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Effect of chitosan nanoparticles on the quality properties of fish burger

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ABSTRACT

In this study, catfish burgers were processed using chitosan and its nanoparticles extracted from shrimp and crab bio-wastes. Chemical composition, physicochemical, microbiological and sensory quality criteria were analyzed. Fatty acids were determined by gas chromatography (CG-FID), while volatile compounds were assessed by CG-MS. Results showed that either chitosan or chitosan nanoparticles significantly decreased pH value, total volatile basic nitrogen (TVBN), trimethylamine (TMAN), thiobarbituric acid (TBA), total bacterial counts and psychrophilic bacteria of catfish burgers; these values did not exceed the acceptable limits for all groups. The incorporation of chitosan and its nanoparticles was effective in controlling the growth of bacteria, and biochemical quality indices, as well as improving the freshness indices of catfish burgers. The above results indicate that it is feasible to obtain fish burgers with improved physicochemical and sensory properties using chitosan nanoparticle pretreatment. Therefore, it could be recommended to advance the field of fish burger production. In addition, this study provided insights for the development of improved processing techniques in the food industry through converting some food additives like chitosan to nanoparticles.

INTRODUCTION

Fish are high in protein with balanced amino acid composition, providing polyunsaturated fatty acids, vitamins (A, B, and D) and minerals (P, Mg, Se, and I), and they are low in cholesterol and easily digested **(Tacon & Metian, 2013; Gökogluand Yerlikaya, 2015)**. Currently, chitosan and chitosan derivatives are getting interest in food science due to its special functional characteristics such as antioxidativeactivity and antimicrobial ability **(Niladri *et al.*, 2015)**. Nowadays, nanotechnology has a promising role in fish preservation and processing technology of seafood products. Nanotechnology involves the manufacturing, manipulating and characterizing of nanosized objects,

particles, with materials with a dimension of approximately 1–100 nm. Nanotechnology provides a range of significant improvements to enhance health, stability and quality of life creating assertive impacts on the environment (**Kuswandi, 2016, 2017)**. Many studies reported that chitosan had antimicrobial and antioxidant effect; **Ramezani *et al*. (2015)** reported that, chitosan nanoparticles are more effective antibacterial agent, when compared with chitosan for cold silver carp fillets. On the other hand, the use of chitosan and its nanoparticles is highly recommended to extend the shelf life and improve the microbiological quality of tilapia fish (**Sorour *et al*., 2021)**. Restructured products prepared from pangasiussurimi with the incorporation of chitosan resulted in reduced increase of TVB-N, FFA, PV, TBA and microbial count of the product during chilled storage (**Jeyakumari *et al*., 2016)**.

African catfish (*Clariasgariepinus*) is a fatty fish classified as a dark muscle fish with a strong muddy odor. All these characteristics have limited its utilization in the food industry. However, washing the minced fish meat can help eliminate lipids and undesirable materials including blood, enzymes and odorous substances, such as trimethylamine oxide and formaldehyde **(Daengprok *et al*., 2021)**. Catfish is an extraordinary nutritious fish that contains large amounts of unsaturated fatty acids, vitamins, proteins and minerals **(Nelson *et al*., 2016)**. In addition, it has little or no saturated fat. However, the African catfish meat has a pale color, mushy texture and a strong fishy odor (**MOAC, 2007)** affecting consumer acceptance. In the African catfish, oxidation of fat is significantly higher and often causes rancid and fishy odor as well as undesirable taste. African catfish is one of the major fish species cultivated in Egypt, but it had low market price as an underutilized fish species because of its soft texture, which could serve as an adequate source for the production of value-added fish product **(Chareonthaikij *et al*., 2018)**.

Fish burgers are typical examples of acceptable fast foods, which are increasing in popularity and have extensively developed in the world food market, and many studies have been conducted to determine their quality **(Tokur *et al*., 2004, 2006; Al-Bulushi *et al*., 2005; HassabAlla *et al*., 2009)**. Fish burger is a ready-to-eat food that is popular among consumers owing to easy processing and rich nutritional value. Fish burger is usually stored under frozen conditions, but long-term frozen storage can denature the proteins in the fish, resulting in a decline in sensory quality (**Zhou *et al*., 2021**). Many researchers worldwide processed fish burger from different fish species e.g., tilapia burger (**Tokur *et al*., 2004)**; Arabian Sea *meagreArgyrosomusheinii* (**Al-Bulushi*et al*., 2005)**; yellow-striped trevally *Selaroidesleptolepis* (**Siah, 2005)**; Gilthead sea bream (*Sparusauratus*) **(Corbo *et al*., 2009a)**; cod hamburgers **(Corbo *et al*., 2009b)**; blue fish **(Del Nobile *et al*., 2009)**; blue fish **(Di Monaco*et al*., 2009)**; catfish **(Hassaballa *et al*., 2009)**; whiting (*M. merlangus*) **(Kose *et al*., 2009)**; deep flounder **(Mahmoudzadeh *et al*., 2010a)**; mackerel **(Ucak *et al*., 2011)**; trout **(Ehsani *et al*., 2014)**; tuna **(Angiolillo *et al*., 2017)**; hake (*Merlucciushubbsi*) **(Asensio *et al*., 2019)**; African catfish

(*Clariasgariepinus*) (**Daengprok *et al*., 2021)**; sturgeon fish burger (**Zhou *et al*., 2021)**; **s**triped catfish and salmon mince **(Ditudompo *et al*., 2021)** and the Nile tilapia **(Mahmoud, 2021)**. However, the effects of chitosan from different marine bio-waste and its nanoparticles on the quality of fish burger have not been reported so far. Thus, the aims of this work were to evaluate the effect of chitosan nanoparticles on the quality of catfish burgers.

