A PROCESS FOR PRODUCING NANO-CAPSULES AND NANO-BEADLETS CONTAINING PHYTONUTRIENTS DERIVED FROM PALM OIL

FIELD OF INVENTION

The present invention relates to a process for producing nano-capsules and nano-beadlets containing phytonutrients derived from palm oil.

BACKGROUND OF THE INVENTION

Palm phytonutrients such as tocotrienols, carotenes and coenzyme Q are highly sensitive towards lights, temperature, pH and air. In room temperature, these phytonutrients get oxidized by air and thus, their half time is normally very short. In addition, these phytonutrients are also destroyed at extreme acidic conditions, resulting in a reduced bioavailability *in vivo*. In order to make up for the reduced bioavailability, larger dosage of these phytonutrients needs to be consumed thus, incurring extra cost. Therefore there is a need for encapsulation of these phytonutrients which will ensure stability, hence longer shelf life and bioavailability of the phytonutrients to reap its full benefits.

SUMMARY OF THE INVENTION

The present invention relates to a process for producing nano-capsules containing phytonutrients derived from palm oil. The process includes the steps of:

i) dissolving the phytonutrients in a non-polar solvent;

ii) adding of at least one shell material and at least one additive into the solution obtained from step (i) to form a mixture;

iii) obtaining stable oil in water emulsion by introducing the mixture obtained from step (ii) to a buffer solution;

iv) removing the solvent from the mixture in step (iii);

v) homogenizing the mixture obtained from step (iv) under pressure of more than 50 bar and/or by using ultrasound;

vi) drying the mixture obtained from step (v).

The phytonutrients derived from palm oil is such as tocotrienols, carotenes, coenzyme Q, γ-tocotrienol, α-tocotrienol, δ- tocotrienol, lycopene and β-carotene.

The present invention also relates to a process for producing nano-beadlets containing phytonutrients derived from palm oil. The process includes the steps of:

i) dissolving the phytonutrients in a non-polar solvent;

ii) adding of at least one shell material and at least one additive into the solution obtained from step (i) to form a mixture;

iii) homogenizing the mixture obtained from step (ii) under pressure of more than 50 bar or by way of sonication;

iv) precipitating the phytonutrients from the mixture obtained from step (iii) to form insoluble nano-beadlets; and

v) drying the insoluble nano-beadlets.

The phytonutrients is precipitated by adding the mixture obtained from step (iii) into a salt solution by using an atomizer. The salt solution is methanolic solution of calcium chloride.

The process for producing nano-beadlets containing phytonutrients derived from palm oil further includes the steps of:

i) introducing the insoluble beadlets into a shell material solution;

ii) adding at least one additive into the mixture obtained in step (i);

iii) stirring the mixture in step (ii);

iv) filtering nano-beadlets obtained from step (iii); and

v) drying the nano-beadlets.

The phytonutrients derived from palm oil is such as tocols, coenzyme Q_{10}, tocotrienol, α-tocotrienol, γ-tocotrienol, δ-tocotrienol and carotenes. The phytonutrients within the nano-beadlets are slowly released over period of 4 – 6 hours.

The present invention also relates to a process for producing nano-capsules containing phytonutrients derived from palm oil, the process includes the steps of:

i) mixing phytonutrients with a mixture of gum and coating material in water;

ii) homogenizing the mixture obtained from step (i) under pressure of more than 50 bar or by way of sonication;

iii) drying of the emulsion obtained from step (ii);

iv) coating of insoluble solids obtained from step (iii) with shell material; and

v) drying of nano-capsules obtained from step (iv).

The phytonutrients derived from palm oil is such as tocotrienol, carotenes, α-tocotrienol, and γ-tocotrienol, coenzyme $Q_{10.}$ The gum is such as arabic gum and acacia gum. The coating material is sugar such as lactose.

