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Microbial chitosan as a biopreservative for fish sausages

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ABSTRACT

Processed fish products are worthy sources to supply man with his main nutritional needs, but they are extremely susceptible to quality loss during storage. Microbial (fungal) chitosan is a bioactive polymer that has numerous applications in health promoting fields. Fungal chitosan was extracted from the grown mycelia of *Aspergillus brasiliensis* (*niger*) to investigate its potential role as antimicrobial, preservative and quality improvement agent, in processed fish sausages from Nile tilapia (*Oreochromis niloticus*). The produced chitosan had a molecular weight of 29 kDa, deacetylation degree of 91% and solubility of 99% in acetic acid solution. Fish sausages supplementation with 1.5% chitosan resulted in sharp reductions of microbial load (Total aerobic microorganisms, coliforms, yeast & molds, *E. coli*, Enterobacteriaceae and *Staphylococcus aureus*) during cold storage, at 4 °C for 28 days. Sensory attributes were notably enhanced in stored chitosan-supplemented sausages, especially the odor and taste characteristics. Captured micrographs, of exposed *S. aureus* to chitosan, exhibited vigorous morphological alterations after 4 h, and complete cell lysis after 8 h of exposure period. *A. brasiliensis* chitosan, however, could be strongly recommended, as a supplement for Nile tilapia fish sausages, to maintain microbiological quality and enhance sensory attributes of the product during storage.

1. Introduction

Fish meat is a worthy source to supply man with his need from protein (15–20%), carbohydrates, vitamins, minerals and water-soluble nutrients [1]. Due to the upsurge in health concerns, seafoods consumption is increasing and red meat usage is diminishing.

Processed fish products' consumption is frequently less than the consumption of other meat sources; this was attributed to the little number of processed products from fish meat and consumers preference for fresh fish eating. Thus, more interests are needed to explore fish meat potentiality in development of food industries [2].

Consumers are progressively aware about the relationship between health and diet. Since seafood was confirmed as nutritious and complete foods, therefore marine-based foods consumption tends to grow constantly. Seafoods were identified as excellent sources of easily digested nutritional proteins and important lipids that contain elevated amounts from polyunsaturated fatty acids (PUFA). These compounds are proved to enhance human health via different mechanisms, e.g. prevention from hypertension, coronary disease and cardiovascular disease. Additionally, seafood products are superb sources for essential minerals and many have been categorized into functional foods or nutraceuticals [3].

Despite these desired properties, processed seafood products are extremely susceptible to quality loss, mainly attributable to lipid oxidation reactions, especially those involving PUFA [4], which are activated with the presence of high protein concentrations. Furthermore, the quality of seafood products is highly affected by bacterial contamination, autolysis, and protein functionality losses [5]. The contamination of processed seafood products with hazardous materials, e.g. industrial wastes, refinery and heavy metals, lead to increasing researchers concerns regarding their consumption [6]. It was reported that the regular heat treatment of fish sausage, in the range of 85–90 °C, could not kill numerous spore-forming bacteria, because fish sausage are considered as an ideal environment for spores and spoilage microorganisms [7]. Accordingly, there is an elevated need to search for effective, safe and suitable preservatives to extend the storage period of fish sausage.

Nisin was one from the most applied biopreservatives in food products to control foodborne pathogens including *Listeria monocytogenes* [8].

Although nisin was effective as antimicrobial agent against many species from Gram-positive bacteria, its antimicrobial activity was ineffective against the Gram-negative bacteria, fungi and yeast [9].

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Chitosan is a polysaccharide derived from chitin, after its deacetylation, and has a cationic nature with many tremendous bioactive properties [10–14]. Technologically, chitosan has been recurrently proposed, in food products, as a powerful antimicrobial agent, preservative, binder agent and texturizer [15–22].

Chitosan has also attracted a great attention regarding its uses in the seafood industry because of its biocompatibility, biodegradability, nontoxicity and mucus adhesiveness characters; chitosan was successfully incorporated into many seafood products for improving their quality and enhancing human nutrition [3].

Chitosan has lately gained more attention, from both consumers and seafood processors, regarding its applications as an antibacterial additive in many seafood products, because of its promising role in reducing seafood preservation with synthetic chemicals [23,24].

The antioxidant capability of chitosan was also reported [25]. From the nutritional viewpoint, chitosan has been recommended as an effective dietary fiber [26], acting as a hypocholesterolemic agent, through diminishing the intestine bile acids [15].

Chitosan could be introduced into foods, to develop their functional properties, in a solubilized form or as a powder, which becomes soluble in the stomach due to its acidic pH. After solubilizing, chitosan could be act as a dietary fiber and will be able to adsorb fats by interacting with cholesterol, bile acids and triglycerides. Afterward, an insoluble complex from them will be formed in the intestine, because of its alkaline pH [27].

