

A PACKAGING MATERIAL AND METHOD OF PREPARATION THEREOF

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application claims the benefit of priority of Singapore patent application No. 10201610373X filed on 9 December 2016, the content of which is incorporated herein by reference in its entirety for all purposes.

TECHNICAL FIELD

[0002] Various embodiments relate to materials and methods in general, and more particularly, to materials and methods for packaging applications, such as packaging materials used in food technology.

BACKGROUND

[0003] Currently, approximately 40 million tons of plastic packaging is used annually around the world, of which food and beverage packaging contributes to an estimated 70 % of the more than \$100 billion packaging market in the United States (US), and more than half of the \$400 billion worldwide market. An increasing, indiscriminate use of non-degradable polymers for food packaging applications is causing serious ecological problems due to their non-degradability, resulting in increasing environmental concerns regarding their disposal.

[0004] Landfill and incineration are the primary methods used in the disposal of these plastic wastes. However, there are limitations associated with these methods of waste disposal. For example, land filling is not sustainable in the long run as non-degradable polymers remain in the environment even after a very long time. As a result, urban planners are always faced with the pressure of having to source for more land space for land filling. On the other hand, burning plastics in incinerators produces toxic by-products that pollute the environment. Physical recycling of the plastic packaging is often impractical and economically inconvenient due to contamination by foodstuff and biological substances.

[0005] Global-wide concerns on environmental impacts caused by extensive usage of polymeric materials have directed much attention to the development of environmentally-friendly degradable polymers for food packaging applications. The use of polymers with a fully-degradable capability serves as a critical step to protect the community from an ever increasing environmental pollution.

[0006] In addition to the above, another important consideration in choice of packing materials for food packaging relates to capability of the packaging materials to maintain safety and quality of food products throughout storage, transportation and end-use. Toxic residues and corrosive solvents left behind during the fabrication process may cause food spoilage, and is harmful to food safety. At the same time, contamination of raw and processed products has always been a serious concern to human health, in particularly, those caused by psychrotrophic pathogens and spoilage micro-organisms.

[0007] One of the conventional methodologies to reduce and control the growth of micro-organism is using antimicrobial dips or sprays on the surface of the products. However, there is minimal success as the antimicrobial substances, when evaporating or diffusing into bulk food, may interact with the food components.

[0008] Other approaches have also been utilized to prolong the storage of green produce. These include high hydrostatic pressure, high intensity ultrasound, and gamma irradiation. However, these treatments have limited success as they can affect the sensory properties of the food products, change structures of the food's proteins, or produce free radicals that affect flavor of food.

[0009] Though the use of films with synthetic preservatives presents a possible solution, to inhibit the proliferation of food spoilage micro-organisms for increased food safety, and an extended shelf-life of food products, consumers have raised concern about the safety of foods coming directly in contact with synthetic preservatives, and there are controversies on the application of chemical additives to food products.

[0010] It has been estimated that despite various preservation methods, foodborne infections in the US have been increasing with time and caused an approximated 76 million cases of illnesses, 325,000 hospitalizations and 5000 deaths annually, of which over 5.2 million cases are attributed to foodborne bacterial pathogens. As a result, consumers are demanding the packaging materials to be formulated from the environmentally-friendly and degradable materials whilst improving food preservation abstaining from the usage of chemical preservatives.

[0011] Therefore, there is a demand for the development of a new packaging system that involves a green fabrication process and generates an environmentally-friendly food packaging film, which may possess an degradation ability in natural environment and at the same time, offers antimicrobial/antifungal properties to inhibit the proliferation of food

spoilage micro-organism, for increased food safety and an extended shelf-life of food products.

SUMMARY

5 [0012] In a first aspect, a packaging material is provided. The packaging material comprises a degradable polymer and a natural antimicrobial agent, wherein the degradable polymer forms a polymeric matrix within which the natural antimicrobial agent is dispersed.

[0013] In a second aspect, a method of preparing a packaging material according to the first aspect is provided. The method comprises providing a natural antimicrobial agent, and
10 dispersing the natural antimicrobial agent in a degradable polymer to obtain the packaging material.

[0014] In a third aspect, use of a packaging material according to the first aspect or a packaging material prepared by a method according to the second aspect in food packaging is provided.

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BRIEF DESCRIPTION OF THE DRAWINGS

[0015] The invention will be better understood with reference to the detailed description when considered in conjunction with the non-limiting examples and the accompanying drawings, in which:

20 [0016] **FIG. 1A** is a schematic diagram showing two-roll milling as a first step in fabricating green packaging films according to an embodiment. In the figure, PCL denotes poly(ϵ -caprolactone).

[0017] **FIG. 1B** is a schematic diagram showing combined sieving and two-roll milling as a second step in fabricating green packaging films according to an embodiment. In the figure,
25 PCL denotes poly(ϵ -caprolactone), CS denotes chitosan, and GFSE denotes grapefruit seed extract.

[0018] **FIG. 1C** is a schematic diagram showing heat pressing as a third step in fabricating green packaging films according to an embodiment. In the figure, PCL denotes poly(ϵ -caprolactone), CS denotes chitosan, and GFSE denotes grapefruit seed extract.

30 [0019] **FIG. 2A** is a schematic diagram showing dissolving step in fabrication of CS/GFSE particulates.

[0020] **FIG. 2B** is a schematic diagram showing freeze drying step in fabrication of CS/GFSE particulates.

[0021] **FIG. 2C** is a schematic diagram showing collection of powder step in fabrication of CS/GFSE particulates.

5 [0022] **FIG. 3A** is an optical image depicting gross appearance and flexibility of the as-fabricated PCL film.

[0023] **FIG. 3B** is an optical image depicting gross appearance and flexibility of the as-fabricated PCL/CS composite film (PCL/CS film: 15 wt.% of CS).

