



(51) International Patent Classification:

A01N 25/10 (2006.01) B65D 81/28 (2006.01)
B29C 47/06 (2006.01)

(21) International Application Number:

PCT/US2019/040454

(22) International Filing Date:

03 July 2019 (03.07.2019)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

62/694,609 06 July 2018 (06.07.2018) US

(71) Applicant: KANSAS STATE UNIVERSITY
RESEARCH FOUNDATION [US/US]; 2005 Research
Park Circle, Suite 105, Manhattan, 66502 (US).

(72) Inventors: TRINETTA, Valentina; 1208 S. Mill Point
Circle, Manhattan, Kansas 66503 (US). YUCEL, Umut;
1208 S. Mill Point Circle, Manhattan, Kansas 66503 (US).

(74) Agent: COOK, Crissa A.; Hovey Williams LLP, 10801
Mastin Blvd., Suite 1000, Overland Park, Kansas 66210
(US).

(81) Designated States (unless otherwise indicated, for every
kind of national protection available): AE, AG, AL, AM,
AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ,
CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO,
DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN,
HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP,
KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME,
MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ,
OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA,
SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN,
TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(54) Title: LIPID NANOEMULSION-DOPED ANTIMICROBIAL PACKAGING FILMS

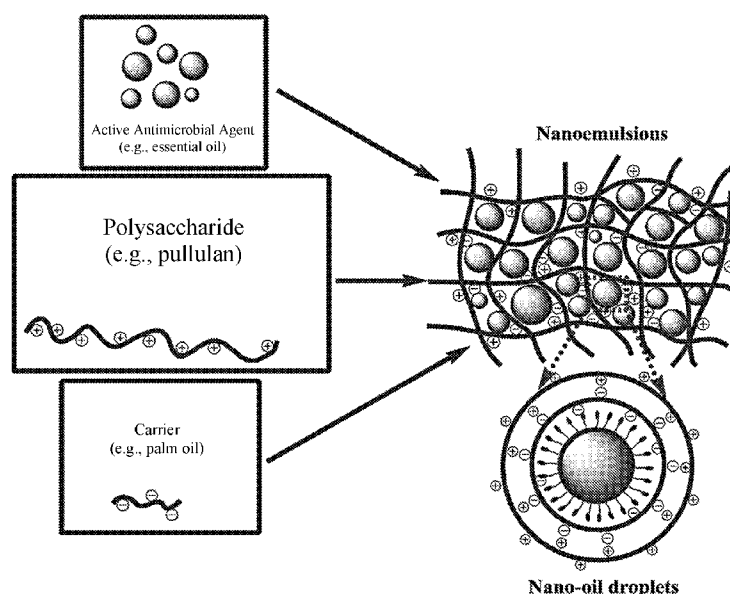


Fig. 1

(57) Abstract: Described herein are antimicrobial food-grade thin films, as well as their methods of manufacture and use. The films generally comprise an antimicrobial agent, such as an essential oil or essential oil mixtures, encapsulated in a carrier and dispersed in a film matrix. The film matrix generally comprises a polysaccharide, such as pullulan, and can be used in packaging systems. The films may comprise components that are edible, generally recognized as safe (GRAS), and/or approved by FDA. The films allow for the slow release of antimicrobial compounds over time, thereby controlling and/or inhibiting postharvest disease and extending the shelf-life of plants, including vegetables and/or small fruit specialty crops.



(84) **Designated States** (*unless otherwise indicated, for every kind of regional protection available*): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

- *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*
- *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

Published:

- *with international search report (Art. 21(3))*

LIPID NANOEMULSION-DOPED ANTIMICROBIAL PACKAGING FILMS

CROSS-REFERENCE TO RELATED APPLICATIONS

The present application claims the priority benefit of U.S. Provisional Application No. 62/694,609, filed July 6, 2018, entitled LIPID NANOEMULSION-DOPED ANTIMICROBIAL PACKAGING FILMS, incorporated by reference in its entirety herein.

BACKGROUND

Field

The present invention is generally directed to packaging films loaded with essential oil (EO) nano-emulsions droplets having desirable mechanical, physical, and anti-fungal properties.

Description of Related Art

Every year 40% of the food items intended for human consumption never reaches the table. The estimated value of food loss in 2010 for the United States was \$161.6 billion. Produce loss represented 19%, or \$30 billion, of that amount. Produce can be lost due to inadequate storage conditions, package failure, spoilage or degradation. Moreover, fruit and vegetables can be wasted when retailers or consumers discard them due to undesirable color or blemish. Berries are very perishable fruit with a short shelf-life; they are susceptible to water loss, bruising and mechanical injuries due to their soft texture and lack of a protective rind. Their high sugar content, high water activity and low pH represents an ideal environment for fungi and molds growth. Postharvest losses during berries harvesting and marketing are mainly caused by fungal disease. Fungi such as *Alternaria alternata*, *Aspergillus niger* and *Rhizopus stolonifera* are the most common cause of berries decay. Fruits that are lost due to these factors between harvest and sale represent a loss to producers that increases per-unit cost of production and decreases profits. Produce growers need cost-effective technologies to help decrease produce loss, preserve quality and safety so that a larger portion of their harvest reaches consumers' tables.

SUMMARY

Food-grade emulsions with sub-micron droplets were used to encapsulate essential oils within a carrier in pullulan-based film packaging systems. The formulation has a slow release of antimicrobial compounds over time. The films described herein are useful as an effective active

packing technology, which includes cooling as a triggering mechanism of antimicrobials release, to control postharvest disease and extend shelf-life of plants, including small fruit specialty crops.

In one embodiment, there is provided an antimicrobial food-grade thin film for food packaging. The film comprises an essential oil encapsulated in a carrier in oil-in-water emulsion droplets. The droplets are dispersed in a thin film matrix. The thin film matrix comprises one or more of a gum, plasticizer, emollient, and/or polysaccharide. In certain embodiments, there is provided an article for containing fruits and/or vegetables after harvest and/or for transport, storage, or sale, said article comprising the thin film. In certain embodiments, there is provided a method or preserving fruits and/or vegetables contained within the article, the method comprising cooling the article, thereby releasing the essential oil from the thin film.