The principal sources, for commercial chitosan production, are the byproducts of seafood processing, e.g. shells of shrimp and crab. The developments in applied biological sciences, e.g. fermentation, endorsed the employment of fungi as promising alternative sources for chitosan production from their biomass [10]. Numerous fungal species were appraised for their capability to produce chitosan including *Absidia orchidis, Aspergillus niger*, *Cunninghamella elegans, Mucor rouxii*, *Penicillium notatum* and *Rhizopus arrhizus* [11,17,28].

Many bioactive attributes of microbial (fungal) chitosans were enhanced, compared to commercial chitosan, regarding their application in food sectors as biopreservatives, antimicrobial agents, adsorbents, chelating agents, clarifying materials or quality enhancers [10,12,19,29].

Thus, the current study aimed to investigate the potential role of microbial (fungal) chitosan, produced from *Aspergillus brasiliensis (niger)*, as antimicrobial, preservative and quality improvement agent, in processed fish sausages.

2. Materials and methods

2.1. Fish meat preparation

Fresh fish of Nile tilapia (*Oreochromis niloticus*), with approximate weight of 500 g/fish, were obtained from the research farm in the Faculty of Aquatic and Fisheries Sciences- Kafrelsheikh University, Egypt. Fish were deheaded, skinned and filleted; the fillets were minced using an electric mincer (Kenwood, Hampshire, UK) with a mincing screen diameter of 4 mm. The minced fish meat was used to prepare the sausage formula (Table 1), which was modified from the formula of Dincer and Cakli [30]. The other ingredients in the formula, e.g. spices, starches, salt, sugar and fats, were purchased from local markets.

2.2. Fungal chitosan

The used fungal chitosan was extracted from the grown mycelia of *Aspergillus brasiliensis* (*niger*), ATCC-16404, according to Tayel et al. [17], using Potato dextrose broth (PDB, Difco Laboratories, Sparks, Md.) as a fermentation medium. Briefly, fungal mycelia were harvested by centrifugation, after their propagation at 28 °C for 9 days, washed with distilled water and treated with 1 M NaOH at 95 °C for 2 h. The insoluble materials were collected by centrifugation, neutralized and treated with 10% v/v acetic acid for 6 h at 65 °C under shaking. The pH of acid soluble fraction was adjusted to 9 using 4 M NaOH, then the precipitated chitosan was collected and washed with distilled water, 95% ethanol and acetone, respectively, then dried at 45 °C for 12 h.

Table 1
Formulation of prepared fish sausage.

Ingredients	Percentages
Minced Tilapia fish meat	70.00
Salt (NaCl)	2.00
Sugar	0.50
Potato starch	2.50
Corn starch	5.00
Red pepper	0.20
Black pepper	0.25
Sodium Polyphosphate	0.20
Coriander	0.30
Cumin	0.25
Cinnamon	0.10
Ginger	0.10
Garlic	0.10
Chitosan	1.50
Sunflower oil	4.00
Beef fat	3.00
Crushed ice	10.00
Total	100.00

The evaluation of microbial chitosan's physico-chemical attributes (solubility, viscosity and color) was conducted according to AOAC [31]. The molecular weight (MW) of chitosan was assessed using gel permeation chromatography (GPC) with a refractive index detector (PN-1000, Postnova, Eresing, Germany), while the deacetylation degree (DD) was determined according to Donald and Hayes [32].

2.3. Preparation and manufacturing of fish sausage

The used fish sausage formula is illustrated in Table 1. All spices, starches, ice and fats were added to minced fish fillet, homogenized well. Cold chitosan solution (10%, at 4 °C) in 1% acetic acid was added to the half of sausage batter, at rate of 15 g/ kg of formulated batter, whereas the other half was mixed with 150 mL acetic acid solution/kg and served as control. The obtained homogenous batters were then covered and kept at 4 °C for 12 h. After that, the batter was packed in cow intestine (coil weight of 250–300 g), that was preliminary softened by fast immersing in hot water [2]. Sausage samples were stored at 4 °C and relative humidity of 80–85% for a storage period of 28 days. The experiments were repeated twice and the obtained values means were calculated.

2.4. Microbiological examination

The prepared fish sausages were aseptically sampled (10 g/sample), mixed with 90 mL of 0.1% buffer peptone water (LAB M, Lancashire, UK) in a stomacher bag and homogenized for 2 min using an electric stomacher. Homogenized samples were serially diluted then the number of various microbial species was determined by plating onto appropriate agar media.

According to the standard methods of analysis, various microbiological examinations were performed to evaluate chitosan effectiveness as a natural preservative in fish sausages. The applied test methods were based on the following standards:

Total aerobic microorganisms enumeration of–colony count at 30 °C [33].

Coliforms enumeration [34].

Yeasts & molds enumeration [35].