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Chemical Composition, Physicochemical, Microbiological and Sensory Quality Attributes of Catfish Pastirma Coated by Crustacean Chitosan and Its Nanoparticles

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ABSTRACT

The present work aimed to evaluate and improve the quality of fish pastirma through using shrimp and blue crab chitosan and their nanoparticles. Catfish pastirma was evaluated through chemical composition, fatty acids, biogenic amines, fatty acids composition, volatile compounds, microbiological, and sensory properties. Catfish pastirma of chitosan or its nano particles showed a slight impact on the chemical content of the samples compared to control. All pastirma samples were charaterized with the high protein (30.15-30.96%) and lipid (15.35-16.17%) content. In addition, all pastirma samples contained slight differences in trimethylamine and total volatile nitrogen, where they ranged between 12.45-12.20 and 2.89-1.50. This result indicated that freshness and proteolytic activity in all pastirma samples were accepted. Furthermore, the bacterial counts of all pastirma samples were below maximum acceptable limits of 6 $\log_{10}$ cfu/g. Regarding fatty acids, pastirma is an excellent source of unsaturated fatty acids, where the highest levels belonged to oleic acid (C18:1n9c), linoleic acid (C18:2n6c) and palmitoleic acid (C16:1), n9 in ranges from 28.09 to 34.78%, 14.44 to 20.40 % and 5.35 to 6.72%, respectively. The bacterial counts of pastirma samples were below the maximum acceptable limits of 6 $\log_{10}$ cfu/g. The nano chitosan has antibacterial ability better than chitosan itself due to the highest activity of fine particles, which are more reactive toward bacteria than chitosan. The volatile compounds of pastirma detected 57 compounds belonging to 9 major groups. These compounds consisted of aldehydes, hydrocarbons, sulfur compounds, terpenes, esters, acids, alcohols, ketones, and furans. The results of biogenic amines in pastirma showed an absence of agmatin and treptamine in all treatments, with the B-phenyl ethyl amine found only in pastirma treated with shrimp chitosan nanoparticles at a level of 2.23mg/ kg samples.

INTRODUCTION

Pastirma product is a cured meat prepared through 4 weeks using whole beef or buffalo muscles. The principal method depends on curing, drying, pressing, cemening

and aging to reach final product **(Çakıcı *et al.*, 2015; Aksu *et al.*, 2020a)**. Cemen paste is prepared by mixing water with some natural material i.e. fenugreek seeds flour, fresh crushed garlic and red pepper powder. **Aksu *et al.*, (2020b)** stated that preparing cemen paste is an important stage in producing pastırma, where it imparts the pastırma sensory attributes such as appearance, color, texture, taste, and arom. Moreover, it protects against microbial contamination, prevents contact with air, delays spoilage and helps to prevent over drying **(Aksu *et al.*, 2022)**. The application of chitosan as food additives is common, but a chitosan nanoparticle is limited. In this respect, **Abdallah *et al.*, (2017)** reported that chitosan can be applied as an alternative to pastirma for improving its sensory quality. Many studies have been conducted on the production of meat pastirma **(Aksu & Kaya, 2005; Kaban, 2009; Ceylan & Aksu, 2011; Abou-Taleb *et al.*, 2018; Erdemir, 2022; Turan & Şimşek, 2022)**. However, producing fish pastirma and using chitosan application as a coating in fish is limited. The aim of this study was to produce and characterize a 1% chitosan solution (derived from shrimp or crab shells), along with its nanoparticles, and evaluate its effect on the levels of biogenic amines, volatile organic compounds, as well as its impact on the fatty acid composition, physicochemical properties, sensory characteristics and microbiological quality characterstics of catfish pastirma.

MATERIALS AND METHODS

Materials

Fish samples

Fresh African catfish (*Clarias gariepinus*) were purchased from local fish Market (El-Obour), Egypt, with an average weight of 3kg and were immediately transferred by using an ice box within 3 hours to Fish Research Station, National Institute of Oceanography & Fisheries, Qaliubia Governorate. Fish samples were prepared directly in Fish Processing and Technology Lab., where fish were washed carefully, beheaded and gutted, then rewashed, filleted, and drained. The fillets with approximately 45% yield were kept frozen at -18°C until used.

Ingredients

The spices, fenugreek flour, garlic and red pepper were purchased from the local market. All chemical reagents (sodium bicarbonate, sodium polyphosphate and commercial chitosan) were of analytical grade and brought from Sigma-Aldrich, GmbH Taufkirchen, Germany.

Chitosan and its nanoparticles

Chitosan was extracted from different marine byproduct (shrimp and crab) and used to prepare nanoparticles. Chitosan and nano-particles chitosan were deemed safe as food additives, as evaluated previously by **Talab *et al.* (2023)**.

Fish pastirma processing

Fish pastirma was produced according to the method of **Abou-Taleb *et al.* (2018)** with some modifications as follows: The frozen fillets were defrosted in refrigerator over night at 4-5°C. The fish fillets were divided into seven groups using chitosan from shrimp and crab and its nanomaterials at a concentration of 1% as follows: the control group without any additives of chitosan (Cont.), commercial chitosan (Com.), commercial chitosan nanoparticles (ComN.), shrimp chitosan (Shr.), shrimp chitosan nanoparticles (ShrN.), crab chitosan (Cra.) and crab chitosan nanoparticles (CraN.), respectively. Then, each fish group individuals were rewashed and drained separately before salting step (20g salt/ 100g fillet) for 6 hours. After which, excess of salt was removed by washing, and then kept hanging up until it dried at room temperature. The obtained dried fillet were pressed using mechanical compressor, and hanged for 4 hrs at room temperature to remove the remaining water. Finally, dried fish fillet was coated with cemen paste that was prepared using 50% fenugreek flour, 35% garlic and 15% red pepper and enough water to make paste. The cemen layer was carried out to obtain 3mm thickness. The coated fillet was hung for one week until the coating layer dried. The final fish pastirma was then wrapped in polyethylene sheets and stored at -18°C until use.