In another embodiment, there is provided a method of forming an antimicrobial food-grade thin film. The method comprises dispersing oil-in-water emulsion droplets in a thin film matrix. The droplets comprise an essential oil encapsulated in a carrier. The thin film matrix comprises one or more of a gum, plasticizer, emollient, and/or polysaccharide. In certain embodiments, there is provided an antimicrobial food-grade thin film formed by the method.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure (Fig.) 1 is a representative drawing showing an antimicrobial food-grade thin film and its component parts, in accordance with an embodiment of the present invention;

Fig. 2 is a drawing showing manufacturing processes in accordance with an embodiment of the present invention;

Fig. 3 is a set of photographs showing antimicrobial testing results;

Fig. 4 is a set of photographs showing a film (A) alone, (B and C) in a clamshell for the field trial, and (D) strawberries in active packaging system;

Fig. 5 is a pair of photographs showing SEM images of (A) film without essential oils and (B) active SLN.

DETAILED DESCRIPTION

Embodiments of the present invention are generally directed to antimicrobial food-grade thin films for food packaging and methods of forming the same. The films comprise an antimicrobial agent, preferably an essential oil or mixture of essential oils, encapsulated in a carrier in oil-in-water emulsion droplets. The droplets are dispersed in a thin film matrix, as best shown in Fig. 1. The thin film matrix comprises one or more of a gum, plasticizer, emollient, and/or

polysaccharide. In certain embodiments, all of the components of the films are edible, generally recognized as safe (GRAS), and/or approved by FDA.

Antimicrobial agents in accordance with embodiments of the present invention include essential oils and mixtures of essential oils. In certain embodiments, the antimicrobial agent is an essential oil or essential oil mixture selected from the group consisting of cinnamaldehyde, eugenol, and/or thymol, and mixtures thereof. In particularly preferred embodiments, the antimicrobial agent is cinnamaldehyde. In certain embodiments, the antimicrobial agent is present in the antimicrobial food-grade thin film at an amount of about 1% to about 40% by weight, preferably about 5% to about 30% by weight, and more preferably about 10% to about 25% by weight, with the total weight of the thin film taken as 100% by weight (after curing).

Carriers in accordance with embodiments of the present invention are used to encapsulate the antimicrobial agent, for example in oil-in-water emulsion droplets (see Fig. 1). The carrier can be selected depending on whether a solid lipid nanoemulsion or (SLN) or liquid nanoemulsion (LNE) is desired. In certain embodiments, the carrier comprises one or more alkanes. In certain such embodiments, the carrier comprises liquid n-tetradecane and/or solid n-eicosane. In certain embodiments, the carrier comprises a lipid. In certain embodiments, the carrier comprises an organic oil. As used herein "organic oil" refers to an oil produced by plants, animals, and other organisms through natural metabolic processes. In certain embodiments, the carrier is a plant oil. In certain such embodiments, the carrier is a plant oil selected from the group consisting of palm oil, coconut oil, and combinations thereof. In certain embodiments, the carrier is present in the antimicrobial food-grade thin film at an amount of about 10% to about 70% by weight, preferably about 20% to about 60% by weight, and more preferably about 25% to about 55% by weight, with the total weight of the thin film (after curing) taken as 100% by weight.

The thin film matrix may comprise one or more of a gum, plasticizer, emollient, and/or polysaccharide. In certain embodiments, the thin film matrix comprises a gum selected from the group consisting of xanthan gum, locust bean gum, and combinations thereof. In certain embodiments, the gum is present in the antimicrobial food-grade thin film at an amount of about 0.1% to about 20% by weight, preferably about 1% to about 15% by weight, and more preferably about 2% to about 15% by weight, with the total weight of the thin film taken as 100% by weight (after curing). In certain embodiments, the thin film matrix comprises an emollient. In certain preferred embodiments, the emollient is glycerol. In certain embodiments, the emollient is present in the antimicrobial food-grade thin film at an amount of about 0.1% to about 10% by weight, preferably about 0.5% to about 8% by weight, and more preferably about 1% to about 5% by

weight, with the total weight of the thin film taken as 100% by weight (after curing). In certain embodiments, the thin film matrix comprises a plasticizer. The amount of plasticizer may be selected as needed or desired. In certain embodiments, the thin film matrix comprises a polysaccharide. In certain embodiments, the polysaccharide is a biopolymer (i.e., a polymeric substance occurring in living organisms), as opposed to a petroleum-based or synthetic polymer. In certain such embodiments, the polysaccharide is pullulan. In certain embodiments, the polysaccharide is present in the antimicrobial food-grade thin film at an amount of about 1% to about 50% by weight, preferably about 10% to about 40% by weight, and more preferably about 20% to about 35% by weight, with the total weight of the thin film taken as 100% by weight (after curing).

Methods of forming the antimicrobial food-grade thin film generally comprise dispersing oil-in-water emulsion droplets in the thin film matrix. In certain embodiments, the oil-in-water emulsion droplets are formed by mixing an antimicrobial agent (e.g., one or more essential oils or essential oil mixtures) in an aqueous solution comprising a carrier. In certain embodiments, the antimicrobial is added to the aqueous solution at about 1% to 30% by weight, preferably about 10% to about 20% by weight. In certain embodiments, the aqueous solution typically comprises about 1% to about 40%, preferably about 10% to about 30% by weight of the carrier. The aqueous solution may comprise one or more additives, which can be used, for example, to assist in forming the emulsion droplets. In certain embodiments, the aqueous solution comprises a phosphoprotein additive. In certain such embodiments, the aqueous solution comprises sodium caseinate (casein). In certain embodiments, the aqueous solution comprises about 0.1% to about 5%, preferably about 1% to about 3% by weight of a phosphoprotein additive. The droplets can then be dispersed in the thin film matrix. In certain embodiments, the thin film matrix is formed by adding one or more of a gum, plasticizer, emollient, and/or polysaccharide directly to aqueous solution. The solution can then be heated to a temperature of at least about 60 °C, preferably from about 60°C to about 90°C, more preferably about 75°C to about 85°C. In certain other embodiments, the thin film matrix is formed separately by mixing one or more of said gum, said plasticizer, said emollient, and/or said polysaccharide in water. The water with the film matrix components can then be heated to a temperature of at least about 50 °C, preferably from about 50 °C to about 90 °C, more preferably from about 60 °C to about 80 °C. The oil-in-water emulsion droplets can then be dispersed in the thin film matrix after the one or more of the gum, the plasticizer, the emollient, and/or the polysaccharide are substantially dissolved in the water. Regardless the method of forming the thin film matrix, the antimicrobial food-grade thin film can then be subsequently formed by cooling

the thin film matrix to a temperature of at least about 30 °C, preferably from about 0 °C to about 30°C, more preferably from about 15 °C to about 25 °C, thereby crystallizing the oil-in-water emulsion droplets dispersed in the thin film matrix.

