

EDIBLE COATING FILMS COMPATIBLE WITH VEGAN, HALAL AND
KOSHER DIETS FOR PRESERVATION OF FRUITS AND VEGETABLES

Cross-Reference to Related Application

- 5 [0001] This application claims the benefit of priority of Singapore Patent Application No. 10201911255T, filed 27 November 2019, the content of it being hereby incorporated by reference in its entirety for all purposes.

Technical Field

- 10 [0002] The present disclosure relates to a film-forming composition for prolonging shelf life of food products, wherein the film-forming composition complies with halal, kosher and vegan dietary requirements. The present disclosure also relates to a method of producing the film-forming composition.

15 **Background**

- [0003] Prevention of microbial spoilage of food is of paramount importance for food safety and security. The quest for sustainable solutions to prolong shelf life of food products has revived a so-called active packaging represented by using edible materials forming a coating film directly on the surface of fruits, vegetables, meat steaks, etc.,
20 wherein the coating film is meant to be capable of preventing microbial contamination and development of pathogenic microflora in the coated products.

- [0004] In the past, one way to reduce the level of microbial contamination in food is adding of artificial chemical preservatives. Such artificial chemical preservatives includes but are not limited to, sulfur dioxide, sodium nitrite, sodium erythorbate,
25 sodium diacetate, sodium succinate, sodium dehydro acetate, parabens, propylphenols, sodium salts of ethylenediaminetetraacetic acid, and polyphosphates. These compounds may inhibit growth of bacteria, mold and insects. However, their usage in food has raised health concerns among consumers, which include incidents of headaches, palpitations, allergies, asthma, skin rashes, urticaria, and various forms cancer. As a
30 consequence, natural antimicrobial agents of plant origin were contemplated as components of pathogen suppressive materials for active packaging.

[0005] A number of natural essential oils possess antimicrobial properties, so these substances were among the first additives used to achieve edible films with antimicrobial functionalities. A few important disadvantages of adding essential oils in antimicrobial films include a limited offer of food grade essential oils in the market, relatively high cost of essential oils and their purified active components, poor durability of the antimicrobial effects due to fast evaporation of volatile active components, pungent smell rendering an undesirable impact to the product taste, and also, incorporation of essential oils into films may render emulsification to result in opaque and visually unappealing coatings. Nanoemulsion techniques may improve the film transparency, but these methods require specific equipment and emulsifiers that unfortunately increases the product cost.

[0006] Utilization of water-soluble polyphenolic phytochemicals helps avoid emulsification and fabricate transparent films with antimicrobial properties and provide a wide range of other biological effects, e.g., antioxidant, antiviral and anti-inflammatory benefits. However, the material cost and their availability/abundance have to be considered, as some of these phytochemical actives are expensive or commercially unavailable.

[0007] Another consideration in multi-national and multi-cultural countries like Singapore is that the product may need to, in various instances, comply with vegan, halal and kosher dietary restrictions/requirements so as to cater to customers of various ethnical, cultural and religious backgrounds. In case of edible coatings for food products, including fruits and vegetables, this requirement signifies that materials of animal origin and alcohol (e.g. ethanol) must be excluded from the coating film compositions. Moreover, alcohol must not be added to the coating formulation at any preparation step.

[0008] Beyond suppressing various pathogens, the ability of active packaging to prolong shelf life of food products by preventing premature over-ripening may be considered a value added feature. Another way for the coating to serve as a sustainable storage solution for these commodities is the prevention of drying caused by the transpiration process. The loss of moisture (weight loss) affects both appearance and texture of food products, thus decreasing their commercial value.

There is thus a need to provide for a solution that addresses one or more of the limitations mentioned above. The solution should at least provide for a film-forming composition that protects and/or preserves food products. The solution should address at least the food security issue regarding the lack of active packaging materials for prolonged shelf life of food products, wherein the packaging material (e.g. a protective film) may be composed of substances approved for food applications and compatible with various dietary restrictions, inhibit pathogen (e.g. active against Gram-positive, Gram-negative bacteria and mold) growth, reduce weight (e.g. moisture) loss of coated food products, inhibit over-ripening and drying, provide an aesthetically appealing look to the coated products, durable, has no undesirable/negative impact to the product taste, and is economical.

Summary

[0009] In a first aspect, there is provided for a film-forming composition which complies with halal, kosher and vegan dietary requirements, the film-forming composition includes:

- a hydrogel including of a polysaccharide; and
- 15 a water-soluble additive including a phenolic phytochemical and an anti-fungal agent, wherein the hydrogel is formed as a matrix incorporated with the water-soluble additive.

[0010] In another aspect, there is provided a method of producing a film-forming composition, wherein the film-forming composition complies with halal, kosher and vegan dietary requirements, wherein the film-forming composition includes

- a hydrogel including of a polysaccharide; and
- a water-soluble additive including a phenolic phytochemical and an anti-fungal agent, wherein the hydrogel is formed as a matrix incorporated with the water-soluble additive, the method including:
 - providing an aqueous solution including the phenolic phytochemical and the anti-fungal agent; and
 - 20 mixing the aqueous solution with the polysaccharide to form the film-forming composition.

Brief Description of the Drawings

[0011] The drawings are not necessarily to scale, emphasis instead generally being placed upon illustrating the principles of the present disclosure. In the following description, various embodiments of the present disclosure are described with reference to the following drawings, in which:

[0012] FIG. 1 is a photo image of films produced from the present film-forming composition, wherein pectin is used. The left film is a pectin control where only pectin was used. The center and right are pectin-based films formed from Pectin 1 and Pectin 2 compositions of the present disclosure, respectively.

