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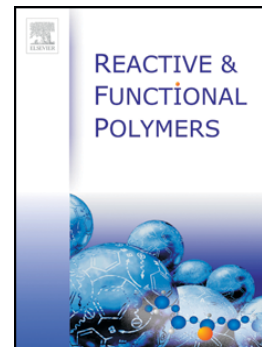
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A novel method for drop in drop edible oils encapsulation with chitosan using a coaxial technique.

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Presented work concerns a novel one step method for oil encapsulation. In this coaxial system the oil constitutes the core of the capsule, while the chitosan solution is the polymer shell surrounding the core to provide separation of the core from the external environment. The paper contains a diagram of the encapsulation equipment and explains the principle of its operation. The experimental results showed the impact of the media flow rate on the size, shape, mechanical properties and the amount of oil in the capsules. conditions of the core solution were determined to be: 2.00 g/h, shell solution: 4.45 g/h. Maximal oil content obtained under these condition was: 45.2 %. The diameters of the capsules collected gravitationally in the nitrogen stream were from 3.7 to 4.4 mm and from 2.8 to 4.3 mm, respectively. The force needed to obtain 50% compression strain of all tested samples was in the range from 0.11 to 0.19 N. In comparison to other encapsulation methods, presented technique is much simpler and can be performed under mild conditions. Moreover, the use of natural polymers as a coating matrix enables the use of the described technique in many branches of industry.

**Keywords:** Chitosan, Coaxial technique, Drop-in-drop capsules, Miroencapsulation, Oil

## 1. Introduction

The microcapsules are an important tool in the delivery of active ingredients whilst protecting unstable compounds. Their versatility offers the possibility of using them, inter alia, in the following industries: pharmaceutical, biomedical, food, plant protection, construction, and also in cosmetics [1-4]. The existing methods that are commonly used for this purpose, such as spray drying, extrusion and coacervation have many

disadvantages. These methods can significantly reduce the possibility of various compounds encapsulation, affect their properties and cause their partial degradation.

This is particularly important in the case of edible oils. A large part of them is a rich source of essential fatty acids (EFA). The EFA are unstable and can easily lose their functional nutritional properties, thus generating a lack of benefits for the body after their consumption. Edible oils, especially those rich in essential polyunsaturated fatty acids, play an important role in many of the mechanisms responsible for the proper functioning of the human body. They have been shown to prevent vascular blood clots, improve heart work, and are the precursors of tissue hormones multidirectional action [5]. Hence, the importance of maintaining the highest possible quality of oil during its processing and storage is essential. This applies not only to the EFA present in the diet but dietary supplements containing oil, the technology of their production and storage. The loss of their quality by oxidation can take place by the action of heat, ultraviolet radiation or the presence of oxygen from the air [6].

One of the approaches for prevention of the above-mentioned effects is to enclose the oils in the carriers using encapsulation process. Most of the encapsulation methods available in the literature are based only on enclosing the oil in the entire volume of carriers (so-called spheres or microspheres) [7-10]. These methods often require the emulsion formation and high temperature to evaporate the solvent, like in the case of spray-drying or extrusion. According to Saleeb and Arora work [11], carriers obtained by extrusion have lower porosity, which better protect oils by adverse factors. On the other hand, the cost of the extrusion compared with spray drying is twice as high. Due to the carriers' large porosity rapid oxidation of oils still remains unavoidable, especially in the layer of microsphere bordering with the external environment containing oxygen. This eliminates the above-mentioned method as a way for the oil supplement delivery.

In recent years, the new works related to the coaxial method were published [12-17]. Two of them included methods of hydrophobic compounds protection [12, 15]. However, in relation to our methods, they present some limitations.

Esquerdo and co-authors presented possibility of encapsulation unsaturated fatty acid in chitosan polymer by emulsification, which is totally different from coaxial method [18]. Unfortunately, it has been recently shown that lipid oxidation, during oils encapsulation and further storage, is attributed to oxygen availability which is introduced into the oil during emulsion formation, at even greater extent than exposure to high



temperature [19]. Compared with this, our method does not require the formation of emulsion, the drying and any additives as surfactants.

Emulsification may also provide a sample preparation step in other methods like electrospray technique [12] or dual coaxial method[20].

