

A METHOD OF PRODUCING AN ENCAPSULATED PRODUCT AND ENCAPSULATED PRODUCT PRODUCED THEREBY

FIELD OF THE INVENTION

5 The present invention relates to a method of producing an encapsulated product and encapsulated product produced thereby. In particular, the invention relates to a method of producing an encapsulated oil-soluble active ingredient dissolved in oil, which is free of additional surfactant and an encapsulated product that is dispersible in aqueous media.

10 BACKGROUND

Oil-soluble active ingredients are not soluble or dispersible in aqueous media. There are many naturally occurring nutrients that are not water soluble. This makes it not easy to formulate the naturally occurring nutrients as active ingredients for use in aqueous-based products, such as foods, cosmetics, supplements and pharmaceutical, that
15 require the active ingredients to be incorporated into aqueous media.

Some nutrients may degrade over time due to oxidation or exposure to heat and light or inappropriate storage environment. Degraded nutrients may lose their nutritional values and may not perform their natural function fully.

20 Attempts have been made to address the above problems. Such attempts include the use of emulsification to emulsify the active ingredient dissolved in oil in water to create oil-in-water emulsion or solubilisation of the oil-soluble active ingredient in oil in aqueous media. However, such methods involve the use of additional surfactants. Surfactants
25 serve the function of emulsifiers and stabilizers and are added to facilitate the formation of the emulsion. Surfactants are widespread in several human activities because of a series of excellent performances like wetting and emulsifying. Due to their excessive use, large amount of surfactants containing wastewater is often discharged into the environment. This causes harmful effects to aquatic life, polluting the water and
30 endangering human health.

It is therefore desirable to provide a method and a product that seek to address at least one of the problems described hereinabove, or at least to provide an alternative.

SUMMARY OF INVENTION

5 The present invention provides a method of producing an encapsulated oil-soluble active ingredient dissolved in oil under high shear condition, without the use of additional surfactant. The encapsulated product produced by the method of the present invention is dispersible in aqueous media.

10 In accordance with a first aspect of this invention, there is provided a method of producing an encapsulated oil-soluble active ingredient dissolved in oil. The method comprises mixing an oil-soluble active ingredient dissolved in oil with a solution of octenyl succinic anhydride (OSA)-modified starch under high shear condition to form an oil-in-water emulsion; mixing the oil-in-water emulsion with a pectin stock solution to
15 obtain a suspension of microcapsules, wherein each of the microcapsules comprises a microcapsule wall formed from the OSA-modified starch from the solution of OSA-modified starch; and adding a solution containing a cross-linker into the suspension of microcapsules to cause a cross-linking reaction so as to form a cross-linked wall on each of the microcapsules, wherein each of the microcapsules comprises the oil-soluble
20 active ingredient dissolved in oil, encapsulated within the cross-linked wall consisting of OSA-modified starch cross-linked with pectin, and wherein the microcapsules are dispersible in aqueous media.

In one embodiment, the method further comprises freeze-drying the microcapsules to
25 obtain the encapsulated oil-soluble active ingredient dissolved in oil, in powder form.

In accordance with another embodiment of the first aspect of this invention, the method comprises mixing an oil-soluble active ingredient dissolved in oil with a solution of octenyl succinic anhydride (OSA)-modified starch and a pectin stock solution under high
30 shear condition to form an oil-in-water emulsion and gradually forming a suspension of microcapsules, wherein each of the microcapsules comprises a microcapsule wall formed from the OSA-modified starch from the solution of OSA-modified starch; and adding a solution containing a cross-linker into the suspension of microcapsules to cause a cross-linking reaction so as to form a cross-linked wall on each of the

microcapsules, wherein each of the microcapsules comprises the oil-soluble active ingredient dissolved in oil, encapsulated within the cross-linked wall consisting of OSA-modified starch cross-linked with pectin, and wherein the microcapsules are dispersible in aqueous media.