[0013] FIG. 2 shows scanning electron microscopy (SEM) micrographs of films based on pectin (i.e. pectin control, Pectin 1 and 2 compositions). The bottom row of images show the cross-section of the resultant pectin based films. Scale bars in top and bottom row images denote 10 μm and 1 μm , respectively.

[0014] FIG. 3 shows the antimicrobial and anti-fungal activity of the three pectin-based films.

[0015] FIG. 4 is a photo image of films produced from the present film-forming composition, wherein κ -carrageenan is used. The left film is a κ -carrageenan control where only κ -carrageenan was used. The center and right are κ -carrageenan-based films formed from κ -carr 1 and κ -carr 2 compositions of the present disclosure, respectively.

[0016] FIG. 5 shows scanning electron microscopy (SEM) micrographs of films based on pectin (i.e. κ -carra control, κ -carra 1 and 2 compositions). The bottom row of images show the cross-section of the resultant κ -carrageenan-based films. Scale bars in top and bottom row images denote 10 μm and 1 μm , respectively.

[0017] FIG. 6 shows the antimicrobial and anti-fungal activity of the three κ -carrageenan-based films.

[0018] FIG. 7A shows photo images of coated and uncoated mango specimens stored at room temperature for 9 days, wherein the coated specimen is coated with a film formed from the present film-forming composition.

[0019] FIG. 7B shows photo images of coated and uncoated tomato specimens stored at room temperature for 10 days, wherein the coated specimen is coated with a film formed from the present film-forming composition.

[0020] FIG. 7C shows photo images of coated and uncoated banana specimens stored at room temperature for 6 days, wherein the coated specimen is coated with a film formed from the present film-forming composition.

5 [0021] FIG. 7D shows photo images of coated and uncoated cherry specimens stored at room temperature for 14 days, wherein the coated specimen is coated with a film formed from the present film-forming composition.

[0022] FIG. 8A demonstrates the antimicrobial and anti-fungal activity of films based on pectin after 7 and 14 days of storage at 40 °C, 80% relative humidity (RH).

10 [0023] FIG. 8B demonstrates the antimicrobial and anti-fungal activity of films based on κ -carrageenan after 7 and 14 days of storage at 40 °C, 80% RH.

[0024] FIG. 8C shows photo images of specimens 1 hr after they were transferred to ambient conditions from +4 °C refrigerator after 14 days of storage.

Detailed Description

15 [0025] The following detailed description refers to the accompanying drawings that show, by way of illustration, specific details and embodiments in which the present disclosure may be practised.

[0026] Features that are described in the context of an embodiment may correspondingly be applicable to the same or similar features in the other embodiments.

20 Features that are described in the context of an embodiment may correspondingly be applicable to the other embodiments, even if not explicitly described in these other embodiments. Furthermore, additions and/or combinations and/or alternatives as described for a feature in the context of an embodiment may correspondingly be applicable to the same or similar feature in the other embodiments.

25 [0027] The present disclosure relates to a film-forming composition for producing edible coating films having the following properties, including antimicrobial activity (e.g. against *Escherichia coli* and *Staphylococcus aureus*), anti-fungal activity (e.g. against *Botrytis cinerea*), reduce weight (moisture) loss of coated fruits and vegetables, absorb light in the UV and/or visible light region, slows down ripening of coated fruits
30 and vegetables, transparent (e.g. opacity of the film with antimicrobial and anti-fungal agents exceeds opacity of the control polysaccharide alone film for less than 30%), adheres well to the surface of fruits and vegetables, durable (e.g. able to preserve

structural integrity and antimicrobial properties) for at least 2 weeks upon storage at ambient conditions, has no pungent smell and are water soluble for easy removal.

[0028] The present film-forming composition and resultant film neither contain ingredients of animal and/or insect origin nor alcohol (e.g. ethanol) and other organic solvents, which are also not involved at any stage of its production. Therefore, the present film-forming composition and resultant film are fully compatible with vegan, halal and kosher diets dietary requirements. A vegan diet requires abstinence from consumption of any ingredient of animal and insect origin. A halal diet complies with Islamic dietary regulations, for example, consuming meat processed and/or prepared in accordance with those requirements. A halal diet is absent of pork-based and alcohol-based products. A kosher diet complies with Jewish dietary regulations, for example, food processed and/or prepared in accordance with those requirements. A kosher diet may exclude food that has a combination of meat-based and dairy-based products, as meat-based and milk-based products may never be mixed (even during processing).

[0029] The present film-forming composition and resultant film may contain an active composition that has a synergetic effect from an antimicrobial agent and an anti-fungal agent. The antimicrobial agent may contain a low-cost food grade plant phenolic phytochemicals (e.g. tannic acid, gallic acid) and the anti-fungal agent may contain food additive salts (e.g. potassium sorbate and sodium benzoate) to provide both antimicrobial and anti-fungal effects via the edible resultant coatings. The present composition is able to inhibit growth of Gram-positive and Gram-negative bacteria as well as mold and other microorganisms, which can be a root-cause of microbial food spoilage and food borne diseases.

[0030] The present film-forming composition and resultant coating film protects food products, including fruits and vegetables, from drying, premature ripening and over-ripening due to a combined effect of the antimicrobial and anti-fungal agents contained therein, as well as a polysaccharide matrix and/or plasticizer of the film-forming composition.

[0031] The present film-forming composition and resultant coating films can be made of ingredients solely of plant origin via a solely water-based process, thus, the film-forming composition and resultant coatings are compatible with vegan, halal and kosher dietary requirements.

[0032] The film-forming composition and hence the resultant coating films are water-soluble, which renders a convenient option of removing the edible film before consumption by simply rinsing the coated food products, such as fruits or vegetables, with water.

5 [0033] Details of various embodiments of the present film-forming composition and method of producing the same, and advantages associated with the various embodiments are now described below.

[0034] In the present disclosure, there is provided a film-forming composition which complies with halal, kosher and vegan dietary requirements.

