Preparation of bio-edible casings from carrageenan enriched with aqueous extract of black pepper

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Abstract:

Researchers focus on creating environmentally friendly packaging materials from natural sources due to the extensive use of petroleum-based polymers and their reputation for posing serious environmental problems and harmful to human health because they are not biodegradable. Films prepared from natural and edible biopolymers have good mechanical and buffer propertie but to a lesser extent than films made from synthetic polymers. In order to increase the availability and competitiveness of biodegradable films used in food packaging, several studies have been conducted, By adding plant extracts that have antimicrobial and anti-oxidant properties. In addition, the majority of plant extracts improve mechanical properties by increasing polymer thickness and tensile strength Reduce water vapor permeability and oxygen permeability, improve water moisture and solubility. In this study, simple and composite edible films were prepared using carrageenan as the base material at a concentration of 2% and glycerol at 40% by the weight of carrageenan (w/w) as a plasticizer (simple film). Then aqueous black pepper extract at a concentration of 0.5 mg/ml was added as a support to the substrate to create a composite film. The active compound piperine was diagnosed in the aqueous and alcoholic extract of black pepper, and the amount of the active compound was higher in the aqueous extract, which amounted to (1524.8)ppm compared to the alcoholic extract, which amounted to (958.9)ppm. When examining the ability of the aqueous and alcoholic extract to inhibit the growth of microorganisms, The aqueous extract has a halo of larger diameter than the alcoholic extract, The diameter of the corona formed around the aqueous extract against bacteria (Staphylococcus aureus) is 16mm in diameter, While the diameter of the inhibitory areola around the alcoholic extract is 8mm, The corona formed around the aqueous extract against (Escherichia coli) is 16 mm in diameter, While the diameter of the inhibitory corona around the alcoholic extract is 13mm and (Bacillus cereus) it was 10mm for the aqueous extract, While the alcohol was 8mm, and finally the diameter of the corona of bacteria (Salmonella spp) was 12mm for the aqueous extract, the alcoholic extract amounted to 8mm. The results showed that the presence of the aqueous extract of black pepper as a reinforcing material enhanced the mechanical properties as the tensile strength increased by 38.10 MPa compared to the simple membrane reaching 29.98 MPa with a decrease in elongation by 23.6% compared to the simple membrane, the elongation percentage was 32.2% and therefore the results of the properties mechanical opposite. As for the physical properties, The water vapor permeability decreased when the aqueous extract of black pepper was added by 2.39 (g.mm/cm²Pa.day) compared to the simple membrane where it was 2.85 (g.mm/ cm²Pa.day) and it has a direct relationship with the solubility assay. The results of the composite films showed asolubility of 15.8% compare d to the simple films that were 20.4%.

*The research is extracted from a master's thesis of the first researcher. casings,

Keywords:

films,

carrageenan

, black pepper, aqueous extract.

1. Introduction:

Natural product development is seeing increased interest and effort due to a rising consumer demand for safe food goods and environmental disturbance brought on by plastic packaging waste (1, 4, 8, 22). Natural polymers are a potential source for the creation of edible materials due to their various benefits, which include quick biodegradability, biocompatibility, regeneration, and the capacity to serve as ideal components for bioactive building blocks(5). Food preservation throughout the entire distribution chain depends on food packaging. The main objectives of packing materials are to prevent food from being contaminated and to extend shelf life (20). This type of packaging's major objective is to make conventional food contact materials as inert as possible,

which means that food and packing shouldn't come into too much touch(25). Scientists becoming more and more interested in finding polymers Biodegradable films and coatings, For example, Can help lessen the damaging effects that using synthetic packaging materials has on the environment(17). Thin layers of edible material are called edible films, and the most biodegradable components of edible films and coatings include lipids, proteins, and polysaccharides, which produce a varietv of improved properties(4). compared membranes However, to constructed from synthetic polymers, those made from natural polymers have less desirable characteristics. Then, research was done to improve the marketability and competitiveness of biodegradable films by adopting active food packaging(15). The availability of synthetic functional food components connected to health and the environment has led to the rise of natural ingredients as an essential ecological alternative to synthetic compounds. They have therefore attracted significant interest in a number of nutritional research investigations(27). Carrageenan was one of

