	0.79
Stearic acid	0.84	nd	0.54	nd	nd	nd	nd
Sulfur containing compounds (14)							
Diallyl sulfide	1.54	nd	1.92	1.94	1.77	2.15	0.81
Diallyl disulphide	2.32	1.02	2.88	3.11	2.84	2.82	2.40
Aromatic compounds							
Benzene	1.13	nd	1.87	nd	3.99	4.70	3.23
Toluene	4.05	1.00	6.32	5.30	4.47	6.27	3.80
o-Xylene	nd	nd	1.26	0.98	0.75	1.06	1.03
Methyl N-hydroxybenzenecarboximidoate	6.35	nd	3.62	4.69	5.19	4.78	2.34
α-Pinene	0.61	0.85	nd	0.78	0.82	0.87	nd
β-Pinene	1.88	nd	1.71	2.42	2.64	2.81	1.21
β-Myrcene	1.81	nd	2.13	2.59	2.54	2.67	1.43
p-Cymene	3.70	nd	4.35	6.06	6.62	6.85	3.03
D-Limonene	4.10	0.94	5.13	7.16	8.28	7.91	3.59
γ-Terpinene	3.48	0.76	3.93	5.55	5.92	6.22	2.75
β-Caryophyllene	3.09	nd	3.06	3.10	3.29	2.81	3.87
Others (9)							

Trimethylhydrazine	4.01	nd	2.00	1.82	nd	nd	2.65
Dihydroxydimethylsilane	nd	0.62	nd	nd	nd	nd	0.29
cis-Thujone	4.45	4.61	3.83	1.38	1.07	1.68	1.31
(+)-Camphor	1.25	1.10	1.56	1.95	1.94	2.09	1.16
Carvone	3.37	6.86	3.83	3.98	3.33	2.86	4.36
α-Terpinyl acetate	1.41	0.90	1.56	1.51	1.57	1.40	2.30
Ethyl Acetate	nd	nd	nd	nd	nd	nd	1.63
Styrene	nd	nd	1.17	nd	nd	1.10	1.32
Copaene	0.40	nd	nd	nd	0.51	0.44	0.57

nd: not detected; **T1:** control sample (without adding chitosan); **T2:** chitosan-based catfish fingers prepared with 1% commercial chitosan; **T3:** chitosan-based catfish fingers prepared with 1% commercial chitosan nanoparticles; **T4:** chitosan-based catfish fingers prepared with 1% shrimp chitosan; **T5:** chitosan-based catfish fingers prepared with 1% shrimp chitosan nanoparticles; **T6:** chitosan-based catfish fingers prepared with 1% crab chitosan; **T7:** with 1% crab chitosan nanoparticles. Each values represent average of three replicate samples ± SD.