10 [0035] The film-forming composition includes a hydrogel formed of a polysaccharide and a water-soluble additive including a phenolic phytochemical and an anti-fungal agent, wherein the hydrogel can be formed as a matrix incorporated with the water-soluble additive. In various embodiments, the phenolic phytochemical and the anti-fungal agent are free of covalent bonding, electrostatic bonding, and/or ionic bonding
15 to the polysaccharide. The chemical structures and properties of the (i) polysaccharide forming the hydrogel matrix, (ii) phenolic phytochemical and (iii) anti-fungal agent, are such that they exclude or not capable of covalent bonding, electrostatic interactions, and/or ionic bonding with each other. However, certain extent of hydrogen bonding may exist between the polysaccharide and the anti-fungal agent.

20 [0036] In various embodiments, the phenolic phytochemical includes a plant-based polyphenol, tannic acid, gallic acid, ferulic acid, or a combination thereof. The phenolic phytochemical may absorb ultraviolet (UV) light, which in turn protects coated specimens and products from premature over-ripening and drying. In various embodiments, the phenolic phytochemical may also include any edible water-soluble
25 phenolic phytochemical, non-limiting examples of which include plant polyphenols, gallic acid, ferulic acid, and/or a combination thereof. The phenolic phytochemical, e.g., plant polyphenols, gallic acid, and ferulic acid, may exist in the pure form or as a part of a plant extract with minimal inhibitory concentration (MIC) equal to or less than 1.5 mg/mL for *Escherichia coli*. The phenolic phytochemical, e.g., plant polyphenols,
30 gallic acid, and ferulic acid, may exist in the pure form or as a part of a plant extract with minimal inhibitory concentration (MIC) equal to or less than 1.75 mg/mL for

Staphylococcus aureus. The phenolic phytochemical, together with the anti-fungal agent, can inhibit growth of Gram-positive bacteria, Gram-negative bacteria, mold, etc.

[0037] In various embodiments, the anti-fungal agent includes benzoic acid, methyl paraben, potassium sorbate, sorbic acid, sodium benzoate, or a combination thereof.

5 The anti-fungal agent, together with the phenolic phytochemical, can inhibit growth of Gram-positive bacteria, Gram-negative bacteria, mold, etc.

[0038] In various embodiments, the polysaccharide includes agar, carboxymethyl cellulose, hydroxypropyl methylcellulose, pullulan, pectin, iota-carrageenan, κ -carrageenan, sodium alginate, or starch.

10 [0039] The present film forming composition may further include a plasticizer, wherein the plasticizer includes glycerol, sorbitol, polyethylene glycol, glucose, or saccharose. In various embodiments, the plasticizer may include a polyol, such as glycerol, sorbitol, polyethylene glycol, and/or a sugar, such as glucose, and saccharose.

[0040] In various embodiments, the polysaccharide, the phenolic phytochemical, and
15 the anti-fungal agent can be present in a weight ratio of 0.5 to 1.5 : 0.1 to 2 : 0.1 to 1. As a non-limiting example, the polysaccharide, phenolic phytochemical and anti-fungal agent can be present in a ratio of 0.5 : 0.1 : 0.1, or 1.5 : 2 : 1, etc. In various embodiments, the phenolic phytochemical includes (i) tannic acid or (ii) tannic and gallic acid. In various embodiments, the anti-fungal agent includes potassium sorbate
20 and/or sodium benzoate. In various embodiments, the polysaccharide, the plasticizer, the potassium sorbate, the sodium benzoate, the tannic acid, the gallic acid, and water, can be present in a weight ratio of 0.5 to 1.5 : 0.5 to 1.5 : 0.1 to 1 : 0.1 to 1 : 0.1 to 2 : 0.1 to 2 : 25 to 75. Other ratios are described in the examples section below.

[0041] If the amount of polysaccharide mixed or present is less than the amount
25 indicated in the above ratio, the film-forming composition may experience difficulty in hardening (becoming a solid) or may not harden to form the film. If a higher amount is used, higher material cost and solubility issues of the other components may arise.

[0042] If the amount of plasticizer mixed or present is less than the amount indicated in the above ratio, the resultant film may be brittle. If a higher amount is used, the film-
30 forming composition or the film may not harden or may not be sufficiently hard as a film.

[0043] If the amount of phenolic phytochemical (e.g. tannic acid) mixed or present is less than the amount indicated in the above ratio, no antimicrobial effect may be observed. If a higher amount is used, undesirable crystallization of the film may occur, the film may turn out to be undesirably dark in colour, and/or solubility issues of the other components may arise.

[0044] If the amount of anti-fungal agent (e.g. sodium benzoate) mixed or present is less than the amount indicated in the above ratio, no antifungal effect may be observed. If a higher amount is used, there may be a danger of exceeding the limit for daily intake. As another example, if the amount of potassium sorbate mixed or present is less than the amount indicated in the above ratio, no antifungal effect may be observed. If a higher amount is used, there may be a danger of exceeding the limit for daily intake.

[0045] If the amount of gallic acid mixed or present is more than the amount indicated in the above ratio, undesirable crystallization may occur when the film-forming composition or the film hardens.

[0046] If the amount of water mixed or present is less than the amount indicated in the above ratio, solubility of the other components may be compromised. If a higher amount is used, the film-forming composition may take a longer time to form into a film or the film may take a longer time to harden.

[0047] The present disclosure also provides for a method of producing a film-forming composition, wherein the film-forming composition complies with halal, kosher and vegan dietary requirements, wherein the film-forming composition includes a hydrogel comprised of a polysaccharide, and a water-soluble additive including a phenolic phytochemical and an anti-fungal agent, wherein the hydrogel is formed as a matrix incorporated with the water-soluble additive. The method includes providing an aqueous solution that includes the phenolic phytochemical and the anti-fungal agent, and mixing the aqueous solution with the polysaccharide to form the film-forming composition.