MATERIALS AND METHODS

Raw materials

Fresh African catfish (*Clariasgariepinus*) samples were purchased from Kafr El-Sheikh Fish Market, Egypt, with an average weight of 2000-3000g and were immediately transferred in an ice box in 3 hours' time to El-Kanater El-Khairia, Qaliubia governorate at Fish Processing and Technology Laboratory, Fish Research Station, National Institute of Oceanography and Fisheries. Fish samples were carefully washed with tap water, manually beheaded, gutted, filleted, rewashed carefully and drained.The fillets with approximately 45% yield were kept frozen at -18°C until used. Before the day of producing the fish burger, the frozen fillets were taken out of the refrigerator and kept at 4-5°C to defrost for 24h. 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 research were of analytical grade, purchased from Sigma-Aldrich, GmbH Taufkirchen, Germany. All other ingredients as onion & garlic were brought from reputed commercial suppliers and were of food grade quality. Chitosan and its nanoparticles were extracted from different marine bio-waste (shrimp and crab), and they were characterized, and their safety as food additives was confirmed upon conducting bioassay studies.

Treatment with chitosan and chitosan nanoparticles

The defrosted fish fillets were cut into 1"×1"×1", and they were classified into 7 groups (n = 12 fish/group). These groups were assigned as follows:

- **T1** formed the control group; it was soaked in distilled water for 45min at 4±1°C;
- **T2** was the group soaked in commercial chitosan 1% for 45min at 4±1°C;
- **T3** was soaked in commercial chitosan nanoparticles 1% for 45min at 4±1°C;
- **T4** was soaked in shrimp chitosan 1% for 45min at 4±1°C;
- **T5** was soaked in nanoparticles shrimp chitosan 1% for 45min at 4±1°C;
- **T6** was soaked in crab chitosan 1% for 45min at 4±1°C, and
- **T7** was soaked in nanoparticles crab chitosan 1% for 45 min at 4±1°C, respectively.

Catfish fillets samples were retrieved from the solution after 45min and were dried on the bench at room temperature for 5min. The treated catfish fillets groups were separately minced with a meat grinder having a 5 mm-hole plate, weighed and then added to other ingredient. Chitosan solution was prepared by dissolving 20g of chitosan in 4973.8ml of distilled water with 6.25g of acetic acid under mechanical stirring for 15min, then heating with constant agitation for 24h **(Qi*et al*., 2004)**.

Fish burger processing

Catfish burgers were produced according to **Bainy*et al.* (2015)**, with some modifications. The burger formulation consisted of 81.83% mince, 1.23% table 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% cold water. All ingredients were thoroughly mixed using a kitchen blender, weighed (80 g each piece), shaped and formed using a conventional burger press (8.5 cm diameter and 1 cm thickness). Then, they were packed, wrapped with polyethylene sheets and stored at -18°C. Seven groups of catfish burgers were produced as described previously. Catfish burger samples were subjected to deep-frying in sunflower oil preheated at 160°C for 5- 6min for sensory evaluation.

Analytical methods

The seven catfish burgers were sampled from each batch and analyzed in triplicates for moisture, ash, total nitrogen and fat in accordance with the method of **AOAC (2003)**. The moisture content was determined by drying samples in a hot air oven at 105°C until having a constant weight. The ash was determined as the remnant weight after the incineration of samples in a muffle furnace at 550°C for 3h. The total nitrogen was determined using Kjeldahl method with a 6.25 nitrogen to protein conversion factor. The crude fat was measured by Soxhlet extraction method using hexane as an organic solvent. The results were expressed as g/ 100g product.

Trimethylamine nitrogen (TMA-N) were analyzed according to **AOAC (2002**). Total volatile basic nitrogen (TVB-N), thiobarbituic acid (TBA) and the pH value **(Pearson 1991)** were analyzed. In addition, *Salmonella* sp., *Escherichia coli*, and total plate count were determined following the methods based on the standard American public health association protocol **(Downes & Ito, 2001)**. Sensory tests **(Feyand Regenstein, 1982)** were evaluated.

Fatty acids composition analysis

Fatty matter extraction from prepared fish samples

The methodology for fat extraction from fish product samples using cold extraction involves, separating the fat content from the rest of the products using a solvent without the need for heating. This technique is preferred over traditional hot extraction methods since it preserves the chemical composition of the fish products, particularly the volatile compounds that contribute to its flavor and aroma. The cold extraction process usually involves the use of n-hexane, which is added to the prepared products and allowed to stand for 15 minutes in a sonicated water bath. The solvent dissolves the fat content, which is then separated from the rest of the samples using filtration. The extracted fat is then further processed and purified for use in identifying fatty acids by gas chromatography. The use of cold extraction methodology not only preserves the quality of the samples but also has lower energy consumption and is more environmentally friendly, compared to traditional hot extraction methods.

