Sea buckthorn juice, tomato juice and pumpkin oil microcapsules/microspheres with health benefit on prostate disease – obtaining process, characterization and testing properties

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FLORINA CSERNATONI1,2, RALUCA MARIA POP2,3, FLORINA ROMACIUC1,2, FLORINELA FETEA1, OANA POP1, CARMEN SOCACIU1,2*
1University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Romania
2Center for Applied Biotechnology CCD-BIODIATECH, Proplanta Cluj-Napoca, Romania
3University of Medicine and Pharmacy Iuliu Hatieganu Cluj-Napoca, Romania
*Corresponding author: University of Agricultural Sciences and Veterinary Medicine, 3-5 Calea Manastur Street, Cluj-Napoca, Romania
Tel. 0040741115673; Fax: 0040264428800; Email: carmen.socaciu@usamvcluj.ro

Abstract
The latest studies show that the active principles of tomatoes, sea buckthorn, and pumpkin oil have beneficial effects on prostate disease. The purpose of this study was to encapsulate the bioactive compounds of seabuckthorn juice, tomato juice and pumpkin oil, to obtain a functional product. Microencapsulation was performed using natural polymers such as sodium alginate to provide stability, bioavailability and bioactive compounds controlled release. The CaCl₂ gelation technique was used to obtain microcapsules with or without chitosan coatings like microspheres and mononuclear microcapsules. Bioactive compounds in microcapsules, microspheres, and ingredients were analyzed using UV-Vis and FT-IR analysis. Total polyphenols and total carotenoids concentration was between (40.90-283.75 mg GAE / 100 g sample; 9.16-19.71 mg carotenoids/100g sample) in the case of the ingredients while total polyphenols and carotenoids content of microcapsules/microspheres was between (14.09-133.93 mg GAE / 100 g sample; 7.13-18.44 mg carotenoids/100g sample). The release rate of the microcapsules and microspheres bioactive compounds was done using simulated gastric juice and simulated intestinal juice and monitored by UV-Vis spectrometry. Therefore the data obtained in this study regarding the phytonutrients presence and their release in the simulated gastric and intestinal juice can be further used to investigate their specific role as natural chemoprotective in prostate disease.

Keywords: Microencapsulation, seabuckhorn juice, tomato juice, pumpkin oil, UV-Vis, FT-IR, prostate protection, simulated gastric juice, simulated intestinal juice

