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Analysis of the Physicochemical Properties of Chitosan Extracted from the Fungus Pleurotus osturatus and its Effect on Inhibiting Some Food Spoilage Organisms

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I. Abstract:

Chitosan was chemically extracted from the oyster fungi *Pleurotus osturatus* by removing acetyl groups, ash, protein, and beta-glycan. Some physicochemical properties were studied, including yield, moisture, ash, protein, and solubility, which were found to be 6.5%, 6.79%, 0.09%, 1.09%, and 73%, respectively. The molecular weight was 329 kDa. The degree of acetyl group removal using FTIR was 79%, and the viscosity was 5.9 centipoes. Three stages of gravimetric pyrolysis were observed. The first stage occurred at temperatures below 170°C, while the second and third stages occurred at temperatures of 201–500°C and 500–800°C, respectively. The efficacy of prepared chitosan solutions in inhibiting some food spoilage organisms was also studied to assess their ability to inhibit the growth of Staphylococcus aureus, Bacillus subtilis, Listeria monocytogenes, Escherichia coli, Salmonella typhimurium, and Pseudomonas aeruginosa using the diffusion burrowing method. The results showed that Gram-positive bacteria were more affected by solutions with high concentrations (0.50%, 1.0%) than Gram-negative bacteria. The efficacy of chitosan solutions was also compared to the antibiotic Ciprofloxacin (5 µg) as a negative control and acetic acid solution as a positive control. Keywords: Chitosan, Pleurotus osturatus, microbiology, physicochemical properties

II. Introduction:

Recently, scientists have faced a number of challenges, namely the extraction of new chemical compounds that can be used in the medical field, in food manufacturing, in preserving food from spoilage microorganisms, and even in medical applications. Therefore, these compounds must possess a set of properties, such as being environmentally friendly, non-toxic, biodegradable, not encountering resistance from the immune response, inexpensive, easy to produce and prepare year-

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round, and contributing to environmental sustainability (Omar et al., 2021). One of the most important of these natural compounds is chitosan, derived from chitin, which is characterized by a set of properties, most notably biocompatibility and biodegradability, in addition to being non-toxic and not causing harm to living tissues and cells. This has enabled it to be used in the pharmaceutical industry, the manufacture of edible food coatings, and even in the cosmetics industry. It is also effective against many types of bacteria and fungi. All these features qualify it as environmentally friendly and non-polluting (Shreya and Subbalaxmi, 2024). Chitin is a fundamental component of the exoskeletons of crustaceans and edible fungi with fruiting bodies, and fungal chitin is a mixture of two types of β α-chains. (Allan et al., 1997). Initially, crustacean shells were the primary source of chitosan, but their year-round availability, high extraction costs, and environmental and other factors led to the search for alternative sources, particularly basidiomycetes, which produce chitin and chitosan within their cell walls (Najm et al., 2020). Furthermore, these fungi can be cultivated under easily controlled conditions and contain a high proportion of soft chitin that can be converted into highly purified chitosan (Birk et al., 2017).

Chitosan is extracted from basidiomycetes through a series of steps that include the removal of proteins, minerals, and lipids, followed by chemical or enzymatic deacetylation to convert the chitin to chitosan. Fungal chitosan is characterized by its lower molecular weight, chemical purity, solubility in acidic solutions, and lack of allergenic components, compared to chitosan extracted from crustaceans (Chopra and Rohi, 2016). Studies have also shown that fungal chitosan possesses excellent inhibitory activity against a wide range of pathogenic microorganisms, including Staphylococcus aureus, Escherichia coli, and Candida albicans. Therefore, this study aimed to analyze the physicochemical properties of chitosan extracted from the oyster mushroom (Pleurotus osturatus) and its effect on inhibiting certain food spoilage organisms. This is crucial for contributing to the development of sustainable and environmentally friendly alternative sources that can be used in diverse applications, such as in the medical, food, and environmental fields, as a natural antimicrobial agent that is safe for humans and the environment (Kaur and Dhillon, 2014).

