Food Research International 99 (2017) 807–814

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Dynamics of unloaded and green tea extract loaded lecithin based liposomal dispersions investigated by nuclear magnetic resonance T_2 relaxation

Emrah Kirtil[a], Damla Dag[a], Selen Guner[a], Kubra Unal[a,b], Mecit H. Oztop[a,*]

[a] Department of Food Engineering, Middle East Technical University, Ankara 06800, Turkiye
[b] Turkish Patent and Trademark Office, 115 Hipodrom Street, Ankara 06330, Turkiye

ARTICLE INFO

Keywords:
Liposomes
TD-NMR relaxometry
T_2 relaxation times
Microfluidization
Stability

ABSTRACT

Liposomes are lipid bilayer vesicles that can be used as encapsulation systems for bioactive agents to provide increased protection against environmental stresses (such as pH or temperature extremes). Time Domain Nuclear Magnetic Resonance (TD-NMR) that is based on differentiation of specimen contents with respect to magnetic relaxation rates provides detailed information on amount, state and distribution of water and oil and provide reproducible results on the samples. These make TD-NMR particularly suitable for time-dependent monitoring of emulsion system dynamics. In this study, spin-spin (T_2) relaxation times and relaxation spectra were used for characterizing green tea extract loaded and unloaded liposomes prepared with soy (S75) and egg lecithins (E80) by different preparation methods (such as homogenization type, pressure and solvent type). Mean particle sizes of liposomes were found to be the most influential factor in shaping mono-exponential T_2 relaxation times. The differences in particle sizes of E80 and S75 samples along with samples with different homogenization pressures could be monitored with T_2 relaxation times. Additionally, T_2 relaxation times were found to be correlated with particle shape irregularity, and chemical instability of samples due to lipid oxidation. With relaxation spectrum analysis, particular components in the sample could be distinguished (internal/external water and lipid bilayers), which gave more elaborate results on mechanisms of instability.

1. Introduction

Encapsulation is a frequently utilized technique in food industry and research, for enhancing functional properties of active agents such as colorings, flavorings, antimicrobials and nutrients. Masking flavor, decreasing permeability of volatile compounds, increasing solubility, targeted delivery and providing the active agent with a protective barrier against degradation are some of the most common functions of encapsulation (Ray, Raychaudhuri, & Chakraborty, 2016; Taylor, Gaysinsky, Davidson, Bruce, & Weiss, 2007). Liposomes are lipid bilayer vesicles that can be used as encapsulation systems. These vesicles are self-assembled by introduction of energy into phospholipid solutions (Taylor et al., 2007). Phospholipids, commonly acquired from egg or soy, are amphiphilic in nature that is they embody both hydrophobic and hydrophilic sections. (Guner & Oztop, 2017).

With energy input, phospholipids curl and take a spherical shape trying to minimize their surface area. During this process, any dissolved active agent inside the medium is encapsulated within the liposomal core. This encapsulation provides increased protection against environmental stresses (such as pH or temperature extremes) and renders it possible to control the release rate and transport of the active agent to specific sites for targeted delivery (Frenzel, Krolak, Wagner, & Steffen-Heins, 2015; Guner & Oztop, 2017; Koloskova, Budanova, & Sebyakin, 2012). However, liposomal encapsulation has the disadvantages of losing entrapped agent during storage via leakage, displaying instability or even total disruption due to external stresses or interaction with certain other food compounds (Frenzel et al., 2015). The fact that their preparation requires energy input causes them to be thermodynamically unstable, which might be observed as increase in liposome dimensions with time (Ge, Möhwald, & Li, 2003a, 2003b; Guner & Oztop, 2017). These potential stability threats, underlines the necessity of elaborate investigation of liposomal system dynamics.

Nuclear Magnetic Resonance (NMR) is a non-destructive analysis method increasingly employed in colloidal science to gain both structural and dynamic information on a molecular level (Leal, Rögnvaldsson, Fossheim, Nilssen, & Topgaard, 2008). Low resolution time-domain NMR Relaxometry instruments has been proven effective in determination of oil and water contents, dispersed phase ratios, particle size distributions, enclosed water volume in W/O/W emulsions as well as polymer gelation and aggregation in other colloidal systems

* Corresponding author.
E-mail address: mecit@metu.edu.tr (M.H. Oztop).

http://dx.doi.org/10.1016/j.foodres.2017.06.064
Received 29 January 2017; Received in revised form 20 June 2017; Accepted 27 June 2017
Available online 29 June 2017

(Mariette, 2009; Vermeir, Sabatino, Balcaen, Van Ranst, & Van der Meeren, 2014). These bench-top affordable NMR instruments, that typically operate at frequencies < 25 MHz, and used to measure the longitudinal (recovery curve time constant, T_1) and transverse (decay curve time constant, T_2) relaxation times (Kirtil et al., 2014; Mariette, 2009; Marigheto et al., 2007). For complex food systems, these relaxation times differ for each food component and even for same components with different proton environments. In numerous applications, the decay (or recovery) of magnetization data are fitted to obtain a multi-exponential behavior thereby providing the distribution of transverse or longitudinal relaxation times ("relaxation time spectrum") coming from different proton pools that constitute the sample. Especially, T_2 relaxation times and relaxation spectra were numerously used in colloidal system analysis for their power to give detailed information on the state and mobility of water and oil. Owing to the non-destructive nature of the method, reproducible results on the same sample can be gathered which makes the method particularly suitable for time-dependent monitoring of emulsion system dynamics (Bernewitz, Dalitz, Köhler, Schuchmann, & Guthausen, 2013; Hashemi, Bradley, & Lisanti, 2010).

