

A METHOD TO PREPARE LUTEIN-ENCAPSULATED POWDER

FIELD OF THE INVENTION

The present invention is directed to a method of preparing lutein-encapsulated powder, more particularly a method of encapsulating lutein and phospholipid in porous starch and then spray drying it. The invention is also directed to the resulting powder composition comprising lutein, phospholipid and porous starch.

BACKGROUND OF THE INVENTION

Lutein, an important carotenoid, and can be found abundantly in egg products, fruits, vegetables, flowers, and various plants. Its molecular structure consists of a lengthy carbon chain comprising 40 carbon atoms, featuring 10 conjugated double carbon bonds and cyclic structures at both ends. Lutein possesses numerous beneficial physiological functions. Its multiple double bonds grant it antioxidant properties and the ability to scavenge free radicals, making it an excellent agent for counteracting oxidative stress in the human body. Additionally, lutein's capacity to filter out blue light makes it effective in protecting the skin and eyes from sun damage. Research has also indicated that lutein exhibits potential effects in preventing or alleviating conditions such as ulcers and strokes. Moreover, its vibrant orange-yellow color renders lutein suitable as a natural food and beverage colorant. Consequently, lutein finds wide-ranging applications in the food, feed, cosmetics, medicine, and other industries. Notably, lutein cannot be synthesized within the body, making dietary intake the sole source. Nevertheless, due to its extensive carbon chain and numerous double bonds, lutein exhibits very low water solubility and poor stability, imposing limitations on its utilization. The inherently poor aqueous solubility of lutein contributes to its relatively lower bioavailability. Hence, it is crucial to explore methods that can mitigate these drawbacks and maximize the utilization of lutein.

Encapsulation has emerged as a highly effective technique for improving the stability and solubility of sensitive bioactive components. In the case of lutein, numerous encapsulation approaches have been investigated, including various emulsion-based systems, nanoparticle- and microparticle-based methods, as well as molecular inclusion encapsulation systems. However, the majority of current systems require the initial solubilization or dispersion of lutein using organic solvents or plant oils prior to encapsulation. For instance, in the preparation of lutein nanoparticles, lutein and polymers are dissolved in the organic solvent acetonitrile. Similarly, oil is utilized in emulsion-based lutein encapsulation to dissolve the lutein. The

inclusion of organic solvents or oils adds complexity to the overall process. In particular, when employing organic solvents, additional steps must be implemented to eliminate any residual traces. The typical process for nano encapsulation involves multiple stages such as solubilization, incubation, agitation, evaporation, washing, and drying. Each stage contributes to production costs and can impact the sustainability of the final product. Therefore, there is a strong demand for a simplified and environmentally friendly method for lutein encapsulation.

Furthermore, the majority of encapsulation methods primarily yield suspensions, whereas the industry typically prefers the use of powder form due to its numerous advantages over liquid form. Powdered encapsulated products offer extended shelf life, reduced transportation costs, ease of handling, and lower chances of incompatibility during formulation. Currently, spray-drying stands as the most prevalent drying technology in the food industry for obtaining powder and is widely employed for the production of microcapsules to encapsulate bioactive compounds. However, spray-drying is notorious for encountering low yield issues with materials that possess a low glass transition temperature, leading to adhesion onto the inner walls of the spray dryer. These adhesion problems also impact the particle size distribution and moisture content of the resulting powder.

SUMMARY OF THE INVENTION

In this study, the inventors have developed a novel, simple, and environmentally friendly process to overcome the aforementioned challenges. The process involves only two steps: mixing and drying. By eliminating the need for organic solvents and oils, the inventors have achieved an overall process yield of up to 90%.

In a first aspect, the present invention provides for a powder composition comprising lutein and hydrogenated phospholipid, encapsulated by porous starch.

In some embodiments, the hydrogenated phospholipid is selected from the group consisting of: phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine, and lecithin.

In some embodiments, the phospholipid is L- α soybean phosphatidylcholine.

In some embodiments, the powder composition comprises a weight ratio of 1-3 g phospholipid: 1 g lutein: 2.5-10 g porous starch.

In some preferred embodiments, the powder composition comprises a weight ratio of 2-3 g phospholipid: 1 g lutein: 5-10 g porous starch.