5 The antimicrobial food-grade thin films formed in accordance with embodiments of the present invention advantageously has desirable mechanical and antimicrobial properties, while still being thin enough for use as a packaging film. In certain embodiments, the thin film has an average thickness of less than about 1 mm, preferably less than about 0.5mm. In certain embodiments, the thin film has a tensile strength of about 1 MPa to about 10 MPa, preferably about 3 MPa to about 7 MPa. In certain embodiments, the thin film has a moisture content of about 1%
10 to about 10%, preferably about 3% to about 8%. The antimicrobial food-grade thin film formed in accordance with embodiments of the present invention advantageously inhibits the growth of undesirable microbes on food, and particularly inhibits the growth of *Alternaria alternata*, *Aspergillus niger* and *Rhizopus stolonifera*.

The antimicrobial food-grade thin films are useful for a number of food storage and
15 preservation applications. In certain embodiments, the films can be used in conjunction with an article for containing fruits and/or vegetables after harvest and/or for transport, storage, or sale, wherein the article comprises the thin film (see, e.g., Fig. 5). The thin films are capable of reducing microbial growth on the fruits and/or vegetables. The articles and thin films can be used to preserve the fruits and/or vegetables contained within the article. In certain embodiments, the
20 article is transported, stored, or sold under refrigerated conditions. In certain embodiments, the article is cooled, thereby crystallizing the emulsion droplets and releasing the antimicrobial agent (e.g., essential oil or essential oil mixture) from the droplets. Without being bound by any theory, it is believed crystallization of the droplets results in expulsion of the encapsulated antimicrobial agent from the carrier droplets into the aqueous phase, thereby increasing available antimicrobial
25 agent and creating interfacial interactions. This can improve the antimicrobial activity of water-insoluble essential oils.

Additional advantages of the various embodiments of the invention will be apparent to those skilled in the art upon review of the disclosure herein and the working examples below. It will be appreciated that the various embodiments described herein are not necessarily mutually
30 exclusive unless otherwise indicated herein. For example, a feature described or depicted in one embodiment may also be included in other embodiments, but is not necessarily included. Thus, the present invention encompasses a variety of combinations and/or integrations of the specific embodiments described herein.

As used herein, the phrase "and/or," when used in a list of two or more items, means that any one of the listed items can be employed by itself or any combination of two or more of the listed items can be employed. For example, if a composition is described as containing or excluding components A, B, and/or C, the composition can contain or exclude A alone; B alone; C alone; A and B in combination; A and C in combination; B and C in combination; or A, B, and C in combination.

The present description also uses numerical ranges to quantify certain parameters relating to various embodiments of the invention. It should be understood that when numerical ranges are provided, such ranges are to be construed as providing literal support for claim limitations that only recite the lower value of the range as well as claim limitations that only recite the upper value of the range. For example, a disclosed numerical range of about 10 to about 100 provides literal support for a claim reciting "greater than about 10" (with no upper bounds) and a claim reciting "less than about 100" (with no lower bounds).

EXAMPLES

The following examples set forth methods in accordance with the invention. It is to be understood, however, that these examples are provided by way of illustration and nothing therein should be taken as a limitation upon the overall scope of the invention.

MATERIALS. Pullulan was selected as the because it is biobased, renewable and sustainable.

ACTIVE INGREDIENTS. The ability of essential oils (such as cinnamaldehyde) to control the growth of pathogen, yeast and spoilage microorganisms has been largely demonstrated, indicating their promising use to assure safety and extend shelf-life of food products. Moreover, these compounds are generally recognized as safe (GRAS) and are already approved by FDA (FDA, 2005). Two concentrations were studied and characterized: 10% and 20% wt. These amounts were determined based on our exploratory studies.

LIPID CARRIER SYTEMS. Preliminary studies show that the antimicrobial effectiveness of active ingredients can be enhanced when encapsulated in emulsion-based delivery systems. Nanoemulsions and solid lipid nanoemulsion (SLN) were designed by using liquid n-tetradecane (C₁₄, m.p. below 0 °C) and solid n-eicosane (C₂₀, m.p. 37 °C) as carrier oils. Carrier systems using alkanes (as they are chemically inert) and demonstrate homogenous composition (pure compounds), giving well-defined crystalline structure contrary to real lipids.

In the present study, nanoemulsions and SLN were prepared using a hot-homogenization technique. The lipid phase was prepared by mixing active ingredients (10 and 20 wt%) with the carrier "lipid." Nanoemulsions and SLN were also formulated to encapsulate active compounds by using Miglyol 812 (i.e., a stable liquid lipid composed of saturated coconut and palm kernel oil-derived caprylic and capric fatty acids with melting point below 0 °C), and hydrogenated palm oil (a stable solid lipid mainly composed of triglycerides of stearic and palmitic acids with its melting point in the range between 50-55 °C). An emulsion premix was prepared by mixing the lipid (10 wt%) with a Na-caseinate solution (2 %wt) using a high-speed mixer (Brinkmann Polytron, Brinkmann Instruments Inc., Westbury, NY). This coarse emulsion was passed through a 2-stage valve homogenizer (Panda Plus 2000, GEA) at pressures of 60 and 600 bars for the first and second valves, respectively at 60-65 °C for 3 times. The pharmaceutical grade homogenizer provides narrow particle size distribution of ca. 0.2 µm for the droplets, which will be measured by using DelsaMax Pro (Beckman Coulter, Indianapolis, IN) equipped with an DelsaMax Assist unit (Beckman Coulter, Indianapolis, IN). Samples were sterilized in the autoclave (120 °C for 20 min) and then used in film and nanofiber preparation. The crystallization and melting profiles of SLN were analyzed and verified by using a modulated differential scanning calorimeter (Q2000, TA Instruments - Waters L.L.C., New Castle, DE). See Fig. 1.

