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Extended Abstract

Preparation of Nano-liposomes Carrying Phycobiliprotein Extracted from Red Algae (*Gracilaria gracilis*) with Chitosan Polymer Coating: Evaluation of Physicochemical, Antioxidant and Antimicrobial Properties

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Abstract

Encapsulation in liposome structure can be used as a protective carrier system for bioactive compounds during processing and storage under different conditions. Phycobiliproteins (PBPs) extracted from algae with antioxidant, antimicrobial, anti-cancer and anti-inflammatory properties can be applied to produce raw materials for functional foods. Therefore, in the present study, phycobiliprotein pigment was extracted from Gracilaria algae and the amounts of pigment was investigated. Also, nanoliposomes containing PBPs coated by chitosan (0, 0.5, 1 and 1.5%) were prepared and their physicochemical properties, antioxidant and antimicrobial activity were evaluated. The mean diameter of nanoliposomes and Polydispersity index (PDI) ranged from 336.9 to 577.7 nm and 0.25 to 0.28 in nanocarriers, respectively. The highest values of nanoliposome encapsulation efficiency of PBPs (83.98%) were obtained under optimal conditions in nanoliposomes with 1.5% chitosan coating. The results showed that the antimicrobial activity of the treatments increased significantly after encapsulation in the nanoliposome. In addition, the antioxidant activity of PBPs increased significantly after nanoencapsulation in liposomes. So that the EC_{50} level decreased to 81.27 and 107.67 ppm in DPPH and ABTS tests in nanoliposomes with 1.5% chitosan coating, respectively. Based on the findings of this study, it can be realized that nanocoating with chitosan effectively increases its stability, antimicrobial and antioxidant properties. Therefore, in order to increase the stability of natural compounds during different processes, it is recommended.

Keywords: Antimicrobial activity, Antioxidant activity, Chitosan, Nanoliposome, Phycobiliprotein

Introduction

The tendency of the production and development of beneficial food products has led to increasing attention to modern natural resources. Algae due to the presence of bioactive compounds including pigments and carotenoids such as astaxanthin, lutein, canthaxanthin, fatty acids, proteins such as phycobiliproteins, polysaccharides, and phenolic compounds are

important and are used in a variety of food products as well as in the formulation of biological products.

The use of phycobiliproteins plays an important role in reducing the incidence of anemia, inflammation, liver disease, and anti-cancer agents (Hosseini *et al.*, 2013). Such compounds are also used as natural colorants and emulsifiers in food products such as icy and non-alcoholic beverages, chewing gums, candies and in the health and cosmetics industries as alternatives to synthetic colorants.

These compounds have a high potential for use as additives and useful compounds due to their nature, unique color and also having biological properties. The use of natural pigments in food has limitations such as the high sensitivity of these compounds to heat, light, chemical reactions, and oxidation, their degradation during processing and storage. Furthermore, their aroma may cause changes in the sensory properties of the food product (El Asbahani *et al.*, 2015). Therefore, the use of the encapsulation technique is a practical way to protect bioactive compounds against digestion by coating and forming capsules. Liposomes are one of the types of lipid carriers used to coat bioactive and food-drug materials. The use of mucosal adhesive polymer systems such as chitosan is the most important step in increasing the liposomal delivery of bioactive protein compounds orally. Thus, the stability of the liposomal carrier and the efficiency of absorption in the gastrointestinal tract can be greatly increased by a layer of chitosan coating, which can be attributed to the binding of chitosan and liposome by electrostatic reactions between chitosan cationic groups and anionic phospholipid groups.

Seyed Yagoubi *et al.* (2018) was coated the phycocyanin pigment of spirulina in solid lipid nanoparticles and nanostructured lipid carriers. Their results showed that the type and amount of lipid and surfactant had a significant effect on particle size, dispersion index and zeta potential.

Therefore, due to the high distribution of seaweed in the southern and northern waters of the country and also the potential for the production of valuable products from these materials, the use of these valuable compounds in the functional food and pharmaceutical industries was considered.

Materials and methods

Phycobiliproteins were extracted according to Moraes *et al.* (2011) with slight modifications. Phycobiliproteins were assayed on the dry powder of Gracylaria red algae according to the method of (Wyman & Fay, 1986).