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In accordance with a second aspect of this invention, an encapsulated product is provided. The encapsulated product comprises a microcapsule comprising an oil-soluble active ingredient dissolved in oil, encapsulated within a cross-linked wall consisting of OSA-modified starch cross-linked with pectin; and wherein the

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BRIEF DESCRIPTION OF THE DRAWINGS

The above advantages and features of a method and resulting products in accordance with this invention are described in the following detailed description and are shown in the drawings:

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Figure 1 is an image showing suspensions of (A) astaxanthin in avocado oil (AAO); (B) FTR-50 (tocotrienols-rich oil, 50%); and (C) FTR-P30 in sunflower oil (tocotrienols-rich powder, 30%) prepared from freeze-dried samples dispersed in deionized water using agitation.

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Figure 2 represents schematically the formation of microcapsule wall of the microcapsule of the present invention.

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Figure 3 are SEM images of three freeze-dried encapsulated products having a core that comprises: (A) 1% astaxanthin in avocado oil; (B) FTR-50 (tocotrienols-rich oil, 50%); and (C) FTR-P30 in sunflower oil (tocotrienols-rich powder, 30%).

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Figure 4 is an image showing (A) a non-encapsulated solution of astaxanthin in avocado oil; and (B) a freeze-dried encapsulated astaxanthin in avocado oil.

Figure 5 is an image showing samples of suspensions of jojoba oil stained with Oil Red dye prepared using different OSA-modified starch to pectin ratios, and the application of different cross-linkers. Numeric sample codes refer to the mix with the specific ratio as

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specified in Table 2. The samples labelled with "a" are cross-linked with adipic acid dihydrazide and the samples labelled with "b" are cross-linked with methylglyoxal. The concentration of Oil Red dye in jojoba oil is 1 mg/ml.

- 5 Figure 6 is an image showing samples of suspensions of jojoba oil stained with Oil Red dye prepared using different OSA-modified starch to pectin ratios, and the application of different cross-linkers. Numeric sample codes refer to the mix with the specific ratio as specified in Table 2. The samples labelled with "a" are cross-linked with adipic acid dihydrazide and the samples labelled with "b" are cross-linked with methylglyoxal. The
10 concentration of Oil Red dye in jojoba oil is 0.5 mg/ml.

DETAILED DESCRIPTION

- The present invention provides a method of producing an encapsulated oil-soluble
15 active ingredient dissolved in oil under high shear condition, without the use of additional surfactant. The method comprises forming a single, oil-in-water type of emulsion under high shear condition, followed by the formation of microcapsules.

- In one embodiment, the method comprises mixing an oil-soluble active ingredient
20 dissolved in oil with a solution of octenyl succinic anhydride (OSA)-modified starch under high shear condition to form an oil-in-water emulsion; and mixing the oil-in-water emulsion with a pectin stock solution to obtain a suspension of microcapsules, wherein each of the microcapsules comprises a microcapsule wall formed from the OSA-modified starch from the solution of OSA-modified starch. This is followed by adding a
25 solution containing a cross-linker into the suspension of microcapsules to cause a cross-linking reaction so as to form a cross-linked wall on each of the microcapsules, wherein each of the microcapsules comprises the oil-soluble active ingredient dissolved in oil, encapsulated within the cross-linked wall consisting of OSA-modified starch cross-linked with pectin, and wherein the microcapsules are dispersible in aqueous media.

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- In another embodiment, the method comprises mixing an oil-soluble active ingredient dissolved in oil with a solution of octenyl succinic anhydride (OSA)-modified starch and a pectin stock solution simultaneously under high shear condition to form an oil-in-water emulsion and gradually forming a suspension of microcapsules, wherein each of the
35 microcapsules comprises a microcapsule wall formed from the OSA-modified starch

from the solution of OSA-modified starch; and adding a solution containing a cross-linker into the suspension of microcapsules to cause a cross-linking reaction so as to form a cross-linked wall on each of the microcapsules, wherein each of the microcapsules comprises the oil-soluble active ingredient dissolved in oil, encapsulated within the cross-linked wall consisting of OSA-modified starch cross-linked with pectin, and wherein the microcapsules are dispersible in aqueous media.