1. Introduction
Microencapsulation is a relatively new technology with large applications in pharmaceutical, cosmetic, food and medical industry [1-3]. Microencapsulation provides product stability, bioavailability and controlled release of active principles [4-5]. Also, it offers the possibility to include a series of compounds in liquid form or solid form (enzymes, micro-organisms, flavors, pigments, vitamins, bioactive molecules, cells) [6-7] in a specific matrix to obtain their protection and stability [8-9]. Thus a barrier is created between the bioactive principles and the environment [10, 11], providing protection against external damage factors (e.g. air, humidity, water, light) and their controlled release [12-14]. That is why the three big stages like the incorporation of the bioactive compounds, preparation and stability research [15] used in microencapsulation are continuously...
evolving to ensure their successful applicability. The most common encapsulation methods used in food and pharmaceutical industry are spray drying technique, microencapsulation by fluidized bed coating and microencapsulation by ionotropic gelation [3,5], while the most important polymers are the natural ones such as sodium alginate, chitosan, starch, pectin, and cellulose. The natural polymers are preferred since they possess low toxicity, constant texture and taste, are easily dispersible and have good emulsifying properties [16-17]. The controlled release of active principles in microcapsules is achieved by changing the external environmental temperature, humidity, pressure or pH [18-19] and is influenced by several factors: density, crystallinity, plasticity, crosslinking pretreatment, solubility, diameter, shell thickness, shape, material, temperature, pH, and water activity [20 – 21, 4]. Recent studies show that tomatoes, sea buckthorn, and pumpkin oil are rich sources of antioxidants like polyphenols, phenolic derivatives, vitamins, carotenoids, tocopherols, fatty acids and sterols [22- 25] with anti-tumor and chemoprotective effects in prostate cancer. Thus these natural chemoprotective sre intensively studied and used in practical usage, mainly due to their minimal side effects compared to synthetic drugs. That is why tomatoes, sea buckthorn, and pumpkin oil were considered both suitable materials for microencapsulation and promising natural chemoprotective to obtain a functional product. Sea buckthorn (Hippophae rhamnoides) is a complex and rich source of bioactive compounds. Among them, the most important are fatty acids like palmitic (23-40%), oleic (20-53%) and palmitoleic (11-27%), sterols with a total content of up to 400 mg/kg fresh weight (FW) [26-27] and carotenoids with concentrations between 119.9-1424.9 µg/g DW. The most representative carotenoids lutein, zeaxanthin, β-cryptoxanthin, lycopene, γ-carotene, β-carotene and esterified carotenoids [28-29, 30]. Other important bioactive molecules are tocopherols with a concentration between 4.6-12.4 mg/100g (FW) with α-, β-, γ-, δ- tocopherol and α-, γ-, δ- tocotrienol as major compounds. The major phenolic derivatives identified in sea buckthorn are leucocyanidin, catechins, flavonols and flavones trace [31-32] while the most important vitamins are B complex, vitaminK,A, C, E [33] and minerals like Ca, Mg, Zn, Se [34]. Tomato (Lycopersicum esculentum) is also an important phytonutrients source like carotenoids (2,6 mg -11.2 mg/100g FW), vitamins, polyphenols, flavonoids and minerals [35-36]. Lycopene and β-carotene are the major carotenoids while lycoxanthin, antheraxanthin, zeaxanthin, lutein, γ-carotene, δ-carotene and phytofluene are minor carotenoids. Among microelements the highest concentration was in case of K, with an average value of 2511 mg/100 g, followed by Mg (141 mg/100 g), P (124 mg/100 g), Ca (60 mg/100 g), Fe (1.39 mg/100 g), Zn (0.97 mg/100 g), Cu (0.57 mg/100 g) and vitamins C, E and B [36-38]. Pumpkin (Curcubita maxima) is a rich source of fatty acids like linoleic, oleic, palmitic and stearic acids found between 35.6% to 60.8%, 21% to 46.9%, 9.5% to 14.5% and 3.1% to 7.4%, respectively. Other fatty acids identified in pumpkin oil are myristic, heptadecanoic, arachidic, eicosenoic and α-linolic acid. Tocopherols (between 16.3 - 46.7 mg/100 g), carotenoids and sterols were also identified as important biomolecules in pumpkin seed oil [39-41]. The major sterols are beta-sitosterol (24.9±1.4mg/100g), stigmasterol (8.4±0.3 mg/100g) and campesterol. According to recent studies, sterols are considered essential in the prevention of prostate disease [22, 42, 43]. Therefore, the major aims of this study were to characterize the chemical compounds from sea buckthorn juice, tomato juice and pumpkin oil with relevant importance in prostate disease, to encapsulate these bioactive compounds in microcapsules and microspheres with stable properties and to obtain the active principles controlled release.
2. Materials and methods

2.1. Samples and chemicals

The sea buckthorn and tomatoes were purchased from a local market while pumpkin seed oil (PSO) was purchased from a specialized company. Further sea buckthorn (SBJ) and tomato juice (TJ) were obtained from fresh fruits, which were crushed and centrifuged at 2500 rpm to separate the seeds and skin. Chitosan and sodium alginate were purchased from FMC Biopolymer, Norway, while calcium chloride (CaCl₂), pepsin, pancreatinin and bile salt were purchased from Merck, Germany.

2.2. Preparation of microspheres and microcapsules

The microspheres and microcapsules were obtained using ionotropic gelation technique with BuchiB 395 PROencapsulator. The obtaining methodology process was specific for both microcapsules and microspheres. Thus, the microspheres were obtained using two types of nozzle: 400 μm for the shell, and 200 μm for the core. The pressure was 293 mbar and the flow rate 1.42 ml/min. The microspheres were obtained using a 450 μm nozzle and a 635 mbar pressure. The combination of ingredients used to obtain microcapsules and microspheres are listed in the next table.