Physico-chemical analysis

Gross chemical composition were determined according to **AOAC (2002)** except carbohydrates were calculated by difference. Total volatile basic nitrogen and Trimethylamine were determined using the method of **AOAC (2002)**. The pH value and Thiobarbituic acid were determined according to **Pearson (1991)**. The portable Rotronic HP23-AW water activity device **(Schiraldi *et al.*, 1996)** was used to measure water activity (aw).

Microbiological analysis

Preparation of homogenate and serial dilution

Ten grams from the center of the meat portion of pastirma were obtained under complete aseptic condition and homogenized with 90-ml sterile of Ringer's solution (Merck, Darmstadt, Germany) in sterile bag for 2min using stomacher (Lab blender 400). A tenfold serial dilution was made from the first homogenate **(Swanson *et al.*, 2001)**.

Aerobic mesophilic bacteria were counted by distributing 0.1ml from each dilution across the surface of double sets of Plate Count Agar (PCA Oxoid CM0463B, Hampshire, England) and incubating it at 35ºC for 48 h **(Dale Morton, 2001)**. A different series of plate count gar plates were held at 7ºC for 8 days to quantify psychotropic bacteria **(Cousin *et al.*, 2001)**. Mesophilic anaerobic spore-forming bacteria were enumerated by inoculating Reinforced Clostridial plates and incubating them anaerobically at 35ºC for 24 hours **(Scott *et al.*, 2001)**. Yeast and molds were counted by inoculating plates of Sabouraud dextrose agar (Merck, Darmstadt, Germany) and incubating at 25ºC for 5 days **(Beuchat & Cousin, 2001)**. All bacterial quantities were

reported as colony-forming units per gram ($cfug^{-1}$) of the sample. Sensory tests were evaluated according to **Fey and Regenstein (1982)**.

Biogenic amines

High Performance Liquid Chromatography (HPLC) was used to isolate and quantify biogenic amines using UV detector calibrated to wavelength 254nm **(Mietz and Karmas, 1977, Ayesh, 2012; Sultan & Marrez, 2014)**. The Agilent Poroshell 120 EC-C18 4µm (4.6mm × 150mm) column was used for biogenic amines separation. The results were calculated using the Chromeleon software application associated with the HPLC system.

Fatty acids composition

Determination of fatty acids was carried out in National Research Center, Giza, Egypt. Cold extraction was used for extraction of fish fat from pastirma samples. Fatty acids were identified by using HP 6890 plus gas chromatography with SP-2380 capillary column and were detected with FID as stated and modified by **Zahran and Tawfeuk (2019)**.

Volatile compounds

Headspace sampling (HS-SPME) coupled to gas chromatography-mass spectrometry (GC-MS) were used for determinaion volatile compounds in fish pastirma samples.

Statistical analysis

Results were presented as a mean values ± standard deviation (SD). One-way analysis of variance (ANOVA) was carried out using statistical software (SPSS Version 12).

RESULTS AND DISCUSSION

Chemical composition of catfish pastirma

The effects of chitosan or nano chitosan of commercial, shrimp or crab source for coating catfish pastirma were evaluated. Table (1) shows that there were no significant difference in the moisture content of control sample (uncoated) and all other coated chitosan types, where they ranged between 44.91- 46.96%. Moreover, all other chemical contents were not affected significantly, where protein, lipid, fat and carbohydrate ranged between 30.15 -30.96%, 15.35-16.17%, 6.11-7.30%, and 0.79-1.76%, respectively. The obtained results agree with **Mahmoud *et al.* (2016)**, who stated that the carp fish pastirma contained 51.80% moisture, 21.27% protein, 13.38% fat, 8.54% ash, and 5.01% carbohydrate.

Table 1. Effect of coating catfish pastirma with chitosan or nano chitosan of commercial, shrimp or crab on their chemical composition (mean±SD g/100g).

Composition (%)	Cont.	Com.	ComN.	Shr.	ShrN.	Cra.	CraN.
Moisture (g/100g)	45.43± 0.21	44.91± 0.15	45.52± 0.08	45.85± 0.18	46.96± 0.40	45.81± 0.16	45.84± 0.19
Protein (g/100g)	30.90± 0.05	30.96± 0.01	30.89± 0.20	30.21± 0.14	30.15± 0.25	30.85± 0.51	30.40± 0.17
Lipid (g/100g)	15.45± 0.15	15.68± 0.86	15.35± 0.65	15.37± 0.55	15.99± 0.29	15.69± 0.06	16.17± 0.27
Ash (g/100g)	6.46± 0.12	7.18± 0.03	7.11± 0.09	7.30± 0.07	6.11± 0.11	6.18± 0.15	6.19± 0.17
Carbohydrates (Calculated g/100g)	1.76± 0.07	1.27± 0.85	1.13± 0.05	1.27± 0.01	0.79± 0.02	1.47± 0.22	1.40± 0.20

Catfish pastirma of: Cont. = Control; Com. = : commercial chitosan; ComN. = commercial chitosan nanoparticles; Shr. = shrimp chitosan; ShrN. = Shrimp chitosan nanoparticles; Cra = Crab chitosan and Cra.N = Crab chitosan nanoparticle.

Physicochemical properties of catfish pastirma

The quality of catfish pastirma that were coated with chitosan or nanochitosan was evaluated by determining some physical and chemical parameters that are good indicators of freshness **(Cheng *et al.*, 2015)**.

Table 2. Effect of coating catfish pastirma with chitosan or nano chitosan on physico-chemical properties (mean±SD g/100g) of commercial, shrimp or crab

Sample	pH value	Water activity (aw)	TVBN (mg N/100 g)	TMA (mg N/100 g)	TBA (mg MA /kg)
Cont.	5.75±0.10	0.980±0.02	12.45±1.23	2.89±0.09	3.22±0.08
Com.	5.70±0.15	0.978±0.03	12.40±1.15	2.60±0.06	2.25±0.09
ComN.	5.69±0.13	0.977±0.02	12.38±1.32	2.59±0.08	2.26±0.07
Shr.	5.71±0.17	0.975±0.01	12.35±1.18	2.33±0.05	2.20±0.06
ShrN.	5.68±0.09	0.970±0.02	12.30±0.82	2.30±0.01	2.15±0.05
Cra.	5.70±0.07	0.974±0.02	12.30±0.80	1.89±0.02	2.18±0.04
CraN.	5.66±0.03	0.965±0.01	12.20±0.69	1.50±0.01	2.13±0.03

Catfish pastirma of: Cont. = Control; Com. = : commercial chitosan; ComN. = commercial chitosan nanoparticles; Shr. = shrimp chitosan; ShrN. = Shrimp chitosan nanoparticles; Cra = Crab chitosan and Cra.N = Crab chitosan nanoparticle.

