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Original research paper

ENCAPSULATION OF RESVERATROL IN SPHERICAL PARTICLES OF FOOD GRADE HYDROGELS

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ABSTRACT: The paper reports about the preparation and characterization of hydrogel particles containing liposomes loaded with resveratrol as an active compound. The materials used for preparation of the particles were chosen to be suitable for food industry. Different polymer concentrations affect particles shape, size, size distribution, as well as the release kinetics of resveratrol. The diameter of particles varied from 360 to 754 μm, while the narrow size distribution was observed for all types of particles. Release studies were performed in Franz diffusion cell and the results showed the prolonged release of resveratrol from all samples, but the sample with the highest content of polymer (2.5% w/w) in particular stood out. The research provides useful information about liposomes containing active compound encapsulated in hydrogel matrices and offers the basis for its application in the food industry.

Key words: *resveratrol, sodium alginate, release, sphericity, particles*

INTRODUCTION

Resveratrol is a natural antioxidant with remarkable antioxidant activity, found in grapes and red wines (Stojanović et al., 2001, Lim et al., 2014). It is also highly sensitive compound and poorly soluble in water surrounding (Lopez-Nicolas et al., 2006; Pineiro et al., 2006). Therefore, its application is uncommon although resveratrol possesses strong antioxidant properties. Nowadays, functional food with addition of highly valuable antioxidants attracts attention of both, consumers and producers, as well as researchers (Đorđevic et al., 2015). In order to use sensitive antioxidants in functional food products it is necessary to protect them in a certain way. For that purpose, encapsulation techniques are found to be convenient (Belščak-Cvitanović et al., 2011; Stojanović et al.,2012; Đorđevic et al., 2015; Istenič et al., 2015) and among them electrostatic extrusion stands out as non-invasive one. In addition, it has been demonstrated that particles obtained via electrostatic extrusion technique have very narrow size distribution (Bugarski et al., 2004), which is important from the application aspect. Unfortunately, due to poor solubility of resveratrol in water, its direct encapsulation in hydrogel particles is not possible. As it was described by Isailović et al. (2013) resveratrol can be

encapsulated in liposomes with high encapsulation efficiency, using simple and easy-scalable cost effective proliposome technique. However, the stability of liposome formulations is often under suspicion so that it constrains their application, especially in food industry. In order to overcome this problem, liposomal emulsion with encapsulated resveratrol can be further incorporated in hydrogel particles. In this work, liposomal suspension with encapsulated resveratrol was mixed with different concentrations of food grade polymer – sodium-alginate (two types of sodium-alginate with different molecular weight) and the mixture was subjected to electrostatic extrusion to obtain micronized particles. The particles were further characterized in terms of size and shape. The release profile of resveratrol from the particles was also determined and discussed. Thus, this study provides useful information for potential application of the multipart system liposome-active-hydrogel into functional food products.

MATERIALS AND METHODS

Materials

Phospholipon 90 NG (more than 90% phosphatidilcholine (PC) was supplied by Natterman Phospholipids (Germany). Trans-resveratrol standard (>99% pure) was obtained from ChromaDex (Irvine, CA, USA). Low viscosity sodium-alginate (LV, molecular weight: 12000- 80000), and medium viscosity sodium-alginate (MV, molecular weight: 80000-120000) ware purchased from Sigma-Aldrich (Germany). Calcium-chloride dehydrate, 99% was obtained from Acros organics, USA. All other chemical used were of analytical grade.

Liposome preparation

Liposomes were prepared using simple proliposome method. Commercial lipid mixture Phospolipon 90NG (P90NG) was used in this step according to Isailović et al. (2013). Briefly, P90NG was mixed with ethanol, resveratrol and small quantity of water and stirred with magnetic stirrer at 60 °C. When the mixture was homogenized, it was further cooled to room temperature and then 50 ml of distilled water was added in small portions. The obtained suspension was stirred for one more hour at 800 rpm. The final concentration of the lipids was 20 mg/ml. The ratio between resveratrol and lipids was 1:20 w/w.

Preparation of calcium alginate particles

Alginate particles containing liposomes were prepared as described by Balanč et al. (2016) with slight modifications. In brief, the solutions were prepared by dissolving sodium-alginate in distilled water and then mixed with previously prepared liposome suspension in volume ratio 2:3 to obtain final concentration of sodium alginate 1.0, 1.5 and 2.5 % w/v. The same procedure was done for both LV and MV alginate. These solutions were then extruded through a blunt stainless steel needle (23 G) using a syringe pump (Razel Scientific Instruments, Stamford, CT, USA) into 2% w/v calcium-chloride under an applied electric field of 6.3 kV. The microbeads were formed in contact with calcium-chloride solution and then left in the solution for 15 min (Bugarski et al., 1994).