10 [0024] **FIG. 3C** is an optical image depicting gross appearance and flexibility of the as-fabricated PCL/CS/GFSE composite film (PCL/CS/GFSE film: 15 wt.% of CS/GFSE (CS:GFSE ratio, 1:1 g/ml)).

[0025] **FIG. 3D** is an optical image depicting gross appearance and flexibility of the as-fabricated CS/GFSE composite film (CS/GFSE film: 1:1 g/ml).

15 [0026] **FIG. 3E** is an optical image depicting gross appearance and flexibility of the as-fabricated PE film (PE denotes polyethylene).

[0027] **FIG. 3F** is an optical image depicting transparency of the as-fabricated PCL film.

[0028] **FIG. 3G** is an optical image depicting transparency of the as-fabricated PCL/CS composite film (PCL/CS film: 15 wt.% of CS).

20 [0029] **FIG. 3H** is an optical image depicting transparency of the as-fabricated PCL/CS/GFSE composite film (PCL/CS/GFSE film: 15 wt.% of CS/GFSE (CS:GFSE ratio, 1:1 g/ml)).

[0030] **FIG. 3I** is an optical image depicting transparency of the as-fabricated CS/GFSE composite film (CS/GFSE film: 1:1 g/ml).

[0031] **FIG. 3J** is an optical image depicting transparency of the as-fabricated PE film.

25 [0032] **FIG. 4A** is an optical image depicting droplet of water before (top) and after (bottom) sliding on PCL film. Contact angle is $74.4 \pm 1.4^\circ$.

[0033] **FIG. 4B** is an optical image depicting droplet of water before (top) and after (bottom) sliding on PCL/CS composite film. Contact angle is $75.0 \pm 2.7^\circ$.

30 [0034] **FIG. 4C** is an optical image depicting droplet of water before (top) and after (bottom) sliding on PCL/CS/GFSE composite film. Contact angle is $50.0 \pm 7.8^\circ$.

[0035] **FIG. 4D** is an optical image depicting droplet of water before (top) and after (bottom) sliding on PE film. Contact angle is $95.3 \pm 4.6^\circ$.

[0036] **FIG. 5A** is a scanning electron microscopy (SEM) image depicting surface morphology of PCL/CS composite film (PCL/CS film: 15 wt.% of CS). Scale bar denotes 50 μm .

[0037] **FIG. 5B** is a scanning electron microscopy (SEM) image depicting surface morphology of PCL/CS composite film (PCL/CS film: 15 wt.% of CS). Scale bar denotes 10 μm .

[0038] **FIG. 5C** is a scanning electron microscopy (SEM) image depicting cross-sectional view of PCL/CS composite film (PCL/CS film: 15 wt.% of CS). Scale bar denotes 100 μm .

[0039] **FIG. 5D** is a scanning electron microscopy (SEM) image depicting cross-sectional view of PCL/CS composite film (PCL/CS film: 15 wt.% of CS). Scale bar denotes 20 μm .

[0040] **FIG. 6** is a graph showing Fourier Transform Infrared spectroscopy (FTIR) spectra of PCL/CS composite film (PCL/CS film: 15 wt.% of CS) showing typical functional groups of PCL and CS.

[0041] **FIG. 7A** is a graph showing UV-vis spectra of PCL/CS composite film as compared to pure PCL film.

[0042] **FIG. 7B** is a graph showing antioxidant property of PCL/CS composite film (DPPH: 1,1-diphenyl-2-picrylhydrazyl; PCL/CS film: 15 wt.% of CS).

[0043] **FIG. 8** is a graph showing degradation profile of PCL/CS composite film in seawater as compared to pure PCL film (PCL/CS film: 15 wt.% of CS).

[0044] **FIG. 9** are optical images depicting preliminary study on fungal growth in bread samples packed in PE, PCL, PCL/CS and PCL/CS/GFSE films and stored at 24 °C for up to 13 days (Control groups: commercialised food packaging polyethylene (PE) film, and pure PCL film; PCL/CS film: 15 wt.% of CS; PCL/CS/GFSE film: 15 wt.% of CS/GFSE (CS:GFSE ratio: 1:1 g/ml); dark arrows: initial fungal growth).

DETAILED DESCRIPTION

[0045] The packaging material according to various embodiments disclosed herein has antimicrobial/antifungal properties due to presence of a natural antimicrobial agent as an additive in the packaging material. As such, in food packaging applications for example, it may be possible to achieve a delay or inhibition in the development of food spoilage micro-organisms by inhibiting undesirable micro-organisms from proliferating on food surfaces upon contact of the packaging material with the food items. In so doing, food safety for

public health and an extended shelf-life of food products may be achieved. Advantageously, the packaging material according to embodiments disclosed herein is an environmentally-friendly, green packaging material, and its preparation involves a green fabrication step free from chemical additives and toxic residues. The packaging material according to
5 embodiments disclosed herein may be particularly suitable as a food packaging material on various food products such as confectionaries, seafood, red meat, and dairy products, to name only a few.

[0046] With the above in mind, various embodiments refer in a first aspect to a packaging material comprising a degradable polymer and a natural antimicrobial agent.

10 [0047] As used herein, the term “packaging material” refers to a material for at least partially or completely encompassing or enclosing an object. By at least partially or completely encompassing or enclosing an object, the packaging material may function as a barrier layer or act an interface between the object and the environment to seal and/or protect the object from degradation and/or contamination by pollutants or contaminants that may be
15 present in the environment. Furthermore, the packaging material may be in direct contact with the object, which may translate into a decrease in the rate at which the object degrades due to action of the natural antimicrobial agent. In some embodiments, the packaging material may be used to form containers that may be used to hold or to accommodate perishable items, such as food items.

20 [0048] In various embodiments, the packaging material is a packaging film. For example, the packaging film may be in the form of a flat sheet or a continuous layer. The packaging material may, for example, be a food packaging film. In some embodiments, the packaging film is in direct contact with food, and may be used as a cling wrap, a cling film, or a food wrap for packaging food, or to seal food items in containers.

