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Fungal chitosan and *Lycium barbarum* extract as anti-*Listeria* and quality preservatives in minced catfish

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ABSTRACT

Listeria monocytogenes is a foodborne bacterial pathogen that causes serious health risks. Chitosan (Ch) is a bioactive polymer that could be effectively applied for foodstuffs biopreservation. *Lycium barbarum* (Goji berry) is ethnopharmaceutical fruit that have diverse health protecting benefits. Chitosan was produced from *A. niger* and employed with *L. barbarum* extract (LBE) as blends for *Listeria* control and quality biopreservation of African catfish mince (*Clarias gariepinus*). Chitosan could utterly control *L. monocytogenes* survival in fish mince and its efficacy was strengthened with added LBE at 0.2 and 0.4%. Blending of fish mince with Ch could effectively reduce the progress of chemical spoilage parameters and this protective effect was greatly enhanced with increased addition of LBE. The sensorial assessment of treated minces indicated panelists preferences for the entire attributes of blended samples with Ch and LBE, particularly with storage prolongation. Scanning micrographs elucidated the antibacterial action of Ch against *L. monocytogenes*. Results recommended the application of fungal Ch/LBE composites as biopreservatives and anti-listerial agents, through their blending with catfish mince, to eliminate bacterial growth, enhance sensory and storage attributes of preserved fish.

1. Introduction

Listeria monocytogenes, the foodborne bacterial pathogen with major health risk, is the principal causative agent of listeriosis disease, which could have a fatality rate of ~30% [1,2]. *L. monocytogenes* is a psychrotrophic and facultatively anaerobic microbe that was associated with many mortality and morbidity outbreaks, mainly involved fast and processed foods [3]. The ability of *L. monocytogenes* to survive under refrigeration temperature make it a very serious threat toward preserved animal foods [2]. Many listeriosis outbreaks, associated with consumption of seafood and processed fish, were reported [3–5].

The production and cultivation of *Clarias gariepinus* (African catfish) is popularly increased in several countries, including Egypt, because of the desirable characteristics of this fish, e.g. resistance to inappropriate propagation conditions, rapid growth rate, ease of harvesting, nutritional requirements and cost effectiveness. However, despite of their low price and abundant supply, the marketing of these fish is still comparatively insufficient; African catfish (fresh, cooked or grilled) were classifies as from the less preferred and less consumed fish species [6]. It was recommended to subject the complete fish to further processing, e.g. minced or surimi-based products, to become more marketable and commercialized [7].

Chitosan, the deacetylated chitin derivative, is a very promising biopolymer that may be naturally found in nature and could be produced in commercial scale from the chemical treatment of natural chitin, from crustaceans' shells. Chitosan was successfully produced from another auspicious source, e.g. fungal species mycelia, as renewable, stable, cost effective and high quality sources [8–10].

Because of their superior antimicrobial activity, fungal chitosan types were efficaciously applied as powerful protectants from the attach of various foodborne bacterial pathogens found in water, animal and plant foodstuffs [11–14].

Furthermore, the usage of fungal chitosan, and its composites with plant extracts, was highly recommended for extending the shelf life and maintaining the overall qualities of many foodstuffs including meat, fruits and fish through blending with minced food or covering with their edible coatings [10,11,15,16].

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Lycium barbarum L. (Goji berry tree) is a deciduous shrubbery belonging to the family Solanaceae that habitually grows many parts of Asia; Goji fruits are ellipsoid berries of 1–2 cm long with bright orange-red color. The fruits of *L. barbarum* have been long traditionally used as medicinal and food plants worldwide, especially in China, as a rich antioxidant compounds source [17,18].

The most purified and investigated chemical components, in *Lycium* fruits, included polysaccharides, flavonoids, phenolic acids, carotenoids and related compounds [17]. *L. barbarum* extracts were proven to possess prosperity biological activities, e.g. anti-ageing effects, increased metabolism, antioxidant properties, anti-diabetics and glucose control, immunomodulation, anti-glaucoma, neuroprotection, anti-fatigue/endurance, cytoprotection and antitumour activity [19].

Numerous studies indicated the powerful antioxidant potentialities achieved from *L. barbarum* molecules, to act as promotions for various health protective effects [20–22].

Regarding its historical safety and importance in Chinese medicine, the usage of *L. barbarum* was recommended as a nutraceutical food and dietary supplement [23,24]. A detailed overview of *L. barbarum* anti-aging and health promoting potentialities was illustrated by Chang and So [25].

Regarding the abovementioned, this research was planned for evaluating the potentiality of natural products, e.g. microbial chitosan (from *Aspergillus niger*) and Goji (*L. barbarum*) extract, to be applied as anti-listerial, biopreservatives and quality maintaining agents in minced catfish during refrigerated storage.