In general, a "microcapsule" is an encompassing structure or a small container that comprises a core material surrounded by a wall, shell or coating, with dimensions in micrometres range. The core material may include: an active ingredient, an active ingredient dissolved in a solvent, an oil-soluble active ingredient, an oil-soluble active ingredient dissolved in oil, a water-soluble active ingredient or a water-soluble active ingredient dissolved in water, etc. In the embodiments of the present invention, "microcapsule" refers to a small encompassing structure that comprises a core material consisting of an oil-soluble active ingredient that is dissolved in oil and surrounded by a wall, shell or coating.

In the present invention, the OSA-modified starch acts as an encapsulation material and as an emulsifier. In one embodiment, the oil-in-water emulsion is formed upon mixing the oil-soluble active ingredient dissolved in oil with the solution of OSA-modified starch under high shear condition. In another embodiment, the oil-in-water emulsion is formed upon mixing the oil-soluble active ingredient dissolved in oil with the solution of OSA-modified starch and the pectin stock solution simultaneously under high shear condition. In yet another embodiment, the oil-in-water emulsion is formed upon mixing the oil-soluble active ingredient dissolved in oil with a solution of OSA-modified starch and a pectin stock solution pre-mixed at specific ratio, under high shear condition. In various embodiments, the solution of OSA-modified starch and the pectin stock solution are mixed or pre-mixed at a ratio of 25:75 to 90:10 wt%. In some embodiments, the solution of OSA-modified starch and the pectin stock solution are mixed or pre-mixed at a ratio of 25:75, 50:50, 75:25 or 85:15 wt%.

"High shear condition" as used herein refers to a condition whereby the solutions or a mixture comprising several components are mixed using suitable homogenizer including, but not limited to, high speed homogenizer, high pressure homogenizer, membrane homogenizer, etc., and including microchannel droplets generators. Any device that can

produce oil droplet of desired size and size distribution can be used without departing from the scope of this invention. In one embodiment, the mixing is carried out at a speed of 20k to 24k rpm. The duration of mixing is dependent on the types of oil-soluble active ingredient and the oil being used.

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The active ingredient can be any suitable active ingredient that is oil-soluble. Examples of such oil-soluble active ingredients include, but are not limited to, astaxanthin, tocotrienols, carotenoids, oil-soluble vitamins, oil-soluble pigments, oil soluble dyes, and other oil soluble molecules.

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The type of oil that is selected is dependent on the type of oil-soluble active ingredient being used for the encapsulation. The oil can be any suitable oil as long as the oil-soluble active ingredient can be completely or substantially dissolved in the oil. Some non-limiting examples of suitable oils include, but are not limited to, any edible oil such as
15 sunflower oil, canola oil, avocado oil, or the like that are suitable for use in food and beverages and non-edible oil such as jojoba oil, essential oil including, but not limited to, lavender oil, eucalyptus oil, etc., that are suitable for use in cosmetic products.

The combinations of the oil-soluble active ingredient and the oil to be encapsulated are
20 not limited to any specific type or combination. In one embodiment, the combination comprises astaxanthin dissolved in avocado oil. In another embodiment, the combination comprises astaxanthin dissolved in jojoba oil. In a further embodiment, the combination comprises tocotrienols dissolved in sunflower oil. The ratio of the oil-soluble active ingredient to the oil to be used is determined by the solubility of the active
25 ingredient and/or the specific requirements requested by the customers. Depending on the viscosity of the oil phase which comprises the active ingredient dissolved in oil, additional emulsifier(s) may be added to the emulsification process if the oil phase is too viscous that makes it not possible to form emulsion.

30 The solution of OSA-modified starch is prepared by dissolving the OSA-modified starch in water. In various embodiments, the OSA-modified starch solution comprises 2.5 to 5% w/v of OSA-modified starch in the solution.

The pectin stock solution is a solution that contains pectin dissolved in water. Pectin is
35 a type of water-soluble polysaccharide. Pectin can be found naturally in fruits and

vegetables. The richest sources of pectin are found in the peels of citrus fruits such as oranges, lemons, grapefruit, limes and passionfruit. In various embodiments, the pectin stock solution comprises 2.5% to 5% w/v of pectin in the solution.