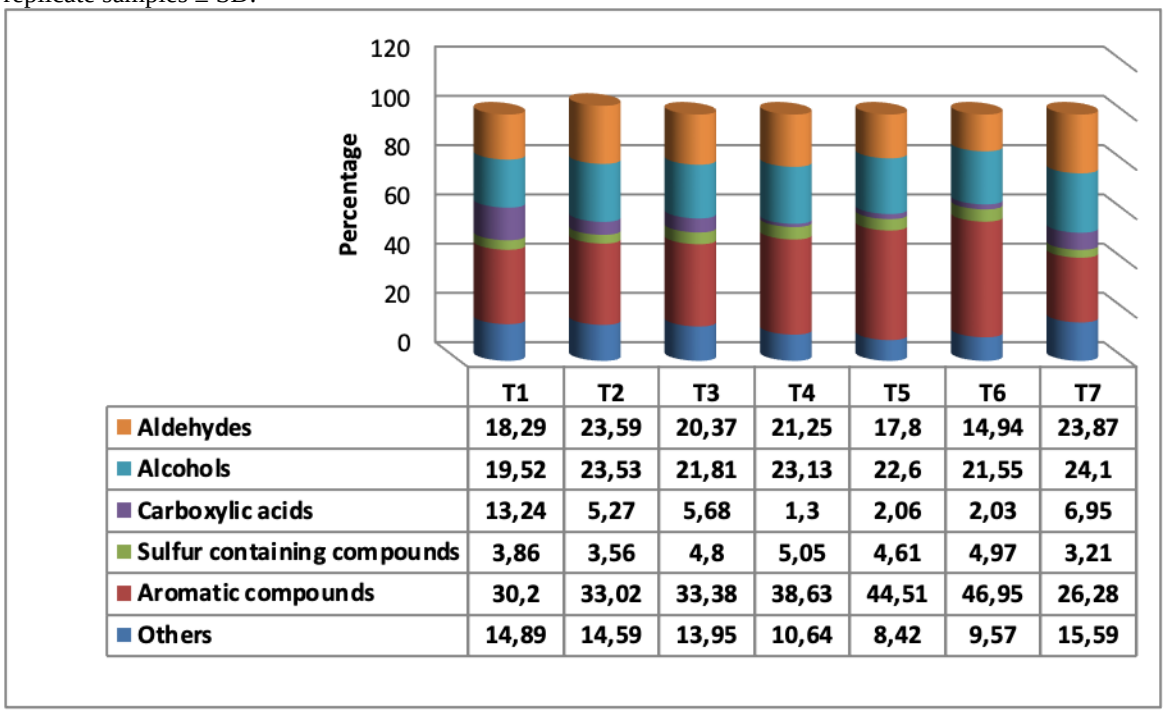

Fig. 1. Percentage of total volatile compounds in catfish fingers samples

Chemical composition of different catfish fingers samples during storage at 4±1°C are clearly shown in Table (4). Slight changes were observed in chemical composition of different catfish fingers during storage at 4±1°C for 15 days, whereas protein content decreased slightly during storage period in all the studied samples. This could be related especially with the addition of 1% chitosan or its nanoparticles to the formula of catfish fingers compared to the control sample. These results agree with those of **Mohamed *et al.* (2015)**, who attributed the decrease in protein contents of the catfish fingers to the effect of change in pH during cold storage, which could increase the

activity of proteolytic enzymes that hydrolyze protein. In general, the proportion of chemical composition and partial degradation of proteins by proteolytic enzymes which were not totally inactivated may be the causes of the decrease in crude protein concentration in various samples through cold storage. These results are in agreement with those obtained by **Talab and Abou-Taleb (2021)**, **Talab *et al.* (2022)** and **Talab *et al.* (2023)**.

Table 4. Chemical composition of different fish fingers samples during storage at 4±1°C for 15 days

Storage (days)	T1	T2	T3	T4	T5	T6	T7
Moisture (%)							
0	56.48	56.47	55.79	55.06	57.70	57.18	56.97
5	55.25	56.11	55.50	54.54	57.32	56.47	56.58
10	55.32	55.57	55.33	54.37	56.12	56.27	56.39
15	55.17	55.29	55.27	54.22	55.85	56.17	56.20
Crude Protein (%)							
0	20.15	20.13	20.11	20.1	20.09	20.12	20.2
5	19.35	19.64	19.2	19.52	19.00	19.08	19.24
10	19.09	18.89	18.78	18.41	18.47	18.15	18.94
15	18.35	18.28	18.25	18.33	18.12	18.09	18.9
Fat (%)							
0	19.84	20.84	21.30	21.91	19.29	19.67	20.46
5	19.25	20.80	21.20	21.82	19.11	19.68	20.50
10	19.85	20.89	21.44	21.92	19.36	19.74	20.63
15	19.90	20.96	21.77	21.97	19.69	19.87	20.70
Ash (%)							
0	2.65	2.54	2.51	2.85	2.32	2.51	2.57
5	2.63	2.48	2.49	2.80	2.30	2.47	2.45
10	2.55	2.45	2.41	2.70	2.25	2.43	2.36
15	2.53	2.42	2.37	2.65	2.20	2.40	2.33
Total (%)							
	T 1	T 2	T 3	T 4	T 5	T 6	T 7
0	99.12	99.98	99.71	99.92	99.4	99.48	98.81
5	96.48	97.03	97.39	98.51	95.73	96.5	97.16
10	96.66	94.8	94.96	95.57	93.2	93.79	94.32
15	95.1	92.95	93.66	94.17	91.16	92.53	93.13