[0048] Embodiments and advantages described for the present film-forming composition in various embodiments of the first aspect can be analogously valid for the present method subsequently described herein, and vice versa. As the various embodiments and advantages have already been described above and examples demonstrated herein, they shall not be iterated for brevity.

[0049] In various embodiments, providing the aqueous solution can include dissolving the phenolic phytochemical and the anti-fungal agent in water. Providing the aqueous solution may further include dissolving the phenolic phytochemical and the anti-fungal agent in water containing a plasticizer, wherein the plasticizer comprises glycerol, sorbitol, polyethylene glycol, glucose, or saccharose.

[0050] In various embodiments, mixing the aqueous solution with the polysaccharide is free of forming any covalent bond and any ionic bond between (i) the phenolic phytochemical and the polysaccharide and (ii) the anti-fungal agent and the polysaccharide.

[0051] The present method may further include heating the aqueous solution at a temperature ranging from 60°C to 100°C, 70°C to 100°C, 80°C to 100°C, 90°C to 100°C, 60°C to 70°C, 60°C to 80°C, 60°C to 90°C, etc., after mixing the aqueous solution with the polysaccharide.

[0052] In various embodiments, heating the aqueous solution can include heating the aqueous solution for a duration ranging from 10 minutes (mins) to 20 minutes, 15 minutes (mins) to 20 minutes, 10 minutes (mins) to 15 minutes, etc.

[0053] In various embodiments, the phenolic phytochemical can include a plant-based polyphenol, tannic acid, gallic acid, ferulic acid, or a combination thereof. Other embodiments of the phenolic phytochemical are described above in various embodiments of the first aspect.

[0054] In various embodiments, the anti-fungal agent can include benzoic acid, methyl paraben, potassium sorbate, sorbic acid, and/or sodium benzoate, or a combination thereof. Other embodiments of the anti-fungal agent are described above in various embodiments of the first aspect.

[0055] In various embodiments, the polysaccharide can include agar, carboxymethyl cellulose, hydroxypropyl methylcellulose, pullulan, pectin, iota-carrageenan, κ-carrageenan, sodium alginate, or starch. Other embodiments of the polysaccharide are described above in various embodiments of the first aspect.

[0056] In various embodiments, mixing the aqueous solution with the polysaccharide can include mixing the polysaccharide, the phenolic phytochemical, and the anti-fungal agent, in water, wherein the polysaccharide, the phenolic phytochemical, and the anti-

fungus agent, are mixed in a weight ratio of 0.5 to 1.5 : 0.1 to 2 : 0.1 to 1. Other ratios for mixing are described in various embodiments of the first aspect.

[0057] In various embodiments, mixing the aqueous solution with the polysaccharide can include mixing the phenolic phytochemical, the anti-fungal agent, the polysaccharide, and the plasticizer, in water. In various embodiments, the phenolic phytochemical can include (i) tannic acid or (ii) tannic and gallic acid. In various embodiments, the anti-fungal agent can include potassium sorbate and/or sodium benzoate. In various embodiments, mixing of the polysaccharide, the plasticizer, the potassium sorbate, the sodium benzoate, the tannic acid, the gallic acid, and the water, can be carried out in a weight ratio of 0.5 to 1.5 : 0.5 to 1.5 : 0.1 to 1 : 0.1 to 1 : 0.1 to 2 : 0.1 to 2 : 25 to 75.

[0058] The word "substantially" does not exclude "completely" e.g. a composition which is "substantially free" from Y may be completely free from Y. Where necessary, the word "substantially" may be omitted from the definition of the present disclosure.

[0059] In the context of various embodiments, the articles "a", "an" and "the" as used with regard to a feature or element include a reference to one or more of the features or elements.

[0060] In the context of various embodiments, the punctuation "~", the term "about" or "approximately" as applied to a numeric value encompasses the exact value and a reasonable variance.

[0061] As used herein, the term "and/or" includes any and all combinations of one or more of the associated listed items.

[0062] Unless specified otherwise, the terms "comprising" and "comprise", and grammatical variants thereof, are intended to represent "open" or "inclusive" language such that they include recited elements but also permit inclusion of additional, unrecited elements.

Examples

[0063] The present disclosure relates to a film-forming composition for producing an edible coating films that includes ingredients of non-animal origin to be utilized as an active packaging for food products, including fruits and vegetables, that prevents

microbiological spoilage, premature over-ripening, and drying during transportation and storage.

[0064] The coating films can be made of a hydrogel polysaccharide matrix (e.g. pectin or κ -carrageenan) with an additive that inhibits growth of Gram-positive bacteria, Gram-negative bacteria, mold, etc. The additive can include an antimicrobial agent and an anti-fungal agent. The antimicrobial agent can include a low-cost phenolic phytochemical (e.g. tannic acid and gallic acid), and the anti-fungal agent can include potassium sorbate and/or sodium benzoate. The combination of the phenolic phytochemical and the anti-fungal agent in the present film-forming composition confer a synergetic effect of antimicrobial and anti-fungal effect without one compromising the effect from one over the other. Both the antimicrobial and anti-fungal agents do not form covalent bonds nor electrostatic bonds with the hydrogel matrix, which maintains and ensures maximal activity of the antimicrobial and anti-fungal agents against microorganisms. The phenolic phytochemical and anti-fungal agent enhance the ultraviolet (UV) absorption properties of the resultant coating, which prevents premature ripening and over-ripening of the coated food products and specimens.

[0065] In addition, the hydrogel matrix includes polysaccharides combined with a plasticizer (e.g. glycerol), which improves retention of water by coated food products and specimens, thus losing moisture (i.e. become dry) significantly slower than uncoated counterparts.

