

# Preparation and comparative characterization of alginate-made microcapsules and microspheres containing tomato, seabuckthorn juices and pumpkin oil

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## ABSTRACT

Recent studies have shown the benefits of tomatoes, seabuckthorn juices and pumpkin oil, rich in bioactives with antioxidant capacity, in the prevention of prostate diseases. To stabilize their antioxidant activity, microencapsulation represent a good technological alternative, improving the stability and bioavailability of bioactive molecules (phenolic derivatives, carotenoids, phytosterols, vitamins). The aim of the study was to prepare and characterize microspheres and microcapsules based on emulsions made of natural polymers like Natrium alginate mixed with tomato and/or seabuckthorn juices, with or without pumpkin oil. The viscosity of emulsions, the morphology of microcapsules and microspheres were characterized comparatively and the bioactives were monitored by UV-Vis spectrometry. In the lipophilic extract there were identified, before and after encapsulation, different classes of compounds, from lipids, to phenolic acid derivatives, flavonoids and carotenoids. Carotenoids were the major components having concentrations from 9.16 up to 19.71 mg/100 g sample. The viscosity of each emulsion including juices, oil and natrium alginate 2%, before encapsulation, showed differences, dependent on the oil addition and speed of homogenization. The macroscopic and microscopic structure of microspheres and microcapsules were comparatively evaluated. Both microspheres and microcapsules had external diameters ranging from 750 to 900  $\mu\text{m}$  and the microcapsules' oily core of 150-180  $\mu\text{m}$ . The results obtained from emulsion's viscosity will be correlated with the rigidity and optimal release rate of bioactive molecules from microcapsules and microspheres. Further studies are directed towards these aspects.

**Keywords:** *microcapsules, microspheres, tomato juice, sea buckthorn juice, pumpkin oil.*

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## INTRODUCTION

Encapsulation is a modern technology which integrates bioactive compounds (vitamins, enzyme, phenols, molecules, cells) in specific matrices in order to protect their activity and to assure a controled release (Ach *et al.*, 2015; Sobel *et al.*, 2014; Narsaiah *et al.*, 2012; Sovilej *et al.*,

2010; Dima, 2009). Microencapsulation has a wide market interest especially in pharmaceuticals, chemicals, food, cosmetics, improving the stability and bioavailability of both liquid and solid ingredients (Donhowe and Kong, 2014; Nazzaro *et al.*, 2012; Zuidam and Shimoni, 2010).

Various encapsulation methods have been proposed until now, spray drying being the most known, converts a liquid substance into a powder for easier processing, it is the most commonly used method for flavor encapsulation since it is economical and efficient (Poncelet *et al*, 2011; Goula *et al*, 2005).

Microencapsulation in natural polymers which act as a matrix with controlled pores is very useful in the biomedical areas. Different types of microcapsules can be produced, based on alginate, cellulose, pectins, casein, etc. where the bioactive ingredient is inserted in the network of the matrix and can be bioavailable under controlled conditions. While a microcapsule includes the bioactive compound in the core, being surrounded by a shell of matrix, a microsphere have a homogeneous structure, the bioactive component being disseminated in the matrix (Poncelet *et al*, 2011; Sergeeva *et al*, 2014; Alexe and Dima, 2014).

This technology develops drugs and dietary supplements with superior qualities and a controlled release of active principles (El-Aassar *et al*, 2014; Lee *et al*, 2013, Partanena *et al*, 2002, Trif *et al*, 2009). In recent years, an increasing interest of population towards food supplements was observed, mainly because of their prophylactic effects on various diseases (Chen *et al*, 2013; Xu *et al*, 1994).