Table 1. The combination of ingredients used to obtain microcapsules and microspheres

<table>
<thead>
<tr>
<th>Sample name</th>
<th>Sea buckthorn juice [%]</th>
<th>Tomato juice [%]</th>
<th>Pumpkin seed oil [%]</th>
<th>Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>INGREDIENTS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P1</td>
<td>100</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>P2</td>
<td>95</td>
<td>-</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>P3</td>
<td>-</td>
<td>100</td>
<td>-</td>
<td></td>
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<tr>
<td>P4</td>
<td>-</td>
<td>95</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>P5</td>
<td>50</td>
<td>50</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>P6</td>
<td>50</td>
<td>50</td>
<td>-</td>
<td>+5% PSO</td>
</tr>
<tr>
<td>P7</td>
<td>-</td>
<td>-</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>MICROSPHERES</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1A</td>
<td>-</td>
<td>100</td>
<td>-</td>
<td>+5% PSO and</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+2% sodium alginate</td>
</tr>
<tr>
<td>1B</td>
<td>100</td>
<td>-</td>
<td>-</td>
<td>+5% PSO and</td>
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<td></td>
<td>+2% sodium alginate</td>
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<tr>
<td>1C</td>
<td>50</td>
<td>50</td>
<td>-</td>
<td>+5% PSO and</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+2% sodium alginate</td>
</tr>
<tr>
<td>1C*</td>
<td>50</td>
<td>50</td>
<td>-</td>
<td>+5% PSO and</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+2% sodium alginate</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>- 0.1% chitosan coating</td>
</tr>
<tr>
<td>MICROCAPSULES</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2A</td>
<td>-</td>
<td>100</td>
<td>-</td>
<td>+2% sodium alginate in the shell</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+5% PSO in the core</td>
</tr>
<tr>
<td>2B</td>
<td>100</td>
<td>-</td>
<td>-</td>
<td>+2% sodium alginate in the shell</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+5% PSO in the core</td>
</tr>
<tr>
<td>2C</td>
<td>50</td>
<td>50</td>
<td>-</td>
<td>+2% sodium alginate in the shell</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+5% PSO in the core</td>
</tr>
<tr>
<td>2C*</td>
<td>50</td>
<td>50</td>
<td>-</td>
<td>+2% sodium alginate in the shell</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+5% PSO in the core</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>- 0.1% chitosan coating</td>
</tr>
</tbody>
</table>

In case of microcapsules 2C* and microsphere 1C* the chitosan coating of 0.1% was obtained in CaCl₂ solution.
2.3. Morphology
The microscopic structure of the microspheres and microcapsules was investigated by optical microscopy (magnification 5X), using a Microscope Carl Zeiss Observer A1, with AxioVision image processing software.

2.4. Extraction of bioactive compounds from ingredients and microcapsules/ microspheres
In order to extract a wide range of bioactive compounds, two types of extraction were performed. The first one was the methanolic extraction specific for polar compounds while the second one was a methanol/ chloroform extraction specific for non-polar.

**Methanolic extraction**
Aliquots of 2 g of each ingredient (P1-P7), microcapsules (2A, 2B, 2C) and microspheres (1A, 1B, 1C) were extracted in 10 ml 96% methanol in water, acidulated with 1% hydrochloric acid. Samples were sonicated for 30 min, centrifuged and filtered using nylon filters (0.2 µm). The clear extracts were kept in the freezer until analysis. The methanolic extract was further analyzed for its total polyphenol content as further described and for their lipids content. The lipids were evaluated using their maximum absorption spectra at 215 nm.

**Chloroformic extraction**
Aliquots of 10 g from each ingredient (P1-P7) microcapsules (2A, 2B, 2C) and microspheres (1A, 1B, 1C) were extracted in a mixture of chloroform:methanol (2:1, v/v). Samples were sonicated for 1 h and stirred for another 30 min using a magnetic stirrer in dark conditions. Further samples were centrifuged for 10 min at 2500 rpm and 4 °C. The pellet and supernatant were recovered. Afterwards, the pellet was reextracted twice using the same procedure. The three supernatants were combined and then separated in a separation funnel using water to induce phase separation (lower lipophilic phase and upper hydrophilic phase). The chloroform phase was further kept in the freezer until analysis and analyzed for its total carotenoids content.

2.5. **Total polyphenols and total carotenoids content**
Total polyphenols content of methanolic extracts was determined using Folin Ciocalteu method. The results were expressed as gallic acid equivalents [mg gallic acid / ml extract]. Total carotenoids content was expressed in mg carotenoids per 100 g sample. The calculation of the concentration was made according to the standardized method [46]. The results expressed are the average of two determinations.

2.6. **FT-IR analysis**
Samples were analyzed on a Shimatzu FTIR spectrophotometer using the Horizontal Attenuated Total Reflection (HATR). The Fourier Transform Infrared spectrum (FTIR) of each extract was recorded in the MIR region, from 4500 to 1000 cm⁻¹, and then the fingerprint region was selected for data analysis [44,45].