III. Materials and Methods:

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Sample Preparation and Source of Chitosan:

The fruiting bodies of the fungus Pleurotus osturatus were obtained from the Plant Protection Department - Organic Agriculture Section, located in Al-Saleh, Baghdad, Iraq. The fruiting bodies were prepared according to the method of Kalbarczy et al. (2008). They were washed with distilled water, sliced, and then dried in a convection oven at 50–60°C. The slices were then ground in a laboratory grinder, and the original and dried weights were recorded. The samples were stored in opaque polyethylene bags.

Demineralization:

According to the method of Rodde (2008), the powdered fruiting bodies were placed in a 4% (v/v) HCl solution at a ratio of 15:1 w/v and left at room temperature for 20–24 hours. The precipitate was then washed with distilled water (pH ≈ 7).

Protein Removal:

According to the method of AOCA (1990), the product from the demineralization stage was treated with a 5% (v/w) sodium hydroxide (NaOH) solution at a ratio of 1:12. V/W for 22-24 hours, washing the precipitate several times until the pH reached 7. The precipitate was then dried for 20 hours.

Beta-glucan removal: After protein and mineral removal, the resulting material was washed several times with distilled water until a pH of approximately 7 was reached. The sample was placed in a 10:1 (v/w) potassium hydroxide (KOH) solution. It was heated to approximately 90°C for 1–2 hours with continuous stirring to facilitate beta-glucan hydrolysis until a yellowish-white precipitate formed. The precipitate was separated by centrifugation at 6000 × g for 5–10 minutes. The resulting precipitate was washed several times with distilled water. Finally, the precipitate was washed with 1% dilute hydrochloric acid to remove any remaining potassium salts. It was then washed several times with distilled water to achieve a pH of 7. The extraction process was repeated using a 10:1 (w/w) 3.5% potassium hydroxide solution to obtain purer chitin. The precipitate was dried at 50–60°C to yield chitin (Alimi et al., 2023).

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Preparation of Chitosan from Chitin Extracted from the Fruiting Bodies of Oyster Mushroom (Pleurotus osturatus):

Chitosan was prepared according to the method of Novikov et al. (2023) by removing the acetyl group from chitin using an alkaline treatment with a 40% (w/w) sodium hydroxide solution at a ratio of 10:1 (w/v). The mixture was heated in an oil bath at 90°C with continuous magnetic stirring for 1–2 hours. The precipitate was washed several times with distilled water until a pH of approximately 7 was achieved. The product was oven-dried at 40–50°C and stored in airtight containers until the following physicochemical analyses were performed:

Estimation of Percentage Yield:

The yield was estimated (Mohanasrinivasan et al., 2014) using the following equation:

% Yield = (weight of chitosan produced) / (weight of mushroom powder before treatment) × 100

2- Estimation of Moisture, Ash, and Protein:

Moisture, ash, and protein were determined according to the standard method (AOAC, 1990).

3- Estimation of the Degree of Acetyl Group Removal:

This was determined according to the method of Abdul-Karim et al. (2017) using Fourier Transform Infrared Spectroscopy (FTIR). Discs were prepared from chitosan samples (obtained from section 5 of the Procedures) by mixing approximately 2 g of chitosan with 100 g of potassium bromide, pressing them into a transparent disc, and measuring within the range of 400-4000 cm^{-1}. The results were analyzed using an FTIR-8400 spectrometer, according to the following equation:

$$DD\% = 100-[(A1655/A3450) \times 100/ 1.33$$

Where:

A1655 = Absorption intensity at wavelength 1655 cm^{-1}

A3450 = Absorption intensity at 3450 cm^{-1}

1.33 = Empirical constant for chitin

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4- Estimation of the viscosity of chitosan produced from the fungus:

A suitable quantity of chitosan (1% w/v) was weighed and dissolved in a 1% (v/v) acetic acid solution with continuous magnetic stirring for several hours until a homogeneous solution free of lumps was obtained. It was then left to stand briefly to remove air bubbles before measurement. The viscosity was measured using a Brookfield viscometer at approximately 25°C with three replicates. The viscosity values were expressed in centipoise (cP) according to Irzoqy (2024) and the following equation:

Relative viscosity (ŋ) = (Chitosan solution viscosity) / (Acetic solution viscosity)

5- Estimation of Molecular Weight:

The molecular weight of the resulting chitosan was estimated according to the method of Kasaai et al. (2000), based on viscosity measurements and using the MHS equation (Mark–Houwink–Sakurada), and relying on the measured viscosity values obtained from section (4) and the equation's constants K and a.