The non-polar solvent used for the processes mentioned above is hexane, heptane, chloroform or any combination thereof. The shell material used for the processes mentioned above is polymer, sugar, gum or any combination thereof. The at least one additive for the processes mentioned above is polymeric. The nano-capsules or nano-beadlets obtained from the above mentioned process having size of 20nm – 500nm.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a process for producing nano-capsules and nano-beadlets containing phytonutrients derived from palm oil. The phytonutrients are such as tocols, tocotrienols, α-tocotrienols, γ-tocotrienols, δ-tocotrienol, coenzyme Q, β-carotene and carotenes. In the present invention, the phytonutrients are encapsulated by three different methods. It should be understood, however, that the disclosed preferred embodiments are merely exemplary of the invention, which may be embodied in various forms. Therefore, the details disclosed herein are not to be interpreted as limiting, but merely as the basis for the claims and for teaching one skilled in the art of the invention.

The first method called for the use of a shell material to encapsulate the phytonutrients derived from palm oil. The shell material may not be of plant origin. The shell material can be of polymer, sugar, gum or any combination thereof. Preferred shell materials include polymer of carbohydrates and phospholipids. Encapsulation of phytonutrients is carried out by way of dissolving the phytonutrients first in a solvent, preferably non-polar solvent. The non-polar solvent is hexane, heptane, chloroform or

any combination thereof. Then adding at least one shell material and at least one additive into the mixture obtained above. The at least one additive is polymeric. Stable oil in water emulsion is achieved by dispersing the dissolved phytonutrients in an aqueous solution (i.e. buffer solution) in the presence of an emulsifier or a surface active agent. The solvent is then removed by distillation under vacuum or flash evaporation after which, homogenization under high pressure, i.e. more than 50bar and/or ultrasound is employed to yield emulsion of very fine particle size. The emulsion is dried and encapsulated phytonutrients is thus obtained. The encapsulated phytonutrients are tested for encapsulation efficiency and stability over time. The encapsulated phytonutrients are in form of nano-capsules with nano range of 20nm-500nm.

The second method of encapsulation involves first, formation of stable oil in water emulsion. The steps involved in forming the stable oil in water emulsion are similar as the first method as mentioned above. The emulsion is then homogenized under high pressure, i.e. more than 50bar or using ultrasound. The homogenized emulsion is then sprayed into a solution of methanolic calcium chloride by using an atomizer. The solution can be of a salt solution. Insoluble nano-beadlets is thus formed. The nano-beadlets are having nano size of 20nm-500nm.

The third method involves formation of encapsulation of the palm phytonutrients using a combination of shell materials. The steps involved forming the stable oil in water emulsion are similar as the first method as mentioned above. The shell materials consist but not confined to a sugar and gum. High pressure, i.e. more than 50bar or ultrasonic homogenization resulted in an emulsion of fine particle size. The emulsion was then dried, resulting in solid encapsulants. The solid encapsulants are having nano size of 20nm-500nm.

Nano-capsules with slow release property over period of 4-6 hours are prepared by mixing or coating the beadlets or solid encapsulants with another polymeric shell material. The polymeric material is thus chosen from edible, food grade materials. The beadlets or solid encapsulants are introduced / dissolved in a shell material solution.

Thereafter at least one additive is added into the mixture obtained above and stirred. The beadlets or solid encapsulants are filtered from the mixture and dried. The beadlets or solid encapsulants are having nano size of 20nm-500nm. Releasing property of the resultant capsules were studied and recorded.

The invention will be further understood from the following non-limited examples.

EXAMPLE 1

1.25g mixture of tocotrienols is dissolved in 2mL dichloromethane at 50^0C. To this mixture, 0.8g phospholipids, 0.15g cholesterol and 0.6g polysorbate 20 are added. The mixture is then injected into 0.01M phosphate buffer solution (pH7.4) and stirred for 30 minutes at 50^0C. Excess dichloromethane is removed by distillation under vacuum. 3.2mL distilled water is then added to the mixture after removal of excess dichloromethane (tocotrienols in water dispersion) and stirred. The mixture is then subjected to homogenization using a probe ultra sonicator for 4 minutes in an ice bath. The mixture is then dried using a spray dryer. Average size of the resultant nano-capsules produced is *ca*. 30nm.

