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Encapsulation of Soy Lecithin Nanoparticles in Gelatine Coating for the Fortification of Gelled Food Products with Phospholipids

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Abstract

Phospholipids are category of nutrients that are essential for growth and health maintenance. They are commonly supplied from animal-derived foods. However, plant-derived phospholipids like soy lecithin seems also to be potential alternative because they are well received by different category of population who have concerns about animal-derived foods. The purpose of the current investigation is to formulate gelatine-based matrix bearing soy lecithin as a model of gelled food product fortified with plant-based phospholipids. First an aqueous colloidal system containing lecithin nanoparticles is formulated. The average particle size of lecithin was 79.0 nm and its zeta-potential was -49.0 mV. Then, lecithin nanoparticles were encapsulated in gelatine coating using the layer-by-layer electrostatic deposition method. That led to the formation of gelatine-encapsulated-lecithin nanoparticles which has an average particle size 204.0 nm and a zeta-potential +4.0 mV. Upon cooling that system at 4ºC, a hydrogel bearing lecithin nanoparticles is formed. The whole process was evaluated using different techniques in order to verify the encapsulation of lecithin nanoparticles within the gelatine matrix. The physical stability of all formulations before and after encapsulation was monitored during and after storage for 3 months. The concept of this investigation can be useful for fortification of some gelatine-based food products with plant-based phospholipids, which can be used as a dietary supplement for health maintenance and promotion among different populations.

Key word: Encapsulation; gelatin; phospholipids; gelled food; fortification

1. Introduction

Food acceptability depends on different physical aspects like appearance, flavour and mouth feel. Nowadays people are more concerned about health and well being, so food functionality arises as another factor that contributes to the acceptability and purchase of food products [1]. Food products fortified with bioactive compounds and nutrients are relevant to this issue due to the health benefits which can be gained through regular consumption of such products [2]. For example, different foods and beverages in the market nowadays are fortified with vitamins and minerals [3,4]. This kind of fortification was meant to compensate for the insufficient intake of these vital nutrients in order to approach the required daily intake. In addition, certain microorganisms like probiotic bacteria are also used for fortification [5,6] due to the health benefits of their secondary metabolites.

Beside the above mentioned, there are another important group of nutrients namely phospholipids which are required on a daily basis to support and maintain health [7]. Phospholipids are the building blocks of the lipid bilayer which contribute significantly to the construction of the cell membranes of all eukaryotes. Phospholipids are also responsible for the bio-synthesis of acetyl choline which is a neurotransmitter of signals between cells. In addition, phospholipids are necessary for memory enhancement and for protection against Alzheimer's disease [8]. Moreover, phospholipids are reported to be anti-aging and anti-cancer agents [9], beside their role in liver cells regeneration [10]. The required daily intake of phospholipids rang from 2-8 gm depending on age, sex, etc. [11]. Phospholipids can be secured by direct consumption of food rich in this vital nutrient such as eggs, veal liver, …etc. [7]. However, such kind of food is also linked to high level of cholesterol and protein which could be unsuitable for cardiovascular compromised people or those who follow a reduced protein diet due to some kidney problems. There are also some groups in the society like vegetarians which have concerns about consuming animal products. Therefore plant-based phospholipids such as soy lecithin could potentially

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be an acceptable alternative to phospholipids from animal sources.

Soy lecithin is obtained as a by-product from the process of de-gumming of crude soybean oil after seed extraction. It contains appreciable collection of different phospholipids like phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine, phosphatidylinositol and phosphatidic acid [12]. Due to that unique collection of phospholipids, soy lecithin is commercialized in the market, *per se*, as a dietary supplement in different forms. That includes capsules, de-oiled powders and granules. These forms can be directly ingested (e.g. capsules) or mixed with the meal (e.g. powders and granules) to enhance its phospholipids content. However, unlike many food products which are fortified with minerals and vitamins, there is no ready-to-eat food product in the market so far which is fortified with phospholipids. Therefore, a previous investigation by the author of the current work [13] developed a method for the formulation of stable water-based soy lecithin nanoparticles for potential fortification of beverages with plant-based phospholipids.