25 [0049] The packaging film may be of a sufficient thickness to provide barrier and mechanical properties as well as ease of handling, and may have a suitable thickness depending on intended application. For example, while thick films, such as films having a thickness of 50 μm or more, have better barrier properties in general, film flexibility and transparency may be compromised for such films. On the other hand, thin films, such as films
30 having a thickness of 10 μm or in the range of about 10 μm to about 20 μm , may result in less material usage, while still providing sufficient barrier and mechanical properties.

[0050] The packaging material comprises a degradable polymer and a natural antimicrobial agent. As used herein, the term “polymer” refers to a large molecule that is built up by repetition of smaller chemical units. The repeat unit of the polymer is usually equivalent or nearly equivalent to the monomer, i.e. starting material from which the polymer is formed. The repeat units of the polymer may be connected to one another via covalent bonds. In some cases, the repetition is linear, such as in the case of a polymer chain having a single backbone with no branches. In some cases, the chains are branched or interconnected to form three-dimensional networks.

[0051] The polymer is a degradable polymer, meaning that the polymer has a molecular structure which may be broken down or decompose into smaller molecules over a period of time (e.g., within days, or months, or years). The time required for degradation may depend on factors such as type of degradable polymer and environmental conditions. In various embodiments, the degradable polymer may degrade over a period of more than 20 years. In some embodiments, the degradable polymer may degrade over a period of up to 20 years, such as up to 15 years, 10 years, 8 years, 5 years, or 2 years. Degradation period of polycaprolactone film in a dry environment at room temperature, for example, may be up to 8 years. The degradation or decomposition of the degradable polymer may take place by various mechanisms due to action of, for example, naturally occurring microorganisms such as bacterial, fungi and algae; light; oxidation; and/or chemicals (such as water). The degradable polymer may accordingly be a biodegradable polymer, a photodegradable polymer, an oxidatively degradable polymer, and/or a hydrolytically degradable polymer.

[0052] Examples of degradable polymers include, but are not limited to, polymers and oligomers of glycolide, lactide, polylactic acid, polyesters of α -hydroxy acids, including lactic acid and glycolic acid, such as the poly(α -hydroxy) acids including polyglycolic acid, poly(DL-lactic-co-glycolic acid) (PLGA), poly-L-lactic acid (PLLA), and terpolymers of DL-lactide and glycolide; ϵ -caprolactone and ϵ -caprolactone copolymerized with polyesters; polylactones and polycaprolactones including poly(caprolactone) (PCL), poly(ϵ -caprolactone), poly(δ -valerolactone) and poly(gamma-butyrolactone); polyanhydrides; polyorthoesters; other hydroxy acids; polydioxanone; and other biologically degradable polymers that are non-toxic or are present as metabolites in the body. Examples of polyaminoacids include, but are not limited to, polylysine (PLL), poly L-aspartic acid, poly L-glutamic acid, and styrene-maleic acid anhydride copolymer. Examples of derivatives of

polyethylene glycol includes, but are not limited to, poly(ethylene glycol)-di-(ethylphosphatidyl(ethylene glycol)) (PEDGA), poly(ethylene glycol)-co-anhydride, poly(ethylene glycol)-co-lactide, poly(ethylene glycol)-co-glycolide and poly(ethylene glycol)-co-orthoester. Examples of acrylamide polymers include, but are not limited to, polyisopropylacrylamide, and polyacrylamide. Examples of acrylate polymers include, but are not limited to, diacrylates such as polyethylene glycol diacrylate (PEGDA), oligoacrylates, methacrylates, dimethacrylates, oligomethoacrylates and PEG-oligoglycolylacrylates. Examples of carboxy alkyl cellulose include, but are not limited to, carboxymethyl cellulose and partially oxidized cellulose.

10 [0053] In various embodiments, the degradable polymer is selected from the group consisting of poly(ϵ -caprolactone), aliphatic polyester, polylactide, poly(glycolide), poly(hydroxy ester ether), poly(hydroxybutyrate), poly(anhydride), polycarbonate, poly(amino acid), poly(ethylene oxide), poly(phosphazene), polyether ester, polyester amide, polyamide, combinations thereof, and copolymers thereof.

15 [0054] In specific embodiments, the degradable polymer comprises poly(ϵ -caprolactone).

[0055] The degradable polymer may have a weight average molecular weight in the range of about 60000 to about 100000, such as about 70000 to about 90000, or about 75000 to about 85000.

[0056] The packaging material also contains a natural antimicrobial agent. As used herein, 20 the term “natural” means that the substance may be found in nature. The term “antimicrobial” as used herein refers to the ability to inhibit, or control the spread or growth of microbes, which are organisms that are unicellular or live in a colony of cellular organisms. Examples of microbes include bacteria, mycobacteria, fungi, yeasts, archaea, viruses, protest, or parasites. The terms “antibacterial” and “antifungal” refer respectively to the ability to inhibit, or 25 control the spread or growth of bacterial and fungi. In some embodiments, the antimicrobial agent is an antibacterial and/or an antifungal agent.

[0057] In various embodiments, the antimicrobial agent is one that is able to inhibit, or control the spread or growth of microbes selected from the group consisting of Gram-positive bacteria, Gram-negative bacteria, fungus, and combinations thereof.