Recent studies have revealed the benefits of seeds, fruits, medicinal plants and some oil extracts in the prevention of prostate diseases (Chen *et al*, 2013; Alfawaz, 2004; Awad and Fink, 2000, Tan *et al*, 2010). Sea buckthorn (*Hippophae rhamnoides*), tomatoes (*Solanum lycopersicum*) and pumpkin seed oil (*Curcubita maxima*) are rich sources of antioxidants and can be used for their chemoprotective and antitumoral effects. This products are a rich sources of lipophilic and hidrophilic compounds like phytosterols, phenolic acids, tocopherols, carotenoids and vitamins (Rabranovic *et al*, 2014; Pop *et al*, 2013; Dulf *et al*, 2010; Chen *et al*, 2013; Trif *et al*, 2009 ).

Sea buckthorn (*Hippophae rhamnoides* L.) grows wild in Eurasia or it is cultivated in many European countries. Sea buckthorn berries are very rich in many antioxidants, from vitamin C (up to 20 g/L) to flavonoids, unsaturated fatty acids, tocopherols, tocotrienols, phytosterols, carotenoids, valuable ingredients for food supplements and cosmetic products (Chen *et*

*al*, 1990; Quirin and Gerard, 1993; Yang and Kallio, 2001; Socaciu *et al*, 2007, 2008, 2009). Microencapsulation can provide the best protection against oxidation of such bioactives.

The sea buckthorn berries have a good chemoprotective and antitumoral effect (Kavitha *et al*, 2014) on prostate diseases because of their high content in bioactive compounds like  $\beta$ -caroten, vitamins , and, phenolic acids and flavonoids (Pop *et al*, 2013; Beveridge *et al*, 1999; Simao *et al*, 2013).

Tomatoes are also excellent source of antioxidant pigments and vitamins, whowing antitumoral effect against prostate diseases, especially for its high content in carotenoids (especially lycopene and  $\beta$ -carotene) and minerals like selenium (Zhang *et al*, 2014; Wan *et al*, 2014; Chen *et al*, 2013; Behara, 2012).

Pumkin seed oil has a proved, good chemoprotective effect against prostate diseases for their high content in phytosterols and unsaturated fatty acids (Prescha *et al*, 2014; Dulf *et al*, 2010; Ryan *et al*, 2007; Alfawaz, 2004).

This study aimed to characterize the ingredients of seabuckthorn and tomato juices, combined with pumpkin oil, to obtain bioactive emulsions to be inserted in microcapsules or microspheres prepared from alginate polymers. The UV-Vis analysis and viscosity of emulsions were comparatively evaluated and correlated with the morphology and stability of microspheres and microcapsules.

## MATERIALS AND METHODS

### Fruit samples and reagents

The raw sea buckthorn juice was obtained after grounding fresh berries, followed by centrifugation at 2500 rpm and separation of skins and seeds. The raw juice was homogenized by high speed centrifugation and after ultrasonication, it was mixed with the matrix (alginate solution) to obtain an emulsion ready for encapsulation.

The tomato juice was obtained similarly, from fresh tomatoes, grounded and homogenized, then centrifuged and separated from seeds and skins. The raw juice was sonicated and used as ingredient to prepare emulsions and to integrate, as such, or mixed with seabuckthorn juice and pumpkin oil. obtain microcapsules or microspheres,

The pumpkin seed oil was purchased from a specialized company and checked preliminary for its authenticity.

The samples used for experiments were marked as follows: 1A- Seabuckthorn juice, 2A - Seabuckthorn juice mixed with 5% pumpkin seed oil, 3A- Tomato juice, 4A- Tomato juice mixed with 5% pumpkin oil, 5A- Mixture of Seabuckthorn and tomato juices, in a ratio 1:1 (w/w), 6A- Mixture of Seabuckthorn juice and tomato juice in a ratio 1:1 (w/w), containing 5% pumpkin oil and 7A- pure Pumpkin oil.

### **Bioactive molecules identified by UV-Vis spectra**

To characterize their composition, aliquots of 20 g from each sample (seabuckthorn or tomato juices) were extracted in chloroform:methanol (2:1). After sonication for 1 h and 30 min stirring, the samples were centrifuged and separated in two phases (lipophilic phase and hydrophilic phase). The clear extracts from the lower phase (chloroform) were kept in the freezer until analysis.

