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ANTIMICROBIAL ACTIVITY OF EDIBLE FILM FROM CHITOSAN ISOLATE INCORPORTED WITH POMEGRANATE PEEL EXTRACT AND ITS USE IN CHICKEN BREASTS COAT IN

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Abstract

The aim of the current research is to study the effect of adding Pomegranate peel to the edible film prepared from the chitosan isolate on the effectiveness of microorganisms and evaluating the of antimicrobial effectiveness of these films on chicken breasts packaging during the Fifteen days of storage. the minimum inhibitory concentration was measured by calculate the diameter of the zone of inhibition on growth of the bacteria and it's included the group of Gram negative bacteria (*Escherichia coli*, *Salmonella spp*, *Pseudomonas aeruginosa*) and the group of Gram positive bacteria (*Staphylococcus Aureus*, *Bacillus spp*) and a yeast (Candida Albican). Where the diameter of the halos formed (16,12,12,20,8,15) respectively. The results of the microbiological tests during the storage period of the two treatments (T_2, T_3) showed a decrease in the total number of bacteria and cold-loving bacteria and colon bacteria by 1-2, 3-2.5, 2–3 logarithmic cycles respectively compared with the control treatment (T_1). Sensory which included phenotypic and gastronomic calendar, superior to chitosan-coated chicken breast supported by pomegranate husk extract in general shape, color, smell and texture compared to chitosan-coated chicken breast and chicken breast without phenotypic casing.

Key words : Edible film, chitosan, pomegranate.

Introduction

Packaging technology has an important role to play in the food processing chain of containment and protection from chemical and microbial contamination from the food environment, physical and biological effects during food production and storage, marketing, transport and distribution until it reaches the consumer without any changes in sensory characteristics that may amount to damage. (Selcuk *et al.,* 2017). Effective food packaging is one of the most innovative technologies in the packaging technology that combines the food and packaging environment and their interaction in order to ensure the preservation of quality and increase the shelf life of the food, in the presence of natural biological materials in natural polymers to ensure the protection of the consumer and the environment by preserving food from pathogenic microorganisms Because of its biodegradability and environmental friendliness, chitosan membranes are ideal because of their properties attributed to the bonds between their molecules along the chain. Chitosan membranes are characterized by their homogeneous, stable structure and mechanical properties. Suitable and biodegradable (Raphaël & Meimandipour, 2017). Poultry meat is one of the most popular meats because it is nutritious and low-fat content, and because chicken meat is perishable and has a relatively short shelf life even when refrigerated, the food industry has moved towards the application of modern preservation techniques to prolong its storage (Ruiz-cruz *et al.*, 2019). The aim of this study was to utilize the residues of foodstuffs such as pomegranate husks and use them in reinforcing prepared membranes to increase their antimicrobial effectiveness and prolong the storage life of chickens.

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Materials and Methods

Chitosan was prepared by Chemsavers / USA. Pomegranate peels collected samples of ripe pomegranate fruits from the local market, washed from the soil with water, and then separated the peels from the rest of the fruit. In an electric mill, after obtaining the dry powder of pomegranate peels, the chemicals mentioned in the

working methods were all intended for chemical analysis.

Preparation of film solution

Film forming solution was prepared according to the method described by (Berizi *et al.,* (2018) with some modifications as follows:

2 g of chitosan was dissolved in 1% of Acetic acid under stirring for 5 minutes. Then the solution was heated to 40°C for 3 h under stirring. Then, the solution left to cool at room temperature. Then Glycerol was added as a plasticizer at the concentration of 1% under stirring for 10 minutes, and then the extract of Pomegranate peels was added to the film solution with a concentration of 3%.Filtration with cloth was performed to avoid any lumps in the solution; pH was adjusted to 7 by NaOH 0.01N. To remove the air bubbles from the film solution the vacuum pump was used for 10 minutes and then the film solution, was stored in the refrigerator in dark conditions to prevent oxidation.