FILMS BY CASTING. For pullulan films production, two film formulations were developed and tested. The formulations and manufacturing processes were as follows (See Fig. 2):

a) Pullulan (10g/l), glycerol (1 g/l), xanthan gum (1 g/l) and locust bean gum (1 g/l) was mixed in water at 70-80 °C for 20 min and then autoclaved at 121 °C for 15 min for faster and complete dissolution of ingredients. During cooling, active nanoemulsions and SLN were added (1:1) to the film solution in a shaking water bath at 80 °C for 30 min.

b) Pullulan (7.5 g/l), plasticizer (0.75 g/l), xanthan gum (0.75 g/l) and locust bean gum (0.75 g/l) were directly added to the active emulsions. The mixture was kept in a shaking water bath at 80 °C for 30 min to allow complete dissolution and mixing of components.

Films were then cast by pouring solutions onto an ultraviolet light-sterilized aluminum sheet pan (17 x 11 inch) and allow to dry for 24 h at room temperature and 40% relative humidity. Films were stored at refrigerated temperatures to allow crystallization of SLN to completion (the crystallization point is 20 °C). Fig. 2 illustrates the casting process: A) mixing of ingredients; B) film solution; C) solution poured in plates or pans.

Each film with and without active lipid solutions was produced in triplicate and characterized as described below.

MECHANICAL PROPERTIES. Thickness and ultimate tensile strength were measured. Thickness was evaluated with a micrometer at 5 random locations and an average measurement for each replicate will be reported at each run. Strips of film 1 cm wide x 5 cm long were used for the measurements. A texture analyzer was used to evaluate ultimate tensile strength following the Standard Test Methods for tensile properties of thin plastic sheeting (ASTM D882-02).

PHYSICAL PROPERTIES. Film moisture content was determined by air oven method (difference in weight) and expressed as percentage.

ANTIMICROBIAL PROPERTIES. The antimicrobial activity of films with and without active lipid solutions was qualitatively determined against *Alternaria alternata*, *Aspergillus niger* and *Rhizopus stolonifera* using the plate overlay assay. Potato Dextrose agar plates were overlaid with 8 ml of semisoft agar (0.5% wt/vol agar) seeded with 100 µl of overnight broth culture of the test microorganisms. Treated samples and controls (specimen dimension: 1 cm x 1 cm) were placed directly on the plate and incubated at the optimal temperature of growth of the test organisms. Plates were scored for inhibition zones after 24 h of incubation. Diameters (mm) of inhibition halos were measured and reported in mm.

Results

Summary of Results

- There was no significant difference ($P < 0.05$) in tensile strength between films loaded with EO emulsions as compared to films without active lipid solutions (CONT) (Table 1).
- Active film combinations presented good elasticity and ductility.
- Films loaded with active SLN and LNE had higher moisture contents as compared to film without EO ($P < 0.05$).
- Control formulations showed no antifungal activity (Table 2).
- Active combinations exhibited significant inhibition zones against the selected post-harvest fungal pathogens ($P < 0.05$).
- Film containing CIN had the biggest inhibition halos against *R. stolonifer*, *Alternaria spp.* and *A. niger* as compared to other active formulations (Table 2).

Table 1. Tensile Strength and moisture content of LNE and SLN formulation 1 and 2 with and without active ingredients.

LNE								
Formulation 1					Formulation 2			
	CIN	EUG	THY	CONT	CIN	EUG	THY	CONT
TS (MPa)	5.67±0.41 ^A	4.38±1.32 ^A	6.79±2.62 ^A	5.43±0.57 ^A	3.93±2.40 ^A	5.90±2.79 ^A	4.50±0.51 ^A	3.34±0.29 ^A
Moisture (%)	4.09±0.68 ^{BCD}	5.33±0.61 ^{AB}	6.23±1.46 ^A	3.39±0.14 ^{CD}	4.12±0.20 ^{BCD}	5.46±0.83 ^{AB}	6.11±0.33 ^A	2.91±0.12 ^D

SLN								
Formulation 1					Formulation 2			
	CIN	EUG	THY	CONT	CIN	EUG	THY	CONT
TS (MPa)	3.12±2.11 ^A	5.09±1.00 ^A	6.29±1.46 ^A	6.75±0.83 ^A	3.27±1.02 ^A	6.08±1.46 ^A	7.20±3.76 ^A	4.28±1.85 ^A
Moisture (%)	5.84±0.65 ^{BCD}	9.50±1.72 ^A	6.34±0.68 ^{BC}	4.11±0.86 ^{CD}	5.45±0.36 ^{BCD}	7.96±1.30 ^{AB}	6.27±0.64 ^{BC}	3.68±0.58 ^D

Table 2. Antifungal activity of LNE and SLN formulation 1 and 2 with and without active ingredients, measured as diameter of inhibition halos (mm).

LNE								
Formulation 1					Formulation 2			
	CIN	EUG	THY	CONT	CIN	EUG	THY	CONT
<i>Alternaria spp.</i>	15.67±2.31 ^A	14.33±2.36 ^A	12.67±1.16 ^A	0±0 ^B	14±4.36 ^A	12.17±1.76 ^A	11±0 ^A	0±0 ^B
<i>A. niger</i>	14.5±2.18 ^A	13±1.32 ^{AB}	11.67±1.15 ^{AB}	0±0 ^C	12±1 ^{AB}	10.67±0.29 ^B	10.83±0.29 ^B	0±0 ^C
<i>R. stolonifer</i>	13.67±0.76 ^A	13.5±1.32 ^A	12±0 ^A	0±0 ^B	12.5±0.87 ^A	11.83±1.44 ^A	11.33±0.58 ^A	0±0 ^B

SLN								
Formulation 1					Formulation 2			
	CIN	EUG	THY	CONT	CIN	EUG	THY	CONT
<i>Alternaria spp.</i>	20.25±6.90 ^A	12.5±0.74 ^A	13.25±1.14 ^A	0±0 ^B	16.83±6.85 ^A	12.33±1.33 ^A	10.58±0.83 ^{AB}	0±0 ^B
<i>A. niger</i>	15.92±2.28 ^A	12.58±0.83 ^{ABC}	11.92±1.16 ^{BC}	0±0 ^D	14.92±0.62 ^{AB}	11.08±0.31 ^C	10.42±0.24 ^C	0±0 ^D
<i>R. stolonifer</i>	18.17±2.321 ^A	13.5±2.13 ^{AB}	11.58±1.59 ^B	0±0 ^C	13.83±1.20 ^{AB}	10.83±1.18 ^B	12.17±2.89 ^B	0±0 ^C

After a preliminary evaluation of feasibility and visual appearance, these two film compositions were studied and characterized both for nanoemulsion and SLN incorporation into pullulan film:

Table 3.