2.7. **UV-VIS analysis**
The methanolic extracts of microcapsules/ microspheres, the simulated gastric and intestinal juice were evaluated spectrophotometrically (200-700 nm) using a Jasco V 530 spectrophotometer. All data were processed with the specific software Shimadzu LC Solution and Spectra Manager for Windows 95/NT.

2.8. **Testing in simulated gastric and intestinal juice**
The simulated juices were prepared according to Brinques et al, 2001[47]. Simulated gastric juices were prepared by suspending pepsin (P7000, 1:10,000) in sterile sodium chloride solution (0.5%, w/v) to a final concentration of 3 g / L. The final pH of 2.0 was obtained using concentrated HCl or NaOH. Simulated intestinal juices were prepared by suspending pancreatin USP (P-1500) in sterile sodium chloride solution (0.5%, w/v) to a final concentration of 1 g/L.
with 4.5% bile salts. The final pH of 8.0 was obtained using NaOH. Further, 2 g of microcapsules/microspheres samples (1A, 2A, 1B, 2B, 1C, 2C, 1C * 2C *) were suspended in 20 mL simulated gastric juice and incubated at 37°C for 30 minutes under continuous stirring (164 RPM). Next, the microcapsules/microspheres were transferred in 20 ml of simulated intestinal juice and incubated at 37°C for 2 hours under continuous stirring (164 RPM, Heidolph Unimax Inkubator 1000).

**2.9. Principal Component Analysis (PCA)**

Advanced chemometrics was applied to discriminate between samples using Unscrambler X 9.7 Software, (CAMO Software AS, Norway). The general FTIR metabolic fingerprints represented by the specific IR absorption spectrum zones (1000-3500 cm⁻¹ for chloroformic samples and 1000-4000 cm⁻¹ for methanolic samples) were further analyzed by principal component analysis (PCA). Spectra were transformed by normalization of the absorbance spectra to the most intense band.

**3. Results and Discussion**

**3.1. Microcapsules and microspheres morphology**

Figure 1 represents the microscopic difference between microspheres and microcapsules.

![Microspheres - 1A](image1)

![Microcapsules - 2A](image2)

**Figure 1.** Optical microscopic image of microspheres vs microcapsules

All microspheres (1A, 1B, 1C) were spherical with sizes ranging between 850 and 900 μm as external diameter, while microcapsules (2A, 2B, 2C) sizes ranged between 750 and 800 μm external diameter with a spherical shape and core sizes between 150-180 μm.

**3.2. Total polyphenols and total carotenoids content**

The next table represents the total polyphenols contents of the ingredients (P1-P7), microcapsules and microspheres (1A, 1B, 1C, 2A, 2B, 2C). Sample identification was previously described under materials and methods section.

**Table 2.** The mean values of total polyphenols content of methanolic sample extracts

<table>
<thead>
<tr>
<th>Sample</th>
<th>[mg GAE/ 100 g sample]</th>
<th>Sample</th>
<th>[mg GAE/ 100 g sample]</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>283.75±0.77</td>
<td>1B</td>
<td>116.96±4.29</td>
</tr>
<tr>
<td>P2</td>
<td>263.75±0.38</td>
<td>1C</td>
<td>56.82±28.49</td>
</tr>
<tr>
<td>P3</td>
<td>46.5±0.7</td>
<td>2A</td>
<td>12.72±1.93</td>
</tr>
<tr>
<td>P4</td>
<td>40.90±1.2</td>
<td>2B</td>
<td>133.93±0.43</td>
</tr>
<tr>
<td>P5</td>
<td>162.42±0.98</td>
<td>2C</td>
<td>49.24±11.14</td>
</tr>
<tr>
<td>P6</td>
<td>138.78±2.1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The results indicated that total phenolic compound was 6 times higher in sea buckthorn juice compared with tomato juice. Also, the addition of 5% pumpkin oil decreases the phenolic compounds concentration with 10-12% for both ingredients and microcapsules/microspheres. Moreover approximately 50% of total polyphenols are found in microcapsules, while the other 50% is considered waste being found in the pellet remaining after centrifugation and filtration.

Table 3 represents the mean values of total carotenoids content for each ingredient (P1-P7) and microcapsules/microspheres (1A, 1B, 1C, 2A, 2B, 2C). Sample identification was previously described under materials and methods 2.2 section.