EXAMPLE 2

Encapsulation of mixture of carotenes is carried out in similar way as described in Example 1. 1.25g mixture of carotenes is used in place of tocotrienols. Average size of the resultant nano-capsules produced is *ca*. 30nm.

EXAMPLE 3

1.25g mixture of coenzyme Q_{10} is dissolved in 2mL dichloromethane at 55^0C. To this mixture, 0.125g phospholipids, 0.015g cholesterol and 0.06g polysorbate 20 are added. The mixture is then injected into 10mL 0.01M phosphate buffer solution (pH7.4) and agitated for 30 minutes at 50^0C. Excess dichloromethane is removed by distillation under

vacuum. The mixture is then subjected to homogenization using a probe ultra sonicator for 4 minutes in an ice bath. The mixture is then dried using a spray dryer. Average size of the resultant nano-capsules produced is *ca*. 30nm.

EXAMPLE 4

0.125g γ-tocotrienol is dissolved in 0.2mL hexane at 50^0C. To this mixture, 0.08g phospholipids, 0.015g cholesterol and 0.06g polysorbate 20 are added. The mixture is then injected into 1mL 0.01M phosphate buffer solution (pH7.4) and stirred for 30 minutes at 50^0C. Excess hexane is removed by distillation under vacuum. 0.3mL distilled water is then added to the mixture after removal of excess hexane (γ-tocotrienol in water dispersion) and stirred. The mixture is then subjected to homogenization using a probe ultra sonicator for 2 minutes in an ice bath. The mixture is then dried using a spray dryer. Average size of the resultant nano-capsules produced is *ca*. 30nm. Encapsulation efficiency is 55%.

EXAMPLE 5

0.13g α-tocotrienol is dissolved in 0.2mL chloroform at 50^0C. To this mixture, 0.08g phospholipids, 0.015g cholesterol and 0.06g polysorbate 800 are added. The mixture is then injected into 1mL 0.01M phosphate buffer solution (pH7.4) and stirred for 30 minutes at 50^0C. Excess chloroform is removed by distillation under vacuum. 0.3mL distilled water is then added to the mixture after removal of excess chloroform (α-tocotrienol in water dispersion) and stirred. The mixture is then subjected to homogenization using a probe ultra sonicator for 2 minutes in an ice bath. The mixture is then dried using a spray dryer. Average size of the resultant nano-capsules produced is *ca*. 30nm. Encapsulation efficiency is 60%.

EXAMPLE 6

Encapsulation of δ-tocotrienol is carried out in similar way as in Example 5 where by δ-tocotrienol is used in place of α-tocotrienol. Average size of the resultant nano-capsules produced is *ca*. 30nm. Encapsulation efficiency is 60%.

EXAMPLE 7

Encapsulation of lycopene is carried out in similar way as in Example 5 where by lycopene is used in place of α-tocotrienol. Average size of the resultant nano-capsules produced is *ca*. 30nm. Encapsulation efficiency is 55%.

EXAMPLE 8

Encapsulation of β-carotene is carried out in similar way as in Example 5 where by β-carotene is used in place of α-tocotrienol. Average size of the resultant nano-capsules produced is *ca*. 30nm. Encapsulation efficiency is 55%.

EXAMPLE 9

1g mixture of tocols is dissolved in 5mL chloroform. This solution is then added into 4% aqueous sodium alginate with 2% polysorbate 80. The mixture is then subjected to sonication using a probe ultra sonicator for 3 minutes in an ice bath. The average size of tocols and sodium alginate (particles) in emulsion at this point is *ca.*300nm. The homogenized mixture is then added into a methanolic solution of calcium chloride with an atomizer. The resultant beadlets is then washed with methanol and dried. Encapsulation efficiency is 70%.

EXAMPLE 10

Encapsulation of 0.5g coenzyme Q_{10} is carried out in similar way as described in Example 9. Average size of emulsion after ultrasonic homogenization is *ca*.200nm. Encapsulation efficiency is 50%.

