Book Chapter

Formulation of Lipoprotein Microencapsulated Beadlets by Ionic Complexes in Algae-Based Carbohydrates

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Abstract

The present study is directed to produce sustained release algae-based carbohydrates microbeadlets of lipoproteins rich-in carotenoids extracted from organic sea buckthorn fruits. β-carotene represented the major compound of the lipoproteins extracts. Emulsification and algae-based carbohydrates, such as sodium-alginate and kappa-carrageenan, provide an inert environment, allowing the embedded targeted bioactive compounds, lipoproteins rich in carotenoids, in our case, to

maintain greater biological activity and to have a better shelf life. Furthermore, the microbeadlets prepared from sodium alginate-kappa carrageenan (0.75%:0.75% w/v) crosslinked with calcium ions showing 90% encapsulation efficiency, have been utilized in HPMC capsules using beadlets-in-a-capsule technology, as a delivery system as finish product. The GI simulated tests under laboratory conditions performed, suggested that the sodium alginate-kappa-carrageenan combination could be useful for the formulation controlled release of microbeadlets containing lipoproteins rich-in carotenoids.

Keywords

Lipoprotein; Carbohydrate; Beadlet; Emulsion; Release; HPMC; Beadlet-in-a-Capsule

Introduction

To enhance the bioavailability of orally delivered bioactive compounds different strategies have been investigated to assure a proper solubility and a good intestinal permeability [1–5].

Algae-based carbohydrates encapsulating structures are continuously developed to ensure that the selected bioactives reach the lower gastrointestinal (GI) tract, and establish protective mechanism to combat metabolic syndrome. Alginate and carrageenan are additives used as texturing. There are a gelling agent and a thickener, which gives the required texture. Theirs ability to form a gel in the presence of calcium ions is used to produce an accurate form of a fibrous texture [6,7]. Epidemiological research data have shown that the dietary supplementation with the natural carotenoids have several benefits, a correlation being made between a high carotenoid intake in the diet with a reduced risk of breast, cervical, ovarian, colorectal cancers, and cardiovascular and eye diseases. It is believed that carotenoids act in a dose-and time- dependent manner [8,9].

Lipid-based formulations solubilized are obtained to improve the gastrointestinal (GI) absorption of poorly water-soluble

bioactives compounds. The nutraceutical and pharmaceutical food products are gaining market share, due to the continuous global population to age, preventive and curative [10–12].
The algal-based carbohydrates, alginate and kappa-carrageenan, besides the benefits of being stabilizers, thickeners or emulsifying agents, are considered as a source of dietary fiber, an indigestible polysaccharide [13].

Ionotropic gelation is a method based on the interaction of polyelectrolytes to crosslink in the presence of divalent ions [14]. Lipoproteins are the "packages" in which cholesterol and triglycerides travel throughout the body. There are four major classes of lipoproteins, and each type helps determine a person's risk for cardiovascular disease more accurately than cholesterol measurement alone. Lipoproteins considered as nanoparticulate carrier systems of endogenous origin, have the responsibility of moving the hydrophobic lipids in the blood toward a different cell [15].

Studies have also shown that tumors have low-density lipoprotein (LDL) overexpressed receptors especially in cancers, and for the proliferation lipid and cholesterol systems are required. Therefore, the key point may be for targeting therapeutics encapsulated with LDLs [16,17]. High-density lipoproteins (HDL) and LDL are natural occurring vehicles attractive for bioactives delivery and targeting tumor cells [18,19].

There is a growing market of new products containing bioactive compounds, which are phytochemicals found in foods. They have the capacity to modulate the metabolic processes resulting in the promotion of better health, and therefore there is an increasing demand for evaluation using different approaches [20,21].

The impact of chemical structures, physicochemical properties and the nature of the food matrix on the release and bioavailability of different bioactive compounds in the gastrointestinal (GI) tract have not been studied in detail. However, it is of crucial importance for health relevant functions

[16,17]. Targeting the bioactives to different regions of the GI is a continuous challenge for the researchers especially with regard to personalized nutrition, healthier diets and plant-based diets [11]. This aligns within the increasing efforts invested nowadays to meet the consumers' expectations and needs on one hand, while on the other in finding ways to differentiate the products. It is considered that the next step in the evolution is personalization, a personalized nutrition concept representing a new opportunity market for food companies [12].