Table (2) shows that the pH values of catfish pastirma were not affected by the use of different types of chitosan or nanochitosan, ranging from 5.66 to 5.75. Furthermore, total volatile basic nitrogen (TVB-N), TMA, and TBA were slightly decreased in chitosan and nanochitosan if compared to the control sample. The obtained

results showed that TVBN, TMA and TBA of coated pastirma with chitosan or nano-chitosan ranged between 12.40-12.20 N/100 g, 150-260 mg MA/kg and 2.13-2.26, respectively. The same trend was noticed in water activity (aw), where it ranged between 0.965-0.978 in coated catfish with chitosan or nano-chitosan. The obtained results agree with previous studies, where the pH and TVB-N values were 5.58 & 5.67 and 17.35 & 15.04 mg/100 g, respectively, for vacuumed and unvacuumed common carp fish pastirma **(Arslan *et al*., 1997; Mahmoud *et al*., 2016; Abou-Taleb *et al*., 2018)**.

Microbial evaluation of catfish pastirma

Fish pastirma samples processed by using chitosan and its nanoparticles were evaluated microbiologically, as shown in Table (3). The microbial activity in fish and fish products is the main reason for the deterioration under different conditions. The microbial counts of fish pastirma in this study was obviously affected by chitosan addition, it showed a decline trend particularly with nano chitosan particles compared to control sample (T1). The highest counts of microbial counts were 5.15, 5.29, 4.16 & 4.20 log_{10} cfu/g in control, which slightly reduced with consecutive trend in treatments contained commercial, shrimp and crab chitosan, respectively, to be 5.11, 5.21, 4.09 and 3.64 log_{10} cfu/g in T2; 5.07, 5.15, 4.05 & 3.45 log_{10} cfu/g in T4 and 5.02, 5.11, 4.01 & 3.30 log_{10} cfu/g in T6 for mesophilic, psychrotrophes, anaerobic bacteria, yeast and mold, respectively. Meanwhile, the nano chitosan particles in fish pastirma samples inhibited the microbial growth to be much lower than control and chitosan treated samples recording 4.35, 4.20 and 4.15 log_{10} cfu/g in T3, T5 and T7 for the mesophilic bacteria, respectively, and <2.00 for psychrotrophes, anaerobes bacteria, yeast and mold.

Table 3. Microbiological evaluation mean±SD (log_{10} cfu/g) of fish pastirma coated by chitosan and its nanoparticles

Trial	Aerobic mesophilic bacteria	Psychrotrophes	Anaerobes	Yeast and moulds
Cont.	5.15±3.15	5.29±3.31	4.16±3.03	4.20±3.19
Com.	5.11±3.09	5.21±3.30	4.09±3.15	3.64±2.57
ComN.	4.35±2.50	<2.00	<2.00	<2.00
Shr.	5.07±3.01	5.15±3.15	4.05±3.11	3.45±2.25
ShrN.	4.20±2.13	<2.00	<2.00	<2.00
Cra.	5.02±2.71	5.11±3.10	4.01±3.05	3.30±2.31
CraN.	4.15±2.05	<2.00	<2.00	<2.00

Catfish pastirma of: Cont. = Control; Com. = : commercial chitosan; ComN. = commercial chitosan nanoparticles; Shr. = shrimp chitosan; ShrN. = Shrimp chitosan nanoparticles; Cra = Crab chitosan and Cra.N = Crab chitosan nanoparticle.

This occurrence was only related to the increment of the exposed surface of fine chitosan particles in fish pastirma, which increase from their inhibitory effect for microorganisms or may be due to the presence of SH groups within the chitosan

structure, and that their effectiveness increases when chitosan is converted into nanoparticles. Generally, the bacterial counts of catfish pastirma samples in this study are below the maximum acceptable limit of 6 $\log_{10}$ cfu/g, as specified by the Egyptian Standard Specification **(ESS, 2019)**, indicating good microbiological quality. These counts were within the range reported by previous studies. For example, **Mahmoud *et al*. (2016)** reported a total bacterial count of 9×10^3 cfu/g in fresh fish pastirma. **Kök *et al.* (2009)** found that the total viable counts in vacuum-packed *Silurus glanis* L. pastirma ranged between 4.40 and 6.30 log cfu/g. The results of the current study are most similar to those of **Arslan *et al.* (1997)**, who reported aerobic, anaerobic, psychrophilic bacteria, yeast, and mold counts in vacuum-packed carp pastirma ranging from 8.1×10^4 to 9.6×10^5 cfu/g, 1.2×10^4 to 2.2×10^5 cfu/g, 3.6×10^2 to 7.6×10^4 cfu/g, and 5.2×10^2 to 2.5×10^3 cfu/g, respectively..

The preservative effect of chitosan clearly appeared in catfish pastirma based on chemical and microbial results, which was discussed by many authors for the ability of chitosan to suppress bacterial growth due to the positively charged chitosan inhibiting the growth of bacteria and fungi **(El Ghaouth *et al.*, 1991)**. The nanochitosan has antibacterial ability better than chitosan itself due to the highest activity of fine particles, which is more reactive toward bacteria than chitosan. The ability of chitosan in the form of nanoparticles also greatly inhibits bacterial growth since it can directly enter the bacterial cell **(Cauerhff *et al.*, 2013)**.