MHS= $\eta = k\,[MW]^{a}$

Where: 1.49×10^{-4}

a = 0.79

ŋ = viscosity in cP units

MW = molecular weight (Daltons)

6- Solubility Assessment: The solubility of the resulting chitosan was assessed according to the method of Ali et al. (2017) by placing 0.1 g of chitosan in a centrifuge tube of known weight after adding 10 mL of 1% (v/v) acetic acid solution. The tube was then placed in a shaking incubator at 240 rpm at 25°C for 30 minutes. Following this, the tube was placed in a boiling water bath for 10 minutes, and the sample was cooled to 25°C. It was then centrifuged at 10,000 rpm for 10 minutes, and the precipitate was separated. The insoluble particles were washed with distilled water and centrifuged at the same speed. The precipitate was separated again, and the insoluble particles were

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dried at 60°C for approximately 22 hours. The solubility ratio was calculated using the following equation:

100 × (B - A) / (C - A) = Solubility Ratio

Where:

A = Weight of the tube and chitosan before treatment

B = Weight of the tube and chitosan after treatment

C = Weight of the tube

7- Thermogravimetric Analysis

Thermogravimetric analysis was performed on the chitosan sample prepared from the fruiting bodies of Pleurotus osturatus, according to the method used in the study by Hosseini (2018), using a thermogravimetric analyzer (TGA). A 10 mg dry sample was placed in an open platinum container under a nitrogen atmosphere flowing at a rate of 20 mL/min, within a temperature range of 25–800 °C, at a heating rate of 10 °C/min.

Thermogravimetric analysis was performed on the chitosan sample prepared from the fruiting bodies of Pleurotus osturatus, according to the method used in the study by Husseini (2018), using a thermogravimetric analyzer (TGA). 8- Studying the effect of chitosan extracted from oyster mushroom (Pleurotus osturatus) on inhibiting some food spoilage organisms:

A- Preparation of Chitosan Solutions

Chitosan solutions were prepared according to the method of Rabia et al. (2003) by dissolving the chitosan powder prepared from the fruiting bodies of oyster mushroom (Pleurotus osturatus). The mushroom fruiting bodies were incubated in a 1% v/v acetic acid solution with continuous stirring for 12–24 hours at approximately 25°C. Chitosan solutions were then prepared at concentrations of 0.25%, 0.5%, and 1% w/v, as shown in the following table: Table 1.

Table 1 represents the prepared chitosan solutions.

Quantity of chitosan/g	Volume of 1% acetic acid	Concentration of chitosan w/v

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	solution/ml	
0.25	100	%0.25
0.5	100	%0.5
1.00	100	%1.0

The pH of the solutions was adjusted to 5.6–6.0. The solutions were then sterilized by membrane filtration (0.45 µm).

b. Bacterial cultures of the microorganisms used in the study (Staphylococcus aureus, Bacillus subtilis, Listeria monocytogenes, Escherichia coli, Salmonella typhimurium, and Pseudomonas aeruginosa) were prepared and grown in Nutrient Broth for 18–24 hours at 37°C. The culture density was adjusted to conform to the McFarland standard of 0.5 CFU/mL (≈10^8).