	Set 1	Set 5
Pullulan (g)	2.5	2.5
Xanthan (g)	0.25	0.25
Locust Bean gum (g)	0.25	0.25

Glycerol (g)	0.25	0.25
Water (mL)	25	0
Emulsion (mL)	25	50
Essential Oil (g)	2	1

Table 4. Mechanical Properties Results

	SLN			NANOEMULSION		
	Set 1	Set 5	Control	Set 1	Set 5	Control
THICKNESS (mm)	0.317±0.025	0.213±0.023	0.247±0.035	0.139±0.021	0.186±0.026	0.151±0.025
Area Positive peak (g/sec)	10.72 ±4.98	19.00±3.25	25.70±6.59	18.21±3.22	19.06±6.20	29.36 ±2.38

A lower tensile strength ($P > 0.05$) was measured for combinations loaded with EO as compared to films without active lipid solutions.

Nevertheless, the active combinations presented good elasticity and ductility.

Table 5. Antimicrobial Activity Results (expressed as zone of inhibition in mm)

	SLN			NANOEMULSION		
	Set 1	Set 5	Control	Set 1	Set 5	Control
<i>Alternaria alternata</i>	20.25±1.90	16.83±1.52	Not detected	15.67± 2.31	14± 4.36	Not detected
<i>Aspergillus niger</i>	15.92±2.28	14.92±0.62	Not detected	14.5± 2.18	12± 1	Not detected
<i>Rhizopus stolonifera</i>	18.17±2.32	13.89±2.85	Not detected	13.67± 0.76	12.5± 0.87	Not detected

As shown in Fig. 3., the control formulations showed no antifungal activity. Conversely, active combinations exhibited significant inhibition zones ($P < 0.05$).

Between active set 1 nanoemulsion film and active SLN film the antimicrobial activity of SLN set 1 film was the greatest.

This formulation was then used for the field trial on strawberries. Fig. 4 shows active SLN set 1 pullulan film: A) alone B) and C) in a clamshell for the field trial; and D) strawberries in active packaging system.

Fig. 5 shows SEM images of A) film without essential oils; and B) active SLN pullulan film with cinnamaldehyde emulsions.

This study demonstrates the potential application of pullulan packaging films loaded with active emulsions against post-harvest disease.

Pullulan is a natural polysaccharide polymer used to create strong, transparent, edible films. In one or more embodiments, essential oils, such as cinnamaldehyde, eugenol and/or thymol within a carrier (such as palm oil) were formulated as active ingredients in pullulan packaging systems. Emulsions were prepared using a hot-homogenization technique. Palm oil (SLN) and coconut oil (LNE) were the carrier systems. The lipid phase was prepared by mixing active ingredients (10 and 20 wt%) with the carrier lipid. An emulsion premix was prepared by mixing the lipid (10 wt%) with a Na-caseinate solution (2 %wt). The active ingredients selected for this study were: cinnamaldehyde, eugenol, and thymol. The crystallization of the droplets (fine oil-in water emulsion with crystalline droplets) results in expulsion of the encapsulated antimicrobial material from the lipid droplets into the aqueous phase and create interfacial interactions that improved the antimicrobial activity of water-insoluble essential oils. Best formulation in terms of tensile strength, moisture content and antimicrobial activity was: 10 % pullulan, 2% essential oils, 50% water and 1% gum.

CLAIMS:

1. An antimicrobial food-grade thin film for food packaging comprising an essential oil encapsulated in a carrier in oil-in-water emulsion droplets, said droplets being dispersed in a thin film matrix comprising one or more of a gum, plasticizer, emollient, and/or polysaccharide.

5

2. The thin film of claim 1, wherein said polysaccharide is pullulan.

3. The thin film of claim 1, wherein said gum is selected from the group consisting of xanthan gum, locust bean gum, and combinations thereof.

10

4. The thin film of claim 1, wherein said emollient is glycerol.

5. The thin film of claim 1, wherein said essential oil is selected from the group consisting of cinnamaldehyde, eugenol, thymol, and mixtures thereof.

15

6. The thin film of claim 1, wherein said carrier is a plant oil.

7. The thin film of claim 6, wherein said carrier is selected from the group consisting of palm oil and coconut oil, and combinations thereof.

20

8. The thin film of claim 1, wherein said thin film has an average thickness of less than about 1 mm.

9. The thin film of claim 8, wherein said thin film has an average thickness of less than about 0.5 mm.

25

10. An article for containing fruits and/or vegetables after harvest and/or for transport, storage, or sale, said article comprising a thin film according to any one of claims 1-9.

11. The article of claim 10, wherein said thin film reduces microbial growth on said fruits and/or vegetables after harvest or during said transport, storage, or sale of said fruits and/or vegetables.

30

12. A method of preserving fruits and/or vegetables contained within the article according to claim 10, said method comprising cooling said article, thereby releasing said essential oil from said thin film.

5 13. A method of forming an antimicrobial food-grade thin film, the method comprising dispersing oil-in-water emulsion droplets in a thin film matrix, said droplets comprising an essential oil encapsulated in a carrier, said thin film matrix comprising one or more of a gum, plasticizer, emollient, and/or polysaccharide.

10 14. The method of claim 13, wherein said oil-in-water emulsion droplets are formed by mixing said essential oil in an aqueous solution comprising said carrier.

15 15. The method of claim 14, wherein said aqueous solution comprises a phosphoprotein.

16. The method of claim 14, wherein said thin film matrix is formed by adding said one or more of said gum, said plasticizer, said emollient, and/or said polysaccharide directly to said aqueous solution and heating to a temperature of at least about 80 °C.