Identification of fatty acids by gas chromatography (GC-FID)

The fatty acid makeup was analyzed using a modified method of **Zahran and Tawfeuk (2019)**. This involves converting the fatty chains to fatty acid methyl esters (FAMEs) through trans-methylation. The FAMEs were then separated using an HP 6890 plus gas chromatography with a Supelco™ SP-2380 capillary column and detected with a flame ionization detector (FID). The injector and detector temperature was set at 250°C, while the column temperature started at 140°C and increased at a rate of 4°C/ min until it reached 240°C, where it was held for 10 minutes. The carrier gas used was helium at a flow rate of 1.2mL/ min, and a sample volume of 1µL (in n-hexane) was injected through a split injector at a splitting ratio of 100:20. The FAMEs were identified by comparing their retention times with those of authentic FAME standards. The fatty acid composition was expressed as a relative percentage of the total peak area.

Volatile compounds analysis

Headspace sampling (HS-SPME) and gas chromatography-mass spectrometry (GC-MS) analysis

Volatile organic compounds of samples were analyzed using solid-phase microextraction (SPME). A quantity of 2g of the sample was weighed into 20mL capacity vials, and then a sodium salt solution of 3% was added. A fused silica SPME fiber covered with 85µm carboxen/polydimethylsiloxane (CAR/PDMS) (Supelco, SIGMA, St. Louis, MO) fiber was used. Vials were heated at 70°C for 20min without stirring. The fiber was exposed to the vial headspace for 10min and then injected into the CG-MS **(Centonze*et al.*, 2019)**. All analyses were performed on an Agilent 8890 GC System, coupled to a mass spectrometer (Agilent 5977B GC/MSD). Volatile compounds were separated on an HP-5ms fused silica capillary column (30 m × 0.25 mm × 0.25 µm), and the oven temperature program was set as follows: the initial temperature was 40°C, held for 3min, then programmed from 40 to 160°C atrate 4°C, maintained for 5 min and increased to 280°C at a rate of 10°C. Helium was used as the carrier gas at a flow rate of 1mL/ min. The volatiles were injected in the GC with a split less mode. The temperature of injection was 270°C. Mass spectra in the electron impact mode (EI) were obtained at 70 eV and scan m/z range from 39 to 500 amu. The isolated peaks were identified by matching them with data from the library of mass spectra (National Institute of Standard and Technology, NIST).

All the obtained results were expressed as mean value of three replicate samples ± SD (Microsoft Office Excel, 2010).

RESULTS AND DISCUSSION

Chemical composition

The effect of different chitosan types and its nanoparticles on the gross chemical composition of catfish burgers are shown in Table (1). Results showed that the moisture, protein, lipid and ash content of control catfish burger sample reached values of 57.89%, 16.38%, 18.55% and 2.29%, respectively. On the other hand, the addition of commercial chitosan, shrimp chitosan and crab chitosan led to a slight significant increase ($P \leq 0.05$) in moisture content from 57.89% in (T1) control burger to 58.64, 57.86 and 58.19 % in fish burger of commercial chitosan (T2), shrimp chitosan nanoparticles (T5) and crab chitosan (T6), respectively. In contrast, the addition of commercial, shrimp and crab chitosan nanoparticles significantly *($P \leq 0.05$)* decreased the moisture content to 57.41, 58.79 and 57.14%, respectively, in comparison with control group. These results are similar to the findings of **Ucak *et al*. (2011)** who reported that, moisture, crude protein, lipid and crude ash contents of mackerel fish burger were 57.97%, 18.10%, 12.75% and 2.18%, respectively.

Table 1. Effect of different chitosan types and its nanoparticles pretreatment on gross chemical composition of catfish burgers (wet weight basis)

Trial	Moisture	Protein	Lipid	Ash
T1	57.89±0.02	16.38±0.01	18.55±0.01	2.29±0.01
T2	58.64±0.02	16.04±0.02	18.68±0.03	2.51±0.02
T3	57.41±0.03	16.50±0.10	18.87±0.02	2.45±0.05
T4	57.86±0.01	16.89±0.05	18.26±0.05	2.38±0.02
T5	58.79±0.01	16.40±0.09	17.79±0.01	2.98±0.01
T6	58.19±0.02	16.33±0.02	18.24±0.03	2.86±0.01
T7	57.14±0.02	16.88±0.08	18.70±0.05	2.22±0.02

Each value represents average of three replicate samples ± SD.

Where, **T1:** Control; **T2:** Commercial chitosan; **T3:** Commercial chitosan nanoparticles; **T4:** Shrimp chitosan; **T5:** Shrimp chitosan nanoparticles; **T6:** Crab chitosan and **T7:** Nanoparticles crab chitosan.