The UV-Vis spectra were recorded for each sample extract (1A-7A) from 200 to 700 nm, using a Jasco V 530 Spectrophotometer. There were identified the regions of maximum wavelengths specific for lipids, phenolic acid derivatives, flavonoids and carotenoids.

### **Extraction efficiency**

To compare the extraction efficiency (EE) of the bioactive molecules in the solvent, there were considered the UV absorptions (A) of each sample (1A-7A) corresponding to phenolic acids (EF-FA), flavonoids (EF-F) and carotenoids (EF-C) and the general formula was applied for EE calculation:  $EE = A (\lambda_{max}) \times D$ , where A ( $\lambda_{max}$ ) represents the absorption values corresponding each molecular category ( according to  $\lambda_{max}$  identified in the UV-Vis spectra) and D represents the dilution factor. The  $\lambda_{max}$  values used for EF-FA, EF-F and EF-C were 240-280, 330-360 and 430-500 nm, respectively.

### **Determination of the emulsion viscosity**

The viscosity was recorded by a rotational viscometer Fungilab using a spider R2 and expressed in cP. This device allows a simple analysis of liquids with low viscosity. The viscosity of 50 ml from each emulsion was determined at 3 speeds

for homogenization (20, 50 and 100 rotations/min). obtained from the samples 1A-tomato juice with 5% pumpkin oil, 2A- seabuckthorn juice, 1B- seabuckthorn juice with 5% pumpkin oil, 2B-tomato juice, 1C- seabuckthorn juice mixed with tomato juice in ratio 1:1 (w/w) with addition of 5% pumpkin oil and 2C- seabuckthorn juice mixed with tomato juice in ratio 1:1 (w/w), mixed with 2 % Natrium alginate.

### **Preparation of microspheres and microcapsules**

Microspheres or mononuclear microcapsules were obtained by ionotropic gelling method applied for alginate polymers. The alginate polymer (Natrium alginate, with G/M ratio <1) was provided from FMC Biopolymer, Norway while chitosan was supplied by Merck, Germany. A solution of calcium chloride 2% was used for gelling, being purchased from Merck, Germany. The chitosan was used as a coating to the alginate microspheres and microcapsules ( only for 1C and 2C samples) being added at a concentration of 0.1% to the calcium chloride gelling solution.

The microspheres and microcapsules were obtained with an encapsulator Buchi B 395 PRO, using two specific types of duses, to obtain microcapsules and microspheres, respectively. The composition of microspheres and microcapsules was similar but the protocol of their preparation was different. Generally, a concentration of 2% Natrium alginate was introduced in the composition.

The microspheres were obtained using a nozzle of 450  $\mu$ m, at a pressure of 635 mPa. The mononuclear microcapsules were obtained using a double wall nozzle (200/400  $\mu$ m core/shell), at a pressure of 293 mPa, the pumping flow of core being 1.42 ml/min.

The microspheres were marked according to their composition in bioactive molecules, as follows: 1A-tomato juice with 5% pumpkin oil and 2 % Natrium alginate; 1B- seabuckthorn juice with 5% pumpkin oil and 2 % Natrium alginate; 1C- seabuckthorn juice mixed with tomato juice in ratio 1:1 (w/w) with addition of 5% pumpkin oil and 2 % Natrium alginate.

The mononuclear microcapsules were marked also according to their composition, as follows: 2A- Pumpkin oil in the core and seabuckthorn juice with 2 % Natrium alginate in the shell 2B

- Core pumpkin oil and tomato juice with 2 % Natrium alginate in the shell. 2C- Core pumpkin oil and seabuckthorn juice mixed with tomato juice in ratio 1:1 (w/w) and 2 % alginic acid sodium shell.

### **Morphology of microspheres and microcapsules**

The macroscopic morphology of microspheres and microcapsules was determined by measuring the mean external diameter, expressed in micrometers.