20 17. The method of claim 13, wherein said thin film matrix is formed by mixing said one or more of said gum, said plasticizer, said emollient, and/or said polysaccharide in water and heating to a temperature of at least about 70 °C.

25 18. The method of claim 17, wherein said oil-in-water emulsion droplets are dispersed in said thin film matrix after said one or more of said gum, said plasticizer, said emollient, and/or said polysaccharide are substantially dissolved in said water.

30 19. The method of claim 13, further comprising cooling said thin film matrix to a temperature of at least about 20 °C, thereby crystallizing said oil-in-water emulsion droplets dispersed in said thin film matrix.

20. An antimicrobial food-grade thin film formed by the method according to any one of claims 13-19.

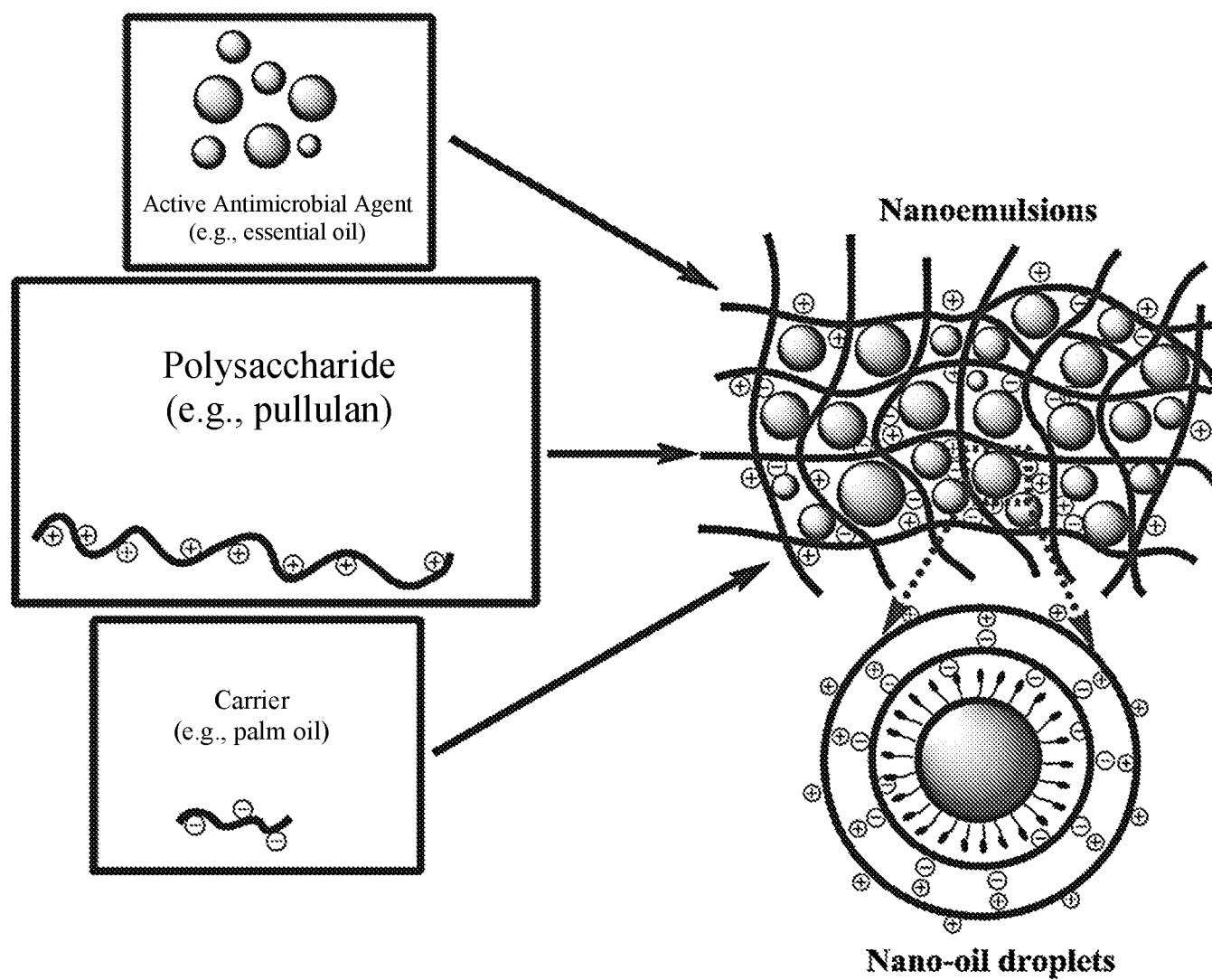


Fig. 1

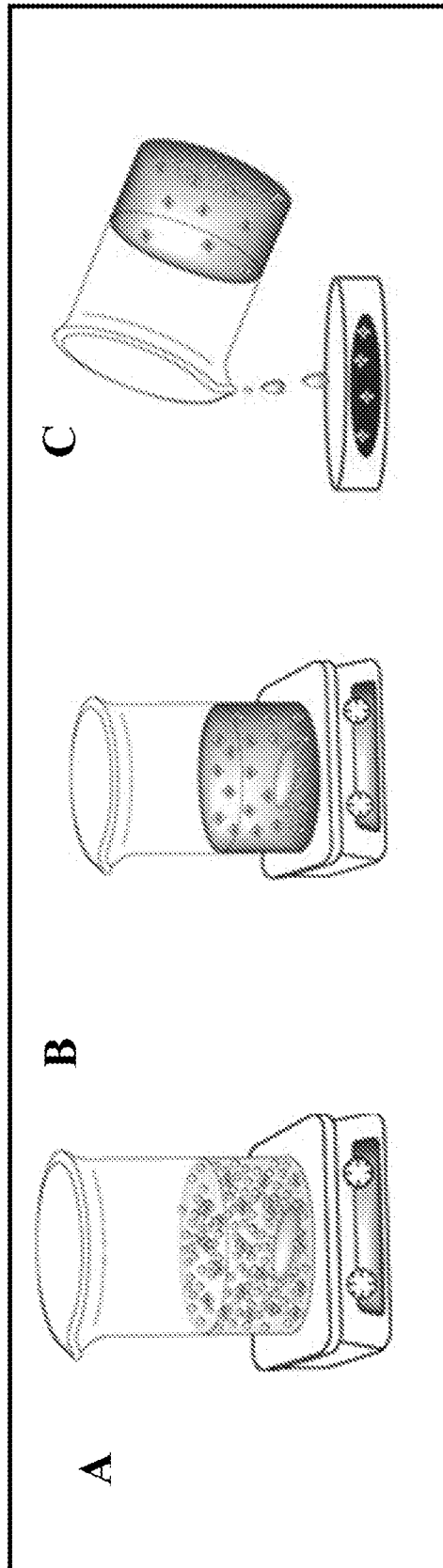
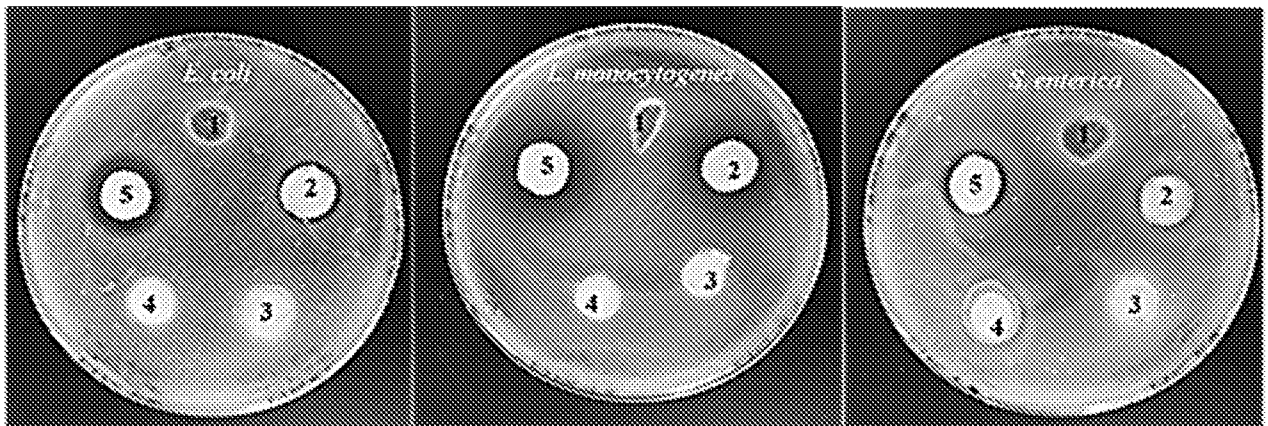
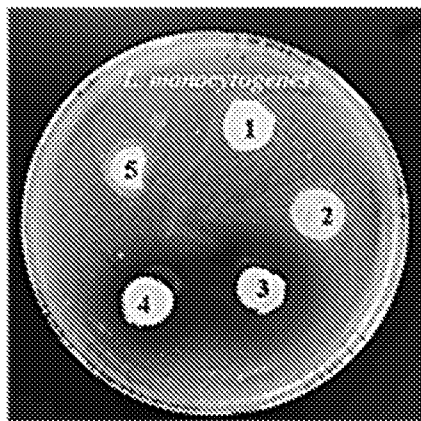


Fig. 2



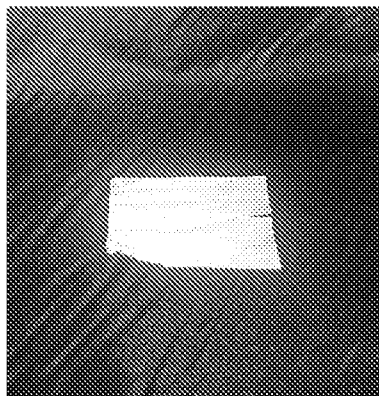
1. pullulan control; 2. nanoemulsion loaded with limonene; 3. nanoemulsion control; 4. SNL control; 5. SNL loaded with limonene.



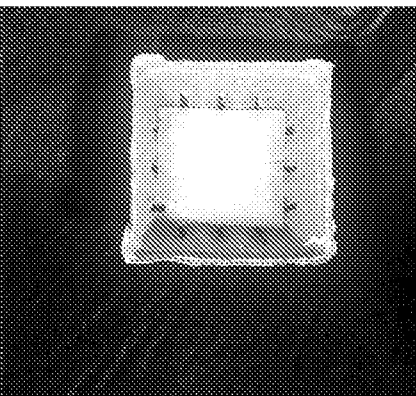
1. pullulan control; 2. nanoemulsion loaded with limonene; 3. nanoemulsion control; 4. SNL control; 5. SNL loaded with limonene.