In the current investigation the author extends the same trend of the previous study [13] to include fortification of gelatine-based food products with soy lecithin as a source of phospholipids from plant origin. Such kind of products includes gummy desserts like jelly, gummy candies, chewable gelatine tablets which depend on gelatine as one of its basic ingredients to give body and texture to the product. These kinds of gelatine products are consumed by large sector of people because they are sweet, flavoured and tasty which make them appreciated among population of different ages. Therefore, many investigators took advantage of these gelatine-based products to use them as delivery vehicle for different nutraceuticals like vitamins, minerals, ω-3-fatty acids, fibers and other bioactive compounds for health maintenance [14-17]. Gelatine-based food products are also convenient nutraceuticals delivery systems to those who are suffering from swallowing problems, because these gelatinous products melt in the mouth and hence become easier to swallow. A detailed review that discusses the opportunities and challenges that encounter fortification of gelatine-based products with bioactive compounds is available [18].

Based on the above mentioned, the current investigation will deal with the formulation and stability of encapsulated soy lecithin in gelatine coating. The layer-by-layer electrostatic deposition method [19, 20] was used for that purpose to deposit a layer of gelatine over lecithin nanoparticles which are dispersed in aqueous phase. This technique has a potential for protecting sensitive food ingredients against oxidation [21] beside other promising food-related applications [22]. The investigation also applied different evaluation methods to follow the encapsulation process and the stability of the formulated gelatine-encapsulated lecithin nanoparticles during storage. Suggestions to deal with some potential problems in practical applications are also posed.

2. Experimental

Materials

Commercial de-oiled lecithin powder (Ultralec® P, ADM Co., Decature, IL, USA) with total phospholipids content 65.0% was used in all formulations. The major phospholipids constituting that powder according to the manufacturer are: phosphatidyl choline (23%), phosphatidyl ethanolamin (20%), phosphatidyl inositol (14%) and phosphatidic acid (8%). Catfish gelatine was obtained from GELITA (North America Inc., Sioux city, IA). Sodium citrate, citric acid and sodium azide (preservative) were obtained from Sigma-Aldrich Chemical Co. Saint Louis, USA.

Formulation of water-borne lecithin nanoparticles (LNP)

The detailed description of the method was revealed in our previously investigation [13]. In brief, soy lecithin powder was slowly and gradually hydrated with 20 mM sodium citrate-citric acid buffer (pH 4.0) using mechanical stirring to end up with 1.0% aqueous dispersions (w/w) of lecithin submicron particles-in-water. Then, that dispersion was homogenized for 2 minutes at 20,000 rpm using high speed homogenizer (Ultra-Turax, IKA, Staufen, Germany) equipped with T25 prop. After that, the whole dispersion was passed 7 rounds through high pressure microfluidizer (M-110Y microfluidic, Newton, MA) at 13920 psi (~ 95.9 MPa). The microfluidized effluent was a translucent colloidal lecithin nanoparticles (LNP) which was received in dark glass bottled and stored at 4ºC until used within 24h. The whole microfluidization process was conducted under cooling conditions using an ice bath. The formulation was repeated twice.

Encapsulation of LNP in gelatine coating

Lecithin nanoparticles (LNP) prepared as described previously were encapsulated in gelatine coating using the layer-by-layer electrostatic deposition technique [22]. A gelatine coating solution (4.0% w/v) was prepared by hydrating fish gelatine granules in 20 mM sodium citrate-citric acid buffer (pH 4.0) using gentle heating (40.0◦C) and stirring.

After gelatine was totally dissolved, the solution was allowed to go back slowly to lukewarm then filtered to remove impurity. The encapsulation process was performed by the quick addition of the whole portion of LNP solution (1.0% w/v) into the gelatine coating solution (4.0% w/v) with high stirring speed for 5 minutes. The appearance of the final dispersion was changed immediately from translucent to opaque, which is a preliminary visual indication of the formation of gelatine-encapsulated lecithin nanoparticles (GE-LNP). The ratio of lecithin to gelatin during the encapsulation process was fixed at 1:4 by weight, respectively. The whole process was repeated twice to get duplicate samples.