The microscopic structure microspheres and microcapsules was investigated by optical microscopy, using a Microscope Carl Zeiss Observer A1, with AxioVision image processing software.

## **RESULTS AND DISCUSSION**

### **UV-VIS fingerprinting of seabuckthorn and tomato juices with/without addition of pumpkin oil**

Fig. 1 represents the comparative UV-VIS fingerprints (180-600 nm) of different individual extracts of seabuckthorn juice, tomato broth and pumpkin oil, to mixtures of these ingredients (seabuckthorn or tomato broth mixed or not with 5% pumpkin oil). Such fingerprints are useful to evaluate the composition of extracts, namely to identify different categories of bioactive molecules, such as phenolic acid derivatives (280 nm), flavonoids (absorptions at 280 and 330-360 nm) and carotenoids (430-470 nm). The UV-VIS spectrometry analysis allowed the identification of three classes of compounds: phenolic acids, with absorptions in the region 220-280 nm, flavonoids with absorptions in the region 330-360 nm and carotenoids with absorptions in the region 430-500 nm. In the tomato juice were found three classes of compounds, namely phenolic acids, flavonoids and carotenoids, while in the buckthorn juice there were identified mainly two classes of compounds (phenolic acids and carotenoids) with minor representation of flavonoids, while in the case of pumpkin oil there were identified lipids (absorptions below 280 nm), phenolic acids and low concentrations of quinones (398-420nm).

### **Extraction efficiency**

The extraction efficiency (EE) values obtained for different extracts are presented in Fig.2.

The graphic representation shows that higher EE were obtained for lipids, phenolic acids and carotenoids. Pumpkin oil and sea buckthorn juice had a better extraction of lipids and phenolic acids, while from tomato juice the extraction of carotenoids was best. Flavonoids were best extracted from tomato juice, while from sea buckthorn juice and pumpkin oil, the extraction was poor. The addition of 5% pumpkin oil to tomato juice or sea buckthorn juice induced a significant increase of lipids and improved the emulsifying capacity.