- 5 After the pectin stock solution is added and mixed with the oil-soluble active ingredient dissolved in oil and the solution of OSA-modified starch, the solution containing the cross-linker is added into the suspension of microcapsules. This causes a cross-linking reaction to take place. The cross-linking reaction involves cross-linking the pectin in the pectin stock solution with the OSA-modified starch that forms the microcapsule wall to
- 10 form a cross-linked wall on each of the microcapsules. Each of the microcapsules at this stage comprises the oil-soluble active ingredient dissolved in oil, encapsulated within the cross-linked wall consisting of OSA-modified starch cross-linked with pectin. The microcapsules thus formed are dispersible in aqueous media.
- 15 Figure 2 represents schematically the formation of microcapsule wall (201) of the microcapsules comprising an oil-soluble active ingredient in oil (202). The cross-linking reaction, facilitated by the cross-linker (203), takes place at a slightly acidic pH and involves predominantly covalently cross-linked the hydroxide groups (-OH) of the modified starch (204) and the pectin (205). At a lower pH, gelation of pectin is induced
- 20 and this provides as a secondary mechanism that stabilizes the microcapsules. In one embodiment, the cross-linking reaction takes place at a pH of less than 4.

The type of cross-linker to be used depends on the final application of the microcapsules, and the cross-linker toxicity that will determine if the cross-linker is suitable for use in

25 producing a food grade or non-food grade encapsulated product. It also depends on whether a hydrophilic or hydrophobic encapsulated product is to be obtained. Suitable cross-linker that can be used includes, but not limited to, adipic acid dihydrazide and methylglyoxal. Methylglyoxal is identified as an active antibacterial molecule naturally present in Manuka honey.

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The method of the present invention further comprises drying the microcapsules to obtain the encapsulated oil-soluble active ingredient in oil in powder form. Any suitable methods of drying can be employed without departing from the present invention. In one embodiment, the method comprises freeze-drying the microcapsules. The method

35 employed should not cause degradation of the active ingredient in the microcapsules.

The microcapsules in powder form can be reconstituted into an aqueous suspension by mixing the powder in aqueous solution. The stability of such suspension can be enhanced by the addition of common additives to the aqueous solution. The size of a microcapsule in powder form is typically one having a diameter in the range from 1 to 100 micrometres. The size is tunable and depends on, among others factors, emulsification process, viscosity of the oil phase, etc. Microcapsules useful in the applications discussed herein typically have a diameter from 0.05 to 100 micrometres. The microcapsule diameter selected depends on a user's intended application or use for the microcapsules. The size of the microcapsules can be adjusted before encapsulation takes place by, for example, adjusting the size of the droplets during the emulsification process. This can be done by using any suitable method including, but not limited to, application of stronger shear, use of membrane to generate droplets of desired size, etc.

The method of the present invention involves encapsulation of an oil-soluble active ingredient that is dissolved in oil to produce microcapsules that are dispersible in aqueous media. The microcapsules comprising the active ingredient dissolved in oil form relatively stable suspension in aqueous media in that the active ingredient encapsulated in the microcapsules do not release spontaneously. The microcapsules form homogeneous suspension in water and the suspension has a neutral fragrance and taste.

In a second aspect of the present invention, an encapsulated product comprising the microcapsules produced by the method of the present invention is provided. The encapsulated product comprising a microcapsule that comprises an oil-soluble active ingredient dissolved in oil, encapsulated within a cross-linked wall consisting of OSA-modified starch cross-linked with pectin, wherein the microcapsule is dispersible in aqueous media. The encapsulated product or the encapsulated oil-soluble active ingredient dissolved in oil obtainable by the method of the present invention is free of additional surfactant.

The oil-soluble active ingredient can be any suitable active ingredient that is oil-soluble. Examples of such oil-soluble active ingredient include, but are not limited to, astaxanthin, tocotrienols, carotenoids, oil-soluble vitamins, oil-soluble pigments and other oil soluble

molecules. The amount of the oil-soluble active ingredient in the microcapsule can be any amount ranging from 1% to 90% w/w or 1% to 75% w/w of the microcapsule.

5 The oil can be any suitable oil as long as the oil-soluble active ingredient can be completely or substantially dissolved in the oil. Some non-limiting examples of suitable oils include, but are not limited to, any edible oil such as sunflower oil, jojoba oil, avocado oil, or the like that are suitable for use in food and beverages; and non-edible oil such as essential oil including, but not limited to, lavender oil, eucalyptus oil, etc. and/or edible oils such as jojoba oil, argan oil, etc. that are suitable for use in cosmetic products.

