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Production of fungal chitosan from date wastes and its application as a biopreservative for minced meat

Ahmed A. Tayel[a,b,*,1], Sami I.A. Ibrahim[a], Mahmoud A. Al-Saman[a], Shaaban H. Moussa[a,c,*]

[a] Genetic Engineering and Biotechnology Research Institute, University of Sadat City, El-Sadat City, Egypt
[b] College of Agriculture and Veterinary Medicine, Qassim University, Buraydah, Saudi Arabia
[c] Promising Research Center in Biological Control and Agricultural Information, Qassim University, Buraydah, Saudi Arabia

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ABSTRACT

Raw and processed meat contaminated with pathogenic microorganisms is a continuing worldwide problem facing health and industry overseers.

Fungal chitosan was extracted, purified and characterized from *Aspergillus brasiliensis* (*niger*) ATCC 16404 grown in date syrup (dips) and applied as a potential meat biopreservative. The main features of produced chitosan were a deacetylation degree of 81.3%, a molecular weight of 31,000 Da, 96% solubility in 1% acetic acid solution and a harmonized IR-spectrum to standard commercial chitosan.

The application of fungal chitosan, as a natural and safe biopreservative for minced meat, was conducted in comparison with potassium sorbate, as a commercial meat preservative.

Treated meat samples with 0.02% chitosan was the least trials in microbial contents, i.e. total count, coliforms, β-glucuronidase-positive *Escherichia coli*, *Enterobacteriaceae*, yeasts and molds, *Staphylococcus aureus* and coagulase positive staphylococci. The antimicrobial activity of fungal chitosan was considerably greater than that of potassium sorbate or their combination at 0.01% from each.

Sensory characteristics, e.g. color, odor and texture, of treated meat with chitosan, were higher than those of control and potassium sorbate treated samples.

Fungal chitosan, however, could be recommended as a powerful, natural and eco-friendly alternative for meat preservation and overall quality maintenance.

1. Introduction

Food researchers and industry overseers continued to seek more and better tools/agents to maintain food safety especially by the use of natural preservatives as antimicrobial agents. Food preservatives and antimicrobials are the added or supplemented agents in foods that kill or hinder growth of microorganisms [1].

Meat processing industry is regularly facing many serious challenges regarding the safety and hygiene of its products [2]. The routes for pathogenic microorganisms' transmission into the product could be throughout handling processes or from the carcass surface [3], decontamination or sterilization of the carcass body is very difficult to be achieved using currently applied antimicrobial agents.

Moreover, most of the commonly used preservatives and antimicrobial agents have chemical and synthetic nature, giving them many potential side effects and risks on the health of consumers [4,5]. Thus, it is believed that natural preservatives and antimicrobial agents will have more efficiency and safety regarding consumers' health and preference [6].

Chitosan is a biopolymer resulting from chitin deacetylation and have nitrogen content above 7% and degrees of acetylation lower than 40% [7]. Chitosan is an astonishing polymer as it may be used in various biological and industrial fields, e.g. food processing and additives [8], cosmetic industries [9], biotechnological approaches [10], agricultural production [11] pharmacology and medicine uses [12,13].

Chitosan is commercially produced via crustacean chitin deacetylation using strong alkali. This process has many disadvantages from the industrial view, e.g. seasonal and limited crustacean

* Corresponding authors at: Genetic Engineering and Biotechnology Research Institute (GEBRI), El-Sadat City, Microbial Biotechnology, El-Sadat City University Egypt, PO box 79/32897, El-Sadat City, Egypt.
Tel.: +00966 509249579/+20 2 37135273; fax: +20 48 2601266 8.
E-mail addresses: shaus2008@yahoo.com, shaus477@yahoo.de (S.H. Moussa).
[1] Current: Promising Research Center in Biological Control and Agricultural Information, Qassim University, Saudi Arabia.

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supply, processing difficulties especially with the large amount of concentrated alkaline solution wastes which lead to environmental pollution and inconsistent product properties [14]. Thereafter, many researches have been carried out for the extraction of chitosan from other alternative sources, mainly from fungi [14–20]. Chitosan production and extraction from fungal cell walls; grown under controlled conditions, offer more consistent and bioactive products with a minimum of environmental pollution [21,22].

The bacteriostatic and bactericidal activities of chitosan were demonstrated to elucidate its antimicrobial activity [23–26]. Chitosan was proven to inhibit the growth of a wide variety of microorganisms including bacteria, yeast and fungi. Moreover, chitosan has a lot of advantages over chemical disinfectants and antimicrobials as it possesses a broader spectrum of activity, a stronger antimicrobial action, a higher microbial killing rate without any toxicity toward mammalian cells [26–28].