T1: control sample (without adding chitosan); **T2:** chitosan-based catfish fingers prepared with 1% commercial chitosan; **T3:** chitosan-based catfish fingers prepared with 1% commercial chitosan nanoparticles; **T4:** chitosan-based catfish fingers prepared with 1% shrimp chitosan; **T5:** chitosan-based catfish fingers prepared with 1% shrimp chitosan nanoparticles; **T6:** chitosan-based catfish fingers prepared with 1% crab chitosan; **T7:** with 1% crab chitosan nanoparticles. Each values represent average of three replicate samples ± SD.

Changes in the ash content (%, ww) of different catfish fingers stored at 4±1ºC are shown in Table (4). Differences were found among the different samples; similar observation was reported by **Khallaf *et al.* (2021)** and **Talab *et al.* (2023)**. The increase in the ash content was mainly due to the salt addition; the other minerals found in recipe components including minerals during formulating process.

Stability of fish finger during cold storage

The pH value

The obtained results showed that there was a significant increase in pH value of all the samples of cold catfish fingers at 4°C±1 for 15 days (Table 4). Similar observation was reported by **Talab and Abou-Taleb (2021)**. The obtained results showed that pH values of fish fingers showed a significant increase due to the glycogen breakdown causing lactic acid formation. Additionally, the decline in pH value during storage was attributed to the formation of lactic acid from glycogen as a result of autolysis **(Aycicek *et al.*, 2004; Kilinc *et al.*, 2008)**. On the other hand, **Osheba *et al.* (2013)** revealed that the reduction in pH values for all fish fingers treatments coated with chitosan or chitosan nanoparticles may be caused by the acidic coatings formed on the surface of fish fingers. **Bazargani-Gilani *et al.* (2015)** endogenous enzymes or microbial activity led to increase the amounts of ammonia and trimethylamine, which caused the increment of pH value during storage. Similar observations were reported by **Rani *et al.* (2017)** and **Lithi *et al.* (2020)**.

Total volatile basic nitrogen (TVB-N)

Changes in the TVB-N content (mg N/100g sample) of different cold catfish fingers at 4±1°C for 15 days are shown in Table (5). The results showed a gradual increase in the TVBN during the cold storage in most samples. This may be due to microbial and enzymatic activity that led to the decomposition of protein and other nitrogenous materials. The decrease in TVBN during storage may be explained by the addition of some nanomaterials in fish fingers mixture. Similar results were reported by **Mohamed *et al.* (2015)** and **Talab and Abou-Taleb (2021)**.

The results indicated that the cold samples of nanomaterials contained lower levels of TVB-N than other samples. According to **EOS (1991)**, the nanomaterials prepared from crab chitosan showed a better effect in reducing the TVB-N than the other nanoparticles (prepared from commercial and shrimp chitosan). The TVBN values were within the standard value and did not exceed the maximum permissible limits during storage periods.