[0066] The present film-forming composition can render a film coating that is transparent with no undesirable nor negative impact on visual appearance (e.g. color, opacity, gloss, etc.) of the coated food product, e.g. fruits or vegetables.

[0067] The present film-forming composition is free of ingredients of animal and insect origin, fully compatible with vegan, halal and kosher dietary requirements. Moreover, all ingredients and components used in the present film-forming composition are approved for use in food applications and available in food grade. For instance, the anti-fungal preservatives, potassium sorbate (E202) and sodium benzoate (E211), are designated as generally recognized as safe (GRAS) by the US Food and Drug Administration, demonstrating that the resultant coatings formed from the present film-forming composition can be safely consumed together with the coated food products.

[0068] The resultant films are water-soluble and can be removed by rinsing the products with water before consumption as an option.

[0069] The present film-forming composition and its method of production are described in further details, by way of non-limiting examples, as set forth below.

5 **[0070] Example 1A: Cost, Materials and Methods**

[0071] Pectin from citrus peel and κ -carrageenan were from Sigma-Aldrich, Denmark, glycerol was Sigma-Aldrich, Austria, tannic acid and gallic acid were from Sigma-Aldrich, China, potassium sorbate was from North Mountain Supply, USA, sodium benzoate was from Sigma-Aldrich, USA. Media for cultivation of microorganisms were
10 as follows: nutrient agar (Sigma-Aldrich, Spain), Luria-Bertani broth (Sigma-Aldrich, USA); potato dextrose agar (Sigma-Aldrich, USA). All chemicals were used as received. Microorganisms *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 6538, *Botrytis cinerea* ATCC 28387 were from ATCC, USA.

[0072] Cost of premix (solids and glycerol) is estimated at SGD 25 per 1 kg. 1 kg of
15 premix is enough to coat ~450 kg of cherry tomatoes.

[0073] Film thickness was measured using a digital micrometer with a resolution of 0.001 mm (Mitutoyo Digimatic Thickness Gage, Japan) at ten randomly selected points. The results are presented as mean $\pm$ standard deviation.

[0074] Moisture content of the films was determined gravimetrically. Pre-weighted
20 pieces of the casted film (2 $\times$ 2 cm) were dried in an oven at 105 °C until a constant weight was achieved.

[0075] To determine water solubility, pre-dried pieces of the films (2 $\times$ 2 cm) were weighted and placed in 40 mL of distilled water at 25 °C and kept for 1 h under constant stirring (60 rpm). The solutions were filtered through a paper filter and pre-dried to a
25 constant weight. The film residues together with the filter were dried at 105 °C for 24 h and weighed.

[0076] Light transmission of the obtained films in the UV and visible regions was determined using a UV-2450 spectrophotometer (Shimadzu, Japan). The films were cut into rectangular pieces (4 $\times$ 1 cm) and placed directly into the cell of the
30 spectrophotometer. An empty test cell was used as a control. The percentage of light transmission (T, %) was measured in the range of wavelengths from 200 to 800 nm.

[0077] The morphology of the films was analyzed using a scanning electron microscope FE-SEM 6700F (JEOL Ltd., Japan) at a voltage of 5 kV. Testing samples were prepared by immersing the obtained film in liquid nitrogen and breaking it into smaller pieces. Even pieces were then collected and fixed on a substrate with a double-sided adhesive tape and covered with a thin layer of gold.

[0078] Antimicrobial and anti-fungal activity were determined by a diffusion method in agar against the strains of *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 6538, *Botrytis cinerea* ATCC 28387 and *Strawberry wild mold*. The film samples were placed on a Petri dish containing nutrient agar (potato dextrose agar for mold), previously inoculated with the test microorganisms.

[0079] Bacterium inocula were prepared in a liquid nutrient medium using a 24 hrs culture of microorganisms incubated in aerobic conditions at 37 °C. The cell concentration was set to 0.5 McFarland (corresponding to 1.5×10^8 CFU/mL).

[0080] Mold inocula were prepared in sterile water. Mold spores were gradually added to sterile water until the light absorption of suspension reached 0.25 at 425 nm (corresponding to 1.0×10^6 spores/mL).

[0081] Petri dishes were inoculated by the spread plate method using 0.1 mL of a bacteria or mold suspension.

[0082] The inoculated petri dishes containing film samples were incubated at aerobic conditions at 37 °C for 24 h (for bacteria) and at 26 °C for 48-72 hrs (for molds), then the diameter of the formed zones of growth inhibition were measured.

[0083] Fruits and vegetables were coated with the developed films by dipping them in the film forming solution and subsequent drying at room temperature.

[0084] Example 1B: Non-limiting Examples of Edible Coating Films

[0085] For the purpose of demonstration and not to limit the scope of the present disclosure, four edible coating films with antimicrobial and anti-fungal properties composed of two different polysaccharides, pectin and κ -carrageenan were developed, each supplemented with defined amounts of potassium sorbate (PS), sodium benzoate (SB), tannic acid (TA), and gallic acid (GA). The following formulations disclosed are based on weight of each ingredient.