Fatty acid composition of fish pastirma

Marine and freshwater fish species possess varying fatty acid compositions. The lipid content and composition in fish differed in species, age, habitat, origin, and environmental factors **(Huss *et al.*, 2004)**. The composition of fatty acids in catfish pastirma made with natural chitosan and its nanoparticles is presented in Table (4). The results indicated that catfish pastirma contained both saturated and unsaturated fatty acids. Among the saturated fatty acids, palmitic acid (C16:0) and stearic acid (C18:0) were predominant. The control sample had the highest percentage of palmitic acid (23.62%), whereas its levels in the treatments containing chitosan and chitosan nanoparticles ranged from 17.30% to 18.84%. Conversely, stearic acid levels followed an opposite trend, with the lowest value (5.72%) observed in the control sample and slightly higher levels in the chitosan-treated samples (6.62% and 6.77%).

The highest level of myristic acid (C14:0) was recorded in the sample treated with commercial chitosan nanoparticles (3.00%), while its concentration remained below 2% in the other treatments.

Regarding unsaturated fatty acids, oleic acid (C18:1n9c), linoleic acid (C18:2n6c), and palmitoleic acid (C16:1n9) were the most abundant, with ranges of 28.09–34.78%, 14.44–20.40%, and 5.35–6.72%, respectively. Additionally, minor

amounts of α-linolenic acid, stearidonic acid, gadoleic acid, and arachidonic acid (C20:4) were detected.

Notably, the concentrations of eicosapentaenoic acid (EPA, C20:5) and docosahexaenoic acid (DHA, C22:6) were the highest in the samples treated with chitosan, compared to the control. This suggests that chitosan may help preserve these polyunsaturated fatty acids by inhibiting lipid oxidation.

Based on these findings, the primary fatty acids in catfish pastirma were oleic, palmitic, and linoleic acids. The product also served as a good source of EPA, DHA, stearic acid, and palmitoleic acid. Variations in fatty acid composition among the different treatments may be attributed to differences in the anatomical parts of the fillets used during pastirma preparation **(Sudirman *et al.*, 2018)**.

Table 4. Fatty acids composition of fish pastirma coated with chitosan or its nanoparticles

Fatty acids (% of total fatty acids)	**Cont.**	**Com.**	**ComN.**	**Shr.**	**ShrN.**	**Cra.**	**CraN.**
Saturated fatty acid (SFA)							
Lauric acid (C12:0)	nd	nd	nd	nd	nd	nd	nd
Myristic acid (C14:0)	0.80	0.41	3.00	1.13	0.80	0.41	0.08
Palmitic acid (C16:0)	23.62	17.30	17.38	18.23	18.84	17.31	17.85
Stearic acid (C18:0)	5.72	6.75	6.62	6.77	6.75	6.75	6.75
Arachidic acid (C20:0)	nd	1.06	1.60	1.84	1.55	1.64	0.77
Unsaturated fatty acid (USFA)							
Palmitoleic acid (C16:1),n9	5.55	6.12	6.72	5.44	5.35	6.12	6.09
Oleic acid (C18:1n9c)	34.78	29.19	28.16	28.09	29.24	30.41	30.36
Linoleic acid (C18:2n6c)	20.40	14.54	14.44	14.77	15.19	14.54	14.95
α- Linolenic acid (C18:3n3)	1.90	2.31	2.36	2.62	2.37	2.31	2.08
Stearidonic acid (C18:4) n3	1.77	2.31	2.31	3.09	2.87	2.30	2.08
Gadoleic acid (C20:1)	nd	0.94	1.86	1.72	1.42	1.06	0.64
Arachidonic acid (C20:4)	nd	1.64	0.94	1.82	1.53	0.94	1.38
Eicosapentaenoic acid (C20:5)	1.72	5.18	4.98	4.82	4.69	5.18	5.10
Docosahexaenoic acid (C22:6)	3.76	12.25	12.19	9.66	9.41	11.04	10.89
Total (%)	100.02	100.00	102.56	100.00	100.01	100.01	99.02
Saturated fatty acids	30.14	25.52	28.60	27.97	27.94	26.11	25.45
Unsaturated fatty acids	69.88	74.48	73.96	72.03	72.07	73.90	73.57

Catfish pastirma of: Cont. = Control; Com. = : commercial chitosan; ComN. = commercial chitosan nanoparticles; Shr. = shrimp chitosan; ShrN. = Shrimp chitosan nanoparticles; Cra = Crab chitosan and Cra.N = Crab chitosan nanoparticle.

Volatile compounds of fish pastirma

The effect of different chitosan types and its nanoparticles on volatile compounds of fish pastirma are shown in Table (5) and Fig. (1a-f). The analysis of volatile compounds for catfish pastirma was detected in 57 compounds belonging to 9 major

groups. The main groups based on chemical structure of identified compounds were subjected to aldehydes, ketones, alcohols, carboxylic acids, phenolics, aromatic compounds, sulfur containing compounds, esters, heterocyclic and other compounds. It is worth mentioning that the irregular appearance of volatile compounds was observed in fish pastirma treatments. Furthermore, the aldehydes group concluded 12 volatile compounds, but the dominant compounds in all fish pastirma treatments were cuminaldehyde, benzaldehyd and hexanal. The similar case, 9 compounds of alcohols were detected, 5 of them (eucalyptol, linalool, eugenol, 1-octen-3-ol and dimethylsilanediol) were extremely found in most of the treatments. The other groups were detected by the same trend, as shown in Table (5).

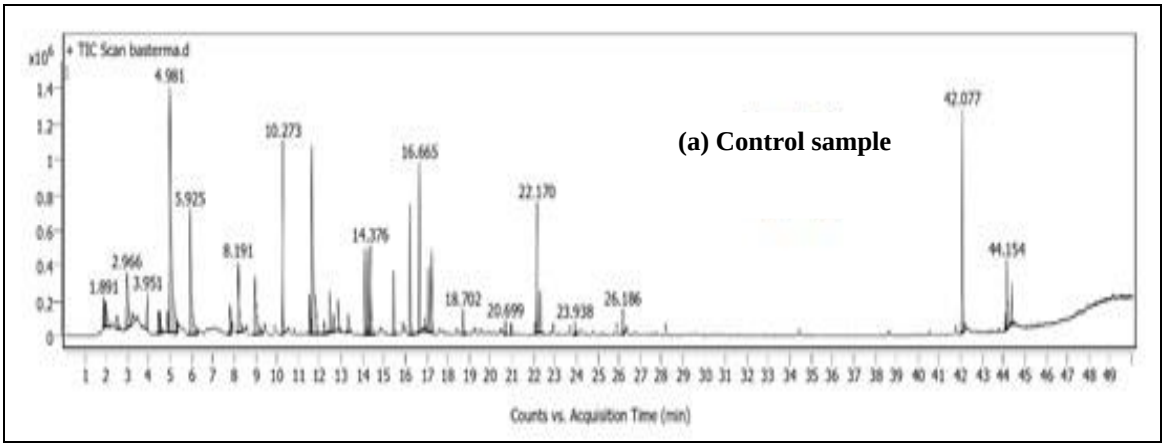
Table 5. Volatile organic compounds of fish pastirma coated with chitosan nanoparticles