Amino acid composition analysis of lyophilized powder of phycobiliproteins was performed by high-performance liquid chromatography (Kenauer, Germany), (Volkmann *et al.*, 2008). For the preparation of nano-liposomes, phycobiliprotein and phospholipid/cholesterol were hydrated in distilled water and heated to 30 °C with glycerol. Crude liposomes with different ratios (0, 0.5, 1, and 1.5% w/v) of chitosan solution were mixed at room temperature for 1 h at about 200 rpm. The liposomal solution was sonicated and stored at room temperature for the next analysis (Hasani *et al.*, 2019; Rasti *et al.*, 2012).

Mean particle size, size distribution (Polydispersity Index; PDI), and zeta potential of nano-liposome were determined using a laser particle size distribution analyzer (Malvern Instruments, UK) at 25 °C.

The encapsulation efficiency was defined as the ratio of encapsulated Phycobiliprotein to free Phycobiliprotein×100 as determined by using the Spectrophotometer (Fathi *et al.*, 2012) DPPH free radical scavenging capacity of the samples was measured according to a modified method of Taheri *et al.* (2014) and ABTS radical scavenging activity was determined using the procedure proposed by Wang *et al.* (2012) to evaluate Antioxidant activities of nano-liposomes.

Broth dilution method was used to evaluate the antimicrobial properties of phycobiliproteins and nanoliposomes with different formulations against Staphylococcus aureus and Escherichia coli by determining the MIC and MBC (Hashemi *et al.*, 2017).

Results and discussion

According to the results, Phycoerythrin had the highest value (1.03±0.27) and the lowest amount was related to Allophycocyanin (0.23±0.01) and then in phycocyanin (0.7±0.0), (Table 1). The results showed that the growth environment and different seasons of the year have significant effects on the concentration of pigments in algae (Pandey *et al.*, 2013).

Table 1. Phycobiliprotein pigments (mg / gdw) in Gracelaria gracilis

Type of pigment	Phycoerythrin	Phycocyanin	Allophycocyanin	Total Phycobiliprotein
Content	1.03± 0.27	0.7± 0.00	0.23± 0.01	1.96±0.18

Reported means (± standard deviations)

The amino acid composition of Phycobiliprotein is presented in Table (2). Phycobiliproteins were rich in alanine (16.23%), valine (9.9%) and leucine (9.12%). Acidic amino acids (glutamic acid and aspartic acid) and alkaline amino acids (arginine, histidine and lysine) were 9.36 and 7.33%, respectively.

Table 2. Amino acid compounds of phycobiliproteins

Amino acid	Content (%)
Glutamic acid	4.12
Proline	2.87
Tryptophan	1.43
Cysteine	0.13
isoleucine	6.14
Threonine	6.32
Tyrosine	0.38
Aspartic acid	5.24
Serine	6.76
Glycine	5.45
Valine	9.9
Alanine	16.23
Methionine	0.63
Lysine	4.45
Leucine	9.12
Phenylalanine	4.97
Histidine	0.68
Arginine	2.20

The size distribution of nano-liposome containing Phycobiliprotein was shown in Table (3). The mean diameter and polydispersity index (PDI) of nano-liposomes were in the range 336.9±21.3 to 557.7±25.9 nm and 0.25 to 0.28, respectively. The mean particle size decreased with the addition of chitosan and then increased with increasing chitosan concentration.

The results of the present study showed that the encapsulation efficiency of liposome structure can be affected by the size and surface area. However, the present study showed a direct relationship between particle size and nanoencapsulation efficiency for liposomes containing phycobiliproteins. According to the results, chitosan molecules can be attached to the surface of the liposome and form a hard skin and limit the fluidity of the lipid membrane, thus making the membrane harder and more resistant (Seyedabadi *et al.*, 2021).

Table 3. Mean particle size, polydispersity index (PDI), encapsulation efficiency (EE %), and zeta potential (mV) values of nano-liposomes (Mean ± Standard Deviation)

	particle size (nm)	Encapsulation Effeciency (EE%)	Zeta potential	PDI
Uncoated nano-liposoms	557.7 ± 25.90^{a}	70.14 ± 1.24^{d}	-10.8 ± 1.85^{c}	0.25 ± 0.00^{a}
Coated with 0.05% CH	336.9 ± 21.30^{c}	75.68 ± 1.20^{c}	22.6 ± 2.70^{b}	0.26 ± 0.02^{a}
Coated with 0.1% CH	406.8 ± 12.70^{b}	80.98 ± 1.12^{b}	30.33 ± 1.45^{a}	0.28 ± 0.00^{a}
Coated with 0. 5% CH	515.5 ± 41.20^{a}	83.98 ± 0.73^{a}	21.31 ± 5.21^{b}	0.26 ± 0.02^{a}

Reported means (± standard deviations)
Lowercase presented differences between treatments in each column.