<table>
<thead>
<tr>
<th>Sample</th>
<th>[mg carotenoids/ 100 g sample]</th>
<th>Sample</th>
<th>[mg carotenoids/ 100 g sample]</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>16.39 ± 0.07</td>
<td>1A</td>
<td>18.44±0.22</td>
</tr>
<tr>
<td>P2</td>
<td>17.31 ± 3.80</td>
<td>1B</td>
<td>8.09±0.16</td>
</tr>
<tr>
<td>P3</td>
<td>19.71 ± 1.21</td>
<td>1C</td>
<td>9.99±0.17</td>
</tr>
<tr>
<td>P4</td>
<td>18.76 ± 0.21</td>
<td>2A</td>
<td>18.14±0.85</td>
</tr>
<tr>
<td>P5</td>
<td>14.44 ± 0.44</td>
<td>2B</td>
<td>8.14±0.21</td>
</tr>
<tr>
<td>P6</td>
<td>9.16 ± 0.03</td>
<td>2C</td>
<td>7.13±0.17</td>
</tr>
</tbody>
</table>

The evaluation of total carotenoids content highlights that tomato juice and seabuckthorn juice are the richest sources of carotenoids among samples. Also the addition of 5% PSO does not significantly influence the amount of total carotenoids. The best carotenoid extraction was achieved from individual samples (TJ or SBJ) and not from mixed samples (TJ + SBJ with or without PSO), suggesting that extraction efficiency decreased when mixtures where used for similar extractions. The microcapsules/microspheres (1A, 2A, 1C, 2C) extraction efficiency was higher than 90%, while the microcapsules/microspheres (1B, 2B) extraction efficiency was approximately 50%.

3.3. Comparative FT-IR general fingerprints of microcapsules and ingredients

Figure 2 represents the comparative FT-IR fingerprints of the microcapsules and their ingredients extracts: methanolic (Fig.2A) and chloroformic (Fig. 2B). The peaks assignment of the extracts were done according to literature [44,48].

![Figure 2A. FT-IR general fingerprint region (1000-4000cm⁻¹) of sample vs ingredients methanolic extracts](image1)

![Figure 2B. FT-IR general fingerprint region (1000-3500cm⁻¹) of sample vs ingredients chloroformic extracts](image2)
The general fingerprint region (4000-1000 cm⁻¹) of methanolic extracts analysis yielded the presence of specific functional groups, included in three areas as follows: area 1 (3300-3600 cm⁻¹) corresponding to stretching vibrations of OH groups from water, alcohols, phenols with signals at: 3311, 3332, 3356, 3358, 3383, 3385 cm⁻¹; area 2 (1600-1750 cm⁻¹), corresponding to bending vibrations N-H (amino acids), C=O stretchings (aldehydes and ketones, esters) as well to free fatty acids and glycerides, with signals at: 1627, 1631, 1635, 1641, 1722, 1724, 1732 and 1741 cm⁻¹; area 3 (1000-1130 cm⁻¹) characterized by stretching vibrations C-O of mono- and oligosaccharides, with signals at 1055, 1072, 1074 and 1076 cm⁻¹. The general fingerprint region (3000-1000 cm⁻¹) of chloroformic extracts analysis was divided into four specific areas as follows: area 1 (2800-2950 cm⁻¹) corresponding to C-H stretching vibrations specific to CH₃ and CH₂ from lipids, with signal at: 2920, 2922 and 2924 cm⁻¹; area 2 (1600-1750 cm⁻¹), corresponding to bending vibrations N-H (amino acids), C=O stretchings (aldehydes and ketones, esters) as well to free fatty acids and glycerides, with signals at: 1743 cm⁻¹ and 1745 cm⁻¹; area 3 (1300-1500 cm⁻¹) corresponding to stretching vibrations C-O (amide) and C-C stretching from phenyl groups, with signals at: 1463 cm⁻¹; area 4 (1150-1270 cm⁻¹) corresponding to stretching vibrations of carbonyl C-O or O-H bendings with signals at: 1157, 1159, 1161, 1163 and 1165 cm⁻¹.

3.4. Principal Component Analysis (PCA)

The normalized FTIR spectra were used in the PCA analysis for both methanolic (Fig. 3A) and chloroformic (Fig. 3B) sample extracts. The first two principal components (PCs) explained 95% (PC1 with 76% and PC2 with 19%) and 99% (PC1 with 94% and PC2 with 5%) of the spectra total variance level in case of methanolic and chloroformic samples extract, respectively.