The aim of this study has been to test how to improve solubility and bioavailability for bioactive compounds by self-emulsions, and carbohydrates-based microbeadlets to be used as controlled release carriers. The release characteristics in a simulated gastrointestinal tract of the lipoproteins contained in the self-emulsified algae-based carbohydrates microbeadlets were measured.

Materials and Methods

Chemicals

Natrium alginate and kappa-carrageenan were purchase from Danisco Ltd. (Cluj-Napoca, Romania). Hydroxypropyl methylcellulose (HPMC) veggie capsules (2.5 cm) was provided by doTerra (induced thermally (no gelling agent added)). Calcium chloride ($CaCl_2$), sodium dodecyl sulfate, pentanol, HCl, KH_2PO_4, NaOH were purchased from Sigma Aldrich. Sanzyme, a commercial product of Pharco Impex 93, contains papain (from *Carica papaya*) and pepsin, two proteolytic enzymes associated with Sanzyme 2000 (a multienzymatic complex containing proteases, amylases, lipases, celluloses and other enzymes). Triferment, a commercial product of Biofarm, contains pancreatin.

Extraction of Lipoproteins

The organic fresh fruits of sea buckthorn (*Hippophae rhamnoides*) were purchase from a Romanian organic producer. The organic fresh fruits were washed, and broken up using a cuter, followed by centrifugation at 6,000 rot/minute at 20 °C,

using a sieve with holes according to the protocol described by Trif et al. [5]. A 1.5 mm diameter holes was.

The following procedure was applied:

- Three phases mainly a dry part, a paste and a clear juice were obtained in a first step. The organic sea buckthorn fruits seeds and skin were remaining in the dry part.
- The paste part was further centrifuged. The three additional fractions were resulting on the second step. The three fractions called: the first fraction, which was the pellet, called "butter" fraction, the second fraction called the lipoproteins fraction, and the third fraction called the juicy fraction.

The "butter" fraction contains a high amount of lipoproteins, and used to extract the lipoproteins for further encapsulation. The lipoproteins fraction were found in a pectin network. The juicy fraction was a clear juice with low content in lipoproteins, and not taken into consideration for further investigations in this study.

The "butter" fraction was washed with distillate water, and centrifuged. The lipoproteins extracted after centrifugation were self-emulsified with sodium-alginate and kappa-carrageenan to obtain beadlets.

Fractions Analysis and β-Carotene Quantification

Acidity Analysis

Of the obtained fractions the total acidity and pH were measured according to protocols described by Trif et al. [5].

HPLC Analysis

For the quantification of carotenoids, the method used was HPLC carried out following the procedure described by Bindea et al., 2018 [20]. HPLC analyses for individual carotenoids were carried out on an Agilent 1200 system with DAD detector (Agilent Tehnologies, USA) using a reversed phase EC 250/4.6

Nucleodur 300-5 C-18 ec. Column (250 × 4.6 mm), 5 µm (Macherey–Nagel, Germany). The mobile phase consisted of mixtures of acetonitrile : water (90/10 v/v (volume/volume) with 0.25% triethylamine (A) and ethyl acetate with 0.25% triethylamine (B). The gradient started with 90% A at 0 min to 50% A at 10 min. The percentage of A decreased from 50% at 10 min to 10% A at 20 min. The flow rate was 1 mL/minute and the chromatogram was monitored at 450 nm. The quantity of β-carotene using a compound standard and a calibration curve was determined. Calibration curves for β-carotene were prepared at seven concentrations in the range 0–300 µg/mL by plotting the peak area recorded by DAD against the known concentration of the standard. The linear regression factor of the calibration curves was greater than 0.98.

UV-Vis Analysis

The absorption spectras were recorded in a UV-Vis spectrometer (PerkinElmer, Cluj-Napoca, Romania). All measurements were performed at room temperature, and the results are the average of 3 runs. The spectrum of the major constituent of lipoproteins fraction, β-carotene, was performed in the range 300–500 nm.