-c Agar plates were prepared. Sterile Nutrient Agar medium was poured into laboratory plates and allowed to freeze. Bacterial cultures were then spread evenly on the surface of the medium using sterile cotton swabs. Circular holes, 6 mm in diameter, were made in the agar medium using a sterile cork drill. These holes were filled with chitosan solution (50–100 µL) in each hole. Acetic acid solution (1%) was used as a negative control, and a standard antibiotic was used as a positive control. The plates were incubated at 37°C for 24 hours. The diameter of the inhibition zone (mm) and around each hole were measured using a fine ruler. The experiment was performed in triplicate, with a broad-spectrum standard antibiotic (Ciprofloxacin, 5 µg) used as a control against all studied microorganisms. (CLSI, (2022) Results were recorded as mean ± standard deviation.

Results and Discussion:

1- Results of the percentage estimation of chitosan yield from the oyster fungi Pleurotus ostreatus:

The results of the percentage estimation of chitosan yield from the oyster fungi Pleurotus ostreatus showed that the chitosan yield obtained was approximately 6.5%. This percentage is relatively high compared to the results obtained in some studies that used different extraction and estimation methods. Gunathilake et al. (2019) found that the percentage of chitosan from the oyster fungi reached 1.22% of the dry weight of the material, which is much lower than the percentage obtained

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in this study. This can be explained by the difference in the extraction method, especially the methods used to remove protein and minerals, which affects the efficiency of isolation and the preservation of larger quantities of chitosan polymer. Other studies indicated that using improved processing methods can significantly contribute to increasing the chitosan yield from the oyster fungi, reaching approximately 4.45% of the dry weight, compared to 2.24% when using classical extraction methods (Couto et al., 2018). The similarity between the results of the current study and some results obtained from studies such as Ssekatawa et al. (2021) is due to the fact that Pleurotus ostreatus fungal cells possess cell walls rich in glycosidic chains such as chitin, which can be converted to chitosan through chemical treatments. The results of studies showing a much lower yield, such as ~1.2%, can be explained by the fact that these studies may have relied on less efficient deacetylation methods, or that the original raw material had a low chitin content, or that the source of the obtained fungus differed, all of which affect the final yield. In addition, the protein content, the demineralization and glycan removal processes, and the relatively high heat treatment time and temperature are all key factors that can contribute to a decrease in the final yield, as they directly affect the efficiency of removing non-chitosan components from the raw materials. For example, using higher concentrations of sodium hydroxide for longer periods... Long, increases the deacetylation coefficient but causes polymer degradation and reduces the yield, especially if the acetylation condition is very harsh (Salman and Zedain, 2018).

2- Results of Moisture, Ash, Protein, and Acetyl Group Removal Estimation:

The moisture content of chitosan extracted from oyster mushroom (Pleurotus ostreatus) was within acceptable limits for chitosan compared to its commercial counterpart, at 6.79%. This result aligns with the requirements for commercial chitosan production, which stipulate a moisture content of less than 10% (Novikov, 2023).

The ash content of the resulting chitosan was remarkably low (0.09%). This low result indicates the effectiveness of the demineralization process, one of the steps in chitosan preparation. This finding is consistent with those of Bhindal et al. (2025), who confirmed an ash content of less than 1%. The low moisture content of the chitosan indicates the effectiveness of the drying steps, which is crucial for preserving the physical properties of chitosan and limiting microbial activity. Moisture control is important for its role in storage stability and subsequent medical, food, and industrial applications

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(Hosseini et al., 2018). The low ash content reflects the effective removal of unwanted minerals, thus improving product purity.

The percentage of residual protein in the chitosan was also low (1.09%), indicating the effectiveness of the removal process. A low protein content in the resulting chitosan (1–2%) suggests higher purity and quality, as chitosan is a saccharide polymer. A protein content exceeding 2% affects its solubility and chemical properties (Da Silva, 2021).