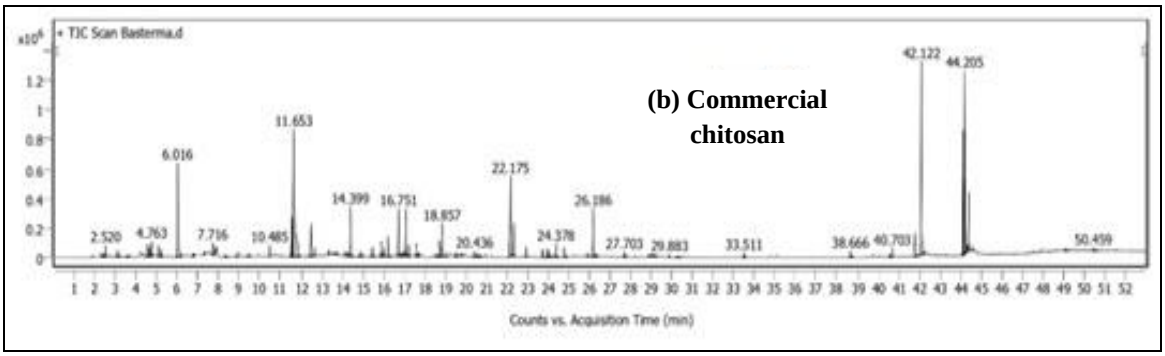
Volatile organic compounds	Cont.	Com.	ComN.	Shr.	ShrN.	Cra.	CraN.
Aldehydes (12)							
Nonanal	4.53	0.72	2.33	1.74	1.62	nd*	2.79
α-Terpinen-7-al	1.03	Nd	Nd	nd	nd	nd	nd
γ-Terpinen-7-al	0.97	Nd	0.38	nd	nd	nd	nd
Cuminaldehyde	12.45	5.78	3.52	2.39	1.13	1.38	5.77
Benzaldehyde	5.12	15.98	11	11.18	10.7	2.87	5.8
2-Methyl-2-butenal	nd	1.62	1.55	0.46	nd	nd	nd
Hexanal	6.33	7.29	6.99	6.41	6.06	1.55	2.23
(Z)-Hept-2-enal	3.07	2.52	1.11	0.43	0.28	nd	1.48
(Z)-2-Decenal	nd	nd	Nd	nd	nd	0.95	nd
trans-2-Decenal	nd	0.52	Nd	nd	nd	nd	nd
Undecanal	nd	nd	Nd	nd	nd	1.95	nd
Decanal	nd	nd	0.32	nd	0.29	nd	0.55
Alcohols (9)							
Eucalyptol	3.07	2.96	2.97	2.88	2.32	nd	2.76
Linalool	2.88	2.79	1.74	1.46	0.63	nd	1.64
Eugenol	2.75	3.22	0.73	0.45	nd	nd	1.2
1-Octanol	nd	nd	Nd	nd	nd	nd	0.97
(Z)-2-octen-1-ol	1.18	1.00	Nd	nd	nd	nd	nd
1-Octen-3-ol	1.87	2.21	1.19	0.98	0.94	nd	0.94
trans-Undec-2-en-1-ol	nd	nd	0.40	nd	nd	0.83	nd
Dimethylsilanediol	nd	0.47	0.77	0.86	2.44	0.77	2.14
β-Mercaptoethanol	nd	nd	Nd	1.06	1.74	nd	6.43
Carboxylic acids (6)							
Palmitoleic acid	nd	1.64	Nd	nd	nd	3.41	nd
Palmitic acid	3.49	14.16	5.21	nd	1.20	24.65	1.67
Linoleic acid	1.36	7.07	2.16	0.40	nd	16.23	nd
Oleic acid	nd	11.65	Nd	nd	nd	23.30	nd
Stearic acid	0.53	2.6	0.84	nd	nd	7.11	nd
Octanoic acid	nd	1.52	Nd	nd	nd	3.31	nd
Sulfur containing compounds (2)							
Diallyl disulphide	7.79	1.31	3.75	2.79	3.19	1.11	5.31
Allyl trisulfide	1.71	nd	Nd	nd	nd	nd	0.93
Ketones (3)							
1-Hydroxy-2-propanone	nd	nd	Nd	nd	nd	0.52	nd