Moghaddam et al. actually provided a method of hydrophobic compounds encapsulation [15]. Unlike our work, their method concerns closing of phase change materials in alginate polymer for textile industry. Moreover, with this method authors can produce capsules with a few hundred-millimeter diameters by using electric field. Their method allows creating a Taylor cone and fragments to form a spray of highly charged droplets [21]. Unfortunately, in this case the choice of matrix for the encapsulation is determined by the electric conductivity of the material.

Chitosan is also an example of the polymer used in recent times for encapsulation techniques. However, it applies already well known methods such as coacervation [16]. Use of chitosan as a separate hydrogel coat for the first time was described in this work.

Others cited works that use the coaxial techniques relate to: extending (retinal pigment epithelial) cells viability [13], construction of biodegradable cell scaffolds [17] and encapsulation of other hydrophilic mixtures or suspensions [14]. There is no relation between these methods and our idea and presented results. In summary, the available scientific literature does not undermine the novelty aspect of our work.

Despite of the trend of seeking new products at the nanoscale and according to our experience in the food industry, the optimum diameter of the capsule intended orally administrated should be approx. 5 mm. Such dimension would facilitate swallowing them, particularly by children. Another advantage of our capsules is their hydrogel form ensuring softness with sufficient resistance to mechanical damage. Thus, this is the answer for harder, larger capsules available in the markets.

In this paper the impact of the media flow rate on the capsules morphology and the amount of oil contained in them were also presented. Obtained results show the great potential of this new method, which can be an alternative technique for encapsulation of active substances.

## **2. Experimental**

### **2.1 Materials**

Medium molecular weight chitosan polymer (MMW) with 75-85% deacetylation degree was obtained from Sigma Aldrich (Iceland). Aqueous lactate acid (CHEMPUR, Poland) solution was used as a solvent for the chitosan polymer. The capsule core was cold

pressed canola oil obtained from Z.T.Kruszwica (Poland). For hardening solution preparation, ethanol (96% v/v, Avantor Performance Materials Poland S.A., Poland) and sodium hydroxide (P.P.H. Stanlab, Poland) were used. Hexane for oil extraction was purchased from Merck KGaA(Darmstadt, Germany). All chemicals were of analytical grade and no further purification was required.

## 2.2 Methods of solutions preparation

As a coat solution, the 2.0% chitosan in lactate acid solution was used. Chitosan with 75-85% deacetylation degree added to 0.1M lactic acid solution was being stirred continuously until a final concentration of 2 g/100 cm<sup>3</sup> solution was obtained. Samples were divided into two parts. One part was not modified. The second part was subjected to microwave irradiation (LG WAVEDOM MB 4337 ARS, 850 W) according to Fuentes et al. method with slight modification [22]. A sequence of 20s MW pulses (540 Watt) was applied to the chitosan suspension until its complete dissolution (300s). To avoid excess heating, temperature of the sample was controlled (<40°C) [22]. Chitosan solution was ready to use after 2 hours of incubation in room temperature (the time required for degassing). Microcapsules were collected using 50% (v/v) ethanol solution containing 5% of sodium hydroxide, having regard to the phenomenon of volume contraction [23]. The oil has not been modified before the beginning of the encapsulation step.

## 2.3 Rheological measurement

The prepared chitosan solutions were introduced into the measuring cell (Brookfield Digital Model DVIII Ultra viscometer (Middleboro, MA, USA)) equipped with temperature-controlled bath and their rheological properties were tested. To determine the rheological properties under constant shear rate conditions, the solution was thermostated in 25°C in the measuring system, kept at this temperature for 30 min, and then shear stress produced by shearing at rates ranging from 0.22 to 44.22 s<sup>-1</sup>, resulting from 1 to 200 rpm of the spindle SC4-25 was recorded. All rheological measurements were performed in triplicates.