EXAMPLE 11

0.1g α -tocotrienol underwent the same process as described in Example 9. Average size of emulsion after ultrasonic homogenization is *ca*. 200nm. Encapsulation efficiency is 50%.

EXAMPLE 12

0.1g γ-tocotrienol underwent the same process as described in Example 9. Average size of emulsion after ultrasonic homogenization is *ca*. 200nm. Encapsulation efficiency is 50%.

EXAMPLE 13

The tocol beadlets prepared in similar way as in Example 9 are added into a solution of 0.5% hydroxypropyl methyl cellulose in water. 40% w/w triethyl citrate purum is then added into this mixture. The mixture is then stirred for 30 minutes and coated beadlets are then filtered from the mixture, washed with water and dried. *In vitro* tests showed that the tocotrienols within the coated beadlets are slowly released over a period of 4 hours.

EXAMPLE 14

4g of the tocol beadlets obtained in Example 9 are added into a solution of 0.2g chitosan in water. 0.8g triethyl citrate purum and 1mL acetic acid are then added into this mixture. The mixture is then stirred for 30 minutes and the coated beadlets is then

filtered, washed with water and dried. *In vitro* tests showed that the tocotrienols within the coated beadlets are slowly released over a period of 3 hours.

EXAMPLE 15

The coenzyme Q_{10} beadlets prepared in similar way as in Example 10 are treated in the same way as described in Example 14. Coenzyme Q_{10} within the beadlets is slowly released over a period of 3 hours.

EXAMPLE 16

The Q_{10} beadlets prepared in similar way as in Example 10 are added into a solution of 0.5% hydroxylpropyl methyl cellulose in water. 40% w/w triethyl citrate purum is then added into this mixture. The mixture is then stirred for 30 minutes and the coated beadlets is then filtered, washed with water and dried. *In vitro* tests showed that the coenzyme Q_{10} within the coated beadlets is slowly released over a period of 4 hours.

EXAMPLE 17

γ-tocotrienol beadlets prepared in similar way as in Example 12 are treated in similar way as in Example 13. γ-tocotrienol within the beadlets is slowly released over a period of 4 hours.

EXAMPLE 18

γ-tocotrienol beadlets prepared in similar way as in Example 12 are treated in similar way as in Example 14. γ-tocotrienol within the beadlets is slowly released over a period of 3 hours.

EXAMPLE 19

1g mixture of tocotrienols is dissolved in 5mL chloroform. This solution is then added into 4% aqueous sodium alginate with 2% polysorbate 80. To this mixture, hydroxypropyl methyl cellulose is added. Ratio of tocotrienols to polymer is 1:9. The mixture is stirred to dissolve for two hours. 0.8mL triethyl citrate purum is added and the mixture is stirred for another 2 hours. The mixture is then subjected to homogenisation using a probe ultra sonicator for 3 minutes in an ice bath. The average size of the particles in emulsion at this point is *ca.*300nm. The homogenized mixture is then added into a methanolic solution of calcium chloride with an atomizer. The resultant beadlets is then washed with methanol and dried. Encapsulation efficiency is 70%. Tocotrienols within the beadlets are slowly released over a period of 3 hours.

EXAMPLE 20

1.25g mixture of tocotrienols was dissolved in 2mL dichloromethane at 50^0C. To this mixture, 0.8g phospholipids, 0.15g cholesterol and 0.6g polysorbate 20 are added. The mixture is then injected into 0.01M phosphate buffer solution (pH7.4) and stirred for 30 minutes at 60^0C. 3.2mL distilled water is then added to the oil in water dispersion and stirred. The mixture is then subjected to homogenisation using a probe ultra sonicator for 4 minutes in an ice bath. The mixture is then subjected to centrifugation at 3000rpm for 10 minutes. Tocotrienols nanocapsules are recovered from the supernatant. The average size of the resultant nanocapsules produced is *ca*. 30nm.