Optical microscopy

Optical microscope was employed for observation of the particles (Olympus CX41RF, Tokyo, Japan). Using ImageJ software the images were analyzed and the sphericity factor (SF) of the particles was calculated according to Chan et al. (2011b):

$$SF = \frac{(l_{\max} - l_{\min})}{(l_{\max} + l_{\min})} \qquad (1)$$

where l_{max} (μm) is the largest diameter and l_{min} (μm) is the smallest diameter perpendicular to l_{max}. (μm). SF was calculated from the 50 beads sample. SF varies from zero for a perfect sphere, to approaching unity as the particle becomes more elongated.

Particles size and size distribution

The size of particles (surface mean diameter) and size distribution were measured by a laser light scattering particle size analyser (PSA) (Mastersizer 2000; Malvern Instruments Ltd., Malvern, Wor-

cestershire, U.K.). The particle size distribution was characterized through the values of SPAN, which was calculated as follows:

$$SPAN = \frac{(d_{90}-d_{10})}{d_{50}} \quad (2)$$

where d_{10}, d_{50} and d_{90} are the intercepts for 10%, 50% and 90% of the cumulative particles number.

Release behaviour

The release studies were performed in Franz diffusion cell (donation of PermeGear, Inc., USA). Two cell compartments were separated with acetatecellulose membrane (pore size of 0.2 μm). Around 2 g of the microbeads was placed in the donor compartment while the receptor compartment was filled with ethanol 50% (20 ml) and constantly mixed at 400 rpm (Klimundova et al., 2006).

The aliquots of 0.5 ml were taken in time intervals during 6 hours and replaced with the same amount of fresh medium. Resveratrol concentration was determined by spectrophotometry at 306 nm as described by Neves et al. 2016.

RESULTS AND DISCUSSION

Particle shape and size

Preparation of particles using electrostatic extrusion depends on several operating parameters which impact its size and shape (Kostić et al., 2012). Hence, viscosity of the starting polymer solution which correlates to its concentration had influence on sphericity and diameter of the droplets generated in the electric field, as described by Prüsse et al. (2008). Concerning, our study showed that polymer solution consisted of 2.5% medium viscosity alginate (MV 2.5%) was not appropriate for particles preparation since it was impossible to process it.

Therefore, this sample was not used in further investigations. On the other hand, the utilization of other MV polymer solutions provided spherical particles with sphericity factor lower than 0.04 (Table 1).

Namely, Chan et al. (2011a) reported that values of SF smaller than 0.05 should be considered as spherical. The results also imply on slight decrement of the SF value with increasing alginate concentration.

This effect could be explained by better molecular packing in samples with higher alginate concentration (Trifković et al., 2014). In addition to particles shape, optical microscopy (Figure 1) also gave visualisation of the particles.

PSA showed that surface mean diameter (D [3,2]) varied from 368 μm to 755 μm (Table 1) and it was also correlated with the concentration of the starting solution. More concentrated solutions provided particles with larger diameter which was expected (Prüsse et al., 2008; Kostić et al., 2012).

The particles size distribution is also important data for their potential application (Prüsse et al., 2008). Therefore, the samples were analyzed by a laser light scattering and the results showed narrow size distribution in all samples as shown in Figure 2 (SPAN< 1). The values of SPAN were smaller (~0.57 for all samples except for LV 2.5% where SPAN was a bit higher (0.745)), and indeed, under optical microscopy, it was visible that the particles were not exactly the same in size.

Table 1.
Sphericity factor and average diameter of the alginate particles

Sample	SF	Surface mean diameter (μm)
LV1%	0.038	368.5
LV1.5%	0.027	429.3
LV2.5%	0.016	754.8
MV1%	0.026	588.9
MV1.5%	0.024	672.1