## 2.4 Preparation of oil-chitosan microcapsules

For the production of capsules, a modified coaxial technique was used, preventing the mixing of the transport solutions. [14]. The experimental setup of drop in the drop coaxial system is shown in Figure 1. (place of Figure 1) The most important element of the equipment is the nozzle (1), made of steel elements free of fat oxidation catalysts. The coaxial nozzle consisted of two stainless steel needles placed one inside the other. The inner (i.d.) and outer diameters(o.d.) of the needles were 0.70 mm (i.d.)/1.25 mm (o.d.) for the inner needle, 2.20 mm (i.d.)/3.00 mm (o.d.) for the outer needle. The nozzle was fitted



with 3 spigots for feeding the respective media. First from the top (1.a) was coupled with the inner needle, used for oil transport. The second spigot(1.b) combined with the outer needle allows introducing the chitosan solution. The last spigot (1.c) was designed to introducing gas blowing drops formed at the end of the needles. To produce the drop in drop microcapsules, oil and chitosan solution in conical flasks were transferred. High pressure nitrogen was used to drive the core and shell solution into and through the nozzle. Flow rate was controlled by two precision rotameters with valves (BROOKS INSTRUMENTS SHO-RATE™250AD6021NMANB). Launch of the equipment consisted of unscrewing the valves of compressed nitrogen bottles and stabilization of the solutions flow-out, so that double layer structure drops were obtained. A glass plate on magnetic stirrer (15 cm diameter) filled with about 250 cm<sup>3</sup> of sodium hydroxide/ethanol hardening solution was kept just below the tip of the compound nozzle. The distance from the tip to the collected plate was fixed at 5 cm during the experiment. Fall of the drop to the collection plate was caused gravitationally or assisted by nitrogen stream regulated by wide range flow rotameter (VEB MLW PRÜFGERÄTE-WERK MEDINGEN Typ TG 300). Distribution of gas holes (8.d) dropping the capsules and needles (8.e, 8.f) in the nozzle was shown in Figure 1. The presence of ethanol [23] and very low solubility of chitosan in an alkaline environment [24] were causing the immediate fall out of solution as a solid and formed spheres with entrapped oil. The formed capsules were collected in the same solution and stirred for 2 hours for full cure. Then microcapsules were thoroughly rinsed with distilled water to its 7.0 pH value. Until further measurement capsules were stored in distilled water at 4°C.

### **2.5 Diameter determination**

The morphology of moist oil-chitosan capsules was observed by optical microscope (DELTA optical EVOLUTION 100) with HDCE-50B Digital Camera. Before the measurements, each capsule was filtered from the excess water on the filter paper and then placed on microscope glass slide. For monitoring of the diameter of the capsules, ScopeImage Plus software with the above mentioned equipment was used. The final diameter is the average value of 20 readings performed on the same sample.

### **2.6 Oil content determination**

Oil content in the samples was determined by using extraction with hexane. Firstly, small amount of hydrogels capsules were weighed and freeze-dried (CHRIST Alpha 2-4 LSC, Osterode am Harz, Germany). Dry capsules were then destroyed quantitatively and transferred to cellulose thimbles. Extraction was carried out in a glass set consisted of a 250



cm<sup>3</sup> round bottom flask and a reflux condenser. The round bottom flask was weighed and in the next step filled with 50 cm<sup>3</sup> of hexane. The cellulose thimble was placed in the same flask above the liquid surface. After reaching the boiling temperature of hexane, extraction was conducted for 90 minutes. After cooling the glass set, extraction solvent was evaporated from the round flask by using a rotary vacuum evaporator (BÜCHI Rotavapor R-134 with BÜCHI Waterbath B-480, Switzerland). In order to remove residual moisture, the inside of the flask was dried further with the nitrogen stream. The flask with oil was then weighed.

### 2.6.1 Applied equations

The oil content calculated in respect of hydrogel capsules weight was determined by the following formula:

$$OC = [(O_2 - O_1)] / C \cdot 100 \text{ (Eq. 1),}$$

Where:

- O<sub>2</sub> is the mass of oil with round-bottomed flask after its extraction and solvent evaporation in grams,
- O<sub>1</sub> is the mass of empty round-bottomed flask in grams
- C is the mass of the capsules before drying used in the dry weight determination in grams.

In order to compare the oil content in all obtained capsules, the flow rate of the gas detaching the capsules also had to be taken into account. Therefore, the results are presented as the ratio of the oil mass to the dry weight of the polymer in the capsule (Table 2). The mass of oil (O<sub>m</sub>) in the capsule and dry polymer mass (P<sub>m</sub>) in shell of the capsule was determined by the equations

$$O_w = (4/3 \cdot (CD/2)^3 \cdot \Pi \cdot \rho_o \cdot OC / 1000), \text{ (Eq. 2)}$$

$$P_w = (4/3 \cdot (CD/2)^3 \cdot \Pi / 1000 - 4/3 \cdot (CD/2)^3 \cdot \Pi \cdot \rho_o \cdot OC / 1000) \cdot 0.02 \text{ (Eq. 3),}$$

respectively.