EXAMPLE 21

2.2g arabic gum is mixed with 1.3g lactose in 9mL water. To this mixture, 0.9g mixture of tocotrienols is thus added. The mixture is then agitated before subjected to homogenization using a probe ultrasonic homogenizer for 4 minutes. Particle size of the emulsion obtained from the homogenization at this point is *ca*. 300nm. The resultant emulsion is then dried to yield dry powder. The resultant dry powder is then washed with

hexane and insoluble solids are then filtered and dried. The dried solids are then added into a solution of 0.5% hydroxypropyl methyl cellulose in water. The mixture is stirred with a rotating pad at room temperature for 1 hour. Coated solids, i.e. tocotrienols nano-capsules, are then filtered from the mixture and dried. The encapsulation efficiency of the Arabic gum is 80% while in vitro test revealed that the tocotrienols within the nano-capsules are slowly released over 5 hours.

EXAMPLE 22

Tocotrienols with slow release property using acacia gum is prepared using method similar to Example 21 with the exception that 2.0g acacia gum is used in place of Arabic gum. The encapsulation efficiency of the acacia gum is 80% while *in vitro* test revealed that the tocotrienols within the nano-capsules are slowly released over 5 hours.

EXAMPLE 23

Coenzyme Q_{10} with slow release property using arabic gum is prepared using method similar to Example 21 with the exception that 0.5g coenzyme Q_{10} is used in place of tocotrienols. The encapsulation efficiency was 80% while *in vitro* test revealed that the coenzyme Q_{10} within the nanocapsules is slowly released over 5 hours.

EXAMPLE 24

Carotenes with slow release property using arabic gum is prepared using method similar to Example 21 with the exception that 0.5g carotenes is used in place of tocotrienols. The encapsulation efficiency was 80% while *in vitro* test revealed that the carotenes within the nano-capsules are slowly released over 5 hours.

EXAMPLE 25

α-tocotrienol with slow release property using arabic gum is prepared using method similar to Example 21 with the exception that 0.2g α-tocotrienol is used in place of mixture of tocotrienols. The encapsulation efficiency was 80% while *in vitro* test revealed that the α-tocotrienol within the nano-capsules is slowly released over 5 hours.

EXAMPLE 26

γ- tocotrienol with slow release property using arabic gum is prepared using method similar to Example 21 with the exception that 0.2g α-tocotrienol is used in place of mixture of tocotrienols. The encapsulation efficiency was 80% while *in vitro* test revealed that the γ-tocotrienol within the nano-capsules is slowly released over 5 hours.

CLAIMS

1. A process for producing nano-capsules containing phytonutrients derived from palm oil, the process includes the steps of:

i) dissolving the phytonutrients in a non-polar solvent;

ii) adding of at least one shell material and at least one additive into the solution obtained from step (i) to form a mixture;

iii) obtaining stable oil in water emulsion by introducing the mixture obtained from step (ii) to a buffer solution;

iv) removing the solvent from the mixture in step (iii);

v) homogenizing the mixture obtained from step (iv) under pressure of more than 50 bar and/or by using ultrasound;

vi) drying the mixture obtained from step (v).

2. The process for producing nano-capsules containing phytonutrients oil derived from palm oil as claimed in claim 1 wherein the phytonutrients is such as tocotrienols, carotenes, coenzyme Q, γ-tocotrienol, α-tocotrienol, δ- tocotrienol, lycopene and β-carotene.

3. The process for producing nano-capsules containing phytonutrients derived from palm oil as claimed in claim 1 wherein the nano-capsules having size of 20nm – 500nm.

4. The process for producing nano-capsules containing phytonutrients derived from palm oil as claimed in claim 1 wherein the non-polar solvent is hexane, heptane, chloroform or any combination thereof.

5. The process for producing nano-capsules containing phytonutrients derived from palm oil as claimed in claim 1 wherein the at least one shell material is polymer, sugar, gum or any combination thereof.

6. The process for producing nano-capsules containing phytonutrients derived from palm oil as claimed in claim 1 wherein the at least one additive is polymeric.