2. Materials and methods

2.1. Fungal chitosan

An identified fungal strain, i.e. *Aspergillus niger* (ATCC-16404), was employed for the production of chitosan. Fungal propagation was implemented in the broth of potato dextrose medium (Merk, Darmstadt, Germany). The applied growth conditions, e.g. shaking incubation at 28 °C for 60 h, was followed to obtain fungal mycelia, which were harvested by centrifugation and subjected to extraction process. Washed mycelia, with distilled water, were homogenized at 100 °C for 90 min using 1 M from NaOH. The separation of insoluble fraction was achieved via centrifugation, and then it was repeatedly washed, with deionized water, re-centrifuged and treated with 10% acetic acid solution, on a rotary shaker, for 6 h at 65 °C, for neutralization. The pH of acid soluble fraction was adjusted to 9 using concentrated NaOH (4 M) and then the precipitated chitosan were separated via centrifugation, washed respectively with distilled water, ethanol (95%) and acetone, then dried at 55 °C and powdered to have the dried chitosan powder [9]. The fungal chitosans characteristics (color, solubility and viscosity) were evaluated as described by AOAC [26], the chitosan molecular weights (MW) was determined using refractive index detector attached to gel permeation chromatography (Postnova, Eresing, Germany). The deacetylation degree (DD) that specifies produced chitosan, was determined according to Donald & Hayes [27].

2.2. Lycium barbarum *fruit extraction*

Packaged dried organic whole Goji fruits (*L. barbarum*), from Ningxia- China origin, were obtained from local market. The fruits were washed and subjected to hot air at 45 °C for 24 h then ground using an electrical grinder to get plant particles size of ~40 mesh. Fruit powder (200 g) was first extracted using 1L of 80% ethanol, under shaking at 120 × *g* for 6 h; the plant particles were then separated by filtration through a Büchner funnel and kept for further extraction, while the filtrate was vacuum evaporated at 45 °C to eliminate most of the extraction solvent. The plant residues, from the first extraction, were re-extracted using boiling water, at 100 °C for 2 h, then left to cool and centrifuged at 1200 × *g* for 10 min to separate the crude polysaccharides [28]. The resultant extracts from the both extraction processes were combined, mixed well and lyophilised to obtain the total crude *L. barbarum* extract (LBE).

2.3. Listeria monocytogenes

Listeria monocytogenes NCTC 7973, possessed antigenic and hemolytic characteristics, was used in experimental inoculation of fish. Bacterial culture was initially inoculated in Brain Heart Infusion (BHI) broth and incubated for 36 h at 37 °C under mild shaking (75 × *g*), the grown cells were harvested by two centrifugation runs (2400 × *g*) separated with washing with physiological serum and removal of broth supernatant. The cell number was spectrophotometrically measured (at optical density of 600 nm) and adjusted to ~10^8 CFU/ml, equivalent to 0.08–0.1 OD.

2.4. Fish mince preparation

Alive African catfish (*C. gariepinus*), with average weight of 450–500 g each, were obtained from the aquaculture research farm, in the Faculty of Aquatic and Fisheries Sciences- Kafrelsheikh University, Egypt. Fish preparation included cutting, gutting, skinning and filleting. Fish fillets were then mechanically minced twice, under sanitary conditions using electric mincer with 1.4 mm diameter hole size. The minced fish meat was inoculated with ~2×10^4 CFU/g from *L. monocytogenes* and then the inoculated fish mince were divided into equal portions (250 g each) and the first portion was appointed as control group. The minced fish treatments included blending with 1% chitosan, dissolved in distilled water, or blending with 1% chitosan + LBE at percentages of 0.2 and 0.4%. All experimental samples were tightly packaged in plastic bags and kept at cooling temperature (4 ± 1 °C) for 14 days.

2.5. Listeria monocytogenes *enumeration*

The microbial detection of *L. monocytogenes* in treated minced fish, throughout the storage period, was performed according to Hegde et al. [29]; five g from minced muscles were added to 45 ml of physiological serum, homogenized well and serially diluted. *L. monocytogenes* cells were counted via spreading 100 μl form culture dilutions onto the selective media (CHROMagar Microbiology, Paris, France), i.e. CHROMagar-Listeria, then inoculated plates were incubated for 24–28 h at 37 ± 1 °C. Appeared *L. monocytogenes* colonies, with blue color surrounded by white halo, were counted and expressed as $\log_{10}$ CFU/g minced muscles. Stored fish minces were sampled, with intervals of 4 days, to evaluate *L. monocytogenes* survival in each treatment during storage.

2.6. Chemical parameters

The approximation of main spoilage chemical indicators, for the treated fish minces, was conducted. The values of total volatile nitrogenous base (TVB-N), expressed as nitrogen (mg)/100 g of minced fish, were determined by micro-diffusion method [30]. The Pearson's methods was followed for the assessment of PV (peroxide values) and TBARS (thiobarbituric acid reactive substances) [31]; PVs were assessed, from the content of peroxide in the fish lipid extracts as oxygen (meq)/lipid, while TBARS were determined colorimetrically at absorbance of 530 nm and expressed as mg malonaldehyde equivalents/kg of minced fish muscle.