Verification of the formation of GE-LNP
Assessment of particle size

Particle size was measured for GE-LNP samples before gelling (in the liquid state) and also after gelling which was achieved by storing the samples at the fridge at 4°C. All measurements were conducted using dynamic light scattering instrument Zetasizer (Nano-ZS model ZEN3600, Nano-series, Malvern Instruments, UK). Measurements were done at 25ºC, with a fixed angle of 172º. The samples were diluted with the buffer as appropriate before measurement and filtered before analysis. Particle sizing of the gelled samples of GE-LNP was conducted after thawing, by taking them out of the fridge to the room temperature to bring them back into their original liquid state. The reported particles size in the results section is the *z*-average mean (dz) ± S.D for the particles hydrodynamic diameter (nm) of six measurements from two preparations, three measurements each.

Assessment of surface charge (zeta-potential)

The surface charge (zeta-potential, mV) of LNP and GE-LNP in all dispersions was measured using the electrophoresis and dynamic light scattering instrument Zetasizer (Nano-ZS). Zeta-potential was calculated from the measurement of the electrophoretic mobility of particles in an applied oscillating electric field using laser Doppler velocimetry. All Measurements were done in duplicates at 25ºC and the values reported were mean ±S.D.

Assessment of light transmittance

The difference in light transmittance (transparency) between the two dispersions of LNP and GE-LNP was measured as one of the verification methods of the encapsulation process and formation of GE-LNP. That was measured by using the instrument for the optical characterization of liquid dispersions (Turbiscan classic MA 2000, formulaction, Toluse, France). The instrument is equipped with two synchronous detectors, (transmission detector and backscattering detectors). The instrument was operated at the transmission mode in which a transmission detector (at 180°) receives the light which goes through the liquid sample (LNP or GE-LNP) placed in flat-bottom cylindrical glass cell. A mobile reading head composed of a pulsed near infrared light diode (λ = 850 nm) and the transmission detector scans the entire height of a flat-bottom cylindrical glass cell containing the sample and acquiring transmission data every certain length (in μm) of the tube. A pattern of the light flux as a function of the sample height is obtained and plotted against transmittance.

Physical stability during storage

Two sets of LNP and GE-LNP (each in duplicate) were stored at room temperature (25.0◦C±2) and at the fridge (4.0±1◦C). The samples were regularly inspected visually every 15 days for three consecutive months for any change in appearance (turbidity or sedimentations). The particle size distribution of these sets of LNP and GE-LNP at the beginning and the end of the storage period was evaluated. In addition, the particle size of the GE-LNP gelled samples which were stored at 4◦C was also evaluated regularly each 15 days for three months period using the same procedure after thawing the samples to the liquid state as previously indicated.

Statistical analysis

Data in the results section are expressed as mean of at least two measurements ± S.D.

3. Results and Discussions

In the current study the author aimed to encapsulate soy lecithin in gelatine matrix for production of functional gelatine desserts rich in plant-based phospholipids to be used as dietary supplements. That can be accomplished by depositing a layer of positively charged gelatine on the surface of lecithin particles that carry a negative charge via the electrostatic layer-by-layer deposition technique. That in turn makes the process potentially suitable for developing different gelatine-based food products fortified with soy lecithin as a dietary source of phospholipids. Based on the above mentioned, it is necessary to trace the steps of the encapsulation process to confirm the formation of stable and homogenous gelatine-encapsulated lecithin nanoparticles (GE-LNP). Therefore, some parameters like particle size, appearance and surface charge of the

formulated GE-LNP were investigated as shown in the next passage.

Particle size, appearance and surface charge

Lecithin nanoparticles (LNP) were obtained by dispersing soy lecithin powder (65.0% phospholipids) in water using the high-pressure homogenizer. Particle size analysis indicated that the nanoparticles have an average size of 79.8±1.0 nm (Fig. 1). This size is in the same range that was reported in our previous study [13] indicating a reproducible result of the formulation process. Encapsulation of LNP in gelatine coating using the electrostatic layer-by-layer deposition method led to an increase in the particle size of LNP from 79.8 to 204.1± 1.1nm (Fig. 1), which is an evidence of the deposition of gelatine coating around lecithin nanoparticles to form gelatine-encapsulated lecithin nanoparticles (GE-LNP). It is necessary to indicate that deposition of the gelatine layer can envelop a cluster of LNP rather than a single particle, and that may justify the ~ 3-fold increase in the particle size after gelatine encapsulation (Fig. 1).