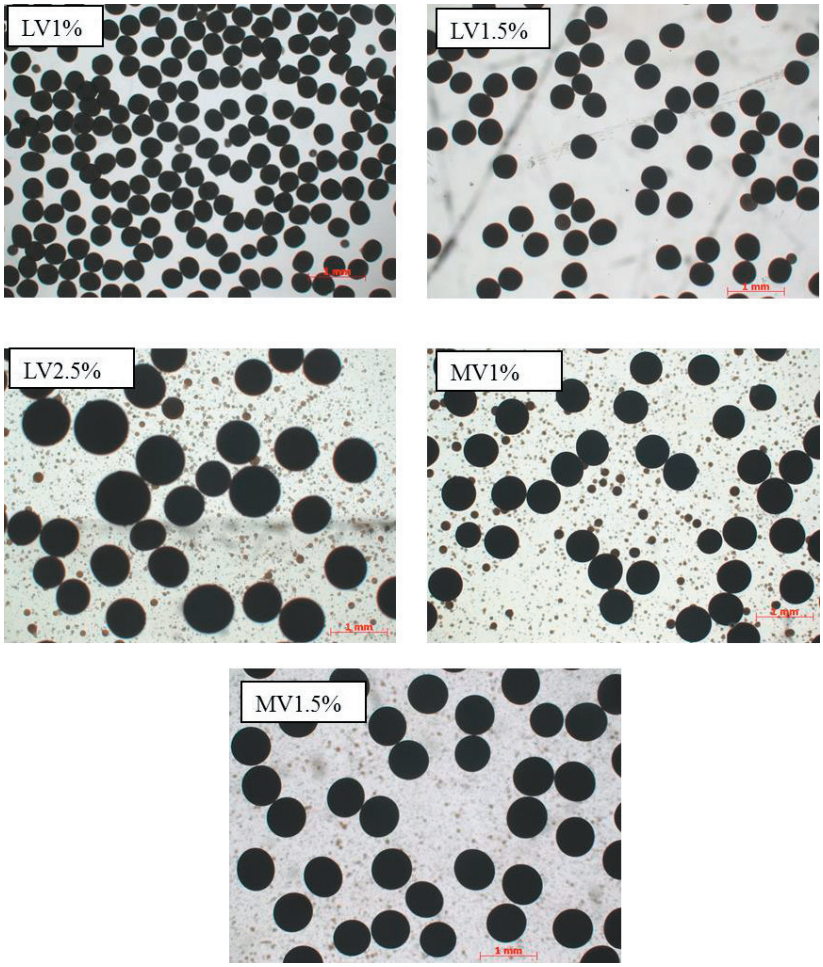

Figure 1. Optical microscopy of the alginate particles containing liposomes with encapsulated resveratrol

Release studies

The release of active compound (resveratrol) was monitored during 6 hours using Franz diffusion cell. Resveratrol concentration in samples was determined by spectrophotometry at 306 nm. The results are presented in Figure 3 and they confirm the extended release of resveratrol from all formulations. The release profiles of resveratrol were compared for all liposomes-containing particles. Particles obtained using more concentrated alginate solution exhibited slower release of encapsulated active compound, which is in accordance with the previous studies (Dini et al., 2001; Dini et al., 2003). Thus, the slowest release of resveratrol was detected in the case of LV 2.5%. For the sake of comparison, less than 40% of the originally entrapped resveratrol was released from LV 2.5% after approximately 330 minutes; while for the same time LV 1% release more than 60% of the initial resveratrol content. Apparently, higher alginate content provides thicker, i.e. less porous polymer network, so higher resistance to mass transfer. Our results are in agreement with literature data showing that the increase in particles porosity leads to poorer control of active compound release (Klose et al., 2006).

Moreover, even after 6 hours, none of the hydrogel particles released the total amount of resveratrol, probably due to interactions between alginate and resveratrol. Namely, the interactions between these two compounds occur via carboxylic and hydroxyl groups as revealed elsewhere (Cho et al., 2014; Istenič et al., 2015, Balanč et al., 2016). Furthermore, looking at the Figure 3, it seems that the impact of alginate type (LV *versus* MV) on release properties was more obvious for the lower concentration (1% *versus* 1.5%).