Table (2) Chemical Composition of Chitosan Prepared from the Fungus Pleurotus ostreatus

Chemical composition of chitosan prepared from the fungus Pleurotus ostreatus %			
Yield	**Moisture**	**Ash**	**Protein**
6.5	**6.79**	**0.09**	**1.9**

Novikov et al. (2023) confirmed that the hydrolysis and removal of protein groups attached to fungal cell walls resulted from alkaline treatment, which also contributed to a high degree of acetyl group removal, reaching 79%. This resulted in a low protein content in the resulting chitosan, encouraging its use in biological and food applications. Agarwal et al. (2018) indicated that the degree of acetyl group removal is directly related to the degree of purity and varies depending on the chitosan source and preparation method. Foster et al. (2015) reported an average acetyl group removal rate of 80%.

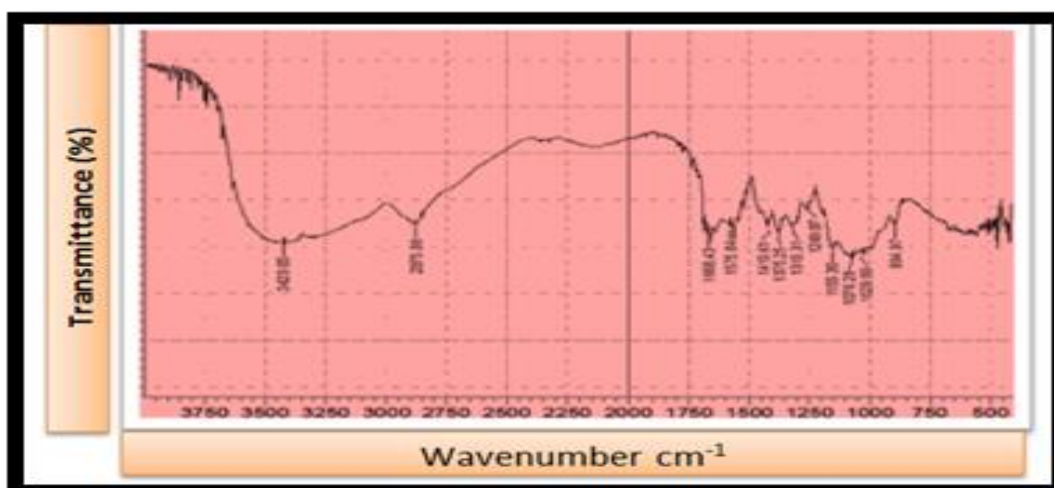

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Figure 1 shows the Fourier transform infrared (FTIR) spectrum of the prepared chitosan.

3- Results of viscosity and molecular weight determination of chitosan prepared from oyster mushroom (Pleurotus ostreatus): The results showed that the viscosity of the chitosan prepared from oyster mushroom was 5.9 centipoise. This result is consistent with the findings of several studies, including the study by Ekinipumeh and Omogbay (2013), which recorded viscosities of chitosan extracted from different fungal species ranging from 3.6 to 7.2 centipoise. In another study by Almasari (2019), a viscosity of 6.7 centipoise was obtained for chitosan prepared from the fruiting bodies and stems of the mushroom (Agaricus bisporus). Bellis et al. (2022) confirmed that chitosan viscosity varies depending on the source and preparation method.

The molecular weight of chitosan extracted from oyster mushroom (Pleurotus ostreatus) was 329 kDa. This low molecular weight reflects the low viscosity of the chitosan extracted in this study. Gonsalves et al. (2021) confirmed a direct relationship between chitosan viscosity and molecular weight, attributing this to the longer polymer chains and increased dispersibility in the medium. Chitosan extracted from crustaceans also exhibited higher viscosity than that extracted from basidiomycetes. These results are largely consistent with those of Sikatawa et al. (2021), who estimated the molecular weight of chitosan prepared from Ugandan mushrooms to be approximately 348 kDa. Mino et al. (2023) determined the molecular weight of chitosan prepared from oyster mushrooms to be 320.7 kDa. The molecular weight of commercially available chitosan was 540 kDa. This slight variation in chitosan values may be attributed to the chitosan source and the separation technique. The molecular weight of the polymer may decrease as a result of the deacetylation process. This explains the lower molecular weight of chitosan extracted from basidiomycetes compared to its counterpart extracted from adult shrimp (Gonsalves et al., 2021).