Preparation of Lipoproteins Self-Emulsion

Different concentrations, 0.5% weight/volum (w/v) and 0.75% w/v of natrium-alginate and kappa-carrageenan in a ratio 1:1 into demineralised water were dissolved by stirring for homogenization for 1 h at 60°C and let to cool down ~37°C until a uniform and bubble free dispersion was obtained.

The obtained homogenized solution of natrium-alginate and kappa-carrageenan was mixed with the extracted lipoproteins (5% volum/weight (v/w)) using the high shear Ultra-Turrax homogenizer at 5,000 rpm, and blended to form an self oil-in-water emulsions.

Evaluation of Lipoproteins Self-Emulsion

Organoleptic Properties

The physical aspects of self-emulsions was checked visually (homogeneity, odor and color). By applying it into the skin surface the greasiness was assessed.

Emulsion Stability

From the prepared oil-in-water self-emulsions an amount of 20 mL aliquots were transferred to graded 25 mL cylinders, were sealed, and for one day stored at room temperature. After 24 h the volume of the aqueous phase was measured. The stability by means of % of separation was measured according to Gangurde and Amin [23]:

$$\% \text{ separation} = H_1/H_0 \times 100 \quad (1)$$

Where
H_0 - represents the emulsion initial height
H_1 – representing the stable emulsion after 24 h (upper phase height)

Stability Under Centrifugation

The self-emulsions stability was evaluated against aggregation of emulsified lipoproteins particles. The centrifugation tubes were filled with 20 mL of oil-in-water self-emulsions, and further at 10,000 rpm for 30 min were centrifuged. The evaluation for phase separation was done [23,24].

Microbeadlets and HMPC Capsules Containing Microbeadlets Preparation

The method used to prepare the sea buckthorn lipoprotein-containing microbeadlets by ionotropically gelation technique. The final dispersion of oil-in-water emulsion was dropped through a 0.4 mm × 20 mm syringe needle into 100 mL of $CaCl_2$ concentration 1mM as hardening bath, and was kept for 30 min under stirring to improve the mechanical strength of the beadlets and also to prevent aggregation of the formed ones. The

formation of small beadlets took place quickly, afterwards were collected from the hardening bath by filtration using a stainless steel sterile sieve (pore size: 0.23 mm), then were transferred on a sterile dry filter paper to absorb moisture.

Afterwards, the microbeadlets have been utilized in two-piece capsule shells made from HPMC (capsule size: 2.5 cm), as a delivery system as finish health product using beadlets-in-a-capsule technology.

Encapsulation Efficiency

The encapsulation efficiency (EE %) by measuring the amount of β-carotene was calculated. The major component of lipoproteins fraction is β-carotene. The content of β-carotene from lipoproteins fraction, before and after encapsulation, was assayed using tetrahydrofuran (THF) spectrophotometrically at 454 nm. To extract β-carotene from microbeadlets, the microbeadlets were crushed using a mortal and THF as solvent. The absorption spectras were recorded in a UV-Vis spectrometer as described in Section 2.3.3 [2,5].

A modified formulae was used:

$$(EE\%) = MB1/(D \times IB2) \times 100 \quad (2)$$

where:

MB1 = concentration of β-carotene in microbeadleats containing lipoproteins

IB2 = initial concentration of β-carotene in lipoproteins

D = dilution (in our case D=1)

Analysis of Microbeadlets

Microscopically

The obtained microbeads size and aspects were characterized with a Zeiss high performance microscope (Cluj-Napoca, Romania).

Fluorescence Method

For labelling the lipoproteins encapsulated NBD-N-3786 (6-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amoni)hexanoyl-1-hexadecanoyl-sn-glycero-3-phosphocholine (NBD C6-HPC) fluorophores was used. A solution 1µM NBD-N-3786 solution was added to 1mL lipoproteins (5%) and followed an incubation at 37°C for 1 h. The sample was centrifuged at 4,000 rpm, the pellet collected and Tris buffer pH 8 was added in a ratio 1:1. The sample was vortexed and microencapsulated in potassium alginate. The microbeads were controlled under a microscope with fluorescence. The absorbance and emission was measured with the lamp with wavelength ranging from 400 to 700 nm.