Volatile organic compounds	Cont.	Com.	ComN.	Shr.	ShrN.	Cra.	CraN.
Menthone	nd	nd	Nd	nd	0.52	nd	1.14
Carvone	2.92	1.95	1.09	0.89	0.39	nd	2.16
Phenols (1)							
Carvacrol	nd	0.93	Nd	nd	nd	nd	nd
Aromatic compounds (9)							
Benzene	nd	nd	3.46	15.41	14.95	nd	5.63
Toluene	5.82	1.03	15.94	17.31	15.98	nd	8.23
o-Xylene	2.30	nd	3.35	3.91	4.44	nd	nd
β-Pinene	nd	nd	0.50	0.51	0.49	nd	0.68
β-Myrcene	1.53	nd	1.03	1.47	2.02	nd	2.14
p-Cymene	3.01	nd	2.53	3.30	4.59	nd	5.01
D-Limonene	2.99	nd	2.49	3.68	4.97	nd	5.20
γ-Terpinene	2.90	nd	2.01	2.53	3.42	nd	4.23
β-Caryophyllene	0.95	nd	Nd	nd	nd	nd	0.63
Esters (2)							
Methyl N-hydroxybenzenecarboximidoate	4.21	0.48	4.41	5.58	2.99	nd	2.09
Vinyl caproate	nd	nd	0.51	0.32	nd	nd	nd
Heterocyclic compounds and others (13)							
2,6-Dihydroxypyridine	nd	0.75	Nd	nd	nd	1.71	nd
p-Xylene	nd	nd	Nd	nd	nd	nd	4.09
2-Propynylbenzene	nd	nd	Nd	nd	nd	nd	1.16
Ethylbenzene	nd	nd	Nd	0.56	nd	nd	nd
2,3-Dihydro-3,5-dihydroxy-6-methyl-4-pyrone	nd	2.59	Nd	nd	nd	3.61	nd
Trimethylhydrazine	3.78	nd	1.23	1.57	2.98	nd	2.39
cis-Thujone	4.47	3.18	4.27	1.46	0.48	1.61	0.81
(+)-Camphor	0.88	0.95	0.73	0.62	0.34	nd	nd
α-Terpinyl acetate	1.13	nd	Nd	nd	nd	nd	0.57
Ethyl Acetate	nd	nd	Nd	nd	nd	nd	2.18
Styrene	2.99	nd	3.81	3.97	3.96	0.94	5.38
Urea	nd	nd	1.01	nd	nd	nd	0.81
Estragole	nd	nd	0.41	0.45	0.43	nd	2.85

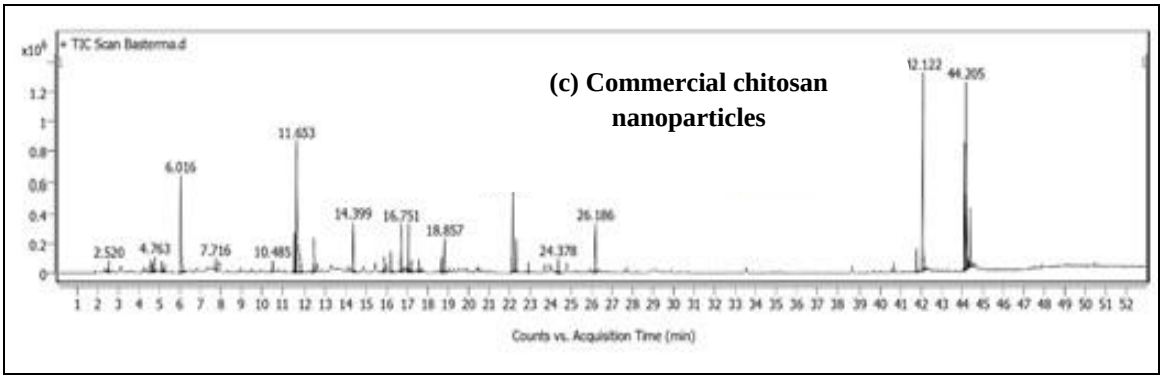
ND* = not detected.

Catfish pastirma of: Cont. = Control; Com. = : commercial chitosan; ComN. = commercial chitosan nanoparticles; Shr. = shrimp chitosan; ShrN. = Shrimp chitosan nanoparticles; Cra = Crab chitosan and Cra.N = Crab chitosan nanoparticle.