The differences in the moisture content between fish burgers may be due to the proximate composition of the various types of fish and nonmeat ingredients contained in the formulations **(Raúl *et al*., 2018)**. The moisture, ash, protein, lipids and fiber of salmon fish burger were 61.08, 1.69, 18.12 and 11.61, respectively (**Cilli *et al*., 2020)**. Additionally, **Cristofel *et al*., (2021)** found that, the raw Nile tilapia burger contained 6.4 pH value, 67.03 moisture, 4.63% ash, 15.29% protein and 7.15 lipids, respectively. The moisture, protein, lipid and ash contents of raw African catfish burger were 67.91, 15.64, 12.40 and 1.84%, respectively **(Daengprok *et al*., 2021)**. In this context, **Mahmoud *et al*. (2021)** reported that, raw Nile tilapia fish burger contained 72.07% moisture, 44.15% protein, 17.78% fat and 7.33% ash, respectively. Whereas, **Abdel-latif *et al*. (2021)** revealed that, moisture, protein, lipid and ash of catfish burger were 69.01, 17.85, 4.64 and 4.03%, respectively.The proximate composition of the fish burgers showed

similarities to the findings of **Tokur *et al*. (2004)**, **Al-Bulushi *et al*. (2005) Hassaballa*et al*. (2009)**, **Mahmoudzadeh *et al*. (2010b)** and **Abdel-latif *et al*. (2021)**.

Physicochemical quality of fish burger

The effect of different chitosan types and its nanoparticles on physicochemical quality of catfish burgers are shown in Table (2). The pH value, TVBN, TMA and TBA of control catfish burgers were 6.92, 16.80, 2.11 mg/100g ww, 0.11 mg/100g ww, and 0.32 mg MDA/kg sample, respectively. The pH value, TVBN, TMA and TBA of catfish burgers showed significant differences (P<0.05) among different treatments, and this might be due to the effect of chitosan types and its nanoparticles. The pH of all fish burger samples complies with the findings of both **Kose*et al*. (2006)** and **Ozyurt*et al*. (2007)**, suggesting that acceptable level of pH of fish should be in the ranges of 6.8−7.0. The results indicated that adding a small amount of sodium chloride salt could lower the anion repulsion between the proteins from the connection of the sodium ion and a higher free hydrogen ion. This is because the sodium chloride salt splits into positive and negative charges (Na+ and Cl−, respectively) and merges with the muscle proteins **(DeMan, 1999)**.

Table 2. Effect of different chitosan types and its nanoparticles pretreatment on physicochemical quality of catfish burgers samples

Trial	pH- value	TVBN (mg/100g)	TMA (mg/100g)	TBA (mg MDA/kg)
T1	6.92±0.04	16.80±0.10	2.11±0.02	0.32±0.01
T2	7.81±0.05	16.80±0.20	1.86±0.02	0.30±0.03
T3	7.84±0.04	15.40±0.03	1.45±0.01	0.58±0.07
T4	6.98±0.02	16.80±0.04	1.70±0.03	1.36±0.03
T5	6.80±0.03	15.40±0.03	1.55±0.02	0.64±0.05
T6	6.82±0.02	14.00±0.10	1.30±0.03	0.45±0.01
T7	7.83±0.03	14.00±0.05	1.01±0.06	0.36±0.04

Each value represents average of three replicate samples ± SD

Where, **T1:** Control; **T2:** Commercial chitosan; **T3:** Commercial chitosan nanoparticles; **T4:** Shrimp chitosan; **T5:** Shrimp chitosan nanoparticles; **T6:** Crab chitosan and **T7:** Crab chitosan nanoparticles.

Our results coincide with those of **Siah (2005)** who elucidated that, the value of TVBN, TMA and TBA of raw *Selaroidesleptolepis* (yellow-striped trevally) fish burgers were 2.32 mg%, 1.02 mg% and 1.63 mg malonaldehyde/kg sample, respectively. Moreover, **Kose *et al*. (2009)** found significant differences (P< 0.05) between each product of whiting burger for TBA, TVB-N and TMA values and recorded 0.21mg MDA/kg sample, 2.57 mg% and 1.04 mg%, respectively.**Ucak *et al*. (2011)** reported that, mackerel fish burger contained TVBN ranging from 13.01- 15.80mg 100 g^{-1} and TBA fluctuating from 0.08- 1.47mg MA Kg^{-1}. On the other hand,TVB-N values ranged from

1.35-1.48 mg N/100 g for a sample of hack burger (**Asensio *et al*., 2019)**. **Saleem *et al*. (2019)** reported that, pH value, TVB-N value, TMA-N and TBA of control catfish burger were 6.38, 9.22mg/ 100 g; 1.35 mg/100 g and 0.62 mg MDA/kg sample, respectively. **Abdel-latif *et al*. (2021)** pointed that, pH value, TVBN, TBA of control catfish burger were 6.31, 9.14 mg/100g and 1.17 mg MDA/kg, respectively.

Total volatile basic nitrogen is proposed as an index of fresh fish quality because its increase corresponds to bacterial spoilage. The concentration of TVB in freshly caught fish is reported to be typically between 5 and 20 mg N per 100 g, whereas levels of 30‑35 mg N per 100 g flesh are generally regarded as the limit of acceptability for iced stored cold water fish **(Huss, 1988; Kose *et al*., 2006)**. Total volatile basic nitrogen (TVB-N) is known as a product of bacterial spoilage and endogenous enzymes action, and its content is often used as an index to assess the keeping quality and shelf-life of products **(EEC, 1995)**. Trimethylamine-oxide is generally present in marine fish, and the product of its decomposition can be used for assessing fish quality. It has been reported that 10‑ 15mg TMA-N per 100 g is usually regarded as the upper limit of acceptability for human consumption **(Huss, 1988)**. TBA is the second breakdown product of lipid oxidation and widely used as an indicator of degree of lipidoxidation **(Aubourg, 1999)**.