Minimum inhibitory concentration

The method described by (Jose, *et al.,* 2016**)** was used to determine the minimum concentration of inhibition by preparing membranes in tablets of 6 mm diameter containing pomegranate husk extract (75%), disinfecting the tablets using ultraviolet light for 5-1 minutes. side. All the media was prepared in the laboratory according to the instructions of the equipped company, sterilized with the sterilizer at 121 ° C and at a pressure of 121 g / cm² for 15 minutes. The tested organisms were activated by taking Loop from the bacterial farm to the center of Nutrient broth under sterile conditions and incubation at 37 ° C for 24 hours. Cram stain (Staphylococcus aureus, Bacillus cereus (both individually. In the case of Candida albicans), active in PDB medium and incubated at 28 ° C for 24 hours (Al-Delaimy and Ali., 1970). Microbiology farms, which included a group of bacteria negative and positive for the dye Cram in addition to yeast, transfer 1 ml of them to Petri dishes The vaccine size was 1 × 10x colony formation unit / ml and using the Muller Hinton Acar culture medium, the prepared membrane tablets were placed at a rate of 4 tablets per dish and two replicates. The concentrations of pomegranate husk extract were added to the membrane (4,3,2,1)% The dishes were incubated at appropriate temperatures for each type of microorganism and for 24 hours for bacteria and 48 hours for yeast. After incubation, the diameter of the transparent corona formed was measured using a sensitive digital micrometer to the nearest 0.01 mm. The lowest inhibitory concentration is defined as the lowest concentration of antimicrobial agent and inhibitor of its growth.

Method of sampling

In the days of chicken breasts (15,12,9,6,3,1) weighed 10 grams of chicken breast model. Meat samples were placed in a sterile plastic dish and stored at refrigerator temperature. The samples were evaluated for microbiological tests.

Microbiology analytical of chicken breasts

The method described was followed (Moghimi *et al*., 2017). In the microbiological examination of chicken breasts, which included the total number of bacteria, the method of casting dishes using the Nutrient agar food medium and incubated at 37 ° C for 24-48 hours, after the lap period was calculated the number of colonies developed using a colony counter The same method was used to calculate the numbers of bacteria in subsequent experiments, taking into account the use of a control plate for each treatment. Nutrient agar was prepared and incubated at 7 ° C for 7-10 days. Coliform bacteria was estimated using MacConkey agar and dishes were incubated at 37 ° C for 48-24 hours.

Sensory evaluation

Samples of chicken breast were evaluated according to a form (Baker & Darfler, 1981) with some modifications. Sensory quality tests were carried out for the packaged and unpacked samples during the storage period of the refrigerator for days (15,12,9,6,3,1) and included (general shape, color, smell, textures). Statistical analysis

Results and Discussion

Minimum inhibitory concentration

The efficacy of chitosan membranes supported by pomegranate peel extract at concentrations of (4,3,2,1) %, was tested as antimicrobials towards some of Cram-positive and Cram-negative bacteria in addition to yeast, in light of recording the diameters of growth-free areas (damping halos) surrounding the tablets. The results are as shown in (Table 1). The addition of pomegranate peel extract at a concentration of 3% is the lowest inhibitory concentration for the growth of all microorganisms tested. Qatar reached the aura of inhibition of bacterial growth (*Escherichia coli, Salmonella typhimurium, Staphylococcus aureus, Bacillus cereus, Pseudomonas aeuroginosa*) 12,20,8,12,16 Mm respectively, The yeast (Candida albicans (The diameter of the transparent corona was 15 mm, and when the extract was used at a concentration of 4%, its inhibitory effect increased. It has an inhibitory effect on the studied organisms except bacteria *E. coli* And bacteria *Bacillus cereus* The diameters of auras (13 and 18) mm were

successive, and when using the extract at a concentration of 1%, the aura of inhibition appeared only on bacteria *Bacillus cereus* The diameter was 13 mm). Numerous studies have shown that pomegranate peel extracts show antimicrobial activity, which can be attributed to its high content of multiple phenolic compounds, which include (punicalagin A, punicalagin B, gallic acid, ellagic acid, chlorogenic acid, caffeic acid, catchin, epicatechin, rutin, quercetin) It has antioxidant and antimicrobial effects (Akhtar *et al*., 2015**)**.