30 [0058] The term “Gram-positive bacteria” refers to bacterial cells which stain violet (positive) in the Gram stain assay. The Gram stain binds peptidoglycan which is abundant in the cell wall of Gram-positive bacteria. In contrast thereto, the cell wall of “Gram-negative

bacteria” is low in peptidoglycan, thus Gram-negative bacteria adopt the counterstain in the Gram stain assay. The bacteria may, for example, be of the genus *Acinetobacter*, *Actinomyces*, *Aeromonas*, *Bordetella*, *Borrelia*, *Brucella*, *Burkholderia*, *Campylobacter*, *Chlamydia*, *Clostridium*, *Corynebacterium*, *Enterococcus*, *Erwinia*, *Escherichia*, *Francisella*,
 5 *Haemophilus*, *Helicobacter*, *Klebsiella*, *Legionella*, *Leptospira*, *Listeria*, *Mycobacterium*, *Mycoplasma*, *Neisseria*, *Pseudomonas*, *Rickettsia*, *Salmonella*, *Shigella*, *Staphylococcus*, *Streptococcus*, *Treponema*, *Veillonella*, *Vibrio* or *Yersinia*. In specific embodiments, the bacteria is selected from the group consisting of *Staphylococcus aureus*, *Mycobacterium smegmatis*, *Pseudomonas aeruginosa*, *Burkholderia cepacia*, *Klebsiella pneumonia*,
 10 *Aeromonas hydrophila*, *Erwinia carotovora*, *Erwinia chrysanthemi*, and *Escherichia coli*.

[0059] The fungus may, for example, be of the species *Candida albicans*, *Candida tropicalis*, *Candida (Clasvispora) lusitaniae*, *Candida (Pichia) guilliermondii*, *Lodderomyces elongisporus*, *Debaryomyces hansenii*, *Pichia stipitis*, *Asperigillus fumigatus*, *Blastomyces dermatitidis*, *Cladophialophora bantiana*, *Coccidioides immitis*, *Cryptococcus neoformans*,
 15 *Fusarium spp.*, *Microsporum spp.*, *Penicillium marneffeii* or *Trichophyton spp.*

[0060] In various embodiments, the antimicrobial agent is one that is able to inhibit, or control the spread or growth of microbes selected from the group consisting of *Escherichia coli*, *Staphylococcus aureus*, *Candida albicans*, and combinations thereof.

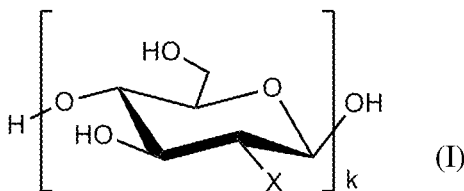
[0061] The natural antimicrobial agents may, for example, be obtained from natural
 20 compounds such as weak organic acids, organic compounds, antimicrobial enzymes, bacteriocins, triclosan, fungicides, nisin, fruit and plant extracts, essential oils, and/or natural polysaccharide. In some embodiments, the natural antimicrobial agent is selected from the group consisting of chitosan, chitin, lignin, derivatives thereof, combinations thereof, and copolymers thereof. Derivatives of lignin may include, for example, lignin esters, lignin
 25 ethers, hydroxyalkylated lignin, acylated lignin, carboxy lignins, or hydroxyalkoxy lignins.

[0062] In specific embodiments, the natural antimicrobial agent comprises chitosan. Chitosan has good biodegradability, biocompatibility, and antimicrobial activity, which render its usefulness for biomedical applications. The term “chitosan”, also referred to as poly-D-glucosamine or polyglucosamine, refers to a biopolymer derived from chitin that
 30 consists of β -1,4-glycosidic linked glucosamine and, optionally, N-acetylglucosamine residues (2-acetamido-2-desoxy- β -D-glucopyranose residues), wherein the ratio of glucosamine to N-acetylglucosamine residues is greater than 1.

[0063] The term "chitosan" as used herein also includes quaternized chitosan, also referred to herein as quaternary ammonium chitosan, which is a derivative of chitosan that is prepared by introducing a quaternary ammonium group on a dissociative hydroxyl group or amino group of the chitosan. As a consequence of the quaternization of the amino group, quaternized chitosan possess a permanent positive charge on the polysaccharide backbone. Due to this permanent positive charge, quaternized chitosan may also be termed as cationic quaternized chitosan.

[0064] In various embodiments, chitosan is represented by formula (I)

[0065]



[0066] wherein each X is independently selected from $-\text{NH}-\text{C}(\text{O})-\text{CH}_3$, $-\text{N}(\text{R}^1)(\text{R}^2)$ and $-\text{N}^+(\text{R}^3)(\text{R}^4)(\text{R}^5)$, provided that at least one X is $-\text{N}^+(\text{R}^3)(\text{R}^4)(\text{R}^5)$, R^1 , R^2 , R^3 , R^4 , and R^5 are independently selected from H and C_{1-18} alkyl, and k is an integer from 3 to 3000.

[0067] The term " $\text{C}_1\text{-C}_{18}$ alkyl" refers to a fully saturated aliphatic hydrocarbon having 1 to 18 carbon atoms, e.g. it means that the alkyl group comprises 1 carbon atom, 2 carbon atoms, 3 carbon atoms etc. up to and including 18 carbon atoms. The $\text{C}_1\text{-C}_{18}$ alkyl group may be straight chain or branched chain, and may be substituted or unsubstituted. Exemplary substituents include, but are not limited to, $\text{C}_1\text{-C}_6$ aliphatic group, hydroxy, alkoxy, cyano, halogen group, nitro, silyl, and amino, including mono- and di-substituted amino groups. Specific exemplary substituents include $\text{C}_1\text{-C}_{10}$ alkoxy, $\text{C}_5\text{-C}_{10}$ aryl, $\text{C}_5\text{-C}_{10}$ aryloxy, sulfhydryl, $\text{C}_5\text{-C}_{10}$ aryl, thio, halogen such as F, Cl, Br, I, hydroxyl, amino, sulfonyl, nitro, cyano, and carboxyl. Examples of alkyl groups may be, but are not limited to, methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, s-butyl, t-butyl, n-hexyl, n-heptyl, n-octyl, n-nonyl or n-decyl and the like.

[0068] Referring to formula (I), k is an integer from 3 to 3000. For example, k may be an integer from 3 to 2800, 3 to 2400, 3 to 1800, 3 to 1600, 3 to 600, 120 to 3000, 600 to 3000, 700 to 2500, 1500 to 2500, 1200 to 2400, 1600 to 2800, or 1800 to 2800.