Fatty acids composition of catfish burgers

Marine and freshwater fish species have a different fatty acid composition. Table (3) illustrates the effect of different chitosan types and its nanoparticles on fatty acids composition of catfish burgers. The contents and composition of fish lipids varied according to the species, age, location, species origin and environmental conditions **(Huss *et al*., 2004)**. The results showed that, the control catfish burgers contain 30.99% oleic acid, 26.52% palmitic acid, 13.55% linoleic acid, 7.15% docosahexaenoic acid, 5.71% stearic acid, 4.27% palmitoleic acid, 4.17% eicosapentaenoic acid, 3.20% myristic acid, 1.83% α- linolenic acid, 1.10% lauric acid, 1.05% stearidonic acid and 0.46% arachidic acid, respectively. Catfish burgers are high in oleic, palmitic and linoleic acid acids. Control fish burgers have 63.01% more unsaturated fatty acids than saturated fatty acids (36.99 %). In both control and treated catfish burgers, oleic, palmitic, linoleic docosahexaenoic, stearic, palmitoleic, eicosapentaenoic and myristic acids were the most prevalent fatty acids (Table 3). On the other hand, α-linolenic, lauric, stearidonic and arachidic contained trace amounts. Linoleic, myristic, erucic, lauric, and arachidic acid levels of the fish burgers increased significantly ($P \leq 0.05$). The investigated catfish burgers processed using crab chitosan and chitosan nanoparticles hadn't any amounts of lauric compared to the control and other trial groups, and this may be due to the action of nanoparticles. Nonsignificant differences ($P \leq 0.05$) were observed in the percentage of most FAs between the control catfish burger and corresponding treated samples. Previously, 19 FAs were identified in hake burgers as follows: palmitic acid (16:0) (221.3 g kg^{-1}) was followed by 22:6 x3 (DHA; with 211.5 g kg^{-1}) and oleic acid (18:1; with

160.1 g kg^{-1}).c-Linolenic acid (18:3x6) was also present at a high amount (57.1 g kg^{-1}) (**Asensio *et al*., 2019)**. While, **Mahmoud (2021)** denoted that, control tilapia burgers contain 10.95 % and 6.69 % α-Linolenic and linoleic acids; respectively. Tilapia burgers are high in oleic and palmitic acids. Control fish burgers have 63.90 % more unsaturated fatty acids than saturated fatty acids (36.10 %).

Table 3.Effect of different chitosan types and its nanoparticles pretreatment on fatty acids composition of catfish burgers

Fatty acids (% of total fatty acids)	T1	T2	T3	T4	T5	T6	T7
Saturated fatty acid (SFA)							
Lauric acid (C12:0)	1.10	0.26	0.26	1.04	0.71	ND	ND
Myristic acid (C14:0)	3.20	0.95	0.96	1.75	1.45	0.97	0.58
Palmitic acid (C16:0)	26.52	29.01	28.11	24.78	25.76	29.40	30.16
Stearic acid (C18:0)	5.71	5.84	5.17	6.62	6.59	5.84	5.79
Arachidic acid (C20:0)	0.46	0.96	0.96	1.74	1.44	0.83	0.66
Unsaturated fatty acid (USFA)							
Palmitoleic acid (C16:1),n9	4.27	3.65	3.44	4.43	4.28	3.31	3.49
Oleic acid (C18:1n9c)	30.99	37.16	36.21	32.94	34.36	35.19	38.44
Linoleic acid (C18:2n6c)	13.55	15.22	15.24	15.01	15.44	15.11	15.66
α- Linolenic acid (C18:3n3)	1.83	1.31	1.31	2.09	1.81	1.31	1.03
Stearidonic acid (C18:4) n3	1.05	0.87	1.87	1.71	1.41	0.77	0.57
Gadoleic acid (C20:1)	ND	1.07	1.37	1.85	1.56	1.19	0.78
Arachidonic acid (C20:4)	ND	0.98	0.98	1.76	1.46	0.98	0.68
Eicosapentaenoic acid (C20:5)	4.17	1.35	1.35	2.13	1.85	1.55	1.07
Docosahexaenoic acid (C22:6)	7.15	1.37	2.77	2.15	1.88	3.55	1.09
Total (%)							
Fatty acids	100	100	100	100	100	100	100
Saturated fatty acids	36.99	37.02	35.46	35.93	35.95	37.04	37.19
Unsaturated fatty acids	63.01	62.98	64.54	64.07	64.05	62.96	62.81

Where, **T1:** Control; **T2:** Commercial chitosan; **T3:** Commercial chitosan nanoparticles; **T4:** Shrimp chitosan; **T5:** Shrimp chitosan nanoparticles; **T6:** Crab chitosan and **T7:** Crab chitosan nanoparticles.

He *et al*. (2021) found that, the percentage of oleic acid in raw and cooked plant-based burgers 1 to 4 was 39.86 and 40.00%, 51.29 and 51.25%, 56.26 and 56.42%, and 48.58 and 48.40%, respectively, while the percentage of linoleic acid in raw and cooked plant-based burgers 1 to 4 was 13.95 and 14.15%, 16.06 and 16.31%, 17.52 and 17.57%, and 24.62 and 23.93%, respectively. Fatty acids profile of catfish burger agree with the finding of **Garcia-Arias *et al*. (2003)**and **Oluwaniyi *et al. (*2010)**. Generally, the greater the number of double bonds in the FA, the greater the ease of its oxidation. For instance, DHA is estimated to be 50-fold more sensitive to oxidation than oleic acid **(Polavarapu *et al*., 2011)**.

