Development of Redispersible Nano-size Dried Liposomes Loaded with Quercetin

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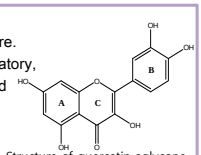
ABSTRACT

Quercetin exerts many beneficial health effects, but it has poor solubility in water and thus is limited bioavailability. The purposes of this study were to develop redispersible nano-sized liposomes loaded with quercetin by lyophilization, and to set up optimized lyophilization conditions minimizing damage of nano-liposomes during lyophilization. The liposomes containing quercetin were prepared by a thin-film hydration method and the size of liposomes was controlled by extrusion. Four cryoprotectants (glucose, sucrose, trehalose and lactose) were applied for freeze-drying process. Lyophilized nano-sized liposomes were reconstituted in distilled water, and the degrees of aggregation, leakage and intactness of the liposomes were evaluated by measuring change of size, PDI and ζ-potential during stored for 7 days at 4℃. The size stability of nano-sized liposome was evaluated in artificial gastric juice for 1 hour. The encapsulated amounts of quercetin were found as 0.15, 0.38, 0.63, 0.68, 0.75 and 0.75 mg with initial amount of 0.2, 0.6, 1.0, 1.2, 1.4 and 2 mg added to 20 mg of phospholipid, respectively. Loading efficiencies were 74.8, 63.6, 63.2, 56.5, 53.3 and 37.6%, and loading amounts were 0.74, 1.85, 3.01, 3.20, 3.49 and 3.42%. Initial quercetin amount of 1 mg was appropriate based on high loading amount and loading efficiency. Average size of liposomes after extrusion was 147 ± 1.07 nm and increased to 162.7 ± 4 nm during stored for 14 days at 4℃. The size of lyophilized nano-liposomes with glucose and lactose was increased about 21-31% while there were little changes in size with sucrose and trehalose during stored for 7 days at 4℃. The sizes of liposomal and lyophilized nano-liposomal quercetin were increased by 3 and 2.3% in artificial gastric juice, respectively. The nano-liposomes, lyophilized with sucrose and trehalose were found to be stable when reconstituted.

INTRODUCTION

• Quercetin

- Quercetin and its derivatives belong to flavonoids and are widely spread in nature.
- Recent researches have discovered that quercetin has anticancer, anti-inflammatory, anti-fibrosis, anti-free-radical, anti-virus and strengthen immunity properties, and can reverse the drug resistance of some tumors.
- It has poor solubility in water and thereby low bioavailability



Structure of quercetin aglycone

• Nano-liposome

- Liposome , a microscopic lipid vesicle, is a simple vesicles in which an aqueous volume is entirely enclosed by a membrane composed of lipid molecules (usually phospholipids).
- Liposomes usually formed from phospholipids, have been used to change the pharmacokinetics profile of, not only drugs, but herbs, vitamins and enzymes.
- It can be used to increase solubility and improving bioavailability of poorly soluble bioactive compounds.
- Nanotechnologies in functional food can offer large surface area to volume ratio of these materials to improve the bioavailability of active ingredients, introduce controlled/target release, improve sensory aspects, and others.

• Lyophilization

- Lyophilization is an approach to ensure the long-term stability of liposomes under normal storage condition.
- For freeze-dried liposomes, two hypotheses based on disaccharide have been proposed; one is the water replacement model and the other is the vitrification model.
- The effect of lyoprotectants in protecting lipid bilayers during lyophilization is usually characterized by size, PDI (polydispersity index) and ζ-potential.

• Objectives

- This study was aimed to develop redispersible nano-sized liposomes loaded with quercetin by lyophilization, and to optimize lyophilization conditions by selecting cryoprotectants which minimize the damage of nano-liposomes during lyophilization.

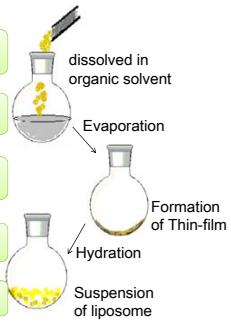
MATERIALS and METHODS

• Main materials and reagents

- Analytically pure grade of phospholipon® 90 G (Phospholipid GmbH, Germany), quercetin (Sigma, Germany) and another reagents were used.

• Method

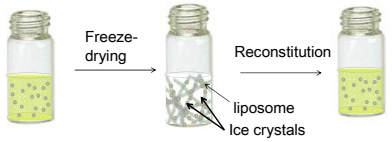
Dissolution of quercetin and phospholipid in organic solvent
↓
Evaporation of solvent under vacuum (formation of lipid film at 37°C)
↓
Hydration (added water or solution of cryoprotectant) and vortexing
↓
Sonication (5 min) and cooling
↓
Centrifugation of liposomal quercetin (3000 g, 3 min)

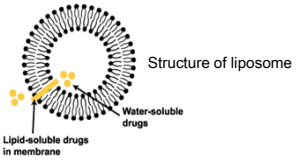


Extrusion by polycarbonate membrane filter (200 nm)
↓
Measurement of size, PDI and ζ-portential
↓
Freeze-drying of nano-liposome
↓
Reconstitution of nano-liposomes in distilled water
↓
Evaluation of size change (7days at 4 °C)

• Measurement of loading amount

Removal of supernatant
↓
Dissolution in methanol
↓
Measurement of absorbance (UV-Visible spectroscopy at 420 nm)