Another evidence of the formation of GE-LNP is the change of the appearance of the dispersion after gelatine encapsulation. Photos accompanying Figure 1, indicates that LNP (79.8 nm) are almost translucent, while GE-LNP (204.0 nm) appeared opaque. That difference in appearance was quantitative assessed by measuring the transmittance of light (T) of the aqueous samples of LNP and GE-LNP using an instrument for optical characterization of liquid dispersions (Turbiscan). Figure (2) indicates that LNP has a (T) value 0.873 indicating more transparent appearance than GE-LNP (T value 0.804) which looked opaquer due to the increase in the particle size after gelatine coating and encapsulation. Based on the above mentioned, the results presented in Fig. 1&2 confirm the increase of particle size due to the deposition of gelatine coating around lecithin nanoparticles and verifies the formation of GE-LNP.

Figure (3) shows the change of the surface charge (zeta-potential) of the of LNP from negative value (-46.0±1.3 mV) before encapsulation, to positive value (+4.3±0.02 mV) after encapsulation in gelatine coating. That finding represents inversion of the sign of the surface charge which is a result of deposition of sufficient layer of the positively charged gelatine around the negatively charged LNP via the electrostatic attraction. The inversion of surface charge sign from negative to positive represents another evidence of encapsulation in gelatine coating and the formation of GE-LNP complex.

Generally, it is worth to indicate that the layer-by-layer deposition technique is essentially considered electrostatic with possibilities of building up assemblies based on hydrogen bonding and sometimes hydrophobic bonding [23]. However, in case of using gelatine, the binding to lecithin particles happen mainly by electrostatic attraction due to the week hydrogen bonding between lecithin and gelatine [24]. It is also important to point out that the freshly prepared GE-LNP in its liquid state at room temperature showed homogenous appearance with no precipitations. That is due to the proper choice of the ratio between lecithin and gelatine (1:4 by weight, respectively) as indicated in the experimental section. Improper choice of the ratios between lecithin and the positively charged biopolymer like gelatine may destabilize the system by forming precipitates which can be called coacervates or agglomerates [22].

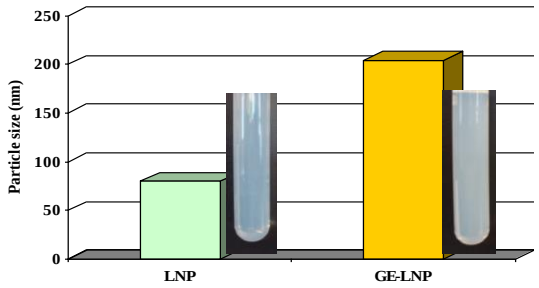

Fig. 1. Change in particle size and appearance of lecithin nanoparticles (LNP) in aqueous dispersion before and after encapsulation in gelatine coating (GE-LNP).

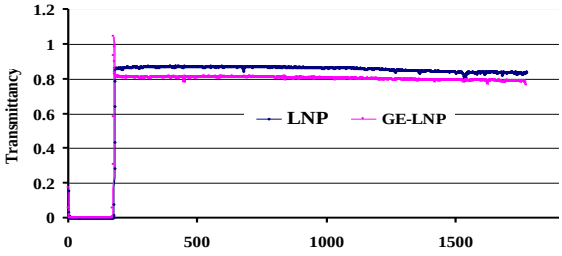

Fig. 2. Change in transparency of lecithin nanoparticles (LNP) in aqueous dispersion before and after encapsulation in gelatine coating (GE-LNP).

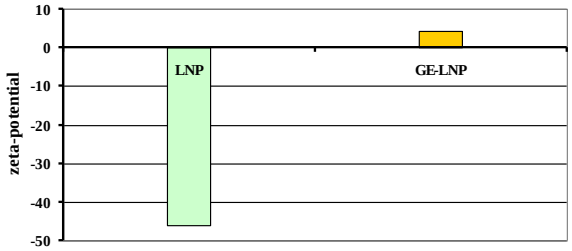

Fig. 3. Change of surface charge (zeta-potential) of lecithin nanoparticles (LNP) in aqueous dispersion before and after encapsulation in gelatine coating (GE-LNP).

