Proceeding Paper

Radical Scavenging Activity of Silymarin Encapsulated in Liposomal Vesicles: Impact of UV Irradiation and Lyophilization †

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Abstract: The radical scavenging activity of silymarin-loaded liposomes after different technological processes (UV irradiation and lyophilization) was investigated using DPPH and ABTS assays. Using the DPPH method, the antioxidant capacity of pure silymarin was 84.03%, while it was lower after encapsulation in liposomes; it was 81.63% after the formulation, 81.15% after UV irradiation, and 79.85% after lyophilization. The anti-ABTS potential was 3.04 μmol Trolox equivalent (TE)/mL for silymarin, 1.68 μmol TE/mL after the liposome preparation, 1.52 μmol TE/mL after UV irradiation, and 2.02 μmol TE/mL after lyophilization. UV irradiation did not cause significant changes in the antioxidant potential of liposomes, while the ABTS scavenging activity was higher after lyophilization. Considering that the two used antioxidant assays are based on different reactions, the obtained data provide a good insight into the overall antioxidant activity of silymarin-loaded liposomes.

Keywords: antioxidants; liposomes; silymarin; lyophilization; UV irradiation

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1. Introduction

Silymarin is a group of polyphenols (silibinin, silydianin, isosilybin, and silychristin) from milk thistle (*Silybum marianum*) [1]. Silymarin exhibits plenty of bioactivities that can promote human health and well-being. It possesses antioxidant, antiviral, antimicrobial, immunomodulatory, anti-inflammatory, and anticancer properties [1–4]. Nevertheless, silymarin is poorly soluble and possesses lower bioavailability; thus, its application is quite limited [1]. Liposomes can increase the stability of encapsulated sensitive compounds and the bioavailability of poorly hydrosoluble components. Liposomal carriers are employed for the encapsulation of drugs, enzymes, phenolic compounds, antioxidants, aromas, vitamins, etc. [5–7]. Concerning potential implementation in various industries, liposomal sterilization, such as UV irradiation, remains a real challenge because of the carriers' particular sensitivity and physicochemical alterations [8]. Lyophilization provides dried products with active compounds that are stable over a long period due to the prevention of hydrolytic and oxidative degradation which can occur in the surrounding water [9].

However, the lyophilization process can result in significant modifications of the liposome characteristics and potential; thus, its effect should be examined as well. Therefore, in the present study, the radical scavenging activity of silymarin-loaded liposomes after different technological processes (UV irradiation and lyophilization) was investigated using DPPH and ABTS assays.

2. Materials and Methods

Soy Phospholipon 90G (phospholipids used for the liposome preparation) was purchased from Natterman Phospholipids (Cologne-Bocklemünd, Germany). The following reagents were used: ethanol (Fisher Scientific, Loughborough, UK) and silymarin, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) or ABTS, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid or Trolox, 2,2-diphenyl−1-picrylhydrazyl or DPPH (Sigma-Aldrich, Hamburg, Germany), and potassium persulfate (Centrohem, Stara Pazova, Serbia). Ultrapure water was prepared using the Simplicity UV® water purification system (Merck Millipore, Darmstadt, Germany).

Liposomes with silymarin were prepared using the proliposome method according to Isailović et al. [6]. Specifically, a mixture of 1 g of phospholipids, 0.02 g of silymarin, and 8 mL of ethanol was stirred and heated to 60 °C for 10 min. After cooling to 25 °C, 20 mL of distilled water was added in small portions. Subsequently, the mixture was stirred for 1 h at 800 rpm.

Silymarin-loaded liposomes were centrifuged, the supernatant was discarded, and the pellet was frozen in the freezer at −80 °C for 1 h and lyophilized at −75 °C and a pressure of 0.011 mbar for 24 h in the device Alpha 2–4 LSCplus (Christ, Osterode am Harz, Germany). The lyophilized liposomal particles with silymarin were reconstructed using ultrapure water to their original volume before further analysis of the antioxidant potential. The liposomes were also exposed to UV irradiation in uncovered Petri dishes for 20 min in a laminar flow cabinet.

The antioxidant potential of non-treated, UV-irradiated, and lyophilized silymarin-loaded liposomes and pure silymarin dissolved in ethanol was determined in the DPPH and ABTS tests.

In the DPPH test [10], the DPPH solution (2 mL) was mixed with liposomes or pure silymarin (20 μL). The absorbance was read at 517 nm (UV-1800, Shimadzu, Kyoto, Japan), after the incubation at 25 °C in the dark (20 min), and calculated using the following equation:

$$\text{radical scavenging capacity (\%)} = (A_c - A_x) \times 100/A_c$$

where A_c is the absorbance of the control (DPPH solution and water) and A_x is the absorbance of the DPPH solution and liposomal sample or extract. All analyses were carried out in triplicate, and the anti-radical potential was expressed as the percentage of neutralization of free DPPH radicals (%).