the polymers investigated. natural Different kinds of sulfuric sugars are produced by red seaweed. That grow between multiple cells of red seaweed's walls (13). Their fundamental cell compositions are based on an alternating disaccharide repeat sequence with B-Dgalactose connected at position 3 and -Dgalactose linked position at 4. Carrageenans(19). They are extracted galactan-based linear water-soluble polymers that are widely used in industry as stabilizers, thickeners, and gelling agents in food and other products or instance toiletries, cosmetics as well as toothpaste(6). Plant extracts have drawn a lot of interest in addition to extracts that feature phenolic components with potent antibacterial activity. Plant extracts are used to create membranes that have improved mechanical, chemical, physical, antioxidant. antibacterial and characteristics For microbes(17).

2. MATERIALS AND METHODS:

2. 1 Raw Materials:

Carrageenan was imported first from The People's Republic of China as a dry, white powder. Black pepper seeds were purchased from a local herb in Baghdad from the origin of Vietnam and cleaned of dirt and dust, then ground and placed in sealed plastic cans, isolated from light and air, and kept in the refrigerator until use.

2.2 Estimation of The Chemical Composition of black pepper Proximate Analysis:

In order to assess moisture, fat, protein, ash, and fiber in black pepper following the methods described in, chemical studies of ,the spice were performed Additionally, using the equation, the percentage of carbohydrates was determined. carbohydrates % = -100 (moisture % + fat % + protein % + ash % + fiber %).

2.3 Identification on the active compound piperine in black pepper extracts by HPLC technology:

The effective compound of dry black pepper extracts (aqueous and alcoholic) was estimated and characterized using high-performance gas-liquid chromatography technology, type SYKMA of German origin, according to the method of (9) in food safety laboratories. Environment and a Water Department / Ministry of Science and Technology, and the dried model was prepared by adding 3ml of methanol for homogenization, and 100 µl was injected using an auto-injector under the following conditions:

- Column type C18-ODS dimensions (25 cm x 4.6 mm x 5µm).

-Mobile phase A is used to separate compounds.

A=(Methanol:D.W : acetic acid)

-Mobile phase B is used to clean the column of suspended compounds.

B= (Methanol:D.W : acetic acid)

- UV detector with a wavelength of 360 nm and a temperature of 25 m C.

- Flow rate is 0.8ml/min.

The concentrations of phenolic compounds were calculated by the following equation:

substance concentration=[a Standard Substance Concentration× area of the curve of the model/Curve area for standard material] × dilution factor

The phenolic compounds in the extract were identified based on the match R.t.

2.4 Extraction:

2.4.1 Water Extraction:

The aqueous extract was prepared by mixing 10 g of black pepper with 300 ml of distilled water at 70 °C and left for 30 minutes on the magnetic stirrer. A filter by means of a Buechner funnel with a vacuum through filter paper (Whatman No.1), then concentrated in a rotary evaporator at 60 °C until the volume of the extract reached 20 ml, then pour the concentrated extract into a Petri dish and put in the electric oven at a temperature of 40 °C/ 24 hours to dry. Skim the dried

powder and collect into clean, dry bottles and store in the refrigerator until use.

2.4.2 Extraction Alcoholic:

The alcohol extract was made by mixing 10 g of black pepper with 100 ml of 60% ethanol and leaving it on a magnetic stirrer for two hours. After that, it was filtered through Whatman No. 1 filter paper using a Buechner funnel, and the extraction was repeated three times. The filter was successively collected for two hours at a time, concentrated in a rotary evaporator at 50°C until the volume reached 20 ml, and then put into a Petri dish and put in an electric oven at 40°C for 24 hours to dry. Until it is needed, skim the dried powder, collect it in clean, dry bottles, and refrigerate.