Coacervates are usually occurs immediately after addition of lecithin to gelatine due to incomplete surface coverage of lecithin particle with the positive

biopolymer while agglomerates are due to the phenomena of depletion flocculation [22].

Effect of storage on the physical stability of LNP and GE-LNP

First: Effect before gelling

After demonstrating the results that confirm the formation of gelatine-encapsulated lecithin nanoparticles (GE-LNP), the study tends to evaluate the effect of three months storage period at room temperature (25ºC) on the physical stability of lecithin nanoparticles (LNP) before and after encapsulation in gelatine coating (GE-LNP). At this storage temperature (25ºC), GE-LNP was in the liquid state and was not become a gel yet because the gelation temperature of catfish gelatine which is used in this study is below that temperature [25]. Results in Fig. (4a) showed that the dispersion of LNP before encapsulation in gelatine coating was not stable for 3 months at room temperature. That can be concluded from the shape of the curve of particle size distribution (Fig. 4a) which shows bi-modal pattern compared with the mono-modal pattern at the zero time of formulation. These observations indicate aggregation of lecithin nanoparticles during storage which can lead finally to precipitation of lecithin from its aqueous solution, which was confirmed visually after the second month of storage. On the contrary, encapsulation of LNP in gelatine coating (GE-LNP), (Fig. 4b) showed higher physical stability during the entire storage period with no precipitations and almost mono-modal particle size distribution similar to that at the zero time of preparation (Fig. 4b).

The physical stability of GE-LNP in its liquid state at 25ºC originates from the film forming properties of gelatin that deposited on lecithin surface and act as a barrier against particles aggregation. It is well known that catfish gelatin, which is used in the current investigation, possessed film-forming properties comparable to those of commercial mammalian gelatin [26]. The protecting effect of the gelatin film seems to compensate for the low zeta potential value (+4.3 mV, Fig. 3) of GE-LNP which is not considered sufficient to justify its observed physical stability during storage for three months at 25ºC.

That is supported by previous investigation [27] which indicated that a zeta potential value not less than +30 mV is required to ensure physical stability of a colloidal dispersion by charge repulsion effect. One should bear in mind that the steric interaction forces exerted by gelatine film molecules [28] should also be taken into consideration as a barrier that participates with the film forming properties of gelatine in stabilizing GE-LNP during the storage at 25ºC.

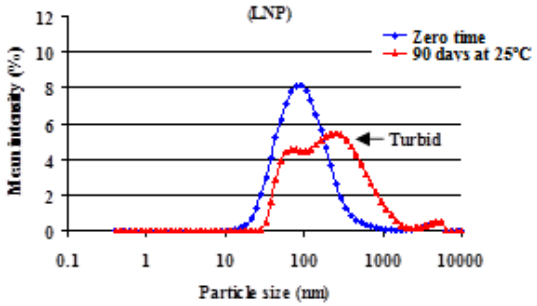

Fig. 4a, Particle size distribution of lecithin nanoparticles (LNP) in aqueous dispersion, at the zero time and after the end of the three-month storage period at 25ºC.

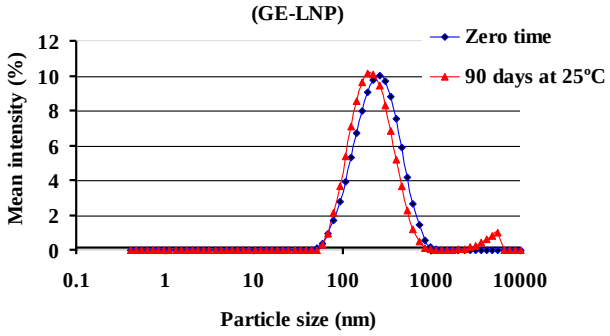

Fig. 4b, Particle size distribution of gelatine-encapsulated lecithin nanoparticles (GE-LNP) in aqueous dispersion, at the zero time and after the end of the three-month storage period at 25ºC.