The zeta potential in the samples ranged from -10.8±1.85 to 30.33±1.45 mV. The reason for the negative surface charge of uncoated nanoliposomes is the presence of lecithin as an ion emulsifier. The coating is applied with the addition of chitosan, due to the negative and positive electrostatic forces between the liposome and chitosan phospholipids. Our findings determined that the free and encapsulated phycobiliprotein in liposomes had good inhibitory activity against DPPH and ABTS free radicals (Fig. 1). The results showed that encapsulated treatments with chitosan coating (1.5%) had the highest antioxidant activity compared to other treatments ($P \geq 0.05$). The obtained results can be related to the presence of chitosan in the composition of the liposome phospholipid wall. Based on the results of both strains of the tested bacteria, the antimicrobial activity of the phycobiliprotein in the form of nanoliposome was higher than in the pure treatment. The levels of MIC and MBC against Escherichia coli were higher than those of Staphylococcus aureus, indicating that Escherichia coli was more resistant to different concentrations of nanoliposomes containing antimicrobial bioactive compounds. In addition, the results of the present study indicated that the MIC and MBC values of both tested strains decreased in the presence of higher levels of chitosan (Table 4).

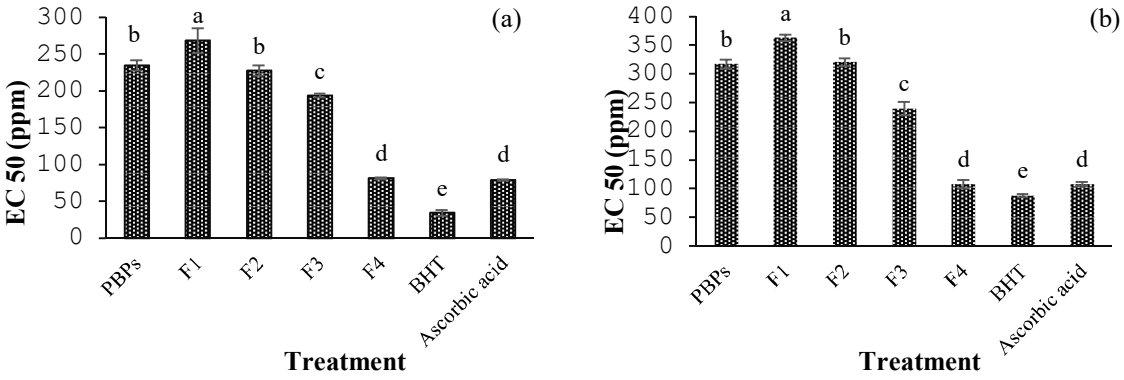

Fig. 1. Comparison of the antioxidant power of free phycobiliprotein protein (PBPs), nanocapsules with different formulations against BHT and ascorbic acid by a) DPPH and b) ABTS. F1: Nanoliposomes containing PBPs without chitosan coating, F2: Nanoliposomes containing PBPs with 0.5% chitosan coating, F3: Nanoliposomes containing PBPs with 1% chitosan coating, F4: Nanoliposomes containing PBPs with 1.5% chitosan coating

Table 4. Minimum inhibitory and bactericidal concentration (MIC and MBC) values for *S. aureus* and *E. coli* for crude and encapsulated in the different formulations of liposomes

Microorganism	Algal extract										Ampicillin	
	Free		Nanoliposome (w/v)									
			0% Chiosan		0.5% Chiosan		1% Chiosan		1.5% Chiosan			
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Staphylococcus aureus	320	1280	640	1280	320	640	80	320	20	80	20	40
E. coli	1280	2560	1280	2560	640	1280	160	640	80	320	40	80

MIC: Minimum inhibition concentration (value in µg/mL)
MBC: Minimum bactericide concentration (value in µg/mL)

Conclusions

The results showed liposomal nanocarriers can be considered as one of the effective systems in nanoencapsulation of bioactive compounds such as phycobiliprotein. Pigment nanoencapsulation in chitosan-coated liposomes effectively enhances the beneficial effects of free pigment, including its antimicrobial and antioxidant properties.