Scanning Electron Microscopy

The physical surface and morphology of the microbeadlets were measured using a scanning electron microscope (Hitachi S-2700, iMOXS micro-X-ray fluorescence spectrometer with BSE detector, Cluj-Napoca, Romania). Microbeadlets were sputtered previous to scanning with gold, and afterwards scanned at an accelerating voltage of 15 kV.

In Vitro simulation of Gastrointestinal Model

For the in vitro simulation of gastrointestinal tests, a combination of the methods described by Trif et al. and Yang, and Chiang, P.-Y was adapted accordingly [5,25]. The stability HPMC capsules containing lipoproteins microbeadlets was measured by evaluation of the physical integrity in different media miming biological fluids with different pH for 24 h at 37ºC under laboratory conditions.

The simulation of gastrointestinal (GI) transit conditions under laboratory conditions was performed as per scheme (Figure 1):

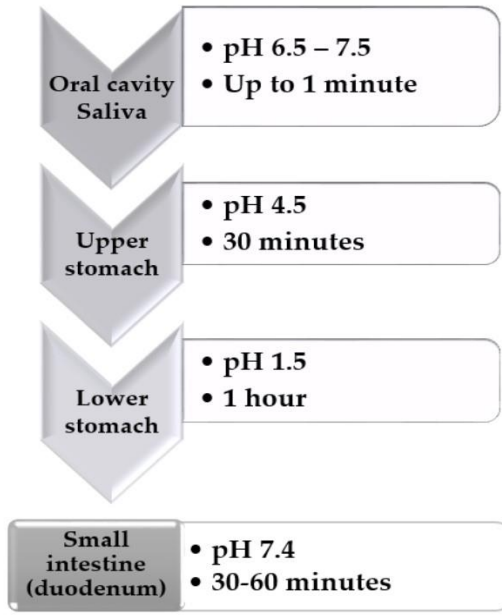


Figure 1: Schematic representation tests in different media miming biological fluids.

The conditions of simulated of GI transit was achieved by using different dissolution media:

- pH 1.5 - consisted of 0.1 N HCl and 5 mL Sanzyme (enzyme syrup containing 80 mg papain, 40 mg pepsin and 10 mg Sanzyme 2000); pH adjusted to 1.5 ± 0.1.
- pH 4.5 - prepared by mixing SGF pH 1.5 and SIF
- pH 7.4 in a ratio 39:61; pH adjusted to 4.5 ± 0.1. pH 7.4 - consisted of KH_2PO_4 1.074g in 30 mL of 0.2 N NaOH, and one tablet of Triferment (containing pancreatin 275 mg, equivalent to a minimum enzyme activity of 2970 amylase units, 3720 lipase units and 250 protease units); pH adjusted to 7.4 ± 0.1.

The experiment was performed into an incubator with a continuous supply of carbon dioxide at 37 °C. The beadlets-in-HMPC capsule and afterwards only the beadlets, *were*

transferred between different media miming biological fluids once after the contact time was over in each media as represented in Figure 1.

Sanzyme syrup is a commercial available product, a recommended balanced combination of digestive enzymes with specific actions, contributing to the complete digestion of food components (proteins, lipids, carbohydrates, and plant fibers).

Statistical Analysis

Results were expressed as a mean value with its standard deviation (mean ± S.D.) of each sample that is repeated three times ($n = 3$). Statistical analysis was performed with student's t-test and differences were considered as significant at p-values under 0.05.

Results

Characterization of Fractions

Acidity Analysis

The acidity was expressed as malic acid, and was in a range 1.82-1.9%. On the other side, the obtained fractions were having a pH value ranging 2.7–3.1 ± 0.2. The pH of fruit juices is generally low, ranging from pH 2.5 to pH 3.7, and it is necessary to be checked as the acid tolerance in the juices it is of high importance for further applications.