5 In some embodiments, the powder composition comprises a water content in the range of 1.5-1.1 wt%, 1.4-1.1 wt%, 1.3-1.1 wt%, or 1.2 wt%.

In some preferred embodiments, the composition is formulated as a tablet or capsule.

10 In a second aspect, the invention provides for a method of producing an encapsulated lutein composition, comprising: i) mixing water and a phospholipid; ii) adding lutein into the mixture of i) and further mixing for a period to obtain a lutein suspension; iii) combining the lutein suspension with a porous starch suspension and mixing for a period to achieve encapsulation; iv) spray drying the encapsulated lutein.

15 In some embodiments, the phospholipid is selected from the group consisting of: phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine, and lecithin.

20 In some preferred embodiments, the phospholipid is L- α soybean phosphatidylcholine.

In some embodiments, the phospholipid in step iii) is at a concentration in the range of 2 g/L to 6 g/L. Preferably, the concentration range is 5-6 g/L.

25 In some embodiments, the lutein in step iii) is at a concentration of 2g/L.

In some embodiments, the porous starch in step iii) is at a concentration in the range of 5 g/L to 20 g/L. Preferably, the concentration range is 10-20 g/L.

30 In some preferred embodiments, in step ii) the further mixing of lutein with the phospholipid is for a period of about 3 hr.

In some preferred embodiments, in step ii) or step iii) the mixing of the lutein suspension with the porous starch suspension is performed at a temperature in the range of 25°C to 60 °C.

35 In some preferred embodiments, in step iii) said phospholipid is in the range of 5-6 g/L; said lutein is at 2 g/L; and said porous starch is in the range of 10-20 g/L.

Encapsulated lutein can be administered as-is in a capsule, or can be formulated with other ingredients, particularly for health supplementary products. In some embodiments, the formulation may include pharmaceutical excipients including, but not limited to, one or more

of surfactants, pH modifiers, diluents, disintegrants, binders and lubricants. The formulation can be in the dosage form of powder, granule, tablet or capsule.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows a molecular structure of lutein.

Figure 2 shows a schematic representation of a process of lutein encapsulation by phospholipid and porous starch using spray-drying.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

Certain terms employed in the specification, examples and appended claims are collected here for convenience.

It must be noted that as used herein and in the appended claims, the singular forms "a", "an", and "the" include plural reference unless the context clearly dictates otherwise.

As used herein, the term "comprising" or "including" is to be interpreted as specifying the presence of the stated features, integers, steps or components as referred to, but does not preclude the presence or addition of one or more features, integers, steps or components, or groups thereof. However, in context with the present disclosure, the term "comprising" or "including" also includes "consisting of". The variations of the word "comprising", such as "comprise" and "comprises", and "including", such as "include" and "includes", have correspondingly varied meanings.

Lutein in the present invention may be lutein in its free form, or as lutein esters.

Having now generally described the invention, the same will be more readily understood through reference to the following examples which are provided by way of illustration, and are not intended to be limiting of the present invention.

EXAMPLES

Example 1 Materials and Methods

Materials

Lutein extract; L- α soybean phosphatidylcholine(hydrogenated); porous starch; and ultrapure water.

Other phospholipids also have similar efficiency and yield, for example: phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine. Lecithin (which main component is phospholipid) also can be used for the similar purpose.

Porous starch is derived from raw starch via chemical or enzymatic method, all sources of porous starch have similar effects in this technology.

Methods

Preparation of lutein-encapsulated powder

30 ml of water and 0-3 g of soybean hydrogenated phosphatidylcholine are added into a 100 ml beaker followed by mixing using a magnetic bar with 1000 rpm. After 5 minutes mixing, 1 gram of lutein extract is slowly added into the beaker and the mixing continued for 3 hours under different temperature (25°C – 60 °C) to obtain a well-dispersed suspension of lutein.

The above suspension is transferred into a 1000 ml beaker with 450 ml of porous starch suspension (0-10 g) and the total volume is topped up to 500 ml with water. The suspension comprises 0-6 g/L hydrogenated phospholipid, 2 g/L lutein, and 0-20 g/L porous starch.