2.5 Estimation of The Inhibitory Activity of Extracts Towards The Growth of Microorganisms: Antimicrobial Activity:

The inhibitory activity of the microorganisms of black pepper extract (alcoholic, aqueous) was estimated by disc method, using different diffusion concentrations of each extract of black pepper extract (aqueous, alcoholic), which are (0.1, 0.3, 0.5) mg/ml to know the effect of adding different concentrations Against a number of Gram-positive and Gramnegative microorganisms, Mueller-Hinton medium was prepared, poured into plates, and bacterial inoculation of (E. Coli Salmonella spp., Staphylococci Soapoccus aureus, Bacillus cereus, Bacillus cereus) were dispensed to each of 100 cotton pads using a cotton swab and sterilized and then paper discs of 6 mm in diameter preprepared and sterilized with a cotton swab. The autoclave was placed on the surface of Mueller-Hinton's medium. Annealing and plates were incubated at a temperature of 37 °C for 24 hours in the incubator, then the diameter of the inhibiting corona formed by the inhibitory force for each concentration of each extract of each type of studied microorganisms was measured.

2.6 Prepare simple edible carrageenan films:

The simple membrane was prepared using a concentration of 2% carrageenan with a weight of 2g of carrageenan with 40% of glycerol as a plasticizer by the weight of carrageenan (w/w). Magnetic stirrer for one hour at a temperature of 70°C, as shown in (Figure1).



Figure 1. Carrageenan Simple Edible Films.

2.7 Preparation of Composite Edible Films:

The composite films were prepared by mixing 2 grams of carrageenan as base material with a small amount of distilled water and mixed well until complete dissolution was obtained, then glycerol was added at 40% by the weight of carrageenan (w/w). As a plasticizer, then add the (aqueous) extract of black pepper at (0.5)%, then supplement the weight with distilled water to 100g, then the solution is homogenized with a homogenizer at 5000 rpm/m for 2 minutes, then mixed with a magnetic stirrer on a hot plate for 1hour At a temperature of 70 degrees Celsius. The solution was allowed to cool to a temperature of 50 \pm 5 °C, as shown in (Figure 2).



Figure 2. Composite Edible Films

2.8 Mechanical Properties(Tensile (TS) & Elongation (EL) Films):

Using the Tensile Strength Test equipment, elongation and tensile strength were evaluated to the extent of cutting the membrane. By creating two rectangular pieces that are each 22 mm long and 17 mm wide.

Elongation % = Casing for cutting tape to tension (mm) / Cover for primary tape length (mm)*100%

2.9 Physical Properties:

2.9.1 Water Vapor Permeability (WVP):

The work supplies (cups) were made locally from Teflon with different diameters (3.4, 3.5, 4.5 (external, (inner), and (depth) respectively, and a dried calcium chloride substance (CaCl₂) was added to it. The water vapour permeability was estimated based on the standard approach represented gravimetric bv ASTM E-96-95(1995) for the American Society for Testing as well as Materials. On top of the cup, silicone grease was used as an adhesive. The cup was then covered with the lid and fastened securely. The cup was then tightly sealed and weighed with all of its contents using a stainless steel ring.It was then put in an oven at a temperature between 25-2°C and a relative humidity of RH 50% for 24 hours until moisture saturation was reached above and below the lid. Seven successive readings were then taken over the course of seven days, every day reading and documenting the results. The weight of the cup increased. The time-slope relationship, which depicts the increase in cup weight with time (g/day) after achieving stability by increasing the weight, was used to compute the water vapour permeability of biofilms. The permeability was then calculated using the following two equations:

• The Calculation of the transfer rate of water vapor to the membranes

$$WVTR = \frac{\Delta m}{\Delta t x A}$$

 Δ m: increase in cup weight over time (g). Δ t: time taken to change in weight (day). A: The area of the permeable membrane (cm)².

• The calculation of water vapor permeability

$$WVp = WVTR\chi \frac{X}{\Lambda P}$$

X : Film thickness (mm)

 ΔP : The molecular pressure of water vapor is equal to (1579.09) pascals, or calculated from the following equation:

 $\Delta \mathbf{P} = \mathbf{S} \ (\mathbf{RH1-RH}).$

S: saturated vapor pressure at 25 °C, equivalent to 3166 pa.

RH1: the relative humidity over the membrane in incubator equal to (50%).

RH2: relative humidity under the membrane $(Cacl_2)$, equivalent to (0%).

