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Food grade liposome systems: Effect of solvent, homogenization types and storage conditions on oxidative and physical stability

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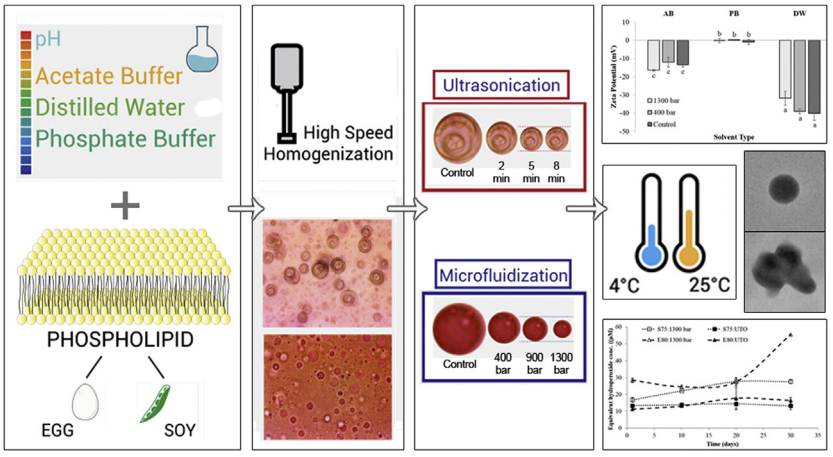
HIGHLIGHTS

- Particle size of the liposomes did not decrease by increasing ultrasonication time.
- Egg sourced liposomes are more prone to oxidation at refrigeration temperature.
- There was a relation between unfolding of the liposomes and increase in oxidation.
- Liposomes at high ionic strength was not stable due to zero zeta potential.
- Presence of dihydrogen phosphate drastically decreased oxidation at pH 7.2.

GRAPHICAL ABSTRACT



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ABSTRACT

Liposomes are the form of phospholipids which could inherently form spherical capsules in aqueous solution by energy. In this study, egg and soy lecithin were used to form liposomes by probe type ultrasonication and high pressure homogenization (microfluidization-MF) methods. To detect physical and chemical changes during storage of liposomes; particle size, zeta potential, transmission electron microscopy, optical microscopy and hydroperoxide formation experiments were conducted for one month. Microfluidization was found to be more efficient than ultrasonication in particle size reduction. When compared with egg lecithin, soy lecithin was found to oxidize less at refrigeration temperature while egg lecithin was better at room temperature, forming less hydroperoxide. Increase in unbound water resulted an increase in hydroperoxide formation with ultrasonication. Disruption of the spherical unity of the liposomes detected by particle size measurements was proposed to increase hydroperoxide formation due to exposure of hydrophobic compartments to the environment while presence of dihydrogen phosphate drastically decreased it.

1. Introduction

Encapsulation could be used to enhance the functional properties of the active agents like flavors, colorings, antimicrobials and nutrients. In food industry, encapsulation is used for several pur-

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poses such as masking flavor, decreasing permeability of volatile compounds, preventing degradation, increasing solubility [1] and forming shield against stresses, which can be either chemical or environmental [2]. Being biocompatible, biodegradable and non-toxic [3], natural capsulation materials are mostly preferred in food and pharmaceutical applications.

Liposomes are the vesicle form of phospholipids that are primarily obtained from egg and soy. Being one of the natural fat based compounds used for encapsulation, liposomes are very popular among their alternatives since they offer preservation as well as targeted delivery and controlled release for further use. Liposomes could be designed to act on specific surfaces and their release behavior can be modified according to field of use.

Another importance of liposomes lies within their self-enclosure mechanism which is triggered by the energy introduced to the system. Structure of a phospholipid contains both hydrophilic and lipophilic compartments. Specifically speaking; phosphatidylcholine – most abundant phospholipid in nature also known as lecithin [2] – has a phosphoric head and a choline group attached to sn-3 position (hydrophilic) and two fatty acid tails in sn-1 and sn-2 positions (lipophilic). Due to this amphiphilic nature, lecithin tends to curl in the presence of energy to decrease its contact with water in an aqueous solution. As they achieve minimum contact area in the spherical shape, resulting structure would be like two nested spheres. Starting from the center, they possess hydrophilic behavior at the very core, following the lipophilic layer in between and another hydrophilic layer exposed to the surrounding. This rearrangement adds up another function to the structure, suggesting hydrophilic agents' encapsulation in the core as well as hydrophobic agents' entrapment within the lipophilic attachment. Since the chemical characteristics of hydrophobic compounds are difficult to deal with, fat based liposomes are very handy with all their functional advantages.