Finally the suspension is stirred under 1000 rpm for encapsulation. The encapsulation continues for 2 hours followed immediately by spray drying.

The spray-drying procedure was conducted utilizing a laboratory-scale spray dryer. The suspension mentioned earlier was converted into a fine spray by the action of air and introduced at the upper section of the drying chamber. The atomizing air, operating at a pressure of 3 bar, and the feed solution, flowing at a rate of 100 ml/min, were independently directed towards a two-fluid nozzle with a diameter of 0.75 mm in a co-current flow arrangement. The inlet temperature of the air was set at 150°C. Subsequently, the dried particles were separated from the hot air stream within a cyclone separator and collected in a receiving vessel.

In some embodiments the powder composition comprises a weight ratio of 1-3 g phospholipid: 1 g lutein: 2.5-10 g porous starch.

Determination of Encapsulation Efficiency

The determination of lutein content involved procedures that exploit the lipophilic nature and hexane solubility of lutein. On the other hand, the wall material starch is water-soluble and insoluble in hexane.

To begin, lutein was dissolved in hexane and formulated into solutions with varying concentrations. The HPLC system equipped with a DAD detector was utilized to measure the peak area of these solutions at 450 nm, using a mobile phase of acetonitrile solution (90%, v/v). A standard curve was established by plotting the peak areas of lutein against the corresponding concentrations of lutein.

Next, 20 mg of accurately weighed lutein microcapsule granules were solubilized in 1 mL of deionized water using an ultrasound-assisted method. The resulting solution was then diluted with hexane to a final volume of 20 mL. Additionally, 20 mg of lutein microcapsule granules were precisely weighed and mixed with lutein to achieve a final volume of 20 mL, while shaking for 1 minute. These two solutions were subsequently centrifuged at 4000 rpm for 10 minutes at 4°C. The lutein concentration in the supernatant was determined using the aforementioned HPLC system and the established standard curve, enabling the determination of both the total lutein content and the lutein content on the surface of the microcapsules. The encapsulation efficiency was quantitatively calculated using the following equation (1):

$$EE (\%) = \left(1 - \frac{M1}{M2}\right) \times 100\% \quad (1)$$

M1: Surface lutein content in the powder

M2: Total lutein content in the powder

Determination of Yield

The yield of lutein microcapsules is determined by the ratio of the final mass of lutein contained within the microcapsules (Mf) obtained after the spray drying process to the initial mass of lutein present in the raw materials (Mi). This yield can be calculated using the following Equation (2).

$$Yield (\%) = \left(1 - \frac{Mf}{Mi}\right) \times 100\% \quad (2)$$

Starch encapsulated lutein in simulated gastrointestinal digestion fluids

The simulated gastric fluid containing pepsin was prepared by dissolving it in sterile NaCl solution, with the pH adjusted to 2.0 using a 1.0 mol/L HCl solution. On the other hand, the simulated intestinal fluid, comprising trypsin and pancreatin, was created by dissolving 10 g/L

of bile salts in phosphate-buffered saline, with the pH maintained at 6.8 through the addition of a 0.1 M NaOH solution.

To initiate the experiment, equal amounts of unencapsulated lutein and starch-encapsulated lutein were combined with the simulated gastric fluid. The mixtures were then subjected to shaking at 200 rpm for 2 hours at a temperature of 37°C, mimicking the process of stomach digestion. Subsequently, the mixtures were mixed with simulated intestinal fluids in equal proportions. The resulting mixtures, now at pH 7.0, were further shaken for 2 hours to simulate intestinal digestion.

At the end of each incubation stage, 0.5 mL of the suspensions were extracted and solubilized using an ultrasound-assisted method. The solubilized samples were then diluted with hexane to a final volume of 20 mL. After thorough mixing and complete protein precipitation, the mixtures were subjected to centrifugation at 20,000× g for 20 minutes. The supernatants obtained from the centrifugation were analyzed to determine the retained lutein content using an HPLC system at a wavelength of 450 nm. The concentration of released lutein was calculated based on a standard calibration curve.

In order to make a comparison, samples without any treatment at time zero also needed to be extracted to calculate the percentage of lutein remaining.