[0069] In various embodiments, R^1 and R^2 are selected from H and C_{1-18} alkyl, and R^3 , R^4 , and R^5 are each independently C_{1-10} alkyl. In specific embodiments, R^1 and R^2 are H, and R^3 , R^4 , and R^5 are each independently C_{1-10} alkyl.

[0070] In various embodiments, R^3 and R^4 are methyl and R^5 is C_{1-10} alkyl, preferably methyl, ethyl, n-propyl, n-butyl, n-pentyl, n-hexyl, n-heptyl, n-octyl, n-nonyl or n-decyl. In specific embodiments, R^3 and R^4 are methyl and R^5 is selected from the group consisting of methyl, ethyl, n-propyl, n-butyl, n-pentyl, n-hexyl, n-heptyl, n-octyl, n-nonyl, and n-decyl.

[0071] In various embodiments, ratio of monomers with $X = -N(R^1)(R^2)$ and $-N^+(R^3)(R^4)(R^5)$ to those with $X = -NH-C(O)-CH_3$ is greater than 1. For example, the ratio of monomers with $X = -N(R^1)(R^2)$ and $X = -N^+(R^3)(R^4)(R^5)$ to monomers with $X = -NH-C(O)-CH_3$ may be in the range of 2:1 to 5:1.

[0072] The ratio of monomers with $X = -N(R^1)(R^2)$ to monomers with $X = -N^+(R^3)(R^4)(R^5)$, on the other hand, may be in the range of 1:4 to 4:1.

[0073] Although the natural antimicrobial agent is able to inhibit, or control the spread or growth of microbes, in various embodiments, the natural antimicrobial agent may further comprise a natural bioactive agent to enhance antimicrobial and/or antifungal properties of the natural antimicrobial agent.

[0074] As used herein, the term “bioactive agent” refers to a substance that has an effect such as a biological effect on a living organism. As in the case for the natural antimicrobial agent, the natural bioactive agent is a substance which may be found in nature. In various embodiments, the natural bioactive agent is selected from the group consisting of grapefruit seed extract, olive leaf extract, natamycin, chinokitiol, lysozyme, bacteriocins, essential oils, tea tree oil, lemon peel, pomelo, cinnamon, and combinations thereof.

[0075] In specific embodiments, the natural bioactive agent comprises or consists of grapefruit seed extract, which refers to one or more antimicrobial components derived from grapefruit seeds, obtainable by extraction using an organic solvent. The organic solvent may, for example, be an organic solvent containing one or more hydroxyl groups such as glycols.

[0076] Advantageously, combined usage of the natural antimicrobial agent and the natural bioactive agent provides an enhanced effect on inhibiting growth of microbes, such as that shown in **FIG. 9** (Group PCL/CS vs Group PCL/CS/GFSE). Ratio of the natural bioactive agent to the natural antimicrobial agent may be in the range of about 1:1 to about 3:1 ml/g to allow ease of fabrication, such as about 1:1 to about 3:2 ml/g, about 1:1 to about 2:1 ml/g,

about 1:1 to about 5:2 ml/g, about 3:2 to about 3:1 ml/g, about 2:1 to about 3:1 ml/g, or about 3:2 to about 2:1 ml/g.

[0077] The degradable polymer forms a polymeric matrix within which the natural antimicrobial agent is dispersed. For example, the natural antimicrobial agent may be in the form of particles. The natural microbial agent may be at least substantially uniformly dispersed in the polymeric matrix. By the term “substantially uniformly dispersed”, it is meant that distribution of the natural microbial agent within the degradable polymer is substantially even or similar in all regions therein, with no particular region in the degradable polymer in which concentration or proportion of the natural microbial agent is substantially greater or lesser as compared to any other region therein.

[0078] In various embodiments, the natural antimicrobial agent comprises particles having a size of 75 μm or less, such as a size in the range of about 5 μm to about 75 μm , about 10 μm to about 75 μm , about 20 μm to about 75 μm , about 30 μm to about 75 μm , about 50 μm to about 75 μm , about 5 μm to about 60 μm , about 5 μm to about 50 μm , or about 30 μm to about 50 μm .

[0079] Amount of the natural antimicrobial agent in the polymeric matrix may be in the range of about 0.01 wt% to about 40 wt%, such as about 0.01 wt% to about 35 wt%, about 0.01 wt% to about 20 wt%, about 0.01 wt% to about 15 wt%, about 1 wt% to about 25 wt%, about 15 wt% to about 40 wt%, or about 25 wt% to about 40 wt%. In some embodiments, amount of the natural antimicrobial agent in the polymeric matrix is in the range of about 0.01 wt% to about 15 wt %, which may be an optimal range to achieve mechanical properties, such as tensile strength and break strain, comparable to those exhibited by commercial polyethylene (PE) packaging films.

[0080] Various embodiments refer in a second aspect to a method of preparing a packaging material according to the first aspect. The method comprises providing a natural antimicrobial agent. Examples of suitable natural antimicrobial agent have already been discussed above.

[0081] As mentioned above, the natural antimicrobial agent may further comprise a natural bioactive agent. Suitable natural bioactive agents have already been mentioned above.

In various embodiments, the natural bioactive agent comprises or consists of grapefruit seed extract. Advantageously, even though natural bioactive agent of grapefruit seed extract has been exemplified herein, the method of preparing the packaging material is not limited to

grapefruit seed extract, and may be adopted for other types of natural bioactive agent such as those mentioned above since the method is not dependent on the chemical properties of the natural bioactive agent.

[0082] In embodiments wherein the natural antimicrobial agent further comprises a natural bioactive agent, providing the natural antimicrobial agent may comprise dissolving the natural antimicrobial agent and the natural bioactive agent in an aqueous solution, such as water or a buffer solution, to form an aqueous mixture, and drying the aqueous mixture to obtain the natural antimicrobial agent.