Table 1: Effect of adding different concentrations of pomegranate peel extract to chitosan membranes on the inhibition of a number of microorganisms.

Type of bacteria	Transparent halo diameter (Mm)				
	Control	Concentration of pomegranate peel extract			
		1 %	2 %	3 %	4 %
Salmonella typhimurium	N.Z	N.Z	N.Z	12	14
Escherichia coli	N.Z	N.Z	13	16	18
Pseudomonas aeuroginosa	N.Z	N.Z	N.Z	12	16
Bacillus cereus	N.Z	13	18	20	22
Staphylococcus aureus	N.Z	N.Z	N.Z	8	14
Candida albicans	N.Z	N.Z	13	15	17

Microbial result

Total count

According to the total number of bacteria of chicken breast slices samples represented by the control model (T_1) not coated, (T_2) the sample coated with chitosan membrane only, (T_3) sample coated with chitosan film + pomegranate peel extract and stored in the refrigerator for a period of 15 days. The results of (Fig. 2) show that the bacterial numbers of the three samples (T_3, T_2, T_1) were 3.8 log cuf/g on day 1, after 9 days of refrigerated storage, the chitosan membranes supported by pomegranate husk treatment (T_3) maintained Acceptable microbial load of 5.3 log cuf / g, and treatments (T_2, T_1) reached (7.7,8.7) log cuf / gm respectively, and at the end of the conservation period the bacterial numbers exceeded the limits allowed for the sample coated with chitosan film + pomegranate crust extract (T_3). The apparent rise in the total bacterial count of T1 may be due to the fact that chicken breasts are not wrapped in chitosan membrane solution and exposed to oxygen directly, thus increasing the number of aerobic bacteria that cause meat

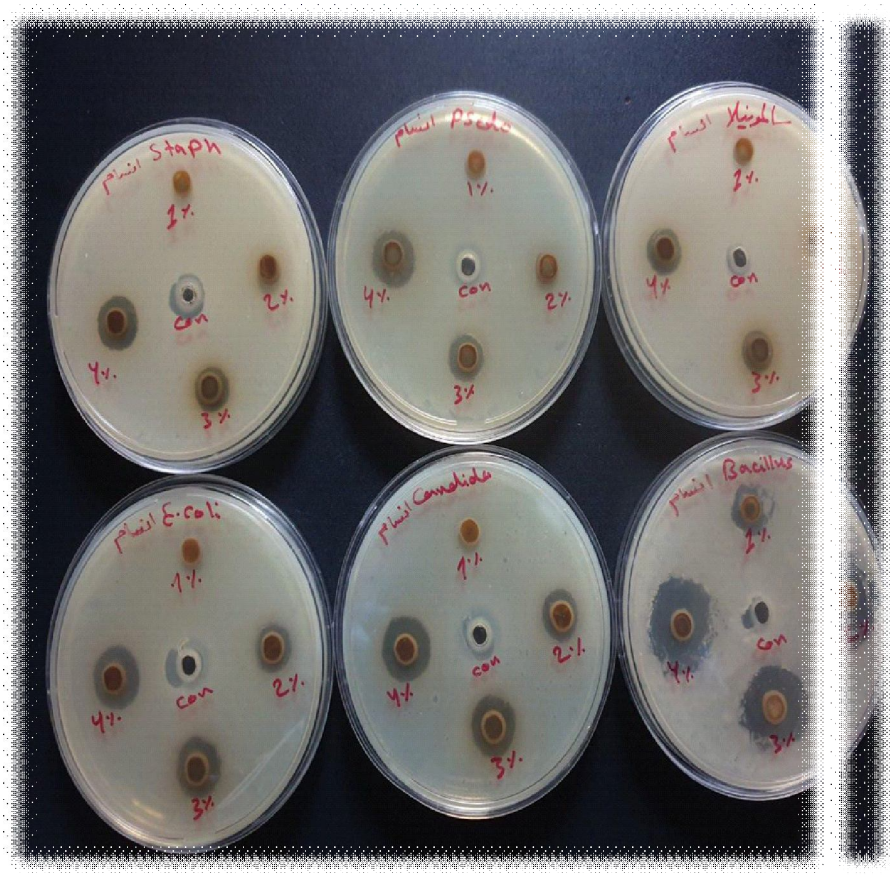

Fig. 1: Inhibiting the growth of different microorganisms with chitosan membranes containing different concentrations of pomegranate peel extract.