Composition of Lipoproteins Fraction Used for Encapsulation by HPLC

The β-carotene from the HPLC chromatogram was measured as a resulting area percent. The calibration curve prepared by using as standard the β-carotene as major compound of lipoproteins fraction, shown that the total content of β-carotene from the lipoprotein fraction was 10% from the total carotenoids content (estimative 4.5 mg\100 g). The presence of the different carotenoids (such as apocarotenoids, lutein, β-cryptoxanthin, zeaxanthin) besides β-carotene was notably.

Self Emulsions Characterization

The self-emulsions were orange in color and found to be smooth, free from grittiness on when applied on the skin surface. The formulations were evaluated for their stability under centrifugation. It was observed that the formulation with sodium alginate-kappa carrageenan (0.5 %:0.5 % w/v) shown an upper phase separation of 10%, and the formulation with sodium alginate-kappa carrageenan (0.75 %:0.75 % w/v) shown no creaming or phase separations under centrifugation, therefore it was decided to further use this formulation in our study.

The light microscopy imaging of the oil-in-water self-emulsion of lipoproteins and sodium alginate-kappa carrageenan (0.75 %:0.75 % w/v), reveals the presence of lipoproteins droplets, whose size varies between 10–60 μM ± 0.15 (Figure 2.).

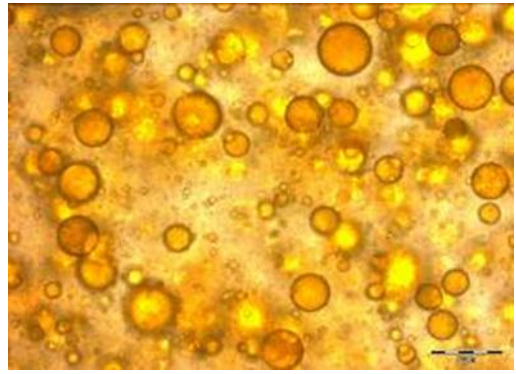


Figure 2: Self-emulsion of lipoproteins and sodium alginate-kappa carrageenan (0.75 %:0.75 % w/v). The scale bar represents 100 μm.

Characterization of Microbeadlets Containing Lipoproteins Encapsulated

Encapsulation Efficiency (EE%)

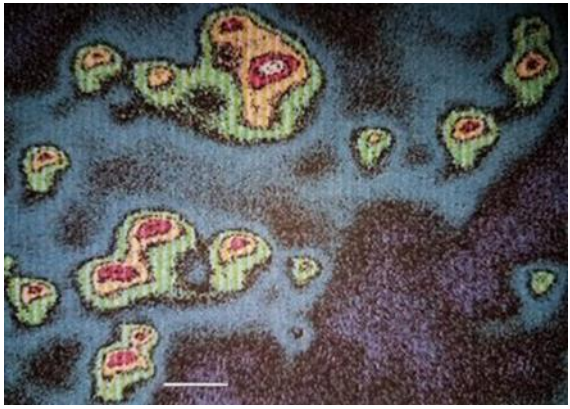
The encapsulation efficiency was 90% determined based on UV-Vis spectroscopy analysis of content of β-carotene as major compound from lipoproteins fraction, before and after encapsulation. The initial β-carotene content in the lipoproteins extracted fraction was 2.035 ± 0.1 mg/DW. After encapsulation

in the sodium alginate-kappa carrageenan (0.75 %:0.75 % w/v) microbeadlets was 1.830 ± 0.1 mg/DW.

Microbeadlets Characterization

The fluorescencent method to show the microbeadlets content was applied. Firstly, the lipoproteins were labelled with the fluorescent NBD-N-3786, encapsulated and then subjected to microscopy. (Figure 3a). It can be observed the orange fluorescence inner tail of the microbeadlets which are the lipoproteins labeled with NDB-N-37862. The microbeadlets's wall is green colored due to the fact that NDB-N-37862 does not label polysaccharides such as alginate and carrageenan. The microbeadlets with a red or\and core can be explained due to the thick microbeadlets' diameter through which the light must pass.

The microbeadlets have the size distribution ranging from 2 to 3 mm ± 0.2, depending on the needle used. The shape is closed to spherical and the surface is smooth (Figure 3b).