[0083] Dissolving the natural antimicrobial agent and the natural bioactive agent in an aqueous solution may, for example, be carried out with ratio of the natural bioactive agent to the natural antimicrobial agent in the range of about 1:1 to about 3:1 ml/g, or any suitable sub-ranges within the specified range. Advantageously, dissolving the natural antimicrobial agent and the natural bioactive agent in an aqueous solution may be carried out at ambient conditions. A substantial portion of or all of the natural bioactive agent and the natural antimicrobial agent may be dissolved in the aqueous solution to form the aqueous mixture.

[0084] The aqueous mixture may be dried using any suitable drying method to obtain the natural antimicrobial agent comprising the natural bioactive agent. In various embodiments, drying the aqueous mixture is carried by a two-step procedure of freeze drying and vacuum drying.

[0085] Various embodiments of the freeze drying technique disclosed herein are based upon principle of sublimation of water. During freeze drying, simultaneous action of vacuum and temperature may facilitate a tight packing order of the natural antimicrobial agent and the natural bioactive agent. At the same time, water present in the aqueous mixture may sublime. In so doing, water may be removed from the aqueous mixture to leave only the natural antimicrobial agent and the natural bioactive agent to allow physical binding of the natural bioactive agent to the natural antimicrobial agent. Advantageously, by controlling pressure and temperature, water may be evaporated or sublimed from the aqueous mixture to form the natural antimicrobial agent comprising the natural bioactive agent. This renders the process green as hazardous and toxic solvents are not used.

[0086] Freeze drying the aqueous mixture may be carried out at any suitable temperature which is sufficient to sublime water that is present in the mixture. In various embodiments, freeze drying the aqueous mixture is carried out at a temperature in the range of about -50 °C

to about 0 °C, such as about -30 °C to about 0 °C, about -10 °C to about 0 °C, about -50 °C to about -10 °C, about -50 °C to about -20 °C, about -50 °C to about -30 °C, about -40 °C to about -10 °C, about -30 °C to about -20 °C. In some embodiments, freeze drying the aqueous mixture is carried out at a temperature of about -40 °C.

5 [0087] The freeze drying may be carried out for a time period in the range of about 1 hour to about 3 hours, such as about 1.5 hours to about 2.5 hours, or about 2 hours. Subsequently, the aqueous mixture which has been subjected to freeze drying may be vacuum dried for a time period in the range of about 1 day to about 3 days, such as about 1.5 days to about 2.5 days, or about 2 days.

10 [0088] In various embodiments, the natural antimicrobial agent is in the form of particles, which may have a size of 75 µm or less.

[0089] The method disclosed herein comprises dispersing the natural antimicrobial agent in a degradable polymer. This may comprise melting the degradable polymer; mixing the natural antimicrobial agent with the molten degradable polymer to form a polymeric mixture;
15 and heat pressing the polymeric mixture.

[0090] Melting the degradable polymer may, for example, be carried out at a temperature above melting point of the degradable polymer using a two-roll milling process, such as that shown in **FIG. 1A**. The rollers may move in opposing directions, and shear force that is created by the rollers may be used to mix or disperse materials that is fed onto the rollers.

20 [0091] Speed at which the rollers rotate may be any suitable speed, such as a speed in the range of about 2 rpm to about 10 rpm. In embodiments wherein the degradable polymer is poly(ε-caprolactone), melting the degradable polymer may be carried out at about 65 °C.

[0092] Mixing the natural antimicrobial agent with the molten degradable polymer may comprise sieving the natural antimicrobial agent onto the molten degradable polymer while
25 the two-roll milling process is carried out, such as that depicted in **FIG. 1B**.

[0093] Upon forming the polymeric mixture, the polymeric mixture may be removed from the rollers and be subjected to a heat-pressing process, such as that shown in **FIG. 1C**. In various embodiments, heat pressing the polymeric mixture is carried out at a pressure in the range of about 20 MPa to about 40 MPa, such as about 25 MPa to about 35 MPa, or about 30
30 MPa. Upon heat pressing the polymeric mixture, the packaging material may be obtained in the form of a packaging film.

[0094] Various embodiments refer in a further aspect to use of a packaging material according to the first aspect or a packaging material prepared by a method according to the second aspect in food packaging. Advantageously, the packaging material according to embodiments disclosed herein have demonstrated suitable optical and mechanical properties as food packaging films, and with sufficient UV-absorption, antioxidant and antimicrobial/antifungal functions, but yet able to degrade naturally. The packaging material disclosed herein have the capability to degrade in natural environment (for example: in seawater), yielding non-toxic products, and with a slow degradation rate for ensuring long-term usage period for prolonged food safety against bacterial/fungal growth, thereby minimizing food wastage.

[0095] The invention has been described broadly and generically herein. Each of the narrower species and subgeneric groupings falling within the generic disclosure also form part of the invention. This includes the generic description of the invention with a proviso or negative limitation removing any subject matter from the genus, regardless of whether or not the excised material is specifically recited herein.

[0096] Other embodiments are within the following claims and non-limiting examples. In addition, where features or aspects of the invention are described in terms of Markush groups, those skilled in the art will recognize that the invention is also thereby described in terms of any individual member or subgroup of members of the Markush group.

EXPERIMENTAL SECTION

[0097] Various embodiments presents a method on the design and fabrication of green packaging films for food technology. Specifically, the films according to embodiments disclosed herein are designed with adequate optical and mechanical properties as food packaging films, and with sufficient UV-absorption, antioxidant and antimicrobial/antifungal functions, but yet able to degrade naturally. The films disclosed herein have the capability to degrade in natural environment (for example: in seawater), yielding non-toxic products, and with a slow degradation rate for ensuring long-term usage period for prolonged food safety against bacterial/fungal growth, thereby minimizing food wastage.