References

El Asbahani, A., Miladi, K., Badri, W., Sala, M., Addi, E. A., Casabianca, H., . . . Renaud, F. (2015). Essential oils: From extraction to encapsulation. *International journal of pharmaceutics*, *483*(1-2), 220-243. https://doi.org/10.1016/j.ijpharm.2014.12.069

Fathi, M., Mozafari, M. R., & Mohebbi, M. (2012). Nanoencapsulation of food ingredients using lipid based delivery systems. *Trends in Food Science & Technology*, *23*(1), 13-27. https://doi.org/10.1016/j.tifs.2011.08.003

Hasani, S., Shahidi Noghabi, M., & Ojagh, S. M. (2019). The Production and Evaluation of Nanoliposomes Containing Bioactive Peptides Derived from Fish Wastes Using the Alkalase Enzyme. *Research and Innovation in Food Science and Technology*, *8*(1), 31-44. https://doi.org/10.22101/jrifst.2019.04.30.813 (in Persian)

Hashemi, M., Ehsani, A., Hassani, A., Afshari, A., Aminzare, M., Sahranavard, T., & Azimzadeh, Z. (2017). Phytochemical, Antibacterial, Antifungal and Antioxidant Properties of Agastache foeniculum Essential Oil. *Journal of Chemical Health Risks*, *7*(2), -. https://doi.org/10.22034/jchr.2017.544170

Hosseini, S. F., Rezaei, M., Zandi, M., & Ghavi, F. F. (2013). Preparation and functional properties of fish gelatin–chitosan blend edible films. *Food Chemistry*, *136*(3), 1490-1495. https://doi.org/10.1016/j.foodchem.2012.09.081

Moraes, C. C., Sala, L., Cerveira, G. P., & Kalil, S. J. (2011). C-phycocyanin extraction from Spirulina platensis wet biomass. *Brazilian Journal of Chemical Engineering*, *28*(1), 45-49. https://doi.org/10.1590/S0104-66322011000100006

Pandey, V., Pandey, A., & Sharma, V. (2013). Biotechnological applications of cyanobacterial phycobiliproteins. *International Journal of Current Microbiology and Applied Sciences*, *2*(9), 89-97.

Rasti, B., Jinap, S., Mozafari, M. R., & Yazid, A. M. (2012). Comparative study of the oxidative and physical stability of liposomal and nanoliposomal polyunsaturated fatty acids prepared with conventional and Mozafari methods. *Food Chemistry*, *135*(4), 2761-2770. https://doi.org/10.1016/j.foodchem.2012.07.016

Seyed Yagoubi, A., Shahidi, F., Mohebbi, M., Varidi, M., & Golmohammadzadeh, S. (2018). Preparation, characterization and evaluation of physicochemical properties of phycocyanin-loaded solid lipid nanoparticles and nanostructured lipid carriers. *Journal of Food Measurement and Characterization*, *12*(1), 378-385. https://doi.org/10.1007/s11694-017-9650-y

Seyedabadi, M. M., Rostami, H., Jafari, S. M., & Fathi, M. (2021). Development and characterization of chitosan-coated nanoliposomes for encapsulation of caffeine. *Food Bioscience*, *40*, 100857. https://doi.org/10.1016/j.fbio.2020.100857

Taheri, A., Sabeena Farvin, K. H., Jacobsen, C., & Baron, C. P. (2014). Antioxidant activities and functional properties of protein and peptide fractions isolated from salted herring brine. *Food Chemistry*, *142*, 318-326. https://doi.org/10.1016/j.foodchem.2013.06.113

Volkmann, H., Imianovsky, U., Oliveira, J. L., & Sant'Anna, E. S. (2008). Cultivation of Arthrospira (Spirulina) platensis in desalinator wastewater and salinated synthetic medium: protein content and amino-acid profile. *Brazilian Journal of Microbiology*, *39*(1), 98-101. https://doi.org/10.1590/S1517-83822008000100022

Wang, B., Li, Z.-R., Chi, C.-F., Zhang, Q.-H., & Luo, H.-Y. (2012). Preparation and evaluation of antioxidant peptides from ethanol-soluble proteins hydrolysate of Sphyrna lewini muscle. *Peptides*, *36*(2), 240-250. https://doi.org/10.1016/j.peptides.2012.05.013

Wyman, M., & Fay, P. (1986). Interaction between light quality and nitrogen availability in the differentiation of akinetes in the planktonic cyanobacterium Gloeotrichia echinulata. *British Phycological Journal*, *21*(2), 147-153. https://doi.org/10.1080/00071618600650171