Trimethylamine (TMA-N)

Changes in the TMA-N content (mg N/100g sample) of different cold catfish fingers at 4±1°C for 15 days are shown in Table (5). The obtained results indicated that the TMA-N in the cold chitosan-based catfish fingers increased to a lower extent in comparison with the control sample, and this could be explained by the fact that the added nanomaterials are able to improve the protein quality in addition to reducing its

rapid changes during storage. This result agrees with the finding of **Mohamed *et al.* (2015)** and **Talab and Abou-Taleb (2021)** for fish burgers formulated with commercial shrimp and crab chitosan mixed with its nanoparticles. The increases of TMA-N may be due to the enzymatic decomposition of trimethylamine oxide (TMA-O) to TMA-N and formaldehyde **(Bekhit *et al.*, 2021; Zaghlool *et al.*, 2023)**. The TMA-N contents of different fish finger treatments didn't exceed the maximum permissible limits (10mg/ 100g sample) set by **EOS (1991)** during storage period.

Table 5. Physiochemical properties of catfish fingers stored at 4±1°C for 15 days

Storage periods (days)	T1	T2	T3	T4	T5	T6	T7
pH value							
0	6.74	6.59	6.6	6.36	6.57	6.55	6.22
5	6.75	6.67	6.63	6.39	6.6	6.57	6.25
10	6.82	6.76	6.7	6.48	6.62	6.69	6.29
15	6.85	6.77	6.76	6.5	6.65	6.75	6.44
Total volatile basic nitrogen (TVB-N)							
0	12.80	11.80	10.60	10.00	11.60	11.85	11.40
5	16.35	14.89	13.76	12.46	12.87	12.90	11.98
10	20.30	16.38	15.23	14.32	15.63	15.67	15.01
15	34.69	29.47	25.22	28.30	23.11	27.46	21.23
Trimethylamine (TMA-N)							
0	2.36	1.75	1.7	1.72	1.71	1.68	1.65
5	5.75	5.86	4.8	5.83	4.79	5.25	3.01
10	8.18	7.65	6.45	7.78	6.2	7.17	5.05
15	10.21	9.13	8.1	9.09	7.23	8.78	6.22
Thiobarbituric acid value (TBA)							
0	0.69	0.76	0.78	0.54	0.48	0.25	0.35
5	1.97	1.80	1.83	1.74	1.69	1.49	1.40
10	3.04	2.94	2.93	2.86	2.88	2.71	2.40
15	4.65	3.90	3.50	3.40	3.25	3.33	3.03

T1: control sample (without adding chitosan); **T2:** chitosan-based catfish fingers prepared with 1% commercial chitosan; **T3:** chitosan-based catfish fingers prepared with 1% commercial chitosan nanoparticles; **T4:** chitosan-based catfish fingers prepared with 1% shrimp chitosan; **T5:** chitosan-based catfish fingers prepared with 1% shrimp chitosan nanoparticles; **T6:** chitosan-based catfish fingers prepared with 1% crab chitosan; **T7:** with 1% crab chitosan nanoparticles. Each values represent average of three replicate samples ± SD.

Thiobarbituric acid value (TBA)

Variations in the TBA content of the different cold catfish fingers at 4 ±1°C are depicted in Table (5). The chitosan-based catfish fingers without nanoparticles had a

higher TBA value than those of the chitosan-based catfish fingers containing nanoparticles during the cold storage period (Table 5). **Ibrahim (1980)** showed that part of lipid is converted into aldehydes and ketones due to the activity of the lipase enzyme and the decomposition and oxidation of fats, which may lead to an increase in TBA values during storage, and the samples containing crab chitosan nanoparticles had the lowest TBA values in comparison with the control and other chitosan-based catfish fingers. TBA values of different fish finger treatments didn't exceed the maximum permissible limits (4.5mg malonaldehyde/ kg sample) set by **EOS (1991)** during the cold storage.

Total plate count (TPC)

Variations in the total plate count of the different cold catfish fingers at 4 ±1°C for 15 days are illustrated in Table (6). The results showed that the total bacterial counts of cold chitosan-based catfish fingers were decreased compared to the control sample. On the other hand, the chitosan-based catfish fingers containing the chitosan nanoparticles had a lower TPC compared to the other samples.