[0098] The green packaging films disclosed herein may comprise two components of:

[0099] (a) a degradable polymeric matrix as a barrier between food product and outside environment, having adequate optical and mechanical properties and at the same time, having

adequate UV-absorption and antioxidant functions. The polymeric matrix may also serve as a support for the natural additive portion; and

[00100] (b) a natural additive portion comprising small particulates that disperses homogeneously within the polymeric matrix, to generate antimicrobial/antifungal properties.

5 [00101] The green packaging films may be fabricated using a three-step physical method without using any solvents/chemicals. A degradable polymer (for example: poly(ϵ -caprolactone) (PCL) depicted in **FIG. 1A**) may first be melted to form a matrix via a two-roll milling technique at an elevated temperature just above the melting point (for example: 65 °C for PCL). Secondly, the matrix may be blended with small particulates (for example: chitosan
10 (CS) powder with a particle size less than 75 μ m) as an additive via a combination of sieving and two-roll milling techniques (**FIG. 1B**).

[00102] The small particulates additive portion may be altered by inclusion of natural component (for example: grapefruit seed extract (GFSE)) via a combination of dissolving and freeze drying techniques (**FIG. 2A** to **FIG. 2C**) to enhance antimicrobial/antifungal
15 properties. Finally, the composite pellets may then be heat-pressed into films (for example: at 65 °C and 30 MPa for PCL/CS and PCL/CS/GFSE films in **FIG. 1C**).

[00103] Incorporation of small amount of additive particulates in the polymeric matrix (for example: 15 wt.% of CS powder in PCL matrix) does not alter the gross appearance and flexibility (**FIG. 3A** and **FIG. 3B**) and transparency (**FIG. 3F** and **FIG. 3G**) of the green
20 packaging film as compared to those of the pure PCL film, respectively.

[00104] The additional incorporation of natural food preservatives (for example: GFSE/CS at a ratio of 1:1 ml/g (GFSE-volume/CS-weight)) also retains the film's gross appearance, flexibility (**FIG. 3C**) and transparency (**FIG. 3H**). In contrast, CS/GFSE film (for example: GFSE/CS, 1:1 ml/g) cannot be bent (**FIG. 3D**) and is totally opaque (**FIG. 3I**). Commonly-
25 used synthetic polyethylene (PE) film also shows a poor handling capability and tends to stick together easily (**FIG. 3E**) although it shows similar transparency to those of the PCL/CS and PCL/CS/GFSE films (**FIG. 3J**).

[00105] The incorporation of small amount of CS particulates (15 wt.%) or CS/GFSE particulates (15 wt.%; CS:GFSE ratio, 1:1 g/ml) results in slight changes to the water contact
30 angles on PCL/CS and PCL/CS/GFSE films as compared to that on pure PCL film (**FIG. 4A** to **FIG. 4D**). Although PE film has a higher water contact angle than those of PCL-based films, they demonstrate similar surface properties against water adhesion (**FIG. 4A** to **FIG.**

4D). With the incorporation of additive particulates at various weight percentages (for example: 0 to 25 wt.% of CS powder within the PCL matrix), mechanical properties of the green packaging films can be changed, and the tensile strength and break strain (for example: up to 15 wt.% of CS) are approaching to that of the PE films (7 to 30 MPa for tensile strength and 50 to 190 % for break strain; **TABLE 1**).

TABLE 1: Mechanical properties of green packaging films at different compositions. (n = 4)

Samples	Yong's Modulus (MPa)	Yield Stress (MPa)	Yield Strain (%)	Ultimate stress (MPa)	Break strain (%)	
PCL/CS film (wt.%)	0	239.1 ± 19.3	11.7 ± 1.2	9.6 ± 2.3	30.3 ± 1.1	1577.8 ± 180.8
	5	268.2 ± 14.4	12.0 ± 0.5	9.6 ± 1.0	15.2 ± 1.2	990.1 ± 90.6
	10	304.1 ± 9.9	13.4 ± 2.5	8.7 ± 0.8	13.6 ± 0.8	555.3 ± 110.7
	15	306.0 ± 19.7	11.9 ± 1.1	8.0 ± 0.3	13.4 ± 1.1	421.8 ± 38.4
	20	319.8 ± 15.0	11.2 ± 0.3	5.6 ± 0.6	12.5 ± 0.2	71.1 ± 17.2
	25	377.4 ± 21.0	11.5 ± 1.1	4.6 ± 0.5	12.9 ± 1.2	31.2 ± 11.3
PCL/CS/GFSE film*	291.0 ± 37.9	11.8 ± 2.2	7.0 ± 1.5	13.0 ± 2.2	194.4 ± 51.8	
PE film**	199.0 ± 24.1	7.8 ± 1.0	4.2 ± 0.1	30.5 ± 1.9	191.5 ± 67.2	
PE film***	182.0 ± 24.0	1.6 ± 2.1	/	6.8 ± 2.2	46.4 ± 2.5	

*: CS/GFSE = 1:1 (g/ml), (GS/GFSE)/PCL = 15:85 (g/g)

**: FDA compliant Diamond® Cling Wrap^{PE} from Reynolds Consumer Products Company

***: Pure PE film as reported in J. Chil. Chem. Soc. 2014,59(2): 2442-2446

The green packaging film exhibits a crack-free topography on its surface (**FIG. 5A** and **FIG. 5B**), and shows additive particulates dispersing uniformly within the polymeric matrix (**FIG. 5C** and **FIG. 5D**). Such film shows a combination of chemical properties from both the matrix and additive particulates, without inducing any changes to the typical chemical bonds of each component during the fabrication process (**FIG. 6**).

The green packaging film (for example: 15 wt.% of CS powder) obtains improved UV-absorption as compared to that of the pure PCL film (**FIG. 7A**), and exhibits a robust antioxidant capability with nearly 90 % of DPPH (DPPH: 1,1-diphenyl-2-picrylhydrazyl; a standard oxidation agent) being scavenged within 72 hr (**FIG. 7B**).