[0086] Pectin 1:

[0087] Pectin : Glycerol : PS : SB : TA : Water = 1 : 1 : 0.3 : 0.4 : 1.5 : 50

[0088] **Pectin 2:**

[0089] Pectin : Glycerol : PS : SB : TA : GA : Water = 1 : 1 : 0.3 : 0.4 : 1.5 : 0.2 : 50

[0090] **κ-carr 1:**

[0091] κ-carrageenan : Glycerol : PS : SB : TA : Water = 1 : 1 : 0.3 : 0.4 : 1.5 : 50

5 [0092] **κ-carr 2 :**

[0093] κ-carrageenan : Glycerol : PS : SB : TA : GA : Water = 1 : 1 : 0.3 : 0.4 : 1.5 : 0.2 : 50

[0094] **Example 2A: Method of Producing Edible Coating Pectin-Based Films**

[0095] The pectin-based film-forming compositions and resultant films were obtained
10 as follows:

[0096] *Pectin 1:* 1.5 mL glycerol, 0.8 g SB, 0.6 g PS and 3.0 g TA were added to 100 mL of deionized (DI) water with constant stirring on a magnet plate. Agitation was continued for 30 min at 150 rotation per minute (rpm) until complete dissolution of all components. Then 2.0 g of pectin was added and stirred for 1 hr at 100 rpm. The
15 obtained film-forming solution was then heated up to 70±5 °C for 15 mins with constant stirring to achieve a homogeneous solution.

[0097] *Pectin 2:* 0.4 g of GA dissolved in 1.5 mL of glycerol was added to 100 mL of DI water. Then 0.8 g SB, 0.6 g PS and 3.0 g TA were added with constant stirring. Agitation was continued for 30 min at 150 rpm until complete dissolution of all
20 components. Then 2.0 g of pectin was added and the mixture was stirred for 1 hr at 100 rpm. The obtained film-forming solution was then heated up to 70±5 °C for 15 mins with constant stirring to achieve a homogeneous solution.

[0098] Physical properties of the developed films and their antimicrobial and anti-fungal activities were studied against a pectin control, i.e. a pectin film containing no phenolic phytochemicals and anti-fungal salts. The pectin control film was obtained by
25 dissolving 2.0 g of pectin powder in 100 mL of DI water using a magnetic stirrer for 1 hr at 100 rpm. Glycerol (1.5 mL = 2 g) was then added as plasticizer. The film-forming solution was heated at 70±5 °C for 15 mins with constant stirring until a homogeneous solution was obtained.

30 [0099] The films were cast by placing 13 mL of a respective chilled solution into a glass petri dish (9 cm in diameter) followed by drying at 55±5° C for 12 hrs.

[00100] Example 2B: Physical, Antimicrobial and Anti-Fungal Properties of Pectin-Based Films

[00101] Sensory aspects of coated food products are a consideration for customer satisfaction and acceptance of the developed technology. In this regard, color is one of the parameters that is controlled. Upon adding the phenolic phytochemicals and anti-fungal salts, pectin polysaccharide films acquired a yellowish tint which was slightly more intense in the case of the composition in Pectin 1 (FIG. 1).

[00102] The data on thickness, moisture content, and water solubility (after 1 hour) of the pectin coating films are presented in Table 1 below.

10 **[00103] Table 1 – Thickness, Moisture Content, and Water solubility of Pectin-Based Films**

Sample name	Thickness (mm)	Moisture content (%)	Water solubility after 1 h (%)
Pectin control	0.057±0.002	44.99±0.49	92.48±0.91
Pectin 1	0.089±0.002	16.82±0.22	89.41±0.75
Pectin 2	0.087±0.003	15.56±0.38	88.39±0.93

15 [00104] Adding phenolic phytochemicals and salts to pectin matrix increased thickness of the forming films by more than 30% and significantly reduced the film moisture content (by more than 60% compare to the Pectin control film). Water solubility of the pectin-based films decreased by less than 5% after adding of pathogen inhibitors. Table 1 also suggests no major difference in physical properties between the Pectin 1 and Pectin 2 film formulations.

20 [00105] Table 2 below shows the results of spectroscopic scanning of pectin-based films in the wavelength range from 200 nm to 800 nm.

[00106] Table 2 – Light Transmission (% T) of Pectin-Based Films

Formulation	Wavelength, nm						
	200	300	400	500	600	700	800
Pectin control	0.054	11.041	69.437	81.579	86.311	88.519	90.360
Pectin 1	0.017	0.005	44.390	74.513	83.987	87.762	89.661
Pectin 2	0.020	0.006	50.228	78.527	86.491	89.477	90.796

25 [00107] The polyphenolic additives and the anti-fungal salts improve the UV protective properties of the resultant pectin films. Compared to the control film, both formulations with pathogen inhibitors are not transparent at 300 nm and less transparent at 400 nm: by 36 % (Pectin 1) and 27 % (Pectin 2). The obtained results indicate that the developed

films are capable of protecting food products from UV irradiation including the potential to slow down ripening of coated fruits and vegetables.

[00108] Surface microphotographs and cross-sections of the pectin-based film are shown in FIG. 2. The presence of phenolic phytochemicals and anti-fungal salts rendered the film's inner structure more smoother and more homogeneous as compared to control samples of the pectin polysaccharide alone.

[00109] FIG. 3 shows the results of antimicrobial activity against *E. coli* ATCC 25922 and *S. aureus* ATCC 6538 and anti-fungal activity against *Botrytis cinerea* ATCC 28387 and *Strawberry wild mold*.

[00110] Phenolic compounds interact with the surface of pathogen cells, causing disintegration of the cell membrane and release of intracellular components, which leads to inhibition of growth or death of bacteria. The developed Pectin 1 and Pectin 2 films exhibited higher antimicrobial activity against Gram-positive *Staphylococcus aureus* bacteria than Gram-negative *Escherichia coli*, albeit there was still antimicrobial activity against Gram-negative microorganisms. Differences in the structure of the cell wall of Gram-positive and Gram-negative bacteria may be the cause of this effect. PS and SB additives in polysaccharide matrix contributed to anti-fungal activity and increased antimicrobial activity of the pectin-based films with added phenolic phytochemicals.