### **Total carotenoids content**

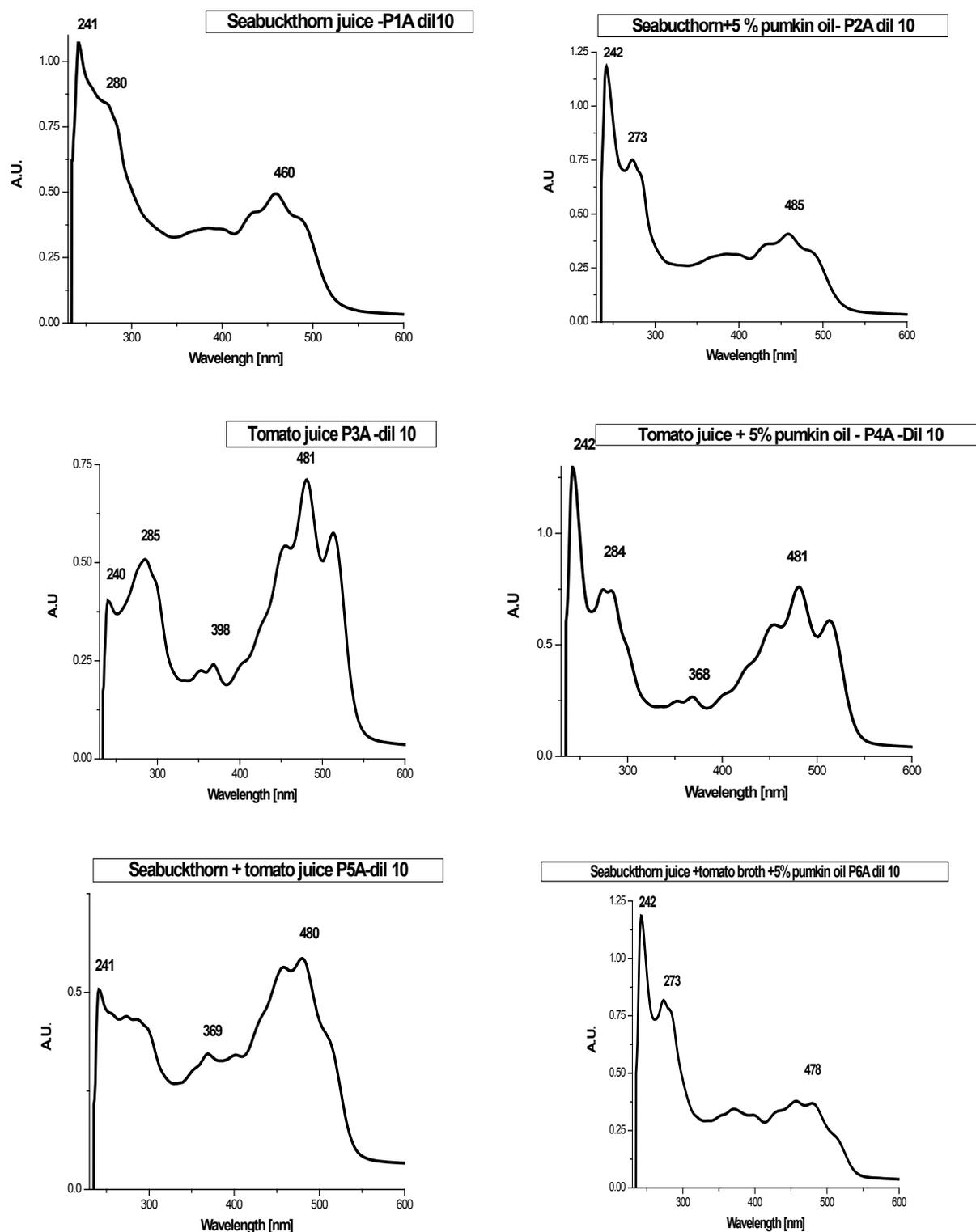
The total carotenoids content identified for each sample are shown in Tab. 1, being expressed in mg carotenoids per 100 g sample. The calculation of the concentration was made according to the standardized method (Britton et al., 1995). The results expressed are the average of two determinations.

The evaluation of total carotenoids content by UV-Vis spectrometry highlights that tomato juice and seabuckthorn juice are rich sources of carotenoids, and the addition of 5% pumpkin oil does not significantly influence the amount of total carotenoids but of other components such as unsaturated lipids. The highest amount of carotenoids are found in samples 3A and 4A, containing tomato juice. Also we observed that best carotenoid extraction was achieved from individual samples (tomato juice or sea buckthorn juice) and not from mixed samples (tomato juice + sea buckthorn juice in a ratio 1:1(w/w) or with pumpkin oils, suggesting that extraction efficiency decrease when mixtures were used for similar extractions.

### **Comparative viscosity of emulsions before encapsulation**

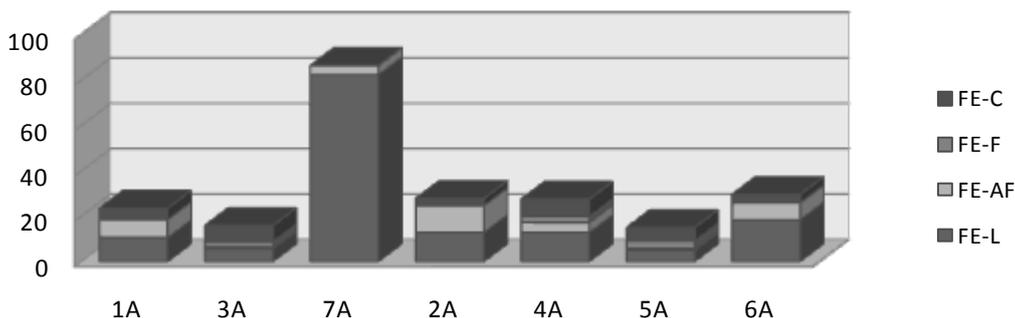
Fig. 3 represents the matrices viscosity used in the process of obtaining the microcapsules, depending on the speed of rotation.