Green tea derived from the dried leaves of the plant *Camellia sinensis* has become one of the most widely consumed beverages in the world, especially due to its numerous health benefits related with its high phenolic content (Ananingsih, Sharma, & Zhou, 2013; Chacko, Thambi, Kuttan, & Nishigaki, 2010; Hosseini, Gorjian, Rasouli, & Shirali, 2015). However, green tea catechins, the major part of polyphenols, are susceptible to degradation with temperature, pH, oxygen availability, the presence of metal ions, concentration of other active compounds and moisture (Komatsu et al., 2014; Kumamoto, Sonda, Nagayama, & Tabata, 2001; Li, Taylor, & Mauer, 2011; Ortiz, Ferruzzi, Taylor, & Mauer, 2008). The stability of green tea polyphenols could be increased by entrapping green tea extract into liposomes that are promising delivery system for phenolic compounds. (Gibis, Vogt, & Weiss, 2012).

In this study, unloaded and green tea extract loaded liposomal systems were produced from either soy or egg lecithin. The dispersions were homogenized with microfluidization and stored at 4 °C for up to 30 days. The main objective of the study was to examine some of the microstructural changes in these systems and gain more information related to the mechanism of these changes as well as the effect of parameters such as lecithin type, homogenization pressure and extract loading on T_2 relaxation times. The study is a follow up to a previous study on the subject and aims to explain some of the observations made through particle size analysis, zeta potential, lipid oxidation, phenolic content measurements, transmission electron microscopy (TEM) and optical microscopy images with T_2 relaxation data.

2. Materials & methods

2.1. Materials

Phospholipids from two different sources with different phosphatidylcholine compositions were used in the study. Soy phospholipid and egg phospholipid, with the commercial names S75 and E80 were kindly provided from Lipoid GmbH (Ludwigshafen, Germany). Green tea extract in the form capsules was purchased from a grocery store (Spring Valley, Bentonville, Arkansas, USA). For buffer preparations; glacial acetic acid, analytical grade disodium hydrogen phosphate dihydrate and potassium dihydrogen phosphate were supplied from Merck KGaA (Darmstadt, Germany). Analytical grade sodium acetate trihydrate was purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). 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).

Table 1
Experimental design parameters for unloaded and green tea extract loaded liposome systems.

Experimental design			
#	Factors	Levels	
		Unloaded liposomes	Green tea extract loaded liposomes
1	Lecithin type	Soya lecithin (S75) Egg lecithin (E80)	Soya lecithin (S75)
2	Solvent type	Acetate buffer (pH = 3.8) Phosphate buffer (pH = 7.2)	Acetate buffer (pH = 3.8) Distilled water (pH ≈ 6.5)
3	Microfluidization Pressure	400, 900 & 1300 bar	1300 bar
4	Time (days)	1st, 4th, 7th, 10th, 14th, 21th, 28th, 30th	1st, 4th, 7th, 10th, 14th, 21th, 28th, 30th

2.2. Methods

2.2.1. Preparation of liposome samples

Both unloaded and green tea extract loaded liposomes were prepared by two-step homogenization, as described in the previous studies (Dag & Oztop, 2017; Guner & Oztop, 2017). The study covers the NMR Relaxation results of two different studies from the same project.

The first study (Guner & Oztop, 2017) focuses on characterization of unloaded liposomes and aims to come up with the best possible combination of parameters that yield the most stable liposomes. In this part, unloaded liposomes were prepared by microfluidization at homogenization pressures of 400, 900 and 1300 bar, using either acetate (AB) or phosphate buffer (PB) as solvents, and storing at 4 °C. At the second part of the study (Guner & Oztop, 2017), most stable liposomal systems (prepared from soy lecithin, microfluidization pressure of 1300 bar were loaded with green tea extract and unstable combinations of phosphate buffer were replaced by distilled water samples.

Table 1 shows the experimental design of unloaded and green tea extract loaded liposome systems. For the interpretation of NMR data, surface mean (d_{32}) particle size results from two different studies (Dag & Oztop, 2017; Guner & Oztop, 2017) were taken as reference to provide correlation in between to enhance discussion.

2.2.1.1. Preparation of extract solution for loaded liposome preparation. The extract solution was prepared by dissolving 0.1% green tea extract (*w/v*) in acetate buffer (pH: 3.8) and distilled water around neutral pH (pH:6.5) separately. A pH of 3.8 was chosen since tea catechins are stable only when pH < 4 while a pH of 6.5 was determined to observe the stability of green tea catechins in neutral pH that is neither alkaline nor acidic (Ananingsih et al., 2013). After the extract solutions were stirred in magnetic stirrer (Multi Hotplate Stirrer, DAIHAN Scientific Co., Ltd.) for 30 min at 300 rpm, then they were filtered by folded cellulose filter paper.

2.2.1.2. Primary processing by high speed homogenization. For primary homogenization, 1% (*w/v*) phospholipid was added to the aqueous solution (extract solution, buffer or distilled water) and hydration of lipids was achieved by using a high-speed homogenizer (IKA $T_2$5 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 high-pressure homogenization (microfluidization). This further process led to formation of small unilamellar vesicles of higher stability.

2.2.1.3. Secondary processing by high pressure homogenization. After primary processing, pre-homogenized samples were fed to the inlet