4. Results of the Solubility Estimation of Chitosan Extracted from Oyster Mushroom (Pleurotus ostreatus):

The solubility of chitosan extracted from animal or fungal sources is a crucial factor in determining its quality. The higher the solubility value, the purer the final product (Bonilla et al., 2019).

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Chitosan has been found to have high solubility in reducing acids such as acetic acid, a characteristic feature of chitosan with a relatively high degree of deacetylation. The solubility of chitosan extracted from oyster mushroom reached approximately 73%, a result that falls within the range of good solubility values for fungal chitosan (30–99%) (Islam et al., 2023). The results of this study are consistent with those of several other studies, such as Al-Kindari et al. (2018). These results are also similar to those of Bhandral et al. In a 2025 study, the solubility of chitosan extracted from the fungus Agaricus bisporus ranged from approximately 75.98% to 75.98%. Bellis et al. (2022) confirmed that several factors influence chitosan solubility, including those affected by the deionization process of chitin, such as temperature, heating duration, and the concentration of the base used. The variation in chitosan solubility is also attributed to the degree of deionization and the optimal pH. Zapata et al. (2023) and Kumari et al. (2016) explained that when chitosan hydrolyzes in acetic acid, it acquires a positive charge by gaining a positive proton (H^+) from the acidic medium, thus becoming NH_3^+. Figure 2 shows the results of thermogravimetric analysis of chitosan extracted from oyster mushroom (Pleurotus ostreatus). The thermogravimetric analysis (TGA) results for both oyster mushroom and commercial chitosan indicate a decrease in chitosan weight with increasing temperature. The figure illustrates this weight loss in three distinct stages. The first stage shows a 14.95% weight loss for both species at 10 and 150 °C. This slight weight loss is likely attributed to the evaporation of free water and volatile components in the chitosan. The second stage begins when the temperature reaches approximately 150 °C and extends to 350 °C, where a further 49.10% weight loss is observed for both species. Initial degradation occurs at approximately 350 °C, followed by a third stage involving a significant 38.03% weight loss for both species within the 350–450 °C temperature range. This weight reduction is most likely attributed to the breakdown of sugars within the molecular structure of the organic matter, in addition to the complete decomposition of the organic material. The thermogravimetric analysis curve also shows a clear weight reduction for both types, indicating the thermal decomposition of the chitosan structure. After this decomposition process, a mass consisting of ash or non-volatile components remains, unaffected by the heating process. This conclusion is supported by the residual weight percentage of 0.1563%, indicating a loss of organic matter at temperatures above 500 °C. It also suggests that the chitosan does not contain any minerals or inorganic materials. The curve in Figure 2 illustrates the stability of the mushroom-derived chitosan at different temperatures and its decomposition strategy. The first stage occurred at a temperature below 170 °C, while the second

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and third stages occurred at temperatures ranging from 201–500 °C and 500–800 °C, respectively. Mass loss for both types during the first stage was 12.9% and 11.4%, respectively, for the fungal and commercial chitosan samples, attributed to moisture or water evaporation. In the second stage, mass loss reached 55.4% and 54.9%, respectively, for the two samples, attributed to chitosan degradation. The remaining mass, consisting mostly of inorganic material, decreased during the third stage to 18.01% and 20.2% for the two samples, respectively. These results are consistent with those of Lianag et al. (2022) on chitosan extracted from white shrimp (Litopenaeus vannamei), which exhibited thermal stability up to 360°C. They also showed that the mass loss of chitosan extracted from shrimp shells occurred in three stages.