In the above equations the formula for the volume of a sphere calculation was used, where radius symbol had been replaced by CD/2 term. CD is the capsule diameter in mm, determined according to point 2.5 point.  $\rho_o$  is an oil density (0.915) in g·cm<sup>-3</sup>, OC – oil content in % determined according to Eq. 1. The 0.02 value is a concentration of chitosan in shell solution at a density 1 g·cm<sup>-3</sup>.

### 2.7 Mechanical determination

The mechanical properties of the obtained capsules were characterized using a universal testing machine (Instron model 5543, USA controlled using the "Merlin" software V 4.42). Each capsule before measurement was filtered from the excess water on the filter paper. Then it was placed in a chamber with 50% humidity at 25°C for 24 hours (Memmert HCP 108,

Germany). Conditioned capsules were compressed until the rupture of the capsule shell at a test speed of 1.0mm/s was reached. Based on the obtained results, the relationship between compression stress and compression strain was determined.

### **2.8 Effect of solution flow rate on diameter and oil content**

The influence of the solution flow rate on the diameter of the capsules and the oil content in the capsules was performed as follows. First the flow rate of the shell and the core solutions allowing formation of the drop in drop capsules was established. Under the microencapsulation head, the beaker with a hardening solution was set. Mass of created capsules was monitored by weight methods [25]. Microcapsules were collected with the following gas flow rates forcing the chitosan solution: 0.6l/h, 1.2 l/h, 1.8 l/h, 2.4 l/h, 3.0 l/h, 3.6 l/h. The value of the oil (core) flow rate was constant: 2.00 g/h in all tests. The capsules diameter, the oil content in the capsules and their susceptibility to mechanical damage were then determined according to point 2.5, 2.6 and 2.7, respectively.

### **2.9 Effect of inert gas flow rate on diameter and oil content**

The influence of the solutions flow rate on the diameter of the capsules and the oil content in capsules was performed as follows. First the flow rate of the shell and the core solutions allowing formation of the drop in drop capsules was established. Under the microencapsulation head, the beaker with a hardening solution was set. Mass of created capsules was monitored by weight methods [25]. As the constant values while creating the capsules were established: the flow rate of the core and shell solutions: 2.00 g/h and 4.45 g/h, respectively. To control the capsules diameter coaxial nitrogen stream was used: 0 l/h, 200 l/h, 400 l/h, 600 l/h and 800 l/h. The capsules diameter, the oil content in the capsules and their susceptibility to mechanical damage were then determined according to point 2.5, 2.6 and 2.7, respectively.

### **3. List of Symbols**

$C(g)$ - mass of capsules before drying used in dry weight determination,

$CD$  (mm) - mean capsules diameter

$F_{max}(N)$  - maximum force under which the external appearance of the sphere or the capsule is destroyed

$F_{50}(N)$  - force needed to 50% compression of the initial diameter value of sample

$K_c^2(cP)$  – Casson plastic viscosity

$P_M(g)$ - dry polymer mass calculated based on Eq. 3

$V_C(g/h)$  – core flow rate

$V_N(l/h)$  – nitrogen flow rate





$V_s(\text{g/h})$  – shell flow rate

$\Pi (-)$  - 3.14159...

$\rho_o(\text{g}\cdot\text{cm}^{-3})$  – oil density at 25°C temperature

$\dot{\gamma}(\text{s}^{-1})$  - shear rate

$\sigma_{oc}(-)$  - yield stress

#### 4. Results and discussion

It should be taken into account that formation of capsules may be affected by many variables in the equipment construction: the inner diameter of the external needle, the inner and the outer diameters of the internal needle, their mutual concentric alignment of the vertical, the distance between the needles and the surface of the hardening solution. Significant are also the physicochemical properties of encapsulated substances and media for their immobilization, like: viscosity, surface tension of the core and shell solution, and interactions between them. Despite of many variables, the tests carried out in order to assess the usefulness of the drop in drop technique confirmed, that with the respective flow rates of the core and the shell solutions, it is possible to obtain capsules in a stable manner as shown in Figure 2. (place of Figure 2)