Example 2

Preparation of lutein-loaded microencapsulates

The microencapsulation technique is widely employed to safeguard active ingredients from degradation, particularly for stabilizing labile compounds. This technology involves packaging solid, liquid, and gaseous materials within small capsules that release their contents at controlled rates over extended periods of time. Various conventional methods, including spray-cooling, spray-drying, freeze-drying, centrifugal extrusion, molecular inclusion, and emulsion freeze-drying, have been utilized in microencapsulation techniques. Among these methods, spray-drying is the most commonly employed due to its cost-effectiveness, availability of equipment, continuous production capabilities, and ease of industrial implementation. Additionally, spray-drying offers compatibility with numerous coating materials and minimal impact on the functional characteristics of the particles after dissolution.

The selection of appropriate wall materials is another crucial aspect of microencapsulation. The efficiency and stability of microcapsules largely depend on the composition of the wall material. Natural polymers, such as gelatin, alginates, proteins, cyclodextrins, starch

maltodextrin, and chitosan, are commonly utilized as wall materials for the microencapsulation of lutein, providing a certain degree of encapsulation for lutein. However, these materials often exhibit limitations, including small pore size, low specific surface area, weak adsorption capability, and poor aggregation behavior, resulting in low lutein content within the microcapsules.

In this study, porous starch was chosen as the raw material for microencapsulation. Porous starch is a modified form of starch obtained through the action of amylases on raw starch below its gelation temperature. With its honeycomb-like structure, porous starch improves the absorptive and adhesive properties of materials. The results of this study demonstrate that microencapsulation using porous starch can enhance the stability of curcumin pigment and lutein, providing improved protection for these compounds.

However, due to lutein's poor solubility in water, organic solvent or oil has to be used to solubilize lutein first in the microencapsulation process. For example, in a study, lutein microencapsulates were manufactured with spray and starch-catching drying technology, in which, lutein and dl-alpha-tocopherol were dissolved as oil phase; modified food starch, water and sucrose were dissolved as aqueous phase; then oil phase and aqueous phase were emulsified, and filtrated, spray granulation and corn starch catching, fluidized drying, sieving and packaging to obtain LM product. In another study, gelatin and porous starch were mixed and dissolved in hot distilled water with stirring to form an aqueous solution. Lutein sample preheated to dissolve in ethanol was dripped into the gelatin and starch solution to form a coarse emulsion at high temperature. Apparently, the aforementioned processes are not economic feasible due to the complexity of the process with the inherent problems brought by organic solvent or oil. A green and simple process is highly anticipated to make the encapsulated product with high competition margin in the market.

Based on the aforementioned analysis, achieving a green process involves dispersing lutein powder in a continuous phase without the use of solvents or oils prior to encapsulation. In our invention, we employ a high concentration of phospholipid water suspension to effectively disperse a large amount of lutein powder. Phospholipids are known to form micelles at their critical micelle concentration (CMC), and previous studies have demonstrated the entrapment of lutein within phospholipid micelles. However, existing methods utilize solvents to dissolve lutein and phospholipids, followed by an antisolvent precipitation technique to obtain encapsulation. Unfortunately, this approach fails to achieve a high concentration of lutein microcapsules in the water suspension, resulting in exorbitant downstream drying costs. Moreover, phospholipids alone are not stable enough for encapsulation.

Therefore, the technology of the invention takes a different approach. Initially a high concentration of phospholipid is utilized to form a well-dispersed emulsion of lutein, which is subsequently subjected to spray-drying with porous starch to obtain encapsulated particles.

- 5 This entire process involves only two steps: mixing and drying. By employing this novel method, the limitations of previous approaches is overcome and a green and efficient process for lutein encapsulation is achieved.

10 In order to develop the technology of lutein encapsulation, it was crucial to optimize the mixing step of the encapsulation process. Experiments were conducted to investigate the impact of various factors on the encapsulation efficiency (EE) and yield of lutein microencapsulation. Specifically, the concentration of phospholipids in the mixture, the incubation temperature, and the starch concentration were focused on, as these factors were found to have significant effects on the encapsulation process. Through our analysis, we were able to demonstrate the
15 profound influence of these factors on the efficiency and yield of lutein encapsulation.