Fig. 3

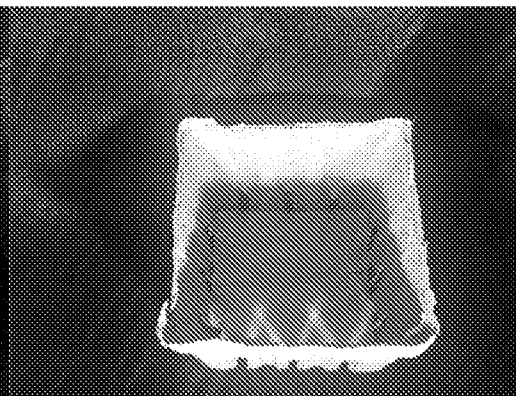
A



B



C



D

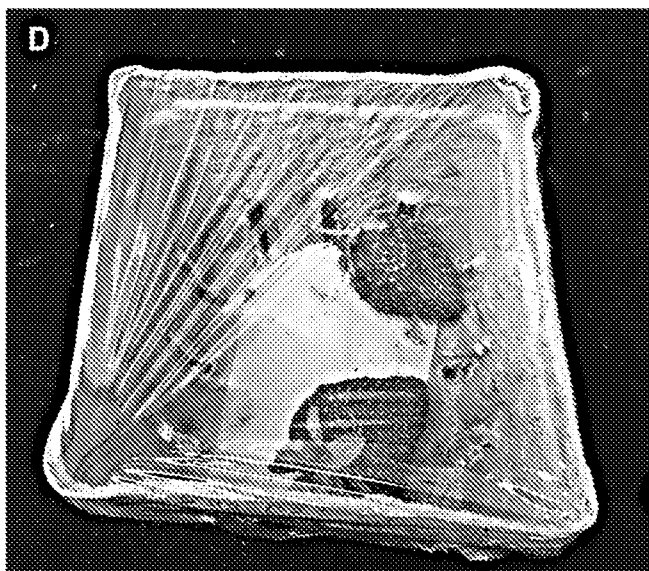


Fig. 4

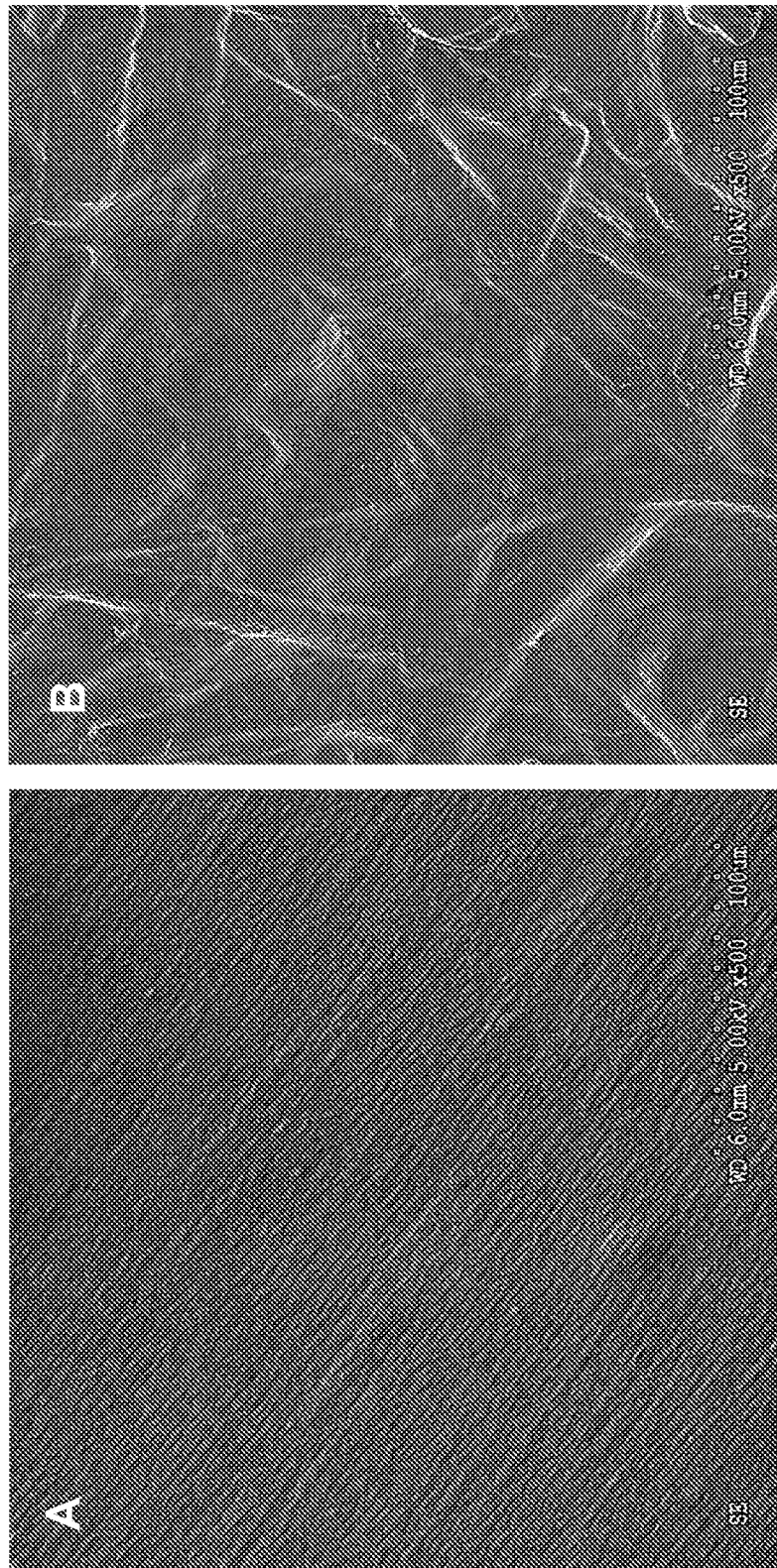


Fig. 5

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 19/40454

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A01N 25/10; B29C 47/06; B65D 81/28 (2019.01)

CPC - A01N 25/10; A01N 25/34; A01N 37/06; B65D 81/28

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History Document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

See Search History Document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y -- A	CHEN et al, 'Preparation, characterization, and properties of chitosan films with cinnamaldehyde nanoemulsions', Food Hydrocolloids, volume 61, 25 June 2016 (25.06.2016), pg 662-671.	1, 5-9, 13-14, 16-18, 20/(13-14, 16-18)
Y	MORSY et al, 'Incorporation of Essential Oils and Nanoparticles in Pullulan Films to Control Foodborne Pathogens on Meat and Poultry Products', Journal of Food Science, volume 79, number 4, 12 March 2014 (12.03.2014), pg M675-M684.	2-4, 10-12, 15, 20/15
Y	WO 2017/178675 A1 (UNIVERSIDAD POLITECNICA DE CARTAGENA), 19 October 2017 (19.10.2017), pg 6-9 and 15-16 [via machine translation into English, retrieved 4 September 2019 (04.09.2019) from ESpaceNet].	19, 20/19
Y	US 6,120,592 A (BRAULT et al), 19 September 2000 (19.09.2000), col 11 ln 50-67, col 23-26 in entirety.	2-4, (10-12)/(2-4)
A	US 3,949,105 A (WIESKE et al), 6 April 1976 (06.04.1976), col 2 ln 24-36.	10-12
A	WIKIPEDIA, 'Medium-chain triglyceride', 14 June 2018 (14.06.2018), pg 1, [retrieved 4 September 2019 (04.09.2019) via < https://en.wikipedia.org/w/index.php?title=Medium-chain_triglyceride&oldid=845849676 >].	15, 20/15
A	US 7,491,406 B2 (LEUNG et al), 17 February 2009 (17.02.2009), entire document.	19, 20/19
		6-7
		1-20

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

5 September 2019 (05.09.2019)

Date of mailing of the international search report

16 OCT 2019

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450
Facsimile No. 571-273-8300

Authorized officer:

Lee W. Young

PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774