In food systems, lipids are generally present in the form of emulsion, not as bulk lipids [4] so the mechanism of oxidation differs from largely oil dominated systems. Presence of the emulsifier as well as a second phase influence the rate of reactions [5], that is why oxidation of lipids significantly depends on where the lipid is located in the emulsion system and the environment of the particles [6]. As an example, when a volatile substance is to be distributed in the bulk oil, it should pass through only one phase while in an emulsion it has to overcome at least three possible phases; aqueous, oil and interface [7]. This difference in mechanism makes liposome or vesicle systems suitable for mimicking the lipid oxidation of heterogeneous structures like tissue or muscles for the medical studies and raw foods as they natively occur in the nature [8].

Being thermodynamically unstable, liposomes alone are fragile entities. They tend to break their folded structure to reach their minimum energy state, which interfere with the stability of the systems. There are various studies in the literature that aim to strengthen the durability of liposomes. For charged liposomes, surface characteristics could be altered by coating with polymers to enhance stability due to electrostatic interactions [9,10]; polypeptides could be incorporated to augment bilayer stability by causing modification on phase behavior of the lipids [11] or simply cholesterol could be used [12]. Utilization of liposomes offers modification for a great number of applications for the industries; however, its nature itself in various environments should be detected first, for a promising setup. Since liposomes have lipid origin, their oxidation status after formation and its change with time is also crucial for the stability of encapsulated food material.

Encapsulation materials and methods vary for each substance to be coated since each compound has its unique molecular characteristics. The main objective of this study is to explain the behavior of phospholipids with different origin for solvents at different pHs. Storage time and temperature were considered for long term oxidative stability of liposomes prepared by high mechanical energy techniques; microfluidization (MF) and ultrasonication.

2. Materials and methods

2.1. Materials

Phospholipid from two different sources with different phosphatidylcholine compositions was used in the study. Soy phospholipid, coded as S75 by the manufacturer was purchased from Lipoid GmbH (Ludwigshafen, Germany). It consisted of 68–74% phosphatidylcholine + lyso-phosphatidylcholine (LPC) and 7–14% phosphatidylethanolamine (PE). Egg phospholipid, E80 was kindly provided by Lipoid GmbH (Ludwigshafen, Germany). Number of the codes on the products denoted the phosphatidylcholine purity. Its ingredient ratios were 80–85% phosphatidylcholine + LPC and 7–9.5% PE. LPC content for both phospholipids were less than 3%. Iodine values indicating the amount of unsaturated bond present in the 100 g of sample reported in the product data sheet by the manufacturer were 85–95 and 65–69 for S75 and E80, respectively.

Two of the three solvents, acetate and phosphate buffers used in the study were prepared manually. For acidic buffer, glacial acetic acid was supplied from Merck KGaA (Darmstadt, Germany) and analytical grade sodium acetate trihydrate was supplied from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). 0.2 M solutions of these chemicals were mixed to achieve a final pH of 3.8 for the acetate buffer (AB). For phosphate buffer (PB), analytical grade disodium hydrogen phosphate dihydrate and potassium dihydrogen phosphate were purchased from Merck KGaA. (Darmstadt, Germany) and their aqueous solutions were mixed properly to give a final pH of 7.2. As the third solvent, distilled water (DW) was used and all the water used in the experiments were obtained using 0.2 μS/cm purity mpMinipure Dest system supplied from mpMinipure Ultrapure Water Systems (Ankara, Turkey).

2.2. Methods

2.2.1. Primary processing by high speed homogenization

In the scope of this study, all liposomes were prepared by a two-step homogenization procedure. For primary homogenization, 1% (w/v) phospholipid was added to the solvent and hydration of lipids was achieved by using a high speed homogenizer (IKA T25 digital Ultra-Turrax, Selangor, Malaysia) at 20,000 rpm for 2 min. This initial homogenization led to formation of multilamellar vesicles which could easily be detected by an optical microscope.

As the secondary homogenization, liposomes were prepared by means of two different mechanical methods, which were high pressure homogenization (MF) and high intensity ultrasound homogenization (direct probe ultrasonication). This further process led to formation of small unilamellar vesicles of higher stability.

2.2.2. Secondary processing by high pressure homogenization

After primary processing, pre-homogenized samples were fed to the inlet chamber of ISA-N-10M Nano Disperser (Ilshin Autoclave, South Korea) equipment. Based on literature and the limits of the equipment, MF pressures were chosen to represent a low (400 bar), medium (900 bar) and a high pressure (1300 bar) respectively [13]. For liposomes prepared in DW, low and high pressures were used. Each sample was subjected to a 5 pass pressurization through the 75 μm diameter nozzle of the interaction chamber. The equipment was installed with a cooling unit which provided temperature control maintained at the range of 20–25 °C, depending on the applied pressure. Both E80 and S75 liposomes were prepared by this method in all three solvents (AB, PB and DW) for all

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