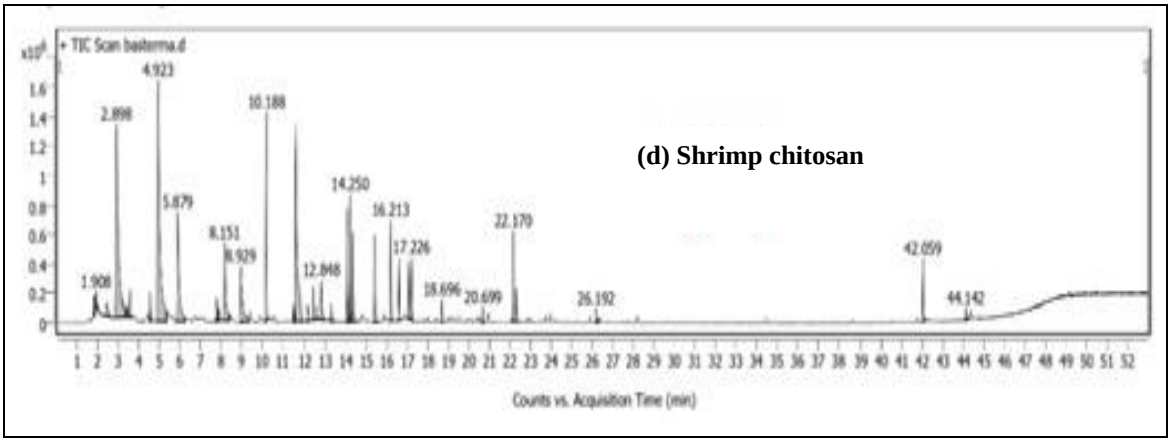




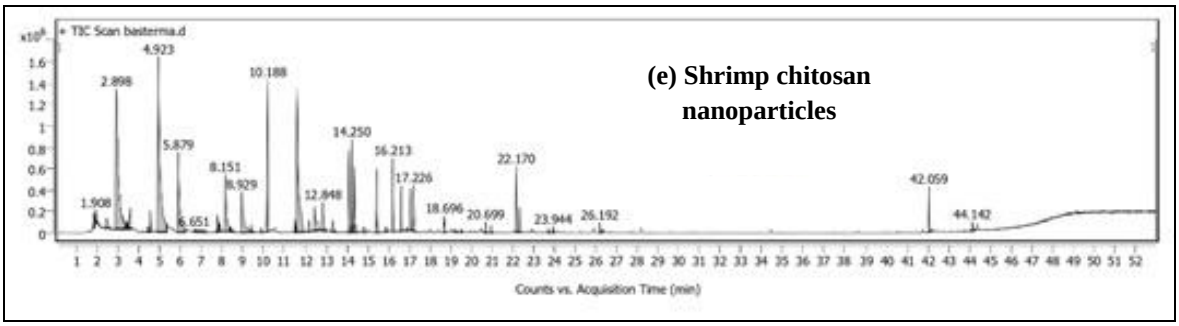
(b) Commercial chitosan



(c) Commercial chitosan nanoparticles



(d) Shrimp chitosan



(e) Shrimp chitosan nanoparticles

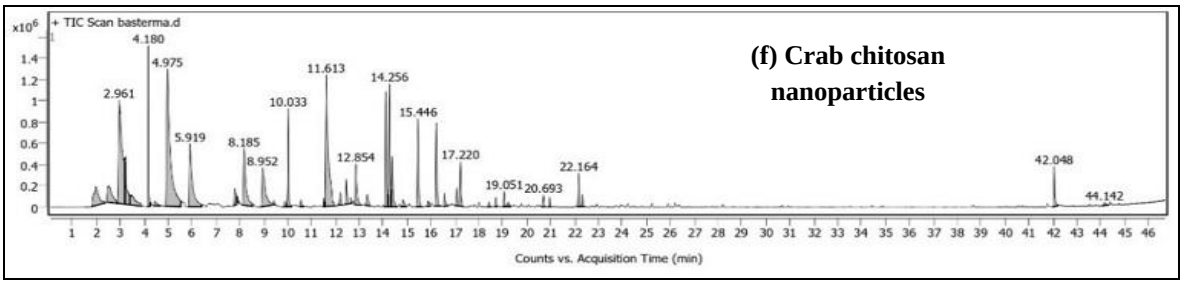


Fig. 1a-f. Volatile compounds compounds of catfish pastirma formulated with chitosan or nanoparticles chitosan

Remarkably, their profiles differed a lot from each other; nevertheless, the fish pastirma had a lot of compounds due to the variety of materials in coating layer such as fenugreek flour, garlic, and red pepper. Although volatile compounds were not detected in the control catfish pastirma, they were present in the treated samples, possibly due to the addition of chitosan and chitosan nanoparticles (Table 5). These findings align with previous research. For instance, **He *et al.* (2021)** reported that pastirma, a traditional Turkish dry-cured, uncooked meat product, has a unique flavor profile influenced by various volatile compounds. These include aldehydes, hydrocarbons, sulfur compounds, terpenes, esters, acids, alcohols, ketones, and furans. The composition of these compounds varies across products due to differences in processing conditions and the types and quantities of ingredients used in pastirma production.

Biogenic amines in catfish pastirma

The key biogenic amines found in fish products include histamine, tyramine, tryptamine, putrescine, and cadaverine, which are derived from the decarboxylation of free amino acids such as histidine, tyrosine, tryptophan, ornithine, and lysine, respectively. Additionally, spermidine and spermine are synthesized from putrescine **(Zarei *et al.*, 2011)**. Table (6) presents the levels of biogenic amines in the different catfish pastirma treatments. The presence of biogenic amines in fish and fishery products is considered a significant food safety concern. Histamine, cadaverine, putrescine, and tyramine, in particular, have been identified as enhancers of histamine toxicity. Their accumulation is primarily attributed to the growth of bacteria possessing amino acid decarboxylase activity **(Arulkumar *et al.*, 2023)**.

The results showed that agmatine and tryptamine were absent in all catfish pastirma samples coated with chitosan or chitosan nanoparticles. However, β-phenylethylamine was detected only in the sample coated with shrimp-derived chitosan nanoparticles, where its concentration reached 2.23mg/ kg. Other types of biogenic amines were detected at varying levels across most treatments.

Putrescine levels ranged from 1.35mg/ kg in the control sample to 4.26mg/ kg in treated samples. Cadaverine was not detected in the control or shrimp chitosan samples,

but was found at a higher level in the shrimp chitosan sample (3.43mg/ kg), and at a much lower level (0.12mg/ kg) in the crab nanochitosan-treated sample.

Histamine, the most critical biogenic amine in fish due to its role in allergic reactions, was the highest in the crab chitosan-treated sample (11.95mg/ kg), while the shrimp chitosan nanoparticle treatment had the lowest level (0.18mg/ kg), suggesting a possible protective effect of nanochitosan against histamine accumulation.

Spermine was present in all samples, ranging from 0.85mg/ kg in the crab chitosan sample to 2.68mg/ kg in the commercial chitosan-treated sample. Similarly, spermidine levels were higher than most other biogenic amines in several treatments: 14.78mg/ kg in T4; 13.67mg/ kg in T2; and 9.48mg/ kg in the control. However, the use of nanochitosan significantly reduced spermidine levels, recording only 0.21mg/ kg in T5 and 0.30mg/ kg in T7.

These results highlight significant variations in the levels and types of biogenic amines among the different catfish pastirma treatments. These differences are likely linked to variations in microbial activity. The formation of biogenic amines in food products is primarily driven by bacterial decarboxylase enzymes acting on free amino acids **(Chong *et al.*, 2014)**.

Table 6. Biogenic amines of fish pastirma formulated with chitosan nanoparticles

Biogenic amines	Cont.	Com.	ComN.	Shr.	ShrN.	Cra.	CraN.
Agmatin	ND	ND	ND	ND	ND	ND	ND
Treptamine	ND	ND	ND	ND	ND	ND	ND
B-phenyl ethyl amine	ND	ND	ND	ND	2.23	ND	ND
Putrescine	1.35	2.11	1.89	4.26	2.16	1.8	2.22
Cadaverine	ND	2.88	2.02	3.43	ND	0.97	0.12
Histamine	9.82	10.89	8.82	4.45	0.18	11.95	7.01
Serotonin	ND	ND	ND	1.5	0.24	0.11	0.7
Tyramine	0.4	ND	0.32	0.22	0.27	0.61	0.36
Spermidine	9.48	13.67	5.28	14.78	0.3	0.53	0.21
Spermine	1.55	2.68	1.02	2.2	2.05	0.85	2.29

Catfish pastirma of: Cont. = Control; Com. = : commercial chitosan; ComN. = commercial chitosan nanoparticles; Shr. = shrimp chitosan; ShrN. = Shrimp chitosan nanoparticles; Cra = Crab chitosan and Cra.N = Crab chitosan nanoparticle.