Volatile compounds of catfish burgers

The effect of different chitosan types and its nanoparticles on volatile compounds of catfish burgers is presented in Table (4). A total of 48 compounds were identified. Six major groups were identified**.** The compounds are grouped according to their chemical structure, into aldehydes, ketones, alcohols, acids, and esters. In total, 48 compounds were detected in these catfish burger samples, including 5 aldehydes, 11 alcohols, 8 carboxylic acids, 3 sulfur-containing compounds, 13 aromatic compounds and 7 other compounds. However, their profiles were highly different from each other, and approximately 15 compounds were detected in all burger samples. On the other hand, 23 compounds were not detected in control catfish burger, while they were detected in the treated groups, and this may be due to the addition of chitosan and nanoparticles. Cuminaldehyde compound recording higher values (21.21) were in the control catfish burger, followed by Eugenol (11.20), Diallyldisulphide (9.00), Palmitic acid 4.43 and Linalool (4.42), respectively **(Table 4 and Fig. 1)**.

Table 4. Volatile compounds (expressed as area units (AU) x $10^8 g^{-1}$ of sample) of catfish burger as affected by chitosan and its nanoparticles pretreatment

Compounds	T1	T2	T3	T4	T5	T6	T7
Aldehydes (5)							
Nonanal	ND	ND	ND	ND	ND	0.62	ND
Undecanal	ND	0.68	ND	ND	ND	ND	ND
α-Terpinen-7-al	2.29	1.98	2.1	1.25	1.83	1.28	1.73
γ-Terpinen-7-al	3.24	2.45	2.83	1.78	2.29	1.79	2.57
Cuminaldehyde	21.21	17.32	15.76	12.09	16.02	11.79	16.34
Total	**26.74**	**22.43**	**20.69**	**15.12**	**20.14**	**15.48**	**20.64**
Alcohols (11)							
Eucalyptol	3.18	12.61	5.85	7.3	5.08	6.88	4.38
1-Hepten-3-ol	ND	ND	ND	ND	ND	0.73	ND
Linalool	4.42	10	6.75	7.06	7.15	6.54	6.4
endo-Borneol	ND	ND	ND	ND	ND	ND	0.82
α-Terpineol	ND	1.17	1.14	0.67	0.88	0.64	0.83
Anethole	ND	ND	ND	ND	ND	ND	0.64
3-Hydroxy-2,3-dihydromaltol	ND	0.97	1.57	ND	ND	ND	ND
Camphol	ND	1.61	1.2	0.84	0.91	0.86	ND
2,5-Dihydropyrrole	ND	0.82	1.76	ND	ND	ND	ND
Eugenol	11.2	9.49	14.69	6.1	6.28	5.12	9.85
Methyleugenol	1.12	0.82	1.28	ND	0.72	0.49	0.95
Total	**19.92**	**37.49**	**34.24**	**21.97**	**21.02**	**21.26**	**23.87**
Carboxylic acids (8)							
Acetic acid	ND	1.33	ND	ND	0.45	0.81	5.52
Allantoic acid	ND	ND	ND	0.86	ND	ND	1.06
Stearic acid	ND	ND	0.74	ND	ND	ND	ND

Compounds	T1	T2	T3	T4	T5	T6	T7
Palmitoleic acid	ND	ND	1.09	ND	ND	ND	ND
Palmitic acid	4.43	3.66	14.92	1.25	0.8	4.73	1.8
Linoleic acid	ND*	ND	1.77	ND	ND	0.34	ND
Oleic Acid	2.19	1.48	7.02	ND	ND	1.6	ND
Stearic acid	ND	ND	2.22	ND	ND	0.7	ND
Total	**6.62**	**6.47**	**27.76**	**2.11**	**1.25**	**8.18**	**8.38**
Sulfur containing compounds (3)							
Ethylvinyl sulfide	ND	ND	0.56	ND	ND	ND	ND
Diallyl sulfide	2.25	1.79	0.82	3.43	2.84	2.6	1.8
Diallyldisulphide	9.00	3.47	2.05	9.43	9.25	7.9	7.54
Total	**11.25**	**5.26**	**3.43**	**12.86**	**12.09**	**10.5**	**9.34**
Aromatic compounds (13)							
Benzene	ND	1.29	ND	4.42	3.17	1.86	4.27
Toluene	3.55	1.5	ND	6.29	5.48	6.42	ND
p-Xylene	ND	ND	ND	ND	ND	ND	1.55
o-Xylene	ND	ND	ND	1.02	1.17	0.99	ND
Methyl N-hydroxybenzenecarboximidoate	3.64	4.04	ND	2.6	3.75	2.39	2.13
α-Pinene	ND	ND	ND	0.95	ND	1	ND
β-Pinene	1.15	0.71	ND	2.64	1.76	2.27	1.38
β-Myrcene	1.42	ND	ND	1.71	2.01	1.68	1.3
p-Cymene	2.6	1.09	ND	5.19	5	4.48	3.59
D-Limonene	2.59	0.95	ND	5.65	5.53	5.02	4.08
γ-Terpinene	2.63	0.94	ND	5.82	5.24	5.08	3.88
β-Caryophyllene	3.21	ND	ND	2.31	3.15	2.31	3.04
Viridiflorene	ND	0.90	ND	ND	ND	ND	ND
Total	**20.79**	**11.42**	**0.00**	**38.60**	**36.26**	**33.50**	**25.22**
Others (7)							
Trimethylhydrazine	2.85	ND	ND	ND	ND	ND	ND
Dihydroxydimethylsilane	ND	0.62	0.3	ND	ND	ND	ND
Urea	ND	ND	1.74	ND	ND	ND	ND
cis-Thujone	3.6	5.48	2.87	2.13	1	3.95	ND
(+)-Camphor	1.2	4.41	2.37	2.6	2.04	2.41	1.82
Carvone	4.74	5.40	5.88	3.00	3.91	3.09	4.14
α-Terpinyl acetate	2.29	1.02	0.73	1.64	2.26	1.58	2.1
Total	**14.68**	**16.93**	**13.89**	**9.37**	**9.21**	**11.03**	**8.06**