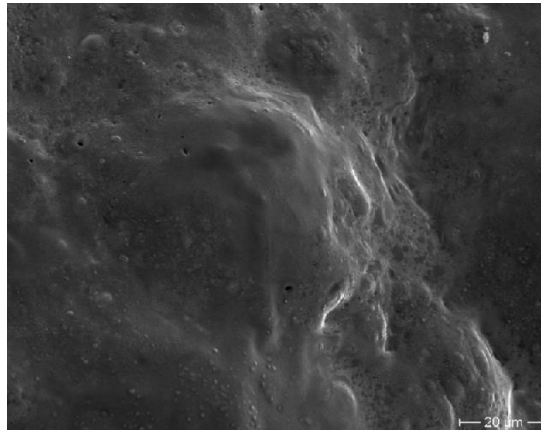


(**a**)

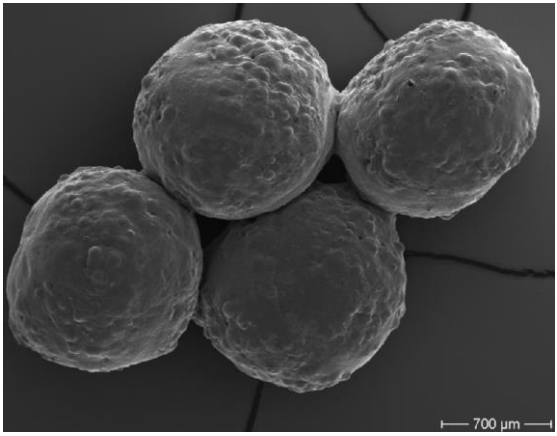
(**b**)

Figure 3: (**a**) Microbeadlets with fluorescence method. The scale bar represents 3 mm; (**b**) Microbeadlets containing lipoproteins.

The surface of microbeadlets obtained show non regular, this is due to the lipoproteins dispersion all over the internal structure (Figure 4a). The SEM pictures of microbeadlets shown a non-porous surface, and a smooth surface morphology is exhibited (Figure 4b). Several studies have shown that kappa-carrageenan enhance thermostability of the networks that of the one-polysaccharide network [26].



(**a**)



(**b**)

Figure 4: Scanning electron micrographs of: (**a**) surface morphology of microbeadlets. The scale represents 20 µm; (**b**) microbeadlets external structure. The scale represents 700 µm. Magnification 70×.

As cross-linking agent $CaCl_2$ containing acetic acid glacial was used in optimum concentration 1mM, resulting in microbeadlets with hard walls.

Sodium-alginate and kappa-carrageenan lead to the cross-linking and aggregation of both in contact with calcium chloride, and due to exchange of divalent ions of calcium (Ca^{++}) during the reticulation process occur the creation of a strong network, which leads to a very strong matrix (Figure 5).

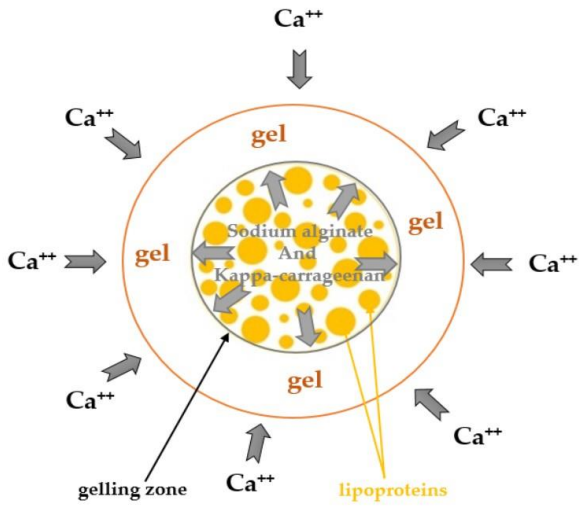


Figure 5: Cross-linking schematic process of sodium alginate-kappa-carrageenan in the presence of calcium ions via diffusion method.