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The combinations of the oil-soluble active ingredient and the oil to be encapsulated are not limited to any specific types or combinations. In one embodiment, the combination comprises astaxanthin dissolved in avocado oil. In a further embodiment, the combination comprises tocotrienols dissolved in sunflower oil.

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The amounts of the oil-soluble active ingredient and the oil present in the microcapsule are dependent on the type of the oil-soluble active ingredient and the oil being used in producing the encapsulated form and the field of application of the encapsulated form. In various embodiments, the oil-soluble active ingredient is in an amount ranging from
20 1% to 90% w/w or 1% to 75% w/w of the overall weight of the microcapsule. The oil is in an amount ranging from 10% to 99% w/w or 25% to 99% w/w of the overall weight of the microcapsule. In various embodiments, the ratio of the oil soluble active ingredient and the oil is determined by the solubility of the active ingredient in oil, and the resulting viscosity of the oil phase with the dissolved active ingredient.

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In various embodiments, the encapsulated product is in dried powder form comprising discrete, solid particles having a diameter of 1 to 100 μ m, depending on the type of the oil-soluble active ingredient and the oil that are to be encapsulated and the wall material that is used to encapsulate the oil-soluble active ingredient in oil. The smaller particle
30 size of the powder aids in full dissolution of the oil-soluble active ingredient in the aqueous media.

The method and the encapsulated product of the present invention provides several advantages. One of which is that the encapsulation method is simple and scalable. The
35 method can be applied to various oil-soluble active ingredients that are suitable to be

encapsulated and have limited solubility in aqueous media. The encapsulation of the oil-soluble active ingredient protects the encapsulated active ingredient from oxidation and degradation due to exposure to heat and light, and helps to promote dispersivity of the oil-soluble active ingredient and oil in aqueous media. Encapsulation promotes stability and flavour-masking and also helps to retain the active ingredient's nutritional properties. The encapsulated product can be incorporated into beverages and forms stable suspensions in aqueous solutions, also in the presence of common food additives. The method allows customisation of the sizes of the microcapsules to cater to different applications of the encapsulated product in different industries including, food and beverages, cosmetics and pharmaceuticals industries. No additional surfactant is required during the emulsification process. This reduces production cost and eliminates any harmful effects that could result from the use of surfactants in food and beverages, and cosmetic products.

To facilitate a better understanding of the present invention, the following examples of specific embodiments are given. In no way should the following examples be read to limit or define the entire scope of the invention. One skilled in the art will recognize that the examples set out below are not an exhaustive list of the embodiments of this invention.

EXAMPLES

Example 1

Materials

In the examples that follow, the following materials were used.

Octenyl Succinate (OSA)-modified starch (N-Creamer 3334) from Ingredion; Pectin from citrus peel (P9135-100g) was purchased from Sigma; Astaxanthin paste (10%) and organic avocado oil were supplied by Innoso Pte Ltd; tocotrienols-rich oil (50%, FTR-50) and tocotrienols-rich powder (30%, FTR-P30(A)) were supplied by Vance Group Ltd; sunflower oil was purchased from a local supermarket. Dialysis membranes (SnakeSkin® dialysis tubing) with cut-off molecular weight of 3.5 kDa was purchased from ThermoFischer Scientific.

Preparation of starch stock solution

An amount of 5.1241g of OSA-modified starch was dissolved in 100 ml of hot, but not boiling, water, under gentle agitation, to form 5% w/v solution. The solution was cooled
5 down to room temperature and used within 2 days.

Preparation of pectin stock solution

An amount of 5.0555g of pectin was dissolved in 100 ml of hot, but not boiling, water,
10 under gentle agitation, to form 5% w/v solution. The solution was cooled down to room temperature and used within 2 days.

Preparation of astaxanthin stock solution

15 Astaxanthin paste, containing 10% astaxanthin, was mixed with avocado oil to form a stock solution of 7.1 mg/ml of pure astaxanthin in oil. This solution was stored in the fridge and was brought to room temperature prior use.