RESULTS and DISCUSSION

• Preparation of liposomes

- Ethanol was used to prepare nano-liposomes, which can be used as food ingredients.
- Loading amount and loading efficiency of nano liposomal quercetin were measured as a function of amount of Initial quercetin doses such as 0.2 ~ 2 mg in phosphatidylcholine 20 mg. Loading amount was increased with increasing quercetin amount, but loading efficiency was decreased (Table 1).
- Initial quercetin dose of 1mg was selected as a condition for liposome formulation.

$$\text{Loading efficiency (\%)} = \frac{\text{loaded quercetin}}{\text{intial quercetin}} \times 100, \quad \text{Loading amount (\%)} = \frac{\text{loaded quercetin}}{(\text{intial quercetin} + \text{lipid})} \times 100$$

Table 1. Loading amount and loading efficiency of liposomal quercetin depending on initial quercetin amount.
mean ± S.D. (n = 3)

Sample NO	Phosphatidyl choline (mg)	Initial quercetin (mg)	Loaded quercetin (μg)	Loading amount (%)	Loading efficiency (%)	Suspension volume (ml)
QL 1	20	0.2	149.6 ± 6.0	0.74 ± 0.03	74.8 ± 3.0	1
QL 2		0.6	381.5 ± 20.9	1.85 ± 0.10	63.6 ± 3.5	1
QL 3		**1.0**	**631.5 ± 13.6**	**3.01 ± 0.06**	**63.2 ± 1.4**	**1**
QL 4		1.2	678.4 ± 47.7	3.20 ± 0.39	56.5 ± 4.3	1
QL5		1.4	746.3 ± 74.6	3.49 ± 0.39	53.5± 4.3	1
QL 6		2.0	751.3 ± 85.1	3.42 ± 0.39	37.6 ± 4.3	1

• Size distribution of nano-liposome and stability test

- The sizes of quercetin loaded nano-liposomes were reduced by extrusion of 7 cycles, and were in the range of 146.8 ± 10.7 ~ 151.8 ± 4.2 nm depending on loading amount.
- Fig. 1 shows the typical size distribution of nano-liposome with initial quercetin dose of 1mg after extrusion cycles, i.e., symmetrical shape (Fig. 1).
- Stability test was carried out for 14 days stored at 4℃, and the sizes of all nano-liposomes tested were increased (Fig. 2). Average size of liposomes after extrusion was increased from147.0 to 161.6 nm (QL 3).

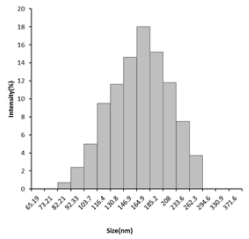


Fig. 1. Size distribution of nano-liposomal quercetin after extrusion as intensity gradients.

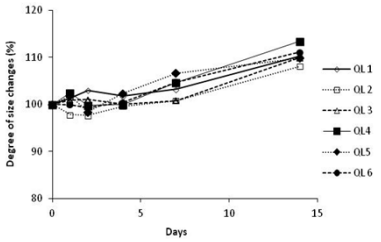


Fig. 2. Changes in particle size ratio of qercetin loaded liposome prepared after extrusion for 14 day storage .

• Freeze-drying and reconstitution of nano-liposomes

- The average size of reconstituted nano-liposome without cryoprotectants was increased from 145.0 to 288.2 nm, because it was physically damaged during freeze-drying process (Fig. 3).
- PDI of reconstituted nano-liposome with glucose was increased from 0.153 to 0.228 and thus glucose would be not an effective cryoprotectant (Fig.4).

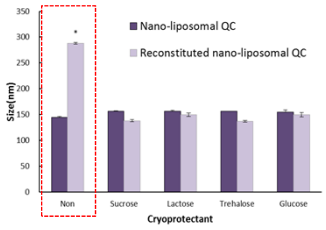


Fig. 3. Changes of particle sizes by reconstitution of freeze-dried liposomal QC with cryoprotectants.

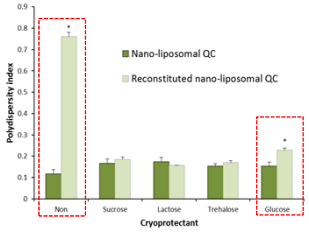


Fig. 4. Changes of PDI by reconstitution of freeze-dried liposomal QC with cryoprotectants.

• Stability test of reconstituted nano-liposomes after freeze-drying

- During 7 days of storage, the sizes of nano-liposomal quercetin were increased with lactose and glucose, but were stable with sucrose and trehalose (Fig. 5).
- The sizes after 7 days of storage were changed from 149.9 to 195.7 nm (31%) with glucose, and from 150 to 182 nm (21%) with lactose, respectively.
- When the liposomal and lyophilized nano-liposomal quercetin were rehydrolyzed in artificial gastric juice, the reconstituted sizes were increased by 3 and 2.3 %, respectively.

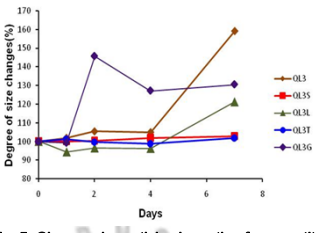


Fig. 5. Changes in particle size ratio of reconstituted lyophilized liposomal quercetin during storage.

• Discussion

- Current attempts provides to the functional food industry new form of oral formulation such as rapidly redispersible liposomal quercetin with an aid of optimized lyophilization processes and this techniques can be applied to various health supplements fields.

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