Elsabagh *et al.* (2023) demonstrated that tuna fillet samples coated with chitosan had a smaller rise in biogenic amine levels compared to the control samples, similar to the effect of chitosan on fish preservation. Among the treated samples, chitosan combined with curcumin exhibited the most significant decrease in biogenic amines production (1.45-19.33, 0.81-4.45, and 1.04-8.14 mg/kg), followed by chitosan with garlic (1.54-21.74, 0.83-5.77, and 1.08-8.84 mg/kg), chitosan with beetroot extract (1.56-31.70, 0.84-6.79, and 1.07-10.82 mg/kg), and chitosan without extract (CH) (1.62-33.83,

0.71-7.82 and 1.12-12.66 mg/kg) when compared to control samples (1.62-59.45, 0.80-11.96, and 1.14-20.34 mg/kg) for histamine, cadaverine, and putrescine, respectively. In summary, edible coatings made from chitosan and infused with plant extracts minimized biogenic amine production, slowed down biochemical spoilage, and prolonged the shelf life of tuna fillets. Histamine serves as a marker for the quality of fish **(Mendes, 1999)**. In the EU, the legal limit for histamine in fish is 100mg/ kg **(EC, 2005)**, while the acute reference dose per meal is around 50mg histamine per meal **(EFSA, 2011)**. The Food and Drug Administration (FDA) set an acceptable histamine upper limit of 50mg/ kg in 2011. Furthermore, in accordance with the Egyptian standards [Egyptian Organization for Standardization and Quality Control **(EOSQC, 2005)**, the maximum permissible level of histamine in frozen fish must not surpass 100mg/ kg. In this research, histamine levels remained below the acute reference dose in all analyzed samples. It is important to note that histamine stayed below 50mg/ kg in the samples. Levels of cadaverine in fish are regarded as an indicator of spoilage **(Al Bulushi *et al*., 2009)**.

Sensory evaluation of fish pastirma

The appearance, color, taste, flavor, texture, and overall acceptability of fish pastirma processed from catfish using chitosan nanomaterials are shown in Table (7).

Table 7. Sensory evaluation of catfish pastirma formulated with chitosan nanoparticles

Organoleptic property	Cont.	Com.	ComN.	Shr.	ShrN.	Cra.	CraN.
Appearance	8.0 ± 0.1	8.2 ± 0.1	8.3 ± 0.1	8.1 ± 0.1	8.1 ± 0.1	8.0 ± 0.1	8.4 ± 0.1
Color	7.5 ± 0.1	7.6 ± 0.1	7.6 ± 0.1	7.7 ± 0.2	7.8 ± 0.1	7.2 ± 0.2	7.9 ± 0.2
Taste	7.2 ± 0.1	7.3 ± 0.1	7.5 ± 0.1	7.5 ± 0.1	7.7 ± 0.1	7.6 ± 0.1	7.9 ± 0.1
Flavor	7.1 ± 0.1	7.3 ± 0.1	7.4 ± 0.1	7.5 ± 0.2	7.7 ± 0.1	7.8 ± 0.2	7.9 ± 0.2
Texture	7.0 ± 0.1	7.1 ± 0.1	7.3 ± 0.1	7.2 ± 0.1	7.2 ± 0.1	7.2 ± 0.1	7.3 ± 0.1
Overall acceptability	7.3 ± 0.2	7.5 ± 0.1	7.6 ± 0.1	7.6 ± 0.1	7.7 ± 0.1	7.5 ± 0.1	7.8 ± 0.1

Catfish pastirma of: Cont. = Control; Com. = : commercial chitosan; ComN. = commercial chitosan nanoparticles; Shr. = shrimp chitosan; ShrN. = Shrimp chitosan nanoparticles; Cra = Crab chitosan and Cra.N = Crab chitosan nanoparticle.

The results showed that all values of sensory properties were over 7 points which indicate being good. The scores of all treatments were between 8 and 8.4 for appearance, 7.2 to 7.9 for color, 7.2 to 7.9 for taste, 7.1 to 7.9 for flavor, 7.00 to 7.3 for texture and 7.3 to 7.8. It must be known the appearance and acceptance of food are affected by its color, flavor, and texture. **Çakici *et al.* (2015)** reported that, the color of pastirma types is one of the most important quality attributes that has an effect on acceptability on both the producers and consumers. The color of pastirma is affected by a lot of factors belonging

to the types and quality of used materials and preparation conditions **(Aksu & Kaya, 2002)**. Similarly, flavor development in pastirma is closely associated with the chemical compounds formed during processing. The extended preparation period allows partial degradation of proteins and fats into desirable flavor compounds. These compounds, in combination with components from the coating layer (e.g., chitosan or its nanoparticles), contribute to the unique flavor profile of pastirma.

Flavor is a complex sensory attribute that combines both aroma and taste. It plays a key role in consumer acceptance and product marketability. According to **Ahmed (2014)**, enzymatic transamination during processing can produce aromatic amino acids derived from protein degradation, which significantly enhance the flavor of the final product. Supporting this, **Dogruer *et al.* (1995)** also noted that pastirma possesses a distinctive taste and palatability.

In addition to flavor, texture is another critical quality parameter. Attributes such as consistency, juiciness, and tenderness are influenced by the curing process, moisture content, and ripening conditions of the product.

The sensory evaluation results in the present study indicated that catfish pastirma received high acceptance scores, which aligns with previous findings. For instance, **Kök *et al.* (2009**) reported that the sensory properties of raw catfish (*Silurus glanis* L.) pastirma stored at ambient temperature (20°C) were rated highly. Similarly, **Arslan *et al.* (1997)** found that *Cyprinus carpio* pastirma remained acceptable during 120 days of refrigerated storage at 4°C. **Mahmoud *et al.* (2016)** further confirmed that refrigerated fish pastirma maintained better overall acceptability compared to products stored at room temperature over the storage period.

CONCLUSION

Through this study, it was concluded that the use of shrimp and crab chitosan and its nanoparticles as a coating for catfish pastirma improves its physical, chemical, microbiological, and sensory properties. Consequently, the produced catfish pastirma is characterized by high quality and nutritional value, safe for health, and is acceptable to consumers. Therefore, it is recommended to use chitosan or their nanoparticles as an antioxidant and antimicrobial substance to preserve the properties and quality of the catfish pastirma.

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