2.9.2 Solubility in Water:

solubility of films made from simple and compound carrageenan was estimated. After being weighed, a 2g piece was submerged in 50 ml of distilled water and allowed to stand for 24 hours at room temperature (352)°C with intermittent shaking. The solution was then filtered through No.1 Wht filter paper.The filter papers and their insoluble biofilm residues were dried in an air oven at a temperature of (105 °C) until the weight was stable after being (pre-weighted) until the weight was stable Solubility:

% solubility = Initial weight of filter paper with the membrane - The final weight of the final paper and the rest of the film on it / the Initial weight of the casing *100 %

3. RESULTS AND DISCUSSION:

The results of the chemical composition of black pepper showed that (protein, oil, fiber, moisture, ash and carbohydrates), the values reached (5.469, 4.909, 4.125, 11.477, 6.122, 67.898)%, respectively, and these results are close to what was found(3), and that the percentage of ashand carbohydrates were different, while the rest of the percentages were close, which amounted to (6.122 and 67.898)%, respectively. The reason for this is attributed to the conditions of cultivation and origin(10) and others. It was noted that the percentage of ash is low, which amounted to (4.971,6.122)%, respectively, and this is clear evidence that the percentage of mineral elements is few, so the higher the percentage of ash, The higher the percentage of mineral elements, and vice versa, as found by (23). The results of the quantitative diagnosis of the active compounds in black pepper extracts (aqueous and alcoholic) by HPLC technology showed that the highest value of the active compounds in the aqueous extract was (1524.8) ppm. Compared with a lower value in the alcoholic extract, which amounted (958.9)to ppm, respectively shown in table (1), the reason is attributed to the black pepper containing many active compounds, which are found in different proportions, and according to what (12) found when separating active compounds from spices and herbs, including pepper The most important of these compounds are Piperine, Cinnamic acid, 13-phenyl-tridecanoic acid, Piperylin, Ascorbic acid, gamma-Sitosterol, and other active compounds that differ in their solubility in aqueous solutions and organic solvents and the nature of the composition of these compounds and what they contain of loving and hate groups water and also to extraction conditions.

Table (2): The quantitative diagnosis of the active compounds in black pepper extracts (aqueous and alcoholic) by HPLC technology

	Piperin
Black pepper	(ppm)
aqueous	1524.8
extraction	
alcoholic	958.9
extraction	

The results also showed that the aqueous extract of black pepper is better than the alcoholic extract based on the results of *bacterial* inhibition shown in table (2). The diameter of the inhibition halo formed by the inhibitory power of the concentration of black pepper extract (aqueous and alcoholic) was measured (0.1, 0.3, 0.5) each mg/ml towards type of microorganisms, where it was noted that the inhibitory force at the concentration 0.5 mg/ml gave the best results compared to the remaining concentrations, which were very weak or almost non-existent. A number of positive and negative microorganisms of the pathogenic grampositive (Escherichia coli, Staphylococcus aureus, Bacillus cereus, Salmonella spp) were superior inhibiting Gram-negative E.coli bacteria and a Staphylococcus positive bacteria. The diameter of the inhibiting aura is 16 mm for each type of bacteria mentioned. which differs significantly from the alcoholic pepper extract, which amounted to (13.8) mm, respectively, compared to the lowest value of (8,10) mm, respectively, for the Inhibition diameter aura, in its effectiveness for other types of bacteria,

and the reason is due to the effectiveness of water and alcoholic black pepper extracts in inhibiting microorganisms as it is rich in active and antimicrobial substances and the concentration of these active what it found (24).

 Table (2): Bacterial inhibition results

An	Turmeric	type of bacteria
Alcoholic	aqueous	
turmeric	extract/ml	
extract/ml		
13	16	Escherichia coli
8	16	Staphyococcus
		aureus
8	10	Bacillus cereus
8	12	Salmonella spp