Second: Effect after gelling

Storing the samples of GE-LNP at 4ºC led to the formation of a consistent gelatine gel (Fig. 5, photo). A continuous follow up study every 15 days was done for three months to monitor the change in particle size of GE-LNP during storage at 4ºC (Fig. 5). We remain the reader that the evaluation of particle size of the gelled GE-LNP took place after thawing the samples at room temperature to return them back into the liquid state as described in the experimental section. From fig. (5) it is evident that the particle size of GE-LNP is almost the same after three months storage period (205±0.8 nm) as it was at the zero time (204.1 nm). That is obviously due to the gelling action of gelatine at 4ºC which immobilize GE-LNP in its three-dimensional network. This action hinders the movement of the particles and prevents their flocculation (gathering together). That ultimately prevents GE-LNP from aggregation and kept the particle size almost constant during storage.

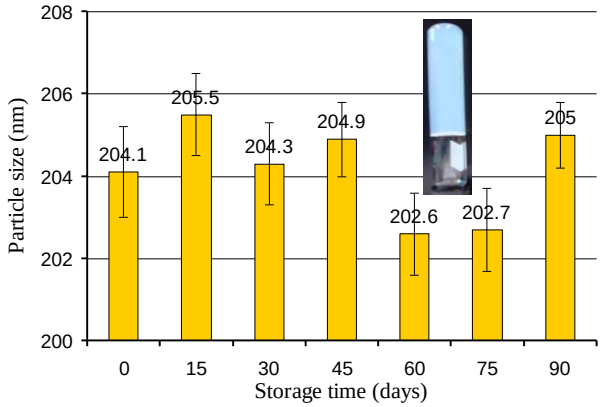

Fig. 5. Gelled sample of GE-LNP (up side down tube) and its particle size evaluated regularly during three-month storage period at 4ºC.

After discussing the process involved formulation and physical stability of GE-LNP, the discussion is now being directed toward the technical challenges that may confront this process in case of practical application for production of gelatine-based gummy desserts fortified with soy lecithin. First, Soy lecithin may have a disagreeable aroma and flavour which may preclude incorporation into edible gelatine-based food products. However, that disadvantage can be overcome by using lecithin powder from sources other than soy, like for instance sunflower lecithin which is know for its milder aroma. In addition, these gelatine-based products are usually acidic and flavoured, which can mask the disagreeable taste of soy lecithin. Another approach may include using specific fraction of soy lecithin with significant biological activity like phosphatidic acid, phosphatidylserine or any other negatively charged fraction. That in return can reduce the amount of soy lecithin required for fortification without compromising the value.

Second, catfish gelatine, which is used in the current investigation for its different advantages [29] over mammalian gelatine, suffers from some draw backs. That includes the low gel strength, a feature which is supposed to be of prime importance in the production of gelled food products. However, this disadvantage can also be overcome during industrial processing by addition of co-enhancers like sucrose to increase gel strength [30]. In the current work, the strength of the GE-LNP gel made of fish gelatine was not evaluated as it is not the main theme of our study. However, other investigators indicated that the strength of gelatine gel from channel catfish can range from 252-276 (expressed in Bloom) [31]. This value is fair enough for commercial application of gelatine which is preferably be around 250-260 [32]. Moreover, the Bloom value of catfish gelatine could be higher than that of some mammalian gelatine which is estimated at 200-240 [33].

Another draw back of using fish gelatine is its low gelling and melting temperatures compared to mammalian gelatine [25, 34]. The melting temperature of catfish gelatine gel can range between 25ºC for channel catfish gelatine [25] to 25.7ºC for African catfish gelatine [35]. Interestingly, in some gelatine-based food applications the low melting temperature can be considered as an advantage because it results in a gel with faster dissolution in the mouth with no residual 'chewy' mouth feel. Mixing fish gelatine at certain ratio with other gelatine having higher gel melting temperature (e.g. from mammalian source) could be one of the options to over come this challenge.

Conclusion

With the growing interest in health promotion through fortification of food with essential nutrients, this investigation presented a theme for using gelatine as delivery systems for supplementation of gummy food products with phospholipids from plant origin like soy lecithin. Specific fractions of lecithin (which carry negative charge) can also be used individually to impart specific biological activity to the gelatine-based product.

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Consent for Publication

Not applicable.

Conflict of Interest

There are no conflicts to declare.

Formatting of funding sources

No funding source to declare

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