Table 1 reveals the crucial role of phospholipids in achieving high encapsulation efficiency (EE). In the absence of phospholipids, the encapsulation efficiency is significantly low (5.4%), despite a high yield (89.0%). This can be attributed to the poor water solubility of lutein, resulting in visible aggregates when lutein is mixed with starch without phospholipids. These
20 large aggregates are unable to penetrate the internal pores of the starch, leading to an extremely low encapsulation efficiency.

On the contrary, when lutein powder is dispersed in a high concentration phospholipid suspension, a homogeneous yellowish emulsion is obtained. Although the underlying
25 mechanism is not fully understood, it is possible that hydrogen bond formation between lutein and phospholipids contributes to the formation of this emulsion. This well-dispersed emulsion allows for efficient encapsulation of lutein, leading to higher encapsulation efficiency.

Table 1 phospholipid concentration effects on lutein microencapsulation

Phospholipid concentration (g/L)	EE (%)	Yield (%)	Moisture content (%)
0	5.4	89.0	1.2
1	30.8	90.5	1.2
2	60.4	91.8	1.3
3	82.4	90.5	1.1
4	89.6	88.3	1.3
5	95.0	90.5	1.2
6	94.8	91.2	1.2

The temperature parameter is considered to be important for emulsion formation. However, as shown in Table 2, our data demonstrates that room temperature is sufficient to achieve high encapsulation efficiency (90.2%) and yield (90.5%). Interestingly, we observed a negative effect on lutein microencapsulation when the temperature was increased to 60°C. It is widely recognized that lutein is prone to instability at high temperatures, especially in an aqueous solution. Our HPLC analysis further confirmed that lutein undergoes degradation when exposed to 60°C for a duration of two hours. Therefore, it is evident that higher temperatures have a detrimental impact on lutein stability and subsequent microencapsulation efficiency.

Table 2 Temperature effect on lutein microencapsulation.

Temperature (°C)	EE (%)	Yield (%)	Moisture content (%)
25	90.2	90.5	1.2
30	95.0	90.8	1.1
50	94.5	92.0	1.4
60	90.1	80.2	1.2

Our experiments revealed another important finding: without the presence of starch as a second layer for lutein encapsulation, the yield of the process is significantly low, as indicated in Table 3. During the drying process, a substantial amount of the materials adheres to the walls of the drying chamber and cyclone, resulting in a much lower yield compared to encapsulation with starch. This can be attributed to the low glass transition temperature of phospholipids. To address this issue, the addition of drying aids like mannitol is a common approach. However, in our innovative method, we successfully encapsulated the phospholipid emulsion within the porous starch, resulting in a remarkably high yield of 90.5%. This approach offers a superior solution to enhance the yield without the need for additional drying aids.

Table 3 Starch concentration effect on lutein microencapsulation.

Starch concentration (g/L)	EE	Yield	Moisture content
0	45.5	20.5	1.5
5	95.0	70.6	1.1
10	94.8	90.5	1.2
20	94.2	90.4	1.2

Example 3

Stability study in simulated gastrointestinal digestion fluids

The results presented in Table 4 depict the percentages of lutein remaining in both free and encapsulated forms. The data clearly demonstrates that over 85% of the lutein content remained within the starch encapsulates, whereas only 40.2% was retained in its free form. This indicates the successful protection provided by encapsulation, preventing significant lutein degradation when exposed to gastric fluid for 2 hours. In other words, less than approximately 15% of the lutein content was lost during the 2-hour exposure to the acidic medium before reaching the intestinal tract.

Furthermore, Table 4 illustrates that approximately 60% of the lutein within the encapsulates was lost after 2 hours of exposure to simulated intestinal fluid. In comparison, without encapsulation, approximately 90% of the lutein was lost under the same conditions. The stability has been increased by 33%. These findings demonstrate the superior stability and protection offered by the encapsulation method. When the lutein encapsulates were subjected to simulated gastric and intestinal fluids, mimicking the *in vivo* contact with these fluids at 37°C, it can be reasonably concluded that the encapsulates remained stable within the gastrointestinal tract, potentially leading to improved bioavailability.