Table 6. Changes in the TPC (cfu/g) of different catfish fingers during storage at 4±1°C for 15 days

Storage period (day)	T1	T2	T3	T4	T5	T6	T7
0	2.44	2.25	2.22	2.19	2.15	2.18	2.13
5	3.98	3.37	3.15	3.25	3.22	3.16	3.11
10	5.88	4.87	4.61	4.66	4.56	4.15	4.09
15	6.29	5.46	5.27	5.23	5.17	5.11	5.05

T1: control sample (without adding chitosan); **T2:** chitosan-based catfish fingers prepared with 1% commercial chitosan; **T3:** chitosan-based catfish fingers prepared with 1% commercial chitosan nanoparticles; **T4:** chitosan-based catfish fingers prepared with 1% shrimp chitosan; **T5:** chitosan-based catfish fingers prepared with 1% shrimp chitosan nanoparticles; **T6:** chitosan-based catfish fingers prepared with 1% crab chitosan; **T7:** with 1% crab chitosan nanoparticles. Each values represent average of three replicate samples ± SD.

The TPC of the control catfish fingers exceeded 6.29 log cfu/g at the end of the storage period, while the treated samples didn't exceed MPL set by **(EOS, 1991)**. These results are in agreement with those reported by **Talab and Abou-Taleb (2021)**. The first TPC for carp fish fingers ranged from 2.25 to 2.65 log cfu/g, respectively.

Organoleptic evaluation

Changes in the organoleptic properties of different cold catfish fingers at 4±1°C for 15 days are shown in Table (7). The addition of nanomaterials did not negatively affect the sensory properties of cold catfish fingers, but rather maintained good qualities during storage, while the quality decreased in samples without nanomaterials. Similar results for cold fish products have been obtained by **Cakli (2005)**, **Talab and Abou-Taleb (2021)** and **Talab *et al.* (2023)**.

Table 7. Organoleptic properties of different catfish fingers during storage at 4±1°C for 15 days