ND* = Not detected

The current outcomes concur with some volatile identified compounds in the study of **He *et al.* (2021)** who detected 64 compounds in burger samples, including 9 aldehydes, 7 ketones, 7 alcohols, 6 carboxylic acids, 4 esters, 7 pyrazine, furan or pyran, 15 sulfur-containing compounds, 5 aromatic compounds, and 4 other compounds. Additionally, **Iacumin *et al.* (2022)** reported that,the addition of bioprotective cultures

avoided bloating spoilage and improved the sensory parameters of the seabass and sea bream fish burgers. **Gänzle (2015)** and **Zotta *et al*. (2017)** reported that, the species used in these trials are facultatively heterofermentative and can ferment pentose sugars present; for example, in nucleotides, producing lactic and acetic acid. Benzene levels were previously reported in raw salmon fillets spoiled with *P. phosphoreum* **(Macé *et al*., 2013)**.

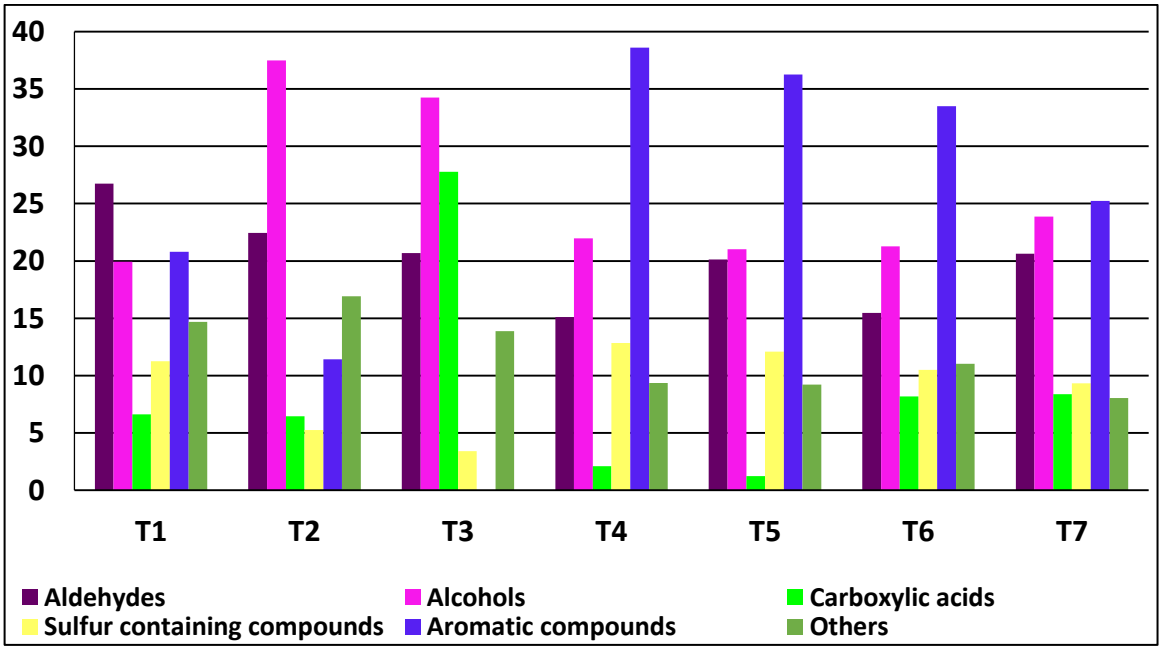

Fig. 1. Volatile compounds groups of catfish burger as affected by chitosan and its nanoparticles pretreatment

Microbiological quality of catfish burger

Total bacterial count is used as an acceptability index for fish products because of the effect of bacteria in spoilage. Effect of different chitosan types and its nanoparticles pretreatment on microbiological quality of catfish burgers are presented in Table (5).