The film also exhibits a degradation ability in natural environment (for example: in seawater), and possesses a slow degradation rate, that can be altered via the incorporation of additive particulates (for example: 15 wt.% of CS powders in **FIG. 8**). Such film (for

example: containing 15 wt.% of CS powder and 15 wt.% of CS/GFSE powder) exhibits a potential to retard the incidence of fungal growth so as to increase the shelf-life of food products (for example: increasing the shelf-life of bread by approximate 2-3 times for the green packaging films as compared to the PE and PCL films in **FIG. 9**), being attributed to the incorporation of the additive particulates.

[00110] While the present invention has been particularly shown and described with reference to exemplary embodiments thereof, it will be understood by those of ordinary skill in the art that various changes in form and details may be made therein without departing from the spirit and scope of the present invention as defined by the following claims.

CLAIMS

1. A packaging material comprising a degradable polymer and a natural antimicrobial agent, wherein the degradable polymer forms a polymeric matrix within which the natural antimicrobial agent is dispersed.
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2. The packaging material according to claim 1, wherein the natural antimicrobial agent is selected from the group consisting of chitosan, chitin, lignin, derivatives thereof, combinations thereof, and copolymers thereof.
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3. The packaging material according to claim 1 or 2, wherein the natural antimicrobial agent comprises chitosan.
4. The packaging material according to any one of claims 1 to 3, wherein the natural antimicrobial agent further comprises a natural bioactive agent.
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5. The packaging material according to claim 4, wherein the natural bioactive agent is selected from the group consisting of grapefruit seed extract, olive leaf extract, natamycin, chinokitiol, lysozyme, bacteriocins, essential oils, tea tree oil, lemon peel, pomelo, cinnamon, and combinations thereof.
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6. The packaging material according to claim 4 or 5, wherein the natural bioactive agent comprises grapefruit seed extract.
- 25 7. The packaging material according to any one of claims 4 to 6, wherein ratio of the natural bioactive agent to the natural antimicrobial agent is in the range of about 1:1 to about 3:1 ml/g.
8. The packaging material according to any one of claims 1 to 7, wherein the natural antimicrobial agent comprises particles having a size of 75 μm or less.
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9. The packaging material according to any one of claims 1 to 8, wherein amount of the natural antimicrobial agent in the polymeric matrix is in the range of about 0.01 wt% to about 40 wt%.

10. The packaging material according to any one of claims 1 to 9, wherein amount of the natural antimicrobial agent in the polymeric matrix is in the range of about 0.01 wt% to about 15 wt %.
- 5 11. The packaging material according to any one of claims 1 to 10, wherein the degradable polymer is selected from the group consisting of poly(ϵ -caprolactone), aliphatic polyester, polylactide, poly(glycolide), poly(hydroxy ester ether), poly(hydroxybutyrate), poly(anhydride), polycarbonate, poly(amino acid), poly(ethylene oxide), poly(phosphazene), polyether ester, polyester amide, polyamide, combinations thereof, and copolymers thereof.
- 10 12. The packaging material according to any one of claims 1 to 11, wherein the degradable polymer comprises poly(ϵ -caprolactone).
- 15 13. The packaging material according to any one of claims 1 to 12, wherein the natural antimicrobial agent is at least substantially uniformly dispersed in the polymeric matrix.
- 20 14. The packaging material according to any one of claims 1 to 13, wherein the packaging material is a food packaging film.
15. A method of preparing a packaging material according to any one of claims 1 to 14, the method comprising
- 25 a) providing a natural antimicrobial agent, and
- b) dispersing the natural antimicrobial agent in a degradable polymer to obtain the packaging material.
16. The method according to claim 15, wherein the natural antimicrobial agent further comprises a natural bioactive agent.
- 30 17. The method according to claim 16, wherein providing the natural antimicrobial agent comprises
- a) dissolving the natural antimicrobial agent and the natural bioactive agent in an aqueous solution to form an aqueous mixture, and

b) drying the aqueous mixture to obtain the natural antimicrobial agent.

18. The method according to claim 17, wherein dissolving the natural antimicrobial agent and the natural bioactive agent in an aqueous solution is carried out at ambient conditions.
19. The method according to claim 17 or 18, wherein dissolving the natural antimicrobial agent and the natural bioactive agent in an aqueous solution is carried out with ratio of the natural bioactive agent to the natural antimicrobial agent in the range of about 1:1 to about 3:1 ml/g.
20. The method according to any one of claims 17 to 19, wherein drying the aqueous mixture is carried by a two-step procedure of freeze drying and vacuum drying.
21. The method according to claim 20, wherein freeze drying is carried out for a time period in the range of about 1 hour to about 3 hours.
22. The method according to claim 20 or 21, wherein vacuum drying is carried out for a time period in the range of about 1 day to about 3 days.
23. The method according to any one of claims 15 to 22, wherein the natural antimicrobial agent comprises particles having a size of 75 μm or less.
24. The method according to any one of claims 15 to 23, wherein dispersing the natural antimicrobial agent in a degradable polymer comprises
- a) melting the degradable polymer;
 - b) mixing the natural antimicrobial agent with the molten degradable polymer to form a polymeric mixture; and
 - c) heat pressing the polymeric mixture.
25. The method according to claim 24, wherein melting the degradable polymer is carried out at a temperature above melting point of the degradable polymer using a two-roll milling process.

26. The method according to claim 25, wherein mixing the natural antimicrobial agent with the molten degradable polymer comprises sieving the natural antimicrobial agent onto the molten degradable polymer while the two-roll milling process is carried out.

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27. The method according to any one of claims 24 to 26, wherein heat pressing the polymeric mixture is carried out at a pressure in the range of about 20 MPa to about 40 MPa.

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28. Use of a packaging material according to any one of claims 1 to 14 or a packaging material prepared by a method according to any one of claims 15 to 27 in food packaging.

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