[00111] **Example 3A: Method of Producing Edible Coating Films Based on κ -Carrageenan**

[00112] κ -carrageenan-based (abbreviated as κ -carr) film-forming compositions and resultant films were obtained as follows:

[00113] κ -carr 1: 1.5 mL glycerol, 0.8 g SB, 0.6 g PS, and 3.0 g TA were added to 100 mL of DI water with constant stirring on a magnet plate for 30 mins at 150 rpm until complete dissolution of all components. Then 2.0 g κ -carrageenan was added to the mixture which was then stirred for another 10 min at 100 rpm. The obtained film-forming solution was heated up to 90 ± 5 °C for 15 mins with constant stirring until a homogeneous solution was achieved.

[00114] κ -carr 2: 0.4 g GA dissolved in 1.5 mL of glycerol was added to 100 mL of DI water. Then 0.8 g SB, 0.6 g PS and 3.0 g TA were added with constant stirring on a magnet plate for 30 mins at 150 rpm until complete dissolution of all components. Then

2.0 g κ -carrageenan was added to the mixture which was then stirred for another 10 mins at 100 rpm. The obtained film-forming solution was heated up to 90 ± 5 °C for 15 mins with constant stirring until a homogeneous solution was achieved.

[00115] Physical properties of the developed films and their antimicrobial and anti-fungal activities were studied against κ -carr control, a κ -carrageenan film containing no phenolic phytochemicals and anti-fungal salts. The κ -carr control film was obtained by dissolving 2.0 g of κ -carrageenan powder in 100 mL of DI water with constant stirring. Glycerol (1.5 mL) was then added as plasticizer. The obtained film-forming solution was heated up to 90 ± 5 °C for 15 mins with constant stirring until a homogeneous solution was achieved.

[00116] The films were casted by placing 13 mL of the film-forming solution into a glass Petri dish (9 cm in diameter) followed by drying at 55 ± 5 °C for 12 hrs.

[00117] **Example 3B: Physical, Antimicrobial and Anti-Fungal Properties of Films Based on κ -Carrageenan**

[00118] Photo images of films based on κ -carrageenan are presented in FIG. 4. The κ -carrageenan polysaccharide control film is colorless but acquires a yellow color after adding the phenolic phytochemicals and anti-fungal salts. Yellow color is visibly saturated in the κ -carr 1 film with the PS+SB+TA additive due to higher concentration of naturally yellowish TA in this mixture relative to the κ -carr 2 film containing PS+SB+TA+GA.

[00119] The thickness, the moisture content, and the water solubility of the κ -carrageenan coating films are shown in Table 3 below.

[00120] **Table 3 - Thickness, Moisture Content, and Water Solubility of films (after 1 hour) Based on κ -Carrageenan**

Sample name	Thickness (mm)	Moisture content (%)	Water solubility after 1 h (%)
κ -carr control	0.115 ± 0.004	47.43 ± 0.65	85.85 ± 0.93
κ -carr 1	0.227 ± 0.023	16.81 ± 0.39	88.22 ± 0.79
κ -carr 2	0.212 ± 0.028	15.84 ± 0.38	87.26 ± 0.27

[00121] Adding phenolic phytochemicals and salts to κ -carrageenan matrix increased the thickness of the forming films by ~50% and significantly reduced the film moisture content (by more than 60% compare to the pectin alone films). The water solubility of

the κ -carrageenan-based films was found slightly higher after adding of the pathogen inhibitors. Table 3 also suggests no major difference in physical properties between the κ -carr 1 and κ -carr 2 film formulations.

[00122] Table 4 below shows the results of spectroscopic scanning of κ -carrageenan-based films in the wavelength range from 200 nm to 800 nm. The polyphenolic additives drastically improved the UV protective properties of the κ -carrageenan-based films by complete blocking of light up to ~380 nm. Adding of the anti-fungal salts further decreased the transmission of the films enabled blocking in almost the entire UV region and reduced transmission of visible light. For instance, the κ -carr 1 film with the PS+SB+TA additive had the lowest level of transmission for UV light and the light transmission at 400 nm was 9.034 %, when the control film of κ -carr alone which displayed 74.208 %. The obtained results indicate the potential of the developed films to protect the food products from the damage caused by the UV irradiation.

[00123] Table 4 – Light Transmission (% T) of κ -carrageenan-based Films

Sample name	Wavelength, nm						
	200	300	400	500	600	700	800
κ -carr control	5.350	42.832	74.208	82.268	85.074	87.123	88.453
κ -carr 1	0.011	0.005	9.034	51.893	73.458	82.578	86.633
κ -carr 2	0.240	0.220	10.638	52.275	72.427	80.456	83.784

[00124] A surface microphotograph and cross-section of each film are shown in FIG. 5. With addition of the phenolic phytochemicals and anti-fungal salts, the film thickness increased as compared with control samples of the κ -carrageenan polysaccharide alone.

[00125] FIG. 6 show the results of antimicrobial activity against *E. coli* ATCC 25922 and *S. aureus* ATCC 6538 and anti-fungal activity against *Botrytis cinerea* ATCC 28387 and *Strawberry wild mold*.

[00126] Phenolic compounds interact with the surface of the cells, causing disintegration of the cell membrane and release of intracellular components, which leads to inhibition of growth or death of bacteria. The investigated κ -carrageenan-based films exhibited higher antimicrobial activity against Gram-positive *Staphylococcus aureus* bacteria than Gram-negative *Escherichia coli*, albeit there was still antimicrobial activity against Gram-negative microorganisms. Differences in the structure of the cell wall of Gram-positive and Gram-negative bacteria may be the cause of this effect. The

addition of PS and SB in the films contributed to the appearance of anti-fungal activity and increased antimicrobial activity.