One can observe that sample 1C- seabuckthorn juice mixed with tomato juice in ratio 1:1 (w/w) with addition of 5% pumpkin oil and 2% Natrium alginate showed the highest viscosity, while single juices without additions of pumpkin oil had lowest viscosity. Generally, the higher rotation speed was correlated with a lower viscosity of the emulsion.



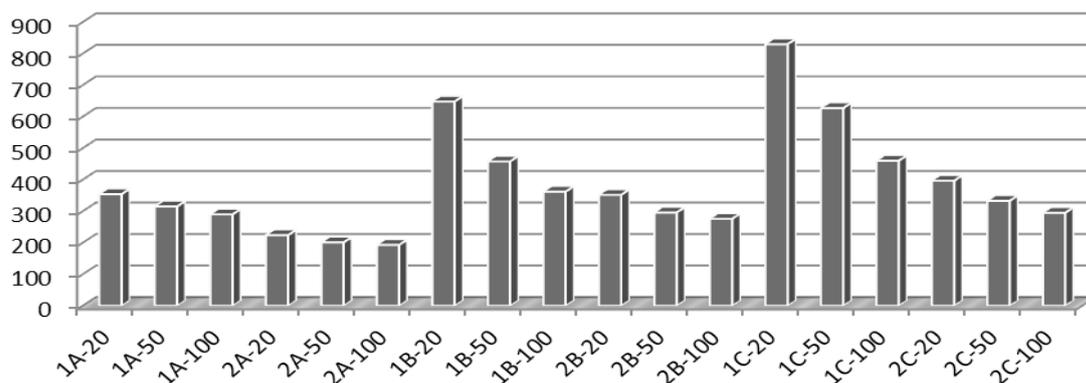
**Fig.1.** Comparative UV-VIS fingerprints of different samples: seabuckthorn juice with/without pumpkin oil (P1A and P2A), tomato juice with/without pumpkin oil (P3A and P4A), seabuckthorn juice mixed with tomato juice with/without pumpkin oil (P5A and P6A).

### Extraction efficiency



**Fig. 2.** Representation of Extraction Efficiency (EE) for carotenoids (FE-C), flavonoids (FE-F), phenolic acids (FE-AF), lipids (FE-L): 1A- Sea buckthorn juice, 3A- Tomato juice, 7A- Pumpkin oil, 2A- Seabuckthorn juice + 5% pumpkin oil, 4A- Tomato juice +5% pumpkin oil, 5A- Sea buckthorn juice + tomato juice, 6A- Seabuckthorn juice + tomato juice + 5% pumpkin oil.

### Viscosity cP



**Fig.3.** Viscosity values (expressed in cP) for the emulsions obtained from 1A-tomato juice with 5% pumpkin oil; 2A- seabuckthorn juice; 1B- seabuckthorn juice with 5% pumpkin oil; 2B-tomato juice; 1C- seabuckthorn juice mixed with tomato juice in ratio 1:1 (w/w) with addition of 5% pumpkin oil and 2C- seabuckthorn juice mixed with tomato juice in ratio 1:1 (w/w). All samples contained 2 % Natrium alginate. The index -20, -50 and -100 represents the speed used for homogenization, expressed in rotations/min.