3.1 Mechanical Properties(Tensile (TS) & Elongation (EL) Films):

The mechanical properties of the films used in packaging are related to the behavior of the material against the forces applied to the surface (15). Where mechanical properties such as tensile strength and film elongation are a critical factor for packaging materials (11, 27). Table (3) shows the mechanical properties of composite films and the their comparison with the simple ones, where the results showed a difference between the normal films and the films reinforced with black pepper extract for tensile strength and elongation, as significant differences observed for were the composite films, as the tensile strength reached (38.1) MPa for the membrane reinforced with black pepper extract. The percentage of elongation was (23.6) % for the membrane reinforced with black pepper extract. The table also indicated that the percentage of elongation is in an inverse relationship with the tensile strength (7). It was noted that when adding the extracts, the tensile strength and elongation of the composite films increased, when compared with the simple carrageenan membrane of at a concentration of (2%). We note that the tensile strength was (29.9) MPa. And the elongation ratio was (20.3)%. The reason for the increase in the tensile strength and elongation of the composite films is attributed to the multiple phenolic compounds that contain hydroxyl groups OH in the black pepper extracts, which form hydrogen bonds with carrageenan that leads to an increase in tensile strength as well as the increase in the thickness of the composite films that Improvement of membrane strength (14).

Table (3): Mechanical properties of simpleand films supported by aqueous blackpepper extract

Elongation %	Tensile strength/M pa	Cover type
20.3	29.9	Carrageenan 2%
23.6	38.1	Carrageenan 2%+ black pepper extract 0.5

3.2 Physical Properties:

The WVP film's water vapour permeability is one of the most crucial aspects of their applicability for food packaging since it limits moisture transfer between food and its surroundings. The estimation and forecasting of a packaged product's shelf life depend on the determination of the polymer barrier qualities. Water vapour permeability is a critical barrier metric that shows the effectiveness of the film against water vapour (28). For food to have a longer shelf life, it is preferable to have lower water vapour permeability because this results in less water vapour transfer between the food and its environment (26). The permeability of the membrane depends on its chemical composition, morphology and environment temperature resulting from the addition of black pepper extract. where the water vapor permeability of the simple films was 2.65 (g. mm/cm^2 . Pa. day), while the percentage of the reinforced film was 2.39 $(g.mm / cm^2 Pa . day)$, In table 4. There was a significant decrease in the water

vapor permeability of the composite films of carrageenan at a concentration of 2% and supplemented with aqueous black pepper extract, which amounted to 2.39, 2.65 (gm.mm/cm². Pa .day) and thus the addition of the extracts reduced the membrane permeability to water vapor and improved the properties of the biofilm. The reason for the decrease was attributed to the nature and type of extract, and to the presence of active compounds (phenols) in the added extracts that contain aggregates. The hydroxyl OH, which binds with carrageenan, forms an interconnected matrix that reduces the membranes' susceptibility to moisture loss and this is confirmed by (16). Water solubility is an important property of biofilms that indicates the affinity of the membrane with water. It is water or moisture resistant to the polymer. Lower solubility leads to more stability and less reaction with moisture (18). The increased of aqueous black pepper extract the solubility of the simple films, which was 20.4% before fortification, while fortification with black pepper extract gave lower results of 15.8%. As shown in a table)4(.

Table (4): physical properties of simpleand films supported by aqueous blackpepper extract

solubilit y rate %	WVP(g.mm/cm ² .Pa .day)	Membrane type
20.4	2.65	simple membrane
15.8	2.39	composite membrane

Conclusion

Food wrappers have been prepared at a concentration of 2% carrageenan with glycerol 40% by the weight of carrageenan as a plasticizer. A composite film was also prepared by adding aqueous black pepper extract as a support to the base material. An alcoholic extract of black pepper was also prepared, which was neglected and relied on the aqueous extract of black pepper only, depending on the inhibitory activity of the extract towards the growth of microorganisms. The active compound piperine was diagnosed in the aqueous and alcoholic extract of black pepper, and the amount of the active compound was higher in the aqueous extract, which amounted to (1524.8) ppm compared to the alcoholic extract, which amounted to (958.9)ppm. The mechanical and physical properties of these membranes were also studied. The results showed that the mechanical properties were improved when an aqueous black pepper extract was used. As the tensile strength increased with the decrease in elongation, the results of the mechanical properties were opposite. As for the physical properties, a decrease in water vapor permeability occurred when an aqueous black pepper extract was added, and it was directly related to the solubility.

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