The cross-linking ions is possible by the two basic methods for a sodium alginate-kappa-carrageenan gel preparation: firstly the diffusion method, and secondly the internal setting method. In the diffusion method the cross-linking ions are allowed to diffuse from a large outer reservoir into a sodium alginate-kappa-carrageenan alginate solution (Figure 5). This diffusion permits a rapid gelling kinetic. In general its utilization is well known for immobilization purposes, resulting one single gel bead with entrapped bioactives. A high-speed setting has a great advantage as in foods restructuring a given size and shape of the final product is targeted.

HMPC Capsules Containing Microbeadlets with Lipoproteins

For further nutraceutical, and dietary and nutritional supplements applications, the food-grade and vegetable-derived microbeadlets have been utilized in HPMC capsules, as a delivery system as finish health product for a specific needs approved for internal use using beadlets-in-a-capsule technology (Figure 6). An extra

advantage of HPMC thermally gelled two-piece capsules used in the present study, is due to HPMC property of unaffected release performance by the presence of calcium ions [27] due to cross-linking of sodium alginate - kappa-carrageenan microbeadlets.

Carbohydrates from algae such as sodium alginate and kappa-carrageenan, and HPMC capsules (cellulose fiber) are considered food additive in accordance with EC regulation No. 1333/2008, are approved for vegetarians by Vegetarian Society [28]. Therefore, are increasingly favored as free animal by-products, due to their properties being preferred for moisture-sensitive active bioactive compounds, and never the last due to the "natural" image the nutritional supplement industry can confer on the product expanding the new possibilities for marketing purposes, as being eligible for organic label language (EU) and suitable for use with other organic bioactive ingredients green extracted as lipoproteins from organic sea buckthorn fruits.

(**a**)

(**b**)

Figure 6: (**a**) and (**b**) Microbeadlets with lipoproteins incorporated in two-piece capsule shells made from HPMC (capsule size: 2.5 cm) using beadlets-in-a-capsule technology.

Stability in Different Simulated Gastrointestinal Fluids under Laboratory Conditions

In our study, the HMPC capsules containing microbeadlets with lipoproteins, obtained using beadlets-in-a-capsule technology (Figure 6), were dissolute in simulated gastric fluid (SGF) of pH 1.2. Further, the microbeadlets were expose to the simulation of gastrointestinal (GI) transit conditions under described laboratory conditions (Figure 7):

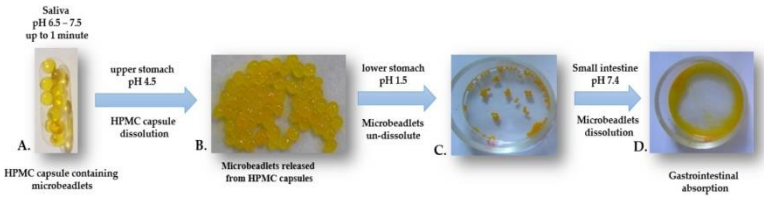


Figure 7: Stability in different simulated gastrointestinal fluids under laboratory conditions.

Along the GI tract, the pH varies. In the first section of the GI tract, oral cavity, the pH of saliva is usually between 6.5–7.5, and last up to 1 min (Figure 7A.). In any dissolution medium with a pH below or equal to 5.8, HPMC capsule shells dissolved rapidly. Therefore, in the oral cavity the HPMC capsules remain intact, and could be given with cold or warm drinks.

The HPMC capsules than access the upper part of the stomach which has a pH between 4.0–6.5 occurring the pre-digestion, and here starts the dissolution of them, releasing the microbeadlets (Figure 7B.). Further, in the lower part of the stomach where the secretion of hydrochloric acid (HCI) and pepsin occur, the pH is between 1.5–4.0. The microbeadlets remain in the lower part of the stomach undissolved (Figure 7C.). After enter the duodenum (small intestine), pH changes to 7.0–8.5. The microbeadlets exhibit visual signs of swelling and erosion. As expected, the swelling volumes of the microbeadlets increased by increasing the pH. At pH > 7 the microbeadlets were completely dissolved as shown in the Figure 7D. According to several studies and supported by different health societies, in the small intestine 90%

of the nutrients absorption is taken in by the body (Canadian Cancer Society).