Preparation of tocotrienols (FTR-P30) stock solution

20 An amount of 2.5000g of tocotrienols-rich powder (30%, FTR-P30) was dissolved in 5 ml of sunflower oil to form stock solution of 500 mg/ml. This solution was stored in the fridge and was brought to room temperature prior use.

Preparation of cross-linker stock solution

An amount of 9.3126g of adipic acid dihydrazide was dissolved in 100 ml of deionized water to form 93.12 g/l (0.53 M) stock solution.

Formation of microcapsules

The process of encapsulation requires two steps: in the first step, a single, oil-in-water emulsion was formed, followed by formation of starch-pectin microcapsules in the second step.

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To avoid overheating, the samples were placed in ice-water bath during homogenization. The oil-in-water emulsion was formed upon mixing (A): 5 ml deionised water, 5 ml of 5% solution of N-Creamer 3334 (OSA-modified starch) in water and 2 ml of stock solution of astaxanthin in avocado oil, or (B): 15 ml deionised water, 15 ml of 5% solution of N-Creamer 3334 (OSA-modified starch) in water, and 6 ml of FTR-50 (tocotrienols-rich oil, 50%) or FTR-P30 (tocotrienols-rich powder, 30%) stock solution. The mixing was executed for 10 min at setting #5 of high pressured homogenizer (Ultra-Turrax, IKA T18 basic), followed by addition of (A): 2 ml, or (B): 10 and 8 ml, respectively, of pectin stock solution in water. The mixing continued for another 2 minutes, followed by crosslinking with an excess adipic acid dihydrazide in solution (5-10 ml). After homogenization at setting #1 of the high pressured homogenizer for another 1 minute, the thus prepared samples were placed on shaker at 150 rpm for 1 to 4 hours. Subsequently, the samples were dialyzed against deionised water for 48 to 72 hours, with deionised water being replaced every 24 hours, to remove unreacted adipic acid dihydrazide, and freeze dried.

Scanning Electron Microscopy was performed with JEOL JSM6700F.

Encapsulation efficiency was calculated based on the amounts of dry ingredients used in the process.

Results

Dried starch microcapsules, containing astaxanthin and avocado oil, and FTR-50 (tocotrienols-rich oil, 50%) and FTR-P30 in sunflower oil (tocotrienols-rich powder, 30%) were examined using SEM to assess their morphology. The results are shown in Figure 1. The properties of the load influence the morphologies of the resulting products. After formulation, the microcapsule can be freeze dried to form powder, and subsequently re-suspended in aqueous media, if required (Table 1). No release of encapsulated material was observed during freeze-drying and re-dispersion in water. There is an excess of encapsulation material (likely pectin) visible in form of flat sheets.

Table 1: Examples of loading of tocotrienols (FTR-P30 (powder), FTR-50 (oil)) and astaxanthin (oil) into pectin microcapsules.

Active ingredient	Loading [% w/w] in freeze-dried product
FTR-P30	12
Sunflower oil + FTR-P30	73
FTR-50	37
Astaxanthin	1
avocado oil + astaxanthin	75

Figure 1 is an image showing suspensions of astaxanthin in avocado oil (AAO) (left), FTR-50 (tocotrienols-rich oil, 50%) (middle) and FTR-P30 (tocotrienols-rich powder, 30%) in sunflower oil (right) prepared from freeze-dried samples dispersed in deionized water using agitation. Concentration of the freeze-dried powders in all three suspensions is the same, 18 mg/ml. The colour of the suspensions is determined by colour of the load.

Figure 3 shows SEM images of the freeze-dried materials that comprises: (A) 1% Astaxanthin in avocado oil; (B) FTR-50 (tocotrienols-rich oil, 50%); and (C) FTR-P30 (tocotrienols-rich powder, 30%) in sunflower oil. After mixing with water, all three materials form homogeneous and stable suspensions at room temperature (as shown in Figure 1), under gentle agitation.

Example 2

Materials

In the example that follows, the following materials were used.