Table 4 Lutein amount at each stage.

	Lutein remaining at incubation beginning (%)	Lutein remaining at simulate gastric digestion (%)	Lutein remaining at simulate intestinal digestion (%)
Free lutein	100	40.2	10.5
Encapsulated	100	85.8	40.4

Summary

The introduction of lutein microcapsules in a carrier mixture composed of phospholipid and porous starch represents a novel achievement. This process is characterized by its simplicity and environmental friendliness, as it only involves two steps: mixing and drying, without the need for any organic solvents or oils. The high concentration of phospholipid in the initial mixing step enables effective dispersion and encapsulation of a concentrated lutein load, while the addition of porous starch provides an additional layer of protection for the encapsulated lutein.

Notably, this process can be carried out at room temperature during the mixing step, resulting in cost savings compared to other encapsulation methods that require heating. *In vitro* studies using simulated gastrointestinal digestion fluids clearly demonstrate the superior stability of

lutein encapsulated in porous starch. Furthermore, the lutein encapsulated powder exhibits an exceptionally high encapsulation efficiency (>95%) and yield (90%). The combination of these favourable attributes, along with the simplicity and sustainability of the process, paves the way for large-scale production of lutein encapsulates. This invention formulates lutein into powders, granules, tablets or capsules which may be used for treating eye problems.

The outcomes of this study hold great promise for the application of lutein in the food and health supplement industries, offering enhanced stability and potential benefits to consumers.

CLAIMS

1. A powder composition comprising lutein and hydrogenated phospholipid encapsulated by porous starch.

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2. The composition according to claim 1, wherein the hydrogenated phospholipid is selected from the group consisting of: phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine and lecithin.

10 3. The composition according to claim 2, wherein the phospholipid is L- α soybean phosphatidylcholine.

4. The composition according to any one of claims 1 to 3, wherein the powder composition comprises a weight ratio of 1-3 g phospholipid: 1 g lutein: 2.5-10 g porous starch.

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5. The composition according to claim 4, wherein the powder composition comprises a weight ratio of 2-3 g phospholipid: 1 g lutein: 5-10 g porous starch.

20 6. The composition according to any one of claims 1 to 5, comprising a water content in the range of 1.5-1.1 wt%, 1.4-1.1 wt%, 1.3-1.1 wt%, or 1.2 wt%, by weight of said composition.

7. The composition according to any one of claims 1 to 6, formulated as a tablet or capsule.

8. A method of producing an encapsulated lutein composition, comprising:

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i) mixing water and a phospholipid;

ii) adding lutein into the mixture of i) and further mixing for a period to obtain a lutein suspension;

iii) combining the lutein suspension with a suspension of porous starch and mixing for a period to achieve encapsulation;

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iv) spray drying the encapsulated lutein.

9. The method according to claim 8, wherein the phospholipid is selected from the group consisting of: phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine, and lecithin.

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10. The method according to claim 9, wherein the phospholipid is L- α soybean phosphatidylcholine.

11. The method according to any one of claims 8 to 10, wherein the phospholipid in step iii) is at a concentration in the range of 2 g/L to 6 g/L.

5 12. The method according to claim 11, wherein the phospholipid in step iii) is at a concentration in the range of 5 g/L to 6 g/L.

13. The method according to any one of claims 8 to 12, wherein the lutein in step iii) is at a concentration of 2 g/L.

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14. The method according to any one of claims 8 to 13, wherein the porous starch in step iii) is at a concentration in the range of 5 g/L to 20 g/L.

15 15. The method according to claim 14, wherein the porous starch in step iii) is at a concentration in the range of 10 g/L to 20 g/L.

16. The method according to any one of claims 8 to 15, wherein in step ii) the further mixing of lutein with the phospholipid is for a period of about 3 hr.

20 17. The method according to any one of claims 8 to 16, wherein in step ii) or step iii) the mixing is performed at a temperature in the range of 25°C to 60 °C.

25 18. The method according to any one of claims 8 to 17, wherein in step iii) said phospholipid is L- α soybean phosphatidylcholine in the range of 5-6 g/L; said lutein is at 2 g/L; and said porous starch is in the range of 10-20 g/L.