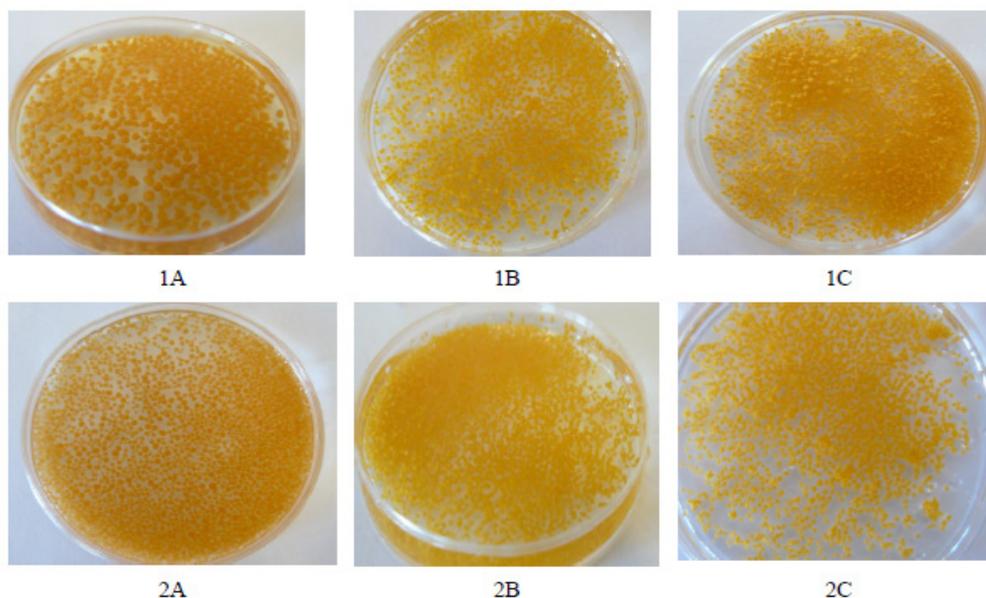
**Tab. 1.** The mean values of total carotenoids contents for each sample

Sample	Total carotenoids [mg/100g sample]
1A	16.39 ± 0.07
2A	17.31 ± 3.80
3A	19.71 ± 1.21
4A	18.76 ± 0.21
5A	14.44 ± 0.44
6A	9.16 ± 0.03

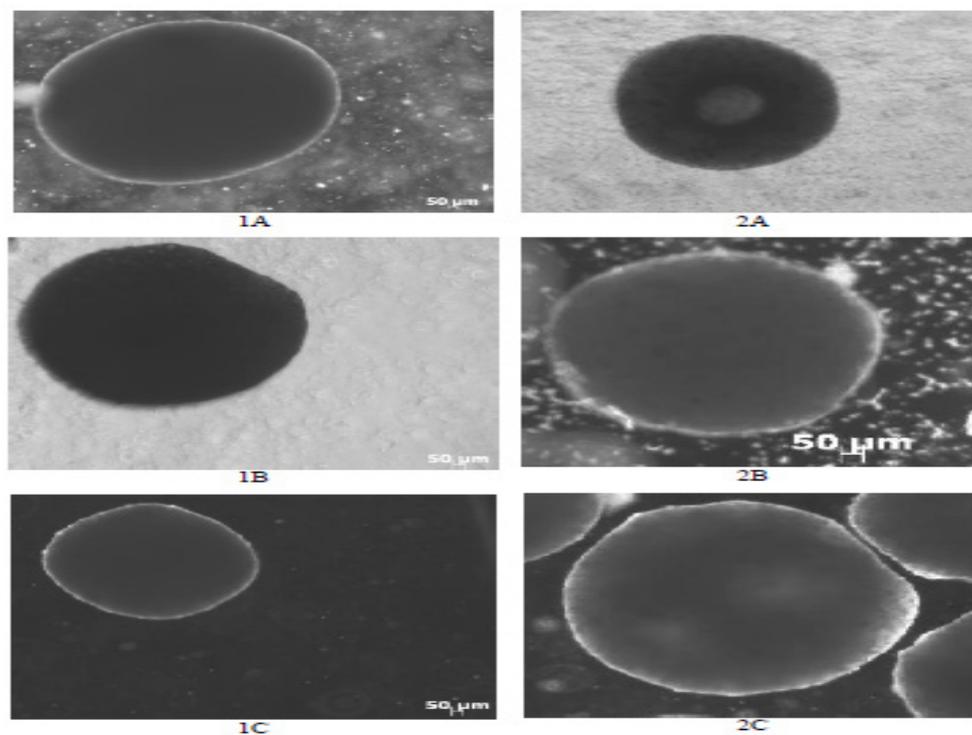
The emulsions obtained from tomatoes juice (1A, 2A) were less viscous, followed by sea buckthorn juice (1B, 2B) and the matrix formed by viscous tomatoes juice and sea bukthorn juice in ratio 1:1 (w/w) (1C, 2C). By the addition of 5% pumpkin oil, the viscosity increased significantly.