spoilage. As for the treatment (T_2) represented by the chicken breasts coated with chitosan membrane unit, it was able to preserve the meat until the sixth day compared to the control treatment is not encapsulated, the bacterial number of 5.8 log cuf / g. These results are consistent with the results of (Shahbazi & Shavisi, 2017) when using chitosan membrane supported with cinnamon extract at a concentration of 1% to maintain the chicken hamburger at 4 ° C, the number of bacteria at the beginning of the storage period 4.39 log cuf / g and after 10 days the number of bacteria For the control model and for the chitosan-treated membrane, the chitosan-treated membrane and cinnamon extract (8.99, 7.2 and 5.36) and cuf / gm respectively. The reason is due to the overlapping action of the nature of membranes with good oxygen retention properties (Vieira *et al.,* 2011; Chiumarelli & Hubinger, 2014), which inhibit aerobic bacteria by reducing the entry of oxygen, which is essential for the growth of microorganisms.

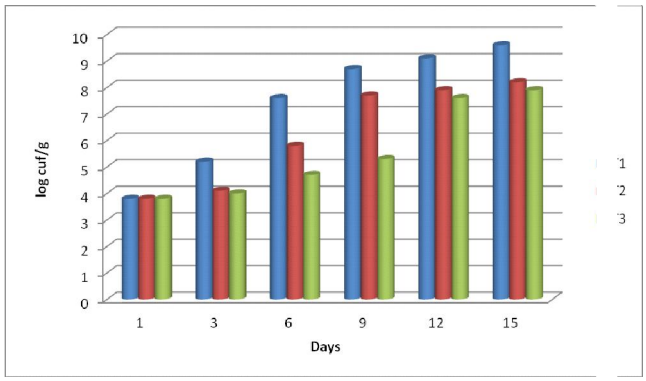

Fig. 2: Total number of bacteria for chicken breast treatments during cold storage.

Number of Sychrotrophic bacteria

Fig. (3) shows the effect of the three coefficients (T_3, T_2, T_1) on the number of cold-tolerant bacteria for chicken breasts stored at 4°C. T_2) compared with the control treatment (T1) which exceeded the allowed limits as it was 10.20 log cuf/ gm. The inhibitory action of chitosan may be due to the presence of a positive charge on the NH*f* z group on the side of the kleososin molecules that interact with large, negatively charged molecules on the surface of the microbial cell, leading to the leakage of components from within the cells of microorganisms. chitosan is associated with the inactivation of polysaccharides of the outer layer of Cram-negative bacteria as the main group of microorganisms responsible for spoilage of meat stored in the refrigerator (Pereda *et al.,* 2011). (Alsaggaf *et al.,* 2017) also attributed the antimicrobial activity of pomegranate peel extract to the sensitivity of phospholipids in cell membranes, curbing microbial enzyme systems and increasing cell permeability

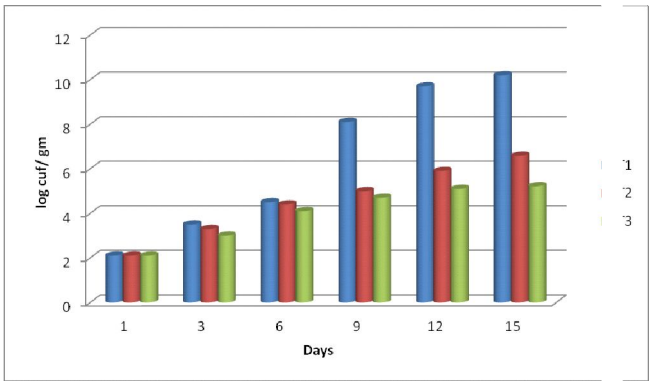

Fig. 3: Preparation of cold-loving bacteria for chicken breast treatments during cold storage.

or excessive leakage of cellular components.