Octenyl Succinate (OSA)-modified starch (N-Creamer 3334) from Ingredion; Pectin from citrus peel (P9135-100g) was purchased from Sigma; Oil Red O (00625-25g) and Methylglyoxal (67028-100ml) were purchased from Sigma-Aldrich, organic jojoba oil (*Simmondsia chinensis*) was purchased from Herbal Sense. Dialysis membranes (SnakeSkin® dialysis tubing) with cut-off molecular weight of 3.5 kDa was purchased from ThermoFischer Scientific. THCP (3,4,9,10-tetra-(hectoxy-carbonyl)-perylene) fluorescent dye was synthesized at home institute following the method described elsewhere (X. Mo , M. M. Shi , J. C. Huang , M. Wang , H. Z. Chen , Dyes Pigm. 2008 , 76, 236).

Preparation of polysaccharides stock solutions

OSA-modified starch was dissolved in 100 ml of hot, but not boiling, water, under gentle
5 agitation, to form 50 mg/ml solution. The solution was cooled down to room temperature
and used within 2 days.

Pectin was dissolved in 100 ml of hot, but not boiling, water, under gentle agitation, to
form 50 mg/ml solution. The solution was cooled down to room temperature and used
10 within 2 days.

Preparation of stained oil stock solutions

Oil Red O dye was mixed with jojoba oil to prepare 1 mg/ml or 0.5 mg/ml solutions.
15 (3,4,9,10-tetra-(hectoxy-carbonyl)-perylene) (THCP) in sunflower was mixed with jojoba
oil to form 2% v/v solution. These solutions were stored in the fridge and were brought
to room temperature prior use.

Preparation of cross-linker stock solutions

20 Adipic acid dihydrazide was dissolved in 100 ml of deionized water to form 10.0331 g/l
stock solution.

Formation of microcapsules

25 4 ml of stained oil was added to a 20 ml of solution of N-Creamer 3334 (OSA-modified
starch) in water mixed with a solution of pectin in water at specific volume ratio, and 10
ml of pure water. The mixture was mixed under high shear condition for 10 min at setting
#5 of high pressured homogenizer (Ultra-Turrax, IKA T18 basic). 10 ml of excess adipic
30 acid dihydrazide in solution was added to cause a cross-linking reaction between the
OSA-modified starch and pectin. Alternatively, 0.25 ml of methyl glyoxal and 0.5 ml of
0.5M HCl were added to facilitate the cross-linking reaction between the OSA-modified
starch and the pectin. After homogenization at setting #1 of the high pressured
homogenizer for another 1 minute, the thus prepared samples were placed on shaker
35 at 150 rpm for 1 to 4 hours. Subsequently, the samples were dialyzed against deionised

water for 48 to 72 hours, with deionised water being replaced every 24 hours, to remove unreacted cross-linker, and freeze-dried. The ratios between OSA-modified starch and pectin amounts used in the thus prepared samples are shown in Table 2 below.

- 5 Table 2: The weight ratios of OSA-modified starch to pectin in samples described in Example 2.

Mix	OSA-modified starch (S)	Pectin (P)
1	0.000	1.000
2	0.013	0.875
3	0.250	0.750
4	0.500	0.500
5	0.750	0.250
6	0.875	0.013
7	1.000	0.000

- 10 The results obtained show that in order to effectively encapsulate the oil-soluble active ingredient dissolved in oil, there should be at least 25 wt% OSA-modified starch in the solution of OSA-modified starch and the pectin solution. If there is less OSA-modified starch, then even after cross-linking, there is partial spontaneous release of oil phase visible. Thus, stable encapsulated products were obtained using a mix comprising OSA-modified starch to pectin ratios shown as Mix 3 to 7 inclusive. There is a benefit of adding
- 15 OSA-modified starch and pectin solutions before the emulsification as this reduces the process steps which does not require addition of pectin solution after emulsification. These tests, as illustrated by the images shown in Figures 5 and 6, include also tests of using different cross-linkers. The results show that the choice of cross-linker can further modify wettability of the surface of the microcapsules.

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A successful and effective method to encapsulate oil-soluble active ingredients has been developed and tested. The method does not require additional surfactant and is carried out without organic solvents. The final encapsulated product can be formulated into powders by means of freeze-drying, or liquids, following purification by dialysis

against required solvent(s). The technology is adaptable to various load ingredients and the encapsulated product obtained is stable and suitable for use in aqueous media.