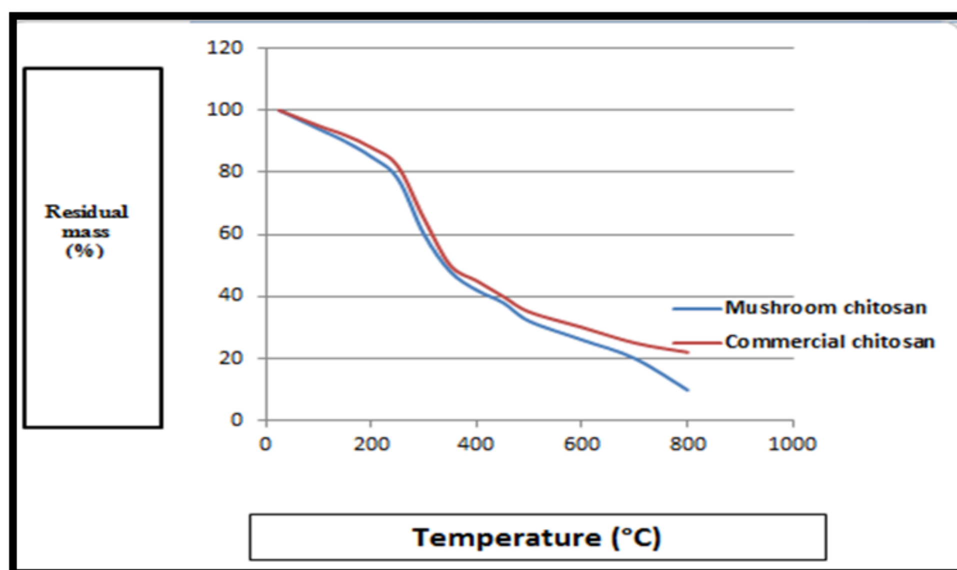

Figure (2) illustrates the thermogravimetric analysis (TGA) of chitosan prepared from the fungus Pleurotus ostreatus and commercial chitosan.

6- Results of the effect of chitosan prepared from the fungus Pleurotus ostreatus on inhibiting some food spoilage organisms:

To evaluate the effect of chitosan prepared from the fungus Pleurotus ostreatus against the food spoilage organisms Staphylococcus aureus, Bacillus subtilis, Listeria monocytogenes, Escherichia coli, Salmonella typhimurium, and Pseudomonas aeruginosa, using different concentrations of the

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chitosan solution, it is observed from Table (3) that the antibacterial activity of the chitosan solutions extracted from the oyster fungus Pleurotus ostreatus depends entirely on the concentration of the solution and the type of bacteria studied. A concentration of 0.25% showed no inhibitory activity against any of the bacterial species studied, indicating that this concentration may be insufficient to induce an active interaction between the positively charged amine groups in chitosan and the negatively charged bacterial cell surface. In contrast, a concentration of 0.50% was moderately effective against bacteria, with the inhibition zones being more pronounced in Gram-positive bacteria, particularly Staphylococcus aureus and Bacillus subtilis, compared to Gram-negative bacteria (Escherichia coli and Salmonella typhimurium) This difference in inhibition efficacy is likely attributed to the structural composition of bacterial cell walls. The presence of a peptidoglycan layer in the cell walls of Gram-positive bacteria facilitates chitosan binding, while the thick peptidoglycan layer of the outer membrane, rich in polysaccharides, acts as a barrier that reduces chitosan permeability (Hillander et al., 2001).

The largest areas of inhibition were observed at a 1% concentration, which showed the highest efficacy against all studied bacteria, with a clear advantage against Staphylococcus aureus, followed by Bacillus subtilis and then Listeria monocytogenes. These results are consistent with those of Shahidi et al. (1999) and Rabia et al. (2003), who demonstrated that increasing the concentration of chitosan solution enhances the positive charge density of the amine groups, leading to impaired cell membrane permeability and leakage of proteins and vital cellular components, ultimately resulting in bacterial cell death.

Studies have shown that increasing the concentration of chitosan solution enhances the positive charge density of amine groups, leading to impaired cell membrane permeability and leakage of proteins and vital cellular components, ultimately resulting in bacterial cell death. These findings support the potential use of chitosan solutions derived from the oyster mushroom (Pleurotus ostreatus) as a natural antibacterial agent in food preservation, particularly for controlling foodborne pathogens.

Table 3: Results of the effect of chitosan prepared from the fungus Pleurotus ostreatus on inhibiting some food spoilage organisms.