Number of coliform

The results of Fig. (4) show a decrease in the number of colon bacteria for samples (T_3, T_2) compared with the control treatment (T_1) as the numbers at the end of the shelf life reached 5.5 log cuf/ gm of the sample chicken meat coated with chitosan membrane supported by extract (T_3) compared with The control treatment (T_1) and the chitosan-coated chicken meat sample (T_2) increased at the end of the shelf life to 6. log cuf/ gm for treatment (T_2) and 6.8 log cuf/ gm for treatment (T_1). (Yuan *et al.,* 2015) stated that the chitosan membrane was effective in reducing the number of negative bacteria of the Cram stain because the outer membrane is composed of lipopolysaccharide (LPS) and bivalent positive ions (Ca2 + and Mg2 +) in the outer membrane play an important role Stabilization of basic anionic negative charges in polysaccharides. It can be assumed that chitosan replaces the divalent positive ions at their binding sites on the cell wall of the bacterium and thus interferes between the positive charges of chitosan and the negative charges on the cell wall of the Cram negative dye, causing the membrane to disintegrate or dissolve and the cellular contents of the bacterium to die.

Sensory evaluation

The sensory qualities of edible food are the main key to accepting the food product definitively, and it is one of the factors that contribute to the perception of the consumer of the quality of food such as flavor, appearance and textures. During handling and storage (Weber, 2001).

(Table 2) shows the sensory characteristics of chicken meat samples during different storage period after (15,12,9,6,3,1) days of preservation. On the first day, all samples had the same scores, but the scores began to

	Storage in days	Overall shape 5 degrees	Virtual color 5 degrees	Odor5 degrees	Textures5 degrees	The final grade
T_1	1	5	5	4	5	19
	3	4	4	3	4	15
	6	2	3	2	4	11
	9	2	1	1	2	6
	12	1	1	1	1	4
	15	1	1	1	1	4
T_2	1	5	5	4	5	19
	3	4	5	3	3	15
	6	3	4	2	3	12
	9	2	3	2	2	9
	12	2	2	1	2	7
	15	1	1	1	1	4
T_3	1	5	5	4	5	19
	3	4	5	4	4	17
	6	4	4	3	4	15
	9	3	4	3	3	13
	12	1	2	1	3	7
	15	1	1	1	1	4

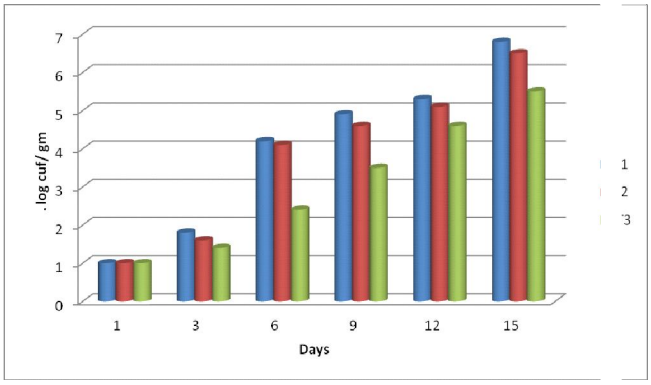

Fig. 4: Logarithm of the number of colon bacteria for chicken breast treatments during storage.

decrease with increasing storage time, but the decrease was slower for the T_3 sample compared to T_1 and T_2. In comparison with This may be due to the acceptance by the arbitrators of the chicken breast coated with extracted chitosan membrane more than the chitosan-coated specimen alone and the non-coated specimen.

Conclusions

1. The possibility of using chitosan for the preparation of edible membranes and use in food packaging, especially as these substances are biodegradable that cause cleanliness of the environment and can be a substitute for plastics in food preservation.
2. Utilization of pomegranate peel residues by extracting and strengthening the chitosan membranes at the minimum inhibition concentrations, which proved its antioxidant effectiveness, which makes it eligible for packaging for conservation purposes.

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