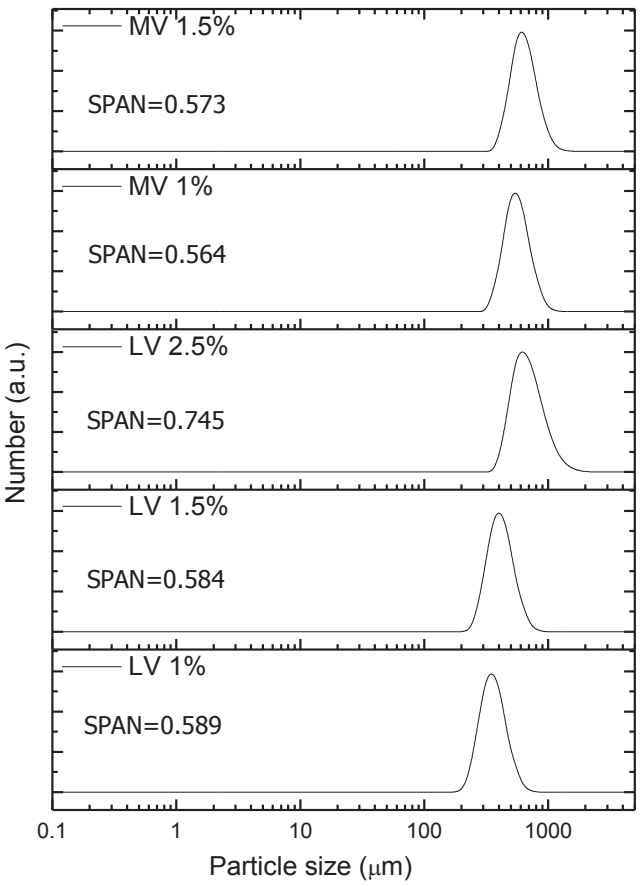

Figure 2. Size distribution of alginate particles containing liposomes with encapsulated resveratrol

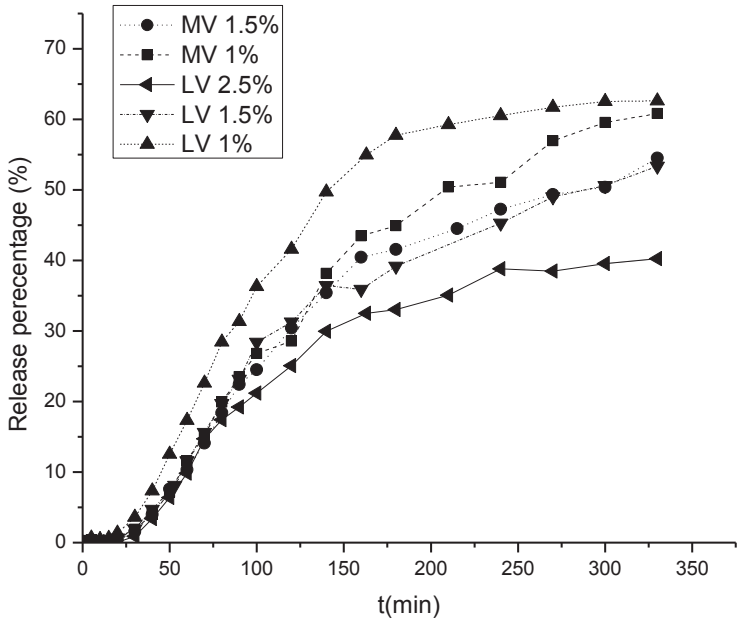

Figure 3. Release profiles of resveratrol from alginate particles containing liposomes

CONCLUSION

Liposomes containing resveratrol were effectively encapsulated in alginate particles using electrostatic extrusion technique. The particles were spherical in shape and their size varied from 360 to 754 μm depending on polymer molecular weight and concentration in the starting solution. Polymer concentration also correlated with release time of resveratrol from the particles where the highest content of alginate induced the slowest release of resveratrol, probably due to the lower particle porosity. All samples had narrow distribution which was confirmed by SPAN values. Accordingly, complex systems such as liposomes containing resveratrol encapsulated in hydrogel particles can be easily prepared by the electrostatic extrusion using low and medium viscosity sodium-alginate up to 2.5% (w/w). These results provide useful information and the basis for further application of these complex systems in the industry of functional food products.

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ИНКАПСУЛАЦИЈА РЕСВЕРАТРОЛА У СФЕРИЧНЕ ЧЕСТИЦЕ НА БАЗИ ХИДРОГЕЛОВА ДОЗВОЉЕНИХ ЗА УПОТРЕБУ У ХРАНИ

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Сажетак: Овај рад даје податке о припреми и карактеризацији честица које садрже липозоме са инкапсулираном активном компонентом ресвератролом. Компоненте које улазе у састав ових честица одабране су тако да могу једноставно да се примене у прехрамбеној индустрији. Приказан је утицај различитих концентрација почетних раствора полимера чија употреба је дозвољена у храни, а самим тим и њихове вискозности на величину формираних честица, њихов облик и расподелу величина, али и на отпуштање ресвератрола из ових сложених система. Пречник честица био је између 360 и 754 μm, док је уска расподела величина детектована у свим узорцима. Отпуштање ресвератрола праћено је у Францовој дифузионој ћелији где су резултати указали на продужено ослобађање ресвератрола у свим узорцима. Ипак, узорак који је имао највећи удео полимера у почетном раствору (2,5% w/w) најспорије је отпуштао активну компоненту. Ови резултати дају корисне податке о комплексним системима где је активна компонента инкапсулирана у липозоме даље обложена полимером чиме доприносе потенцијалној апликацији ових и сличних система у прехрамбене производе.

Кључне речи*: ресвератрол, алгинат, отпуштање, сферичност, честице*

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