Discussion

The carotenoids, as a supplement, have to be presented in a ready-absorbed form through the gastrointestinal tract to be able to exert their function on a targeted part of the human body. Commercially available carotenoids supplements are in the form of soft gel capsules obtained from gelatin as soft shell or two piece hard gel capsules. Nowadays the attention is draw on developing products suitable for vegetarians, vegans and to be allergen-free [29].

Carbohydrates from algae and plant-based encasing bioactive ingredients such as carotenoids are of interest. In our study the lipoproteins rich in carotenoids as bioactive ingredients were encased microbeadlets obtained from carbohydrate algae-based by ionic complexes.

The lipoprotein self-emulsions were obtained without adding an emulsifier. We examined this possibility having in mind the presence of pectin as structural natural matrix found in sea buckthorn fruits (can be noticed the presence of in Figure 2). The extraction of a free-pectin matrix lipoproteins using green methods cannot be achieved. Therefore, the presence of some left pectin surrounding the lipoproteins influence the stability of an emulsion being its self-considered an natural emulsifier [30,31].

The size of the injector is having a decisive role in the formation of microbeadlets, which further influences the final filling of the HPMC capsules. When using a 0.4 mm × 20 mm size injector the capacity of HPMC capsules could not be filled completely by the microbeadlets (Figure 6). Further research will address the evaluation of smaller injectors, and their capacity to produce stable microbeadlets capable to completely fill the HPMC capsule.

The carotenoids absorption is facilitated by fats [32], therefore the system of lipoproteins fraction extracted from sea buckthorn encased in microbeadlets make this system to be considered as an ideal supplement formulation, and a much higher bioavailability of carotenoids is expected. Besides, the enhancement of carotenoids stability, their physical properties are considerable improved due to lipoproteins and encapsulation technique used. The obtained microbeadlets are suitable for use in food supplements and incorporated in food products, and for further medical applications. A β-carotene of up to 50% superior bioavailability has been given by encapsulation of it in beadlets than in oil suspension in softgel capsules [33,34].

The encapsulation of a well-known anticancer agent curcumin with lipoproteins, as natural vehicles, has been attractive for bioactives delivery and targeting tumor cells, and has shown an improved anticancer treatment [35,36].

The sodium alginate and kappa-carrageenan have been investigated as dietary with health benefits, and have been incorporated in different products showing amazing results, as reducing the blood sugar level (glucose absorption rates reduced) [37,38]. Even comparing with other dietary carbohydrates such as guar gum in products like snack bars, sodium alginate has reduced postprandial peak glucose concentrations and total glucose uptake over 3 h [39–41].

The proposed cross-linked combination of algae-based carbohydrates, sodium alginate and kappa-carrageenan, can considered as new delivery for plants bioactives loading and controlled delivery systems. Their further utilization in HPMC capsules, as a delivery system as finish product, make them a good candidate for nutraceutical, and dietary and nutritional supplements applications. Nowadays the naturally occurring biomaterials (biopolymers) and the bioactives extracted from organic plants are economically attractive and an innovative approach for food industry [42] and other practical applications (following the advantages of the capsule-in-a-capsule technology, tablet-in-a-capsule technology or duo-capsule) [43]

especially in personalized food and feed diets, and medical nutrition [11,12,16,44–46].

Conclusions

In this study a successful lipoproteins rich in carotenoids fraction extracted from organic sea buckthorn fresh fruits has been encapsulated into a mixture of algae-based carbohydrates (sodium alginate-kappa carrageenan, ratio 1:1), microbeadlets being obtained by crosslinking method with calcium chloride. A 90% encapsulation efficiency has been achieved. The microbeadlets have been utilized in HPMC capsules, as a finish product delivery system, for further nutraceutical, dietary and nutritional supplements applications.

The GI simulated tests performed under laboratory conditions, suggested that the sodium alginate-kappa-carrageenan combination could be useful for the formulation with controlled release of microbeadlets containing lipoproteins rich-in carotenoids. This represents a potential further applications, enabled by the using of the microbeads either in present form or as duo-capsule. Through the beadlets-in-a-capsule method applied, the bioactives can be easily targeted to different regions of the GI, with potential implications in personalized nutrition and plant-based diets.

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