Table 5. Effect of different chitosan types and its nanoparticles pretreatment on microbiological quality of catfish burgers samples

Trial	*Salmonella* sp.	*Escherichia coli* (cfu/g)	Total Plate count (cfu/g)
T1	Absence	Absence	$2.2x10^3$
T2	Absence	Absence	$1.4x10^3$
T3	Absence	Absence	$1.2x10^3$
T4	Absence	Absence	$1.8x10^3$
T5	Absence	Absence	$1.4x10^3$
T6	Absence	Absence	$1.5x10^3$
T7	Absence	Absence	$1.3x10^3$

Where, T1: Control; **T2:** Commercial chitosan; **T3:** Commercial chitosan nanoparticles; **T4:** Shrimp chitosan; **T5:** Shrimp chitosan nanoparticles; **T6:** Crab chitosan and **T7:** Crab chitosan nanoparticles.

The results showed that, none of the samples presented *Salmonella* or *Escherichia coli*. Total bacterial count of fish burgers did not exceed the limit for all groups (<5 log cfu g^{-1}). Significant differences ($P< 0.05$) were observed among groups. The control group contained high level of total plate counts compared to treatment groups. These results indicate that ingredients of the burgers could contribute high amounts of bacteria since the ingredients were not sterilized. For fresh water and marine species, the microbiological limit recommended by the **ICMSF (2000)** for TVC at 30°C is 7 log g^{-1} or log cm^{-2}. Initial total viable counts of fish burger was 4.47 log cfu g^{-1}, which was higher than those reported for the Arabian Sea meagre by **Al-Bulushi *et al*. (2005)**, the whiting burgers in the study of **Kose *et al*., (2009)**, and the cod burgers in the work of **Corbo *et al*. (2009)**. **Saleem *et al*. (2019)** argumented that, TBC, yeast and mold counts of raw catfish burger were 3.22 and 0.60 log10 cfu/g, respectively.

Sensory evaluation of catfish burger

Sensory values are more acceptable criteria for judging shelf life of the products in terms of consumer preference **(Kose *et al*., 2009)**.

Table 5. Sensory evaluation offish burger samples

Trial	Sensory parameters					
	Color	Tenderness	Juiciness	Taste	Flavor	Overall acceptability
T1	9.25±0.05	8.11±0.08	8.20±0.05	9.90±0.05	8.50±0.04	9.00±0.05
T2	9.69±0.04	8.17±0.06	8.28±0.07	9.80±0.04	8.60±0.03	9.20±0.02
T3	9.75±0.05	8.02±0.04	8.19±0.06	9.85±0.03	8.53±0.03	9.35±0.05
T4	9.18±0.08	8.45±0.05	8.48±0.08	9.17±0.03	8.70±0.04	9.40±0.03
T5	9.11±0.07	8.67±0.03	8.74±0.10	9.20±0.02	8.92±0.04	9.25±0.05
T6	9.10±0.05	8.87±0.03	8.89±0.07	9.49±0.04	8.78±0.02	9.10±0.03
T7	9.47±0.05	8.14±0.06	8.17±0.09	9.79±0.05	8.69±0.06	9.50±0.04

Where, **T1:** Control; **T2:** Commercial chitosan; **T3:** Commercial chitosan nanoparticles; **T4:** Shrimp chitosan; **T5:** Shrimp chitosan nanoparticles; **T6:** Crab chitosan and **T7:** Crab chitosan nanoparticles.

According to the statistical analysis, there were no significant differences ($P> 0.05$) in sensory evaluation of all groups. In terms of sensory analysis (Table 5), it was observed that the average scores for each attribute in the acceptance test did not show statistical difference ($P> .05$) between the samples, with an average of 7.0 (like moderately for all parameters). This result demonstrated that the addition of different chitosan types and its nanoparticles did not significantly interfere with the sensory characteristics of the final product in relation to all attributes analyzed. Similar results were obtained from the other studies (**Cristofel *et al*., 2021)**. Our results corroborate previous findings, which showed that chitosan nanoparticles pretreatment had significant effects on the quality of fish products.

CONCLUSION

This study addressed the effect of chitosan and chitosan nanoparticles on the quality properties of catfish burgers. The results demonstrated that both chitosan and chitosan nanoparticles significantly improved the physicochemical and microbiological characteristics of the burgers. Parameters such as pH value, total volatile basic nitrogen, trimethylamine, thiobarbituric acid, total bacterial counts, and psychrophilic bacteria were effectively reduced without exceeding the acceptability limit. This suggests that the incorporation of chitosan and its nanoparticles can effectively control bacterial growth and enhance the biochemical quality and freshness of catfish burgers. However, the sensory properties of the burgers were not significantly affected by the chitosan and chitosan nanoparticles treatment. While this may indicate that the addition of chitosan does not impart noticeable sensory changes, it also suggests that the improved quality properties of the burgers can be achieved without compromising the overall sensory experience.The successful application of chitosan and chitosan nanoparticles as pretreatment technologies for catfish burgers holds promise for both home cooking and commercial production. The findings of this study support the production of high-quality fish burgers with enhanced physicochemical and microbiological attributes. Future research should focus on exploring the treatment mechanism of chitosan nanoparticles to further improve the quality properties of different cooked fish burgers.These findings encourage the food industry to consider producing frozen fish burgers from catfish flesh on a commercial scale. By utilizing chitosan and chitosan nanoparticles as pretreatment agents, manufacturers can enhance the quality and safety of fish burgers, meeting consumer demands for healthier and safer food options. Overall, this research contributes to advancing the field of fish burger production and provides valuable insights for the development of improved processing techniques in the food industry.

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