[00127] Example 4: Prototyping - Coating of Fruits and Vegetables with Pectin-Based Films

5 **[00128]** Fruits of mango, tomato, banana, and cherry were coated with the developed Pectin 2 film and examined upon storage at ambient conditions. Different fruits and vegetables may differ in terms of their rate of transpiration (loss of moisture), ripening, and microbial spoilage. In the present examples, the fruits and vegetables were chosen in such a way to demonstrate the potential of the developed coating to tackle
10 undesirable/negative effects of each process thus prolonging the storage time for the coated specimens.

[00129] Specimens were immersed in the Pectin 2 coating film-forming solution for 1 min hanged up and air-dried at ambient conditions for 12-14 hrs. Coated specimens and uncoated controls were then observed for several days upon storage at room
15 temperature until the effect of the coating film became prominent.

[00130] a) Mango (prevention of loss of moisture)

[00131] Coated fruits of mango and uncoated controls were observed for 9 days upon storage at room temperature (FIG. 7A). Coated and uncoated specimens became visually different after 5 days of storage: whereas a coated specimen still had a smooth
20 and shiny skin, an uncoated specimen appeared dull and wrinkly. With a longer storage time, the observed difference between two specimens gradually became more significant. Beyond wrinkles, the surface of the uncoated sample acquired dark brown spots, which number was increasing upon storage. In contrast, the coated specimen had a spotless skin even after 9 days of storage at room temperature.

25 **[00132]** The difference between the initial and final weights of tested fruits was considered as the total weight loss during storage and was expressed as a percentage related to initial weight. At the end of the storage period, the total weight loss in the mango fruit coated with Pectin 2 was about 15.36% compared with 24.36% in the uncoated specimen. This reduction in weight loss indicates the effectiveness of the
30 developed coating film to prevent evaporation of moisture from mango fruits due to transpiration process characteristic to fruits and vegetables.

[00133] b) Tomatoes and bananas (prevention of over-ripening)

[00134] Three coated and three uncoated tomato specimens of different ripeness (red (fully ripen), brown (under-ripe), green (unripe)) were observed for 10 days upon storage at room temperature (FIG. 7B). FIG. 7B suggests that the initially unripe coated specimen reached full ripeness two days later than its uncoated counterpart. The developed coating preserved smooth and shiny skin of the specimens of all stages of ripeness over the whole observation period, whereas the ripen uncoated specimens displayed wrinkly skin already after the second day of storage. Visible sign of microbial spoilage appeared on the initially fully ripen uncoated specimen after 10 days of storage. At the same time, all coated specimens were visually of good quality without visible evidence of the weight loss or microbial contamination.

[00135] Coated banana specimens and uncoated controls were observed for 6 days upon storage at room temperature (FIG. 7C). After four days of storage, the uncoated specimens were fully spoiled turning brown unlike their coated counterparts, which did not reach that stage even after six days of storage.

15 **[00136] c) Cherry berries (prevention of microbial spoilage)**

[00137] 16 coated cherry specimens were compared with uncoated control group containing the same amount of berries over 14 day of storage at ambient conditions (FIG. 7D).

20 [00138] The Pectin 2 coated group revealed 25 % berries with mold vs 75 % of molded berries in the control group after 14 days of observations indicating the effectiveness of the developed coating film to prevent microbial spoilage.

[00139] Example 5: Stability of Resultant Films From Present Film-Forming Composition

25 [00140] Film stability (structural integrity and antimicrobial effect) was checked at different temperatures and relative humidity to determine their actual operating conditions.

[00141] To determine durability of antimicrobial and antifungal activity of the developed films, they were kept in a climate control chamber at 40 °C and 80 % RH for 7 and 14 days. Then their physical appearance and antimicrobial and anti-fungal activities were tested (FIG. 8A and 8B). Both pectin and κ -carrageenan films acquired darker shade at the storage conditions described above. Antimicrobial activity of the

films remained almost unchanged. However, no anti-fungal activity of the films stored for 7 days and onwards was observed.

[00142] Film structural stability to water condensation was checked 1 hr after coated specimens were transferred to ambient conditions from +4 °C refrigerator after 14 days of storage. No visible defects in the coatings were observed (FIG. 8C). The surface of coated specimens also had no sticky feel and the coating remained intact upon touching of specimens transferred from refrigerator. No signs of mold or microbial spoilage were detected on the coated specimens stored in the fridge for 14 days.

[00143] The film stability studies suggest their actual operating conditions for the best aesthetic appearance and pathogen inhibition effect are room temperature and ambient humidity or +4 °C refrigerator.

[00144] **Example 6: Summary, Commercial and Potential Applications**

[00145] In summary, the present disclosure describes the formulations of edible film-forming compositions for producing coating films on food products, not limited to fruits and vegetables, and possessing antimicrobial and anti-fungal properties. Beyond that, the films provide an aesthetic appearance to the coated commodities, protect them from UV light (premature over-ripening) and prevent evaporation of moisture (drying) caused by the process of transpiration characteristic to fruits and vegetables. Antimicrobial and anti-fungal activity of the films is based on a pathogen suppressive mixture of low-cost food grade antimicrobial phenolic phytochemicals (e.g. tannic acid, gallic acid) and two anti-fungal food additives, potassium sorbate and sodium benzoate, incorporated into a film matrix made of plant polysaccharides (pectin or κ -carrageenan). Synergy of the phytochemicals and salts provides the obtained films can inhibit both Gram-positive and Gram-negative bacteria and mold. The developed formulation contains no ingredients of animal origin and produced through a fully water-based method, thus the coating films are compatible with vegan, halal and kosher dietary requirements.

[00146] While the present disclosure has been particularly shown and described with reference to specific embodiments, it should be understood by those skilled in the art that various changes in form and detail may be made therein without departing from the spirit and scope of the present disclosure as defined by the appended claims. The scope of the present disclosure is thus indicated by the appended claims and all changes

which come within the meaning and range of equivalency of the claims are therefore intended to be embraced.