7. The process for producing nano-capsules containing phytonutrients derived from oil as claimed in claim 1 wherein the nano-capsules having size of 20nm – 500nm.

8. A process for producing nano-beadlets containing phytonutrients derived from palm oil, the process includes the steps of:

i) dissolving the phytonutrients in a non-polar solvent;

ii) adding of at least one shell material and at least one additive into the solution obtained from step (i) to form a mixture;

iii) homogenizing the mixture obtained from step (ii) under pressure of more than 50 bar or by way of sonication;

iv) precipitating the phytonutrients from the mixture obtained from step (iii) to form insoluble nano-beadlets; and

v) drying the insoluble nano-beadlets.

9. The process for producing nano-beadlets containing phytonutrients derived from palm oil as claimed in claim 8 wherein the phytonutrients is such as tocols, coenzyme Q_{10}, tocotrienol, α-tocotrienol, γ-tocotrienol, δ-tocotrienol and carotenes.

10. The process for producing nano-beadlets containing phytonutrients derived from palm oil as claimed in claim 8 wherein the phytonutrients is precipitated by adding the mixture obtained from step (iii) into a salt solution by using an atomizer.

11. The process for producing nano-beadlets containing phytonutrients derived from palm oil as claimed in claim 10 wherein the salt solution is methanolic solution of calcium chloride.

12. The process for producing nano-beadlets containing phytonutrients derived from palm oil as claimed claim 8 wherein the process further includes the steps of:

i) introducing the insoluble beadlets into a shell material solution;

ii) adding at least one additive into the mixture obtained in step (i);

iii) stirring the mixture in step (ii);

iv) filtering nano-beadlets obtained from step (iii); and

v) drying the nano-beadlets.

13. The process for producing nano-beadlets containing phytonutrients derived from oil as claimed in claim 8 wherein the nano-beadlets having size of 20nm – 500nm.

14. The process for producing nano-beadlets containing phytonutrients derived from palm oil as claimed in claim 8 wherein the non-polar solvent is hexane, heptane, chloroform or any combination thereof.

15. The process for producing nano-beadlets containing phytonutrients derived from palm oil as claimed in claims 8 and 12 wherein the shell material is polymer, sugar, gum or any combination thereof.

16. The process for producing nano-beadlets containing phytonutrients derived from palm oil as claimed in claims 8 and 12 wherein the at least one additive is polymeric.

17. The process for producing nano-beadlets containing phytonutrients derived from palm oil as claimed in claim 8 wherein the phytonutrients within the nano-beadlets are slowly released over period of 4 – 6 hours.

18. A process for producing nano-capsules containing phytonutrients derived from palm oil, the process includes the steps of:

i) mixing phytonutrients with a mixture of gum and coating material in water;

ii) homogenizing the mixture obtained from step (i) under pressure of more than 50 bar or by way of sonication;

iii) drying of the emulsion obtained from step (ii);

iv) coating of insoluble solids obtained from step (iii) with shell material; and

v) drying of nano-capsules obtained from step (iv).

19. The process for producing nano-capsules containing phytonutrients derived from palm oil as claimed in claim 18 wherein the phytonutrients is such as tocotrienol, carotenes, α-tocotrienol, and γ-tocotrienol , coenzyme $Q_{10.}$

20. The process for producing nano-capsules containing phytonutrients derived from palm oil as claimed in claim 18 wherein the gum is arabic gum.

21. The process for producing nano-capsules containing phytonutrients derived from palm oil as claimed in claim 18 wherein the gum is acacia gum.

22. The process for producing nano-capsules containing phytonutrients derived from palm oil as claimed in claim 18 wherein the coating material is sugar such as lactose.

23. The process for producing nano-capsules containing phytonutrients derived from palm oil is claimed in claim 18 wherein the shell material is polymer.

24. The process for producing nano-capsules containing phytonutrients derived from palm oil is claimed in claim 18 wherein the nano-capsules having size of 20nm – 500nm.