### Morphology of microcapsules and microspheres

Fig. 4 represents the macroscopic image of the six types of microspheres (1A-1C) and microcapsules (2A-2C) obtained from seabuckthorn and tomato juices with/without pumpkin oil and alginate as gelling agent.



**Fig. 4.** Macroscopic image of microcapsules and microspheres, freshly prepared. Images 1A-1C represents microspheres: 1A-tomato with 5% pumpkin oil and Natrium alginate 2%); 1B- seabuckthorn juice with 5% pumpkin oil and Natrium alginate 2%); 1C- seabuckthorn juice+ tomato juice (1:1) with 5% pumpkin oil and Natrium alginate 2%); Images 2A-2C represents microcapsules 2A- core of pumpkin oil and shell of seabuckthorn juice mixed with 2% Natrium alginate; 2B- Core pumpkin oil and shell of tomato juice mixed with 2% Natrium alginate; 2C- core of pumpkin oil and shell of seabuckthorn juice+ tomato juice (1:1 w/w) mixed with 2% Natrium alginate.



**Fig. 5.** Microscopic images of the different microspheres and microcapsules obtained according to the protocol presented in Materials and methods. For legend see Fig.4.

The microscopic structure (size and shape) of microcapsules comparing with microspheres was done by optical microscopy (magnification 5x) and shown in Fig.5.

All microspheres 1A, 1B, 1C were spheric and had sizes ranging between 850 and 900  $\mu\text{m}$  as external diameter, while microcapsules 2A, 2B, 2C had sizes ranging between 750 and 800  $\mu\text{m}$  as external diameter and spherical shape. In the microcapsules 2A one can see highlighted the core of pumpkin oil having 150-180  $\mu\text{m}$  diameter, while the shell dimension had between 750-800  $\mu\text{m}$ . In the microcapsules 2B, the core can not be observed due to the high pigment intensity of the capsule shell. In the case of 2C microcapsules, the shell consisted on a mixture of sea buckthorn and tomato juices 1:1 (w/w), the core being hardly observed, less than in sample 2A.

## CONCLUSION

The seabuckthorn and tomato juices with/without addition of pumpkin oil, rich in bioactive ingredients were chosen to be encapsulated in the perspective to obtain microencapsulated nutraceuticals with controlled release, for prostate disease prophylaxy. The bioactive molecules from the ingredients (juices and oil) were identified (lipids, to phenolic acid derivatives, flavonoids and carotenoid) and quantified by UV-Vis spectrometry, carotenoids being the major components, with concentrations from 9.16 up to 19.71 mg/100 g juice sample.

There were prepared and characterized comparatively microspheres and mononuclear microcapsules based on tomato and/or seabuckthorn juices, mixed or not with 5% pumpkin oil, to form emulsions including 2% Natrium alginate, then gelified in calcium choride with or without coating of chitosan.

The viscosity of emulsions including juices, oil and natrium alginate 2%, before encapsulation was determined and correlated with the morphology of microcapsules and microspheres. The viscosity showed differences between emulsions, dependent on the oil addition and speed of homogenization. The macroscopic and microscopic structure of microspheres and microcapsules were comparatively evaluated, both microspheres and microcapsules having external diameters ranging from 750 to 900  $\mu\text{m}$

and the microcapsules' oily mononucleated core of 150-180  $\mu\text{m}$ .

Considering the results obtained by viscosity mesurements, from the morphological data of microcapsules and microspheres, the procedure will be further optimized, to identify the optimal viscosity for stable microencapsulated structures, with good release in time, under physiologic conditions.

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