Organoleptic properties	Storage time (days)	T1	T2	T3	T4	T5	T6	T7
Appearance	0	8.1 ± 0.1	8.6 ± 0.1	8.0 ± 0.1	8.6 ± 0.1	8.0 ± 0.1	8.5 ± 0.1	8.5 ± 0.1
	5	7.9 ± 0.8	8.3 ± 0.5	7.8 ± 0.5	8.2 ± 0.8	7.7 ± 0.6	8.3 ± 0.5	8.5 ± 0.5
	10	7.4 ± 0.5	8.1 ± 0.6	7.7 ± 0.7	8.0 ± 0.4	7.3 ± 0.8	8.1 ± 0.7	8.2 ± 0.7
	15	6.2 ± 0.4	7.5 ± 0.6	7.5 ± 0.6	7.3 ± 0.2	7.7 ± 0.2	8.0 ± 0.6	7.9 ± 0.2
Color	0	8.0 ± 0.1	8.0 ± 0.1	8.1 ± 0.1	8.4 ± 0.2	8.4 ± 0.1	8.2 ± 0.2	8.2 ± 0.2
	5	7.6 ± 0.1	7.8 ± 0.1	7.9 ± 0.1	7.7 ± 0.1	7.5 ± 0.4	7.5 ± 0.1	7.5 ± 0.1
	10	7.4 ± 0.2	7.5 ± 0.1	7.6 ± 0.2	7.4 ± 0.2	7.3 ± 0.1	7.4 ± 0.2	7.5 ± 0.2
	15	6.7 ± 0.1	7.3 ± 0.2	7.4 ± 0.1	7.2 ± 0.1	7.1 ± 0.3	7.2 ± 0.2	7.4 ± 0.3
Taste	0	8.2 ± 0.1	8.3 ± 0.1	8.5 ± 0.1	8.5 ± 0.1	8.7 ± 0.1	8.6 ± 0.1	8.9 ± 0.1
	5	7.5 ± 0.4	7.7 ± 0.4	7.6 ± 0.4	7.7 ± 0.4	8.4 ± 0.5	8.5 ± 0.4	8.6 ± 0.4
	10	7.2 ± 0.4	7.5 ± 0.4	7.5 ± 0.3	7.5 ± 0.4	7.9 ± 0.5	7.8 ± 0.3	8.3 ± 0.2
	15	6.8 ± 0.4	7.2 ± 0.8	7.1 ± 0.4	7.4 ± 0.1	7.6 ± 0.4	7.5 ± 0.4	7.8 ± 0.1
Flavor	0	8.1 ± 0.1	8.3 ± 0.1	8.4 ± 0.1	8.5 ± 0.2	8.7 ± 0.1	8.8 ± 0.2	8.9 ± 0.2
	5	7.5 ± 0.2	8.1 ± 0.3	7.9 ± 0.5	7.8 ± 0.1	8.3 ± 0.1	7.9 ± 0.1	8.6 ± 0.2
	10	7.0 ± 0.2	7.6 ± 0.4	7.8 ± 0.1	7.0 ± 0.1	7.4 ± 0.6	7.3 ± 0.6	7.8 ± 0.1
	15	6.4 ± 0.5	7.4 ± 0.6	7.5 ± 0.2	6.9 ± 0.1	6.7 ± 0.5	6.6 ± 0.6	7.1 ± 0.1
Texture	0	8.0 ± 0.1	8.1 ± 0.1	8.3 ± 0.1	8.2 ± 0.1	8.2 ± 0.1	8.2 ± 0.1	8.3 ± 0.1
	5	7.3 ± 0.5	7.4 ± 0.5	7.6 ± 0.5	7.8 ± 0.5	8.0 ± 0.6	8.1 ± 0.5	8.3 ± 0.5
	10	7.2 ± 0.6	7.3 ± 0.6	7.5 ± 0.6	7.7 ± 0.6	7.9 ± 0.5	7.8 ± 0.6	8.1 ± 0.6
	15	7.1 ± 0.6	7.1 ± 0.6	7.4 ± 0.2	7.6 ± 0.2	7.8 ± 0.2	7.6 ± 0.1	7.9 ± 0.1
Overall acceptability	0	8.1 ± 0.4	8.3 ± 0.1	8.3 ± 0.1	8.4 ± 0.1	8.4 ± 0.1	8.5 ± 0.1	8.6 ± 0.1
	5	7.6 ± 0.3	7.9 ± 0.3	7.8 ± 0.5	7.8 ± 0.5	8.0 ± 0.6	8.1 ± 0.5	8.3 ± 0.2
	10	7.2 ± 0.1	7.6 ± 0.5	7.6 ± 0.4	7.5 ± 0.2	7.6 ± 0.4	7.7 ± 0.6	8.0 ± 0.2
	15	6.6 ± 0.4	7.3 ± 0.2	7.4 ± 0.3	7.3 ± 0.2	7.4 ± 0.3	7.4 ± 0.1	7.6 ± 0.3

T1: control sample (without adding chitosan); **T2:** chitosan-based catfish fingers prepared with 1% commercial chitosan; **T3:** chitosan-based catfish fingers prepared with 1% commercial chitosan nanoparticles; **T4:** chitosan-based catfish fingers prepared with 1% shrimp chitosan; **T5:** chitosan-based catfish fingers prepared with 1% shrimp chitosan nanoparticles; **T6:** chitosan-based catfish fingers prepared with 1% crab chitosan; **T7:** with 1% crab chitosan nanoparticles. Each values represent average of three replicate samples ± SD.

CONCLUSION

The addition of chitosan and its nano-particles improved the physical, chemical, microbial, and sensory properties of fish fingers. Moreover, chitosan and chitosan nano-particles trials had a shelf-life of up to the end of storage compared to control sample, according to biochemical quality indices. Furthermore, chitosan nano-particles improved the quality attributes of fish fingers samples.

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