5 Although an embodiment of the present invention have been shown and described, the present invention is not limited to the described embodiments. Instead, it would be appreciated by those skilled in the art that changes may be made to the embodiments without departing from the scope of the invention, the scope of which is set forth in the following claims.

CLAIMS

1. A method of producing an encapsulated oil-soluble active ingredient dissolved in oil, the method comprising:

5 mixing an oil-soluble active ingredient dissolved in oil with a solution of octenyl succinic anhydride (OSA)-modified starch under high shear condition to form an oil-in-water emulsion;

mixing the oil-in-water emulsion with a pectin stock solution to obtain a suspension of microcapsules, wherein each of the microcapsules comprises a microcapsule wall formed from the OSA-modified starch from the solution of OSA-
10 modified starch; and

adding a solution containing a cross-linker into the suspension of microcapsules to cause a cross-linking reaction in the suspension of microcapsules so as to form a cross-linked wall on each of the microcapsules, wherein each of the
15 microcapsules comprises the oil-soluble active ingredient in oil, encapsulated within the cross-linked wall consisting of OSA-modified starch cross-linked with pectin, and wherein the microcapsules are dispersible in aqueous media.

2. A method of producing an encapsulated oil-soluble active ingredient dissolved in oil, the method comprising:

20 mixing an oil-soluble active ingredient dissolved in oil with a solution of modified starch and a pectin stock solution simultaneously under high shear condition to form an oil-in-water emulsion and gradually forming a suspension of microcapsules, wherein each of the microcapsules comprises a microcapsule wall formed from the OSA-
25 modified starch from the solution of OSA-modified starch; and adding a solution containing a cross-linker into the suspension of microcapsules to cause a cross-linking reaction in the suspension of microcapsules so as to form a cross-linked wall on each of the microcapsules, wherein each of the microcapsules comprises the oil-soluble active ingredient dissolved in oil, encapsulated within the cross-linked wall consisting of
30 OSA-modified starch cross-linked with pectin, and wherein the microcapsules are dispersible in aqueous media.

3. The method according to claim 2, wherein the solution of OSA-modified starch and the pectin stock solution are pre-mixed prior to mixing with the oil-soluble active
35 ingredient dissolved in oil.

4. The method according to claim 2 or 3, wherein the solution of OSA-modified starch and the pectin stock solution are mixed at a ratio of 25:75 to 90:10 wt%.
- 5 5. The method according to claim 1 or 2, further comprising freeze-drying the microcapsules to obtain the encapsulated oil-soluble active ingredient dissolved in oil in powder form.
- 10 6. The method according to claim 1 or 2, wherein the cross-linker is selected from the group consisting of adipic acid dihydrazide and methylglyoxal.
7. The method according to claim 1 or 2, wherein the oil-soluble active ingredient is selected from the group consisting of astaxanthin, tocotrienols, carotenoids, oil-soluble vitamins, oil-soluble pigments and other oil soluble molecules.
- 15 8. The method according to claim 1 or 2, wherein the oil is selected from the group consisting of jojoba oil, sunflower oil, avocado oil.
9. An encapsulated product comprising:
20 a microcapsule comprising an oil-soluble active ingredient dissolved in oil, encapsulated within a cross-linked wall consisting of OSA-modified starch cross-linked with pectin;
wherein the microcapsule is dispersible in aqueous media.
- 25 10. The encapsulated product according to claim 9, wherein the encapsulated product comprises a plurality of microcapsules.
11. The encapsulated product according to claim 9, wherein the encapsulated product is in powder form.
- 30 12. The encapsulated product according to claim 9, wherein the oil-soluble active ingredient is selected from the group consisting of astaxanthin, tocotrienols, carotenoids, oil-soluble vitamins, oil-soluble pigments, and other oil soluble molecules .

13. The encapsulated product according to claim 9, wherein the oil is selected from the group consisting of jojoba oil, sunflower oil, avocado oil.
14. The encapsulated product according to claim 9, wherein the oil-soluble active
5 ingredient in oil is astaxanthin dissolved in avocado oil.
15. The encapsulated product according to claim 9, wherein the oil-soluble active ingredient in oil is tocotrienols dissolved in sunflower oil.