Figure 3. Sample methanolic (A) and chloroformic (B) extracts score plots of the first two principal components, PC1 and PC2 of the IR general fingerprint region (1000-4000 cm⁻¹)
The principal IR bands which characterize the specific absorptions of functional groups from methanolic extracts were identified in the score loadings plots (data not shown). Thus the wavelengths that influenced sample grouping P2, P6, P4 located along the PC2 axis and in the negative PC1 axis (Fig. 3A) ranged from 1020 to 1080 cm\(^{-1}\). The major identified signals 1055, 1072, 1074 and 1076 cm\(^{-1}\) corresponded to CO mono- and oligosaccharides bands vibration. Further the group represented by sample 2B is situated at the top of PC2 axis and middle of PC1 axis, the group represented by samples 1B, 1A and 2A is located near the center of the score plot and the group represented by samples 1C, 2C is placed almost opposite to P2, P6, P4 samples along the PC1 axis on the right side. The distribution of the three groups along PC1 and PC2 axes was largely influenced by the wavelength ranging from 1700 to 1732 cm\(^{-1}\) in case of group 2B and 3300–3460 cm\(^{-1}\) in the case of group 1C, 2C. The specific vibration bands identified in the first region corresponded to NH (amino acids), aldehydes, ketones, esters, fatty acids and glycerides while the specific vibration bands identified in the second region where the OH vibration bands of water, alcohol or phenol compounds with major signals at 3311, 3332, 3356, 3358, 3383, 3385 cm\(^{-1}\). In case of chloroformic extract (Fig. 3B) the PCA score plot indicated the formation of three major groups as follows: 1B, 1A, 2A group, 1C, 2B, 2C group located in the lower and upper PC2 axis and P2, P4 and P6 group situated opposite to the other two groups on the right end side of PC1 axis. The wavelengths that influenced the first two groups distribution were represented by the lipids specific vibration bands like CH, CH\(_3\), and CH\(_2\) from the two regions: 2700–2800 cm\(^{-1}\) and 2920 cm\(^{-1}\). The wavelengths that influenced the third group ranged between 600 and 1750 cm\(^{-1}\) with major signals at 1743 cm\(^{-1}\) and 1745 cm\(^{-1}\) of NH (amino acids), aldehydes, ketones and esters vibration bands as well as fatty acids and glycerides vibration bands.

### 3.5. Testing in simulated gastric and intestinal juice

The comparison between extraction efficiency of methanolic extracts with simulated gastric and intestinal juice are presented in Figure 4 and 5, respectively.

![Figure 4](image1.png)

**Figure 4.** Comparison of extraction efficiency of metanolic extracts (M) with simulated gastric juice (MG).

![Figure 5](image2.png)

**Figure 5.** Comparison of an extraction efficiency of metanolic (M) extracts with simulated intestinal juice (MI).
The UV-VIS spectra recorded for both gastric juice and methanolic extract indicated the presence of two major classes of compounds like lipids (absorption at 210 nm) and phenolic acids (absorption at 280 nm). To compare the extraction efficiency the maximum absorbance values of the extracts were compared in accordance with dilution factor.

In general methanolic extracts had a better extraction yield excepting samples 1A and 2A which had better extraction yield in simulated gastric juice (Fig. 4).

In the case of microcapsules/ microspheres tested in the intestinal juice, the UV-VIS spectra indicated the presence of two major classes of compounds like phenolic acids (absorption at 280 nm) and carotenoids (absorption at 450 nm). The presence of flavonoids was also observed (absorption at 340 nm). In case of carotenoids over 50% were released in intestinal juice 1B, 2B, 1C, 2C, 1C* and 2C* samples, while for 1A and 2A samples only 10% were released in the intestinal juice. This can be explained by the fact that these types of microcapsules/microspheres were not disintegrated in intestinal juice.

4. Conclusions

The use of ionotropic gelation technique yielded microspheres with sizes between 850-900μm, with a spherical shape and to microcapsule with peripheral diameter between 750-800 μm and the core between 150-180 μm. Regarding the bioactive compounds content, sea buckthorn microcapsules had the highest concentration of phenolic compounds while tomato microcapsules had the highest carotenoid concentration. The FT-IR analysis provided relevant data which allowed Principal Component Analysis to identify significant differences and similarities between samples based on the vibration and absorption bands. The evaluation of microcapsules/microspheres methanolic extraction, gastric and intestinal juice extraction indicated that the methanolic extract and gastric juice extract were rich in lipids and phenolic acids while the intestinal juice extract was rich in carotenoids, phenolic acids and traces of flavonoids.

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