Large quantities of agricultural wastes are annually produced from date trees, especially in Arab and Islamic countries; date pits resulted in a percentage of 10% from the grown date fruits, whereas fruits by-products and wastes are not properly utilized and are regularly burned in farms which cause a hazardous environmental threat [29,30]. In Saudi Arabia, only about 50% of the annual date's fruit production are consumed as human food or exported abroad. From the remaining, a small part is used as animal feed whereas the larger remaining part is considered as a leftover which necessitate suitable means of economic utilization [31].

The present study, however, was planned to produce fungal chitosan from dates syrup and apply it as a natural preservative and antimicrobial agent to maintain the microbiological and sensory quality of minced meat.

2. Materials and methods

2.1. Extraction of chitosan

2.1.1. Cultivation media and growth conditions

Aspergillus brasiliensis (*niger*) ATCC 16404 was used in this study for the production of chitosan. Potato dextrose broth and potato dextrose agar (PDB and PDA, Difco Laboratories, Sparks, Md.) were used for the cultivation, growing and maintenance of the fungal culture.

Date syrup extraction was carried out according to Al-Jasass et al. [32] using wasted non-edible date fruits for extraction. Dates were de-pitted and the flesh were dried and ground, then an equal amount of hot water (80 °C) was added and homogenized for 30 min. The mixture was filter pressed through 4 layers of cheese cloth to remove impurities and insoluble matters. Finally the extract was concentrated to 75° Brix at low temperature (80 °C) by vacuum drying to give the syrup named dips.

Diluted dates' syrup (10%), after the addition of 0.5% NH_4NO_3, was sterilized at 121 °C for 15 min and 10 mL of fungal spores suspension (10^6 spores/mL) were inoculated into 500 mL of composed medium, then stirred and rotated incubation at 28 °C for 6 days.

2.1.2. Chitosan extraction

After the period of cultivation, fungal mycelia were filtrated and washed by distilled water then was dried in oven.

Dried mycelia was treated with sodium hydroxide solution (1:30 w/v) and then the content was sterilized in autoclave at 121 °C till 20 min, followed by separation of alkali insoluble material by centrifugation, these fractions were washed by distilled water and re-centrifuged to a neutral pH (pH 7). The residue was extracted by acetic acid (1:40 w/v) at 90 °C for 6–8 h. The extracted slurry was centrifuged and the acid insoluble materials were discarded. The pH of supernatant was adjusted to 9–10 with 2 N NaOH, and then chitosan was obtained by precipitation through centrifugation, washed with distilled water, 95% ethanol and acetone, and dried at 60 °C to a constant weight [33].

2.2. Characterization of the physiochemical characteristics of fungal chitosan

IR spectrum was recorded with a Shimadzu FTIR-4200 spectrometer using a disk of KBr as a reference. The maximum intensity of the IR absorption band was determined by the baseline method [12].

The degree of deacetylation calculated from FT-IR spectroscopy by using the following equation [34]:

$$\mathbf{DDA} = 97.67 - \left[26.486\left(\frac{A_{1655}}{A_{3400}}\right)\right]$$

The molecular weight of fungal chitosan was determined by gel permeation chromatography (GPC) using refractive index detector (PN-1000, Postnova Analytics, Eresing, Germany).

2.3. Application of chitosan as a natural food preservative

2.3.1. Preparation of samples

Raw meat samples were pre-washed and treated by disinfectant (sodium hypochlorite) through immersing meat slices (100 g each) in 100 mL sterilized distilled water containing 10 ppm of disinfectant till 60 min, and then meat was rinsed twice with distilled water.

Meat slices were them minced, homogenized and divided into four groups:

Group A: Control meat sample.
Group B: Meat sample treated with 0.02% chitosan.
Group C: Meat sample treated with 0.02% potassium sorbate.
Group D: Meat sample treated with 0.01% potassium sorbate + 0.01% chitosan.

Under aseptic condition, 10 g from each group were immersed in 90 mL of diluents peptone-salt solution and homogeneously mixed in blender stomacher to make initial suspension [35]. The same examination was repeated after meat storage for 7 days at 5 °C.

2.3.2. Examination of samples

Different microbiological examinations were carried out for all samples according to standard test methods of analysis for evaluating the effectiveness of chitosan as a natural preservative compared to potassium sorbate which is widely used as a chemical preservative as follow:

Enumeration of microorganisms–colony count technique at 30 °C [36].
Enumeration of *coliforms* [37].
Enumeration of yeasts & moulds [38].
Enumeration of β-glucuronidase-positive *Escherichia coli* [39].
Detection & enumeration of *Enterobacteriaceae* [40].
Enumeration of *Staphylococcus aureus* and coagulase positive staphylococci [41].

2.3.3. Sensory characteristics evaluation

Eleven-member trained sensory panel was employed to judge the effect of meat treatment, with 2% chitosan or potassium sorbate, on the sensory color, odor and texture characteristics, compared to untreated minced meat samples. Panelists were trained by an experienced panel leader according to the American Meat Science Association guidelines [42,43]. Sensory evaluation was conducted, on a 1–5 scale, after 7 days of samples storage at 4 °C and relative