In the ABTS test [11], the ABTS solution (2 mL) was mixed with liposomes or pure silymarin (20 μL). The absorbance was read at 734 nm (UV-1800, Shimadzu, Japan), after the incubation at 25 °C in the dark (6 min), and calculated using the following equation:

$$\Delta A = A_0 - A_x$$

where A_0 is the absorbance of the ABTS solution, while A_x is the absorbance of the ABTS solution and the liposomes or pure silymarin. Trolox was used for the preparation of the calibration curve. All analyses were carried out in triplicate, and the anti-radical potential was expressed as μmol Trolox equivalent (TE)/mL.

The statistical analysis was performed employing one-way ANOVA and Duncan's post hoc test (STATISTICA 7.0). The differences were considered statistically significant at $p < 0.05$ (n = 3).

3. Results and Discussion

The anti-radical capacity of silymarin-loaded liposomes measured immediately after preparation and different technological processes (UV irradiation and lyophilization) in the DPPH and ABTS assays is presented in Table 1.

Table 1. Radical scavenging activity of silymarin-loaded liposomes (non-treated, UV-irradiated, and lyophilized samples) and pure silymarin determined in the DPPH and ABTS assays.

Sample	Anti-DPPH Potential (%)	Anti-ABTS Potential (μmol TE */mL)
Non-treated liposomes	81.63 ± 1.20 ^{b*}	1.68 ± 0.52 ^{b}
UV-irradiated liposomes	81.15 ± 1.33 ^{b}	1.52 ± 0.36 ^{b}
Lyophilized liposomes	79.85 ± 1.24 ^{b}	2.02 ± 0.21 ^{b}
Silymarin	84.03 ± 0.70 ^{a}	3.04 ± 0.31 ^{a}

* TE, Trolox equivalent; the same letter indicates the absence of the differences that were considered statistically significant (analysis of variance, i.e., one-way ANOVA and Duncan's post hoc test; $p < 0.05$, n = 3).

As can be seen from Table 1, the antioxidant potential of pure silymarin determined in the DPPH assay was 84.03 ± 0.70%, while it was significantly lower after the encapsulation in liposomes, probably due to the prolonged release of the active compound from liposomal vesicles. Namely, a longer incubation period is necessary for the complete neutralization of free radicals. Specifically, the anti-DPPH activity of the non-treated liposomes with silymarin was 81.63 ± 1.20%, 81.15 ± 1.33% after UV irradiation, and 79.85 ± 1.24% after lyophilization. The anti-ABTS potential was 3.04 ± 0.31 μmol TE/mL for pure silymarin dissolved in ethanol (statistically significantly higher in comparison to liposomal samples), 1.68 ± 0.52 μmol TE/mL after the liposome preparation, 1.52 ± 0.36 μmol TE/mL after UV irradiation treatment, and 2.02 ± 0.21 μmol TE/mL after lyophilization. Köksal et al. [12] also showed the effective DPPH and ABTS scavenging ability of silymarin. According to Anthony and Saleh's study [13], taxifolin is the most effective component of silymarin due to its free radical scavenging potential. As can be seen from the presented results, UV irradiation treatment did not cause significant changes in the radical scavenging capacity of liposomes with silymarin. The data obtained are in agreement with the results of our previous study, where UV treatment did not affect the antioxidant activity of silibinin-loaded liposomes using both DPPH and ABTS methods [14]. On the other hand, the ABTS scavenging potential of the liposomes with silymarin was significantly higher after lyophilization than that of non-treated and UV-irradiated parallels. The reason for this could lie in the fact that the lyophilization process can cause leakage of the encapsulated compounds and the consequently faster reaction of a free antioxidant compound (non-encapsulated fraction) with free radicals. In addition, the measurements of the antioxidant potential were taken after the reconstruction of lyophilized liposomes in ultrapure water. Therefore, the mentioned step can cause the rearrangement of the bilayer membrane and leakage of silymarin, resulting in a higher, i.e., more rapid, antioxidant effect [15]. According to the literature data, antioxidant liposomes hold great potential in the treatment of various disorders in which oxidative stress plays an essential role [16]. The beneficial properties of silymarin in human and animal health highlight its application in liposomal vesicles as carriers followed by potential implementation in food and pharmaceutical products.

4. Conclusions

In the present research, the anti-radical potential of pure silymarin and developed silymarin-loaded liposomes was investigated. Pure silymarin showed significantly higher radical scavenging activity compared to the encapsulated form, while UV irradiation did not decrease the antioxidant activity of liposomes, confirming the protective effect of the liposomal bilayer. In the case of anti-ABTS effects, silymarin-loaded liposomes showed higher capacity after the lyophilization process. The employed antioxidant tests are based on different reactions; thus, the presented results provide a good insight into the radical scavenging capacity of silymarin-loaded liposomes before and after modification treatments (UV irradiation and lyophilization). The obtained data related to the antioxidant potential of developed liposomes with silymarin qualify them for application in food, functional food, and pharmaceutical formulations. Nevertheless, future experiments should focus on other biological properties of the developed liposomes, as well as their changes during the storage period.

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Conflicts of Interest: The authors declare no conflicts of interest.

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