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Chitosan solution concentration	**Diameter of the inhibition zone (mm)**					
	Microorganisms that cause food spoilage					
	Escherichia coli	*Salmonella typhimurium*	*Pseudomonas aeruginosa*	*Listeria monocytogenes*	*Bacillus subtilis*	*staphylococcus aureus*
0.25	--	--	--	--	--	--
0.5	9	8	6.5	10	11	12
1.00	14	12	10	16	17	19

The symbol – indicates the absence of an inhibition zone.

7- Results of the effect of the negative control (1% acetic acid) and the positive control (Ciprofloxacin 5 µg) on the studied bacteria:

The data shown in Table (4) indicates that acetic acid at a concentration of 1%, used as a negative control, did not produce any inhibition zones with any of the studied bacteria, as the diameters of all inhibition zones were zero millimeters. This confirms that the solvent used has no effect on the bacteria at a concentration of 1%, which supports its use as a negative control in the bioavailability tests of chitosan extracted from the fungus Pleurotus osturatus. It also confirms that any observed inhibitory activity is due to the active ingredient, chitosan, and not the solvent.

On the other hand, a high and clear efficacy was observed against all studied bacterial species, resulting from the use of Ciprofloxacin (5 µg) as a positive control. The largest inhibition diameters were recorded for Gram-positive bacteria, particularly Bacillus subtilis and Listeria monocytogenes. This is attributed to the simple chemical structure of the cell wall in Gram-positive bacteria; they lack the outer membrane found in Gram-negative bacteria. This provides protection for Gram-negative bacteria and facilitates the penetration of the antibiotic into the Gram-positive bacteria. The results in Table (3) also showed that Gram-negative bacteria, such as Pseudomonas aeruginosa,

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exhibited greater resistance and relatively smaller inhibition zone diameters than their Gram-positive counterparts. This can be explained by their outer membrane being rich in lipopolysaccharides (LPS), which act as an additional barrier reducing antibiotic permeability, in addition to possessing efficient efflux pumps. These results are consistent with several studies indicating the high efficacy of Ciprofloxacin as a broad-spectrum antibiotic. It can be used as a positive control in antibacterial activity tests, especially when evaluating biomaterials such as chitosan or its derivatives. (Kong et al., 2010)

Table No. (4) Effect of the negative control (1% acetic acid) and the positive control (Ciprofloxacin 5 µg) on the studied bacteria

Bacteria	Gram staining type	Diameter of inhibition zone (mm) – Acetic acid 1%	Diameter of inhibition zone (mm) – Ciprofloxacin (5 µg)
Staphylococcus aureus	Positive	**0.0**	**32**
Bacillus subtilis	Positive	**0.0**	**35**
Listeria monocytogenes	Positive	**0.0**	**33**
Pseudomonas aeruginosa	Negative	**0.0**	**25**
Salmonella typhimurium	Negative	**0.0**	**27**
Escherichia coli	Negative	**0.0**	**29**

Values represent mean ± standard deviation (n = 3).

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Ciprofloxacin is a fluoroquinolone antibiotic that works by inhibiting DNA gyrase and topoisomerase IV enzymes in bacteria. This prevents DNA replication and bacterial cell proliferation, ultimately leading to cell death. In contrast, chitosan, a naturally occurring organic compound, is positively charged in acidic environments. This enhances the electrostatic interaction with the negatively charged bacterial cell membranes, increasing membrane permeability and leakage of cell contents, thereby reducing intracellular activity. Several studies have demonstrated that chitosan alone possesses antibacterial activity, but a more pronounced effect is typically observed at higher concentrations (Akbarzadeh et al., 2023). In conclusion, based on the findings of the current research and comparative studies, oyster mushroom (Pleurotus osturatus) represents a promising source of bioavailable chitosan, and its production can be improved by modifying the chemical processing stages and extraction conditions. This aligns with the results of previous studies, taking into account the differences in experimental conditions and extraction techniques. This encourages its inclusion in the food and pharmaceutical industries.

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