##### 4.1 Effect of solution viscosity and flow rate on diameter and oil content

Canola oil was used as the model compound in the assessment of the possibility of forming capsules. That choice was dictated by its hydrophobic properties. The core, in combination with a hydrophobic core, hydrophilic shell and a hydrophilic hardening solution allowed to create a form similar to the micelle, further preventing leakage of oil out of the capsule before it was cured in solution. The most important restriction of the core in terms of its properties has proven to be viscosity. The low viscosity of the oil (despite its delivery by the internal needle with only 0.7 mm diameter), resulted in a low content of the core in capsule (below 5% for a flow rate of less than 1 g/min) or piercing of the capsule shell layer during its formation (for share rate of greater than 3 g/min). Therefore, in the presented research for all tests, the constant value of core flow rate, 2 g/min, has been applied. This is also the optimum value determined empirically. To create the shell of the capsules, chitosan polymer was used. The choice of this biomaterial was caused by a number of its advantageous properties such as: biocompatibility, biodegradability, non-toxicity and its lethal or static activity against selected bacteria and fungi strains [24]. This allows for its wide use e.g. in pharmaceutical industry, water and waste treatment, cosmetics or agriculture. More importantly, chitosan can also be used in food [26], particularly for protection of edible oils due to its inert



nature[27, 28]. Creation of a coat insulating oil from the external environment may suggest the use of a polymer solution in the highest possible concentration. Unfortunately, the small diameter of the outer needle transporting the coat (2.20 mm) and the high viscosity of chitosan solution, already at several percent concentration, limited concentration of the polymer in the solution, and thereby in the coat of prepared capsules. Therefore, based on Fuentes et al. studies [22], to dissolve the polymer in an acid solution, the microwave radiation was used. This method allows reducing the viscosity of the chitosan solution with respect to the mechanical dissolution, which was also confirmed by our research team. The rheological properties of 2% solutions of chitosan were analyzed in the Casson model and are defined by the relations:

$$\sqrt{\sigma} = K_{oc} + K_c \sqrt{\dot{\gamma}} \quad (\text{Eq. 4}),$$

in which  $(K_c)^2$  is the Casson plastic viscosity ( $\eta_c$ ),  $\dot{\gamma}$  is the shear rate ( $s^{-1}$ ). The Casson model provides determination of the yield stress ( $\sigma_{oc}$ ). The latter was determined as the square of the intercept ( $K_{oc}$ ) from linear regression of square roots of shear rate - shear stress data (Table 1). The application of microwaves for the procedure of preparing chitosan solutions reduced the viscosity of the pressed coat solution. This allowed gaining the lower pressure in the encapsulation set without the need to reduce the polymer concentration. Probably the use of microwaves resulted in the degradation of chitosan as was suggested by Fuentes et al.[22]. By using microwaves it is possible to adjust optimal viscosity of a solution containing the appropriate concentration of chitosan. Using the same preparation of the chitosan it is possible to modify the viscosity of the solution with precisely defined concentration.

As shown by the results, the shape, diameter and the oil content of the capsules are strongly dependent on the flow rate of the media used in the method. According to Table 2, each gas flow rate forcing the shell solution corresponds to substantially less real flow rate of the solution. Although a higher gas velocity also increases the actual flow rate of the shell solution, this relationship is not linear. The reason for this can be the pressure changes in the system associated with the compression of the gas forcing. Figure 3 showed the mean value of the capsule diameter obtaining with a different shell solution flow rate, described in point 2.8. (place of Figure 3) It was shown that the increase of the chitosan flow rate resulted in a decrease of the capsules diameter, which were in the range from 3.7 to 4.4 mm. The differences are the result of the lack of full sphericity of obtained capsules. Deviations from the model spherical shape are dictated by factors such as the high viscosity of the solution - responsible for the elongation of the sphere before

obtaining the mass necessary for its detachment from the needle, and flattening the capsule during drop into the hardening solution. Moreover, the excess gas velocity forcing the solution jacket equal to 3 l/h resulted in the formation of so-called multi-core structures, which were located in the tail of the capsule (Figure 3. E, F).

The largest amount of the oil was reached for the capsule formed at the shell solution flow rate equal to 4.45 g/h (Table 2) Further coat flow rate increase caused a decrease the oil content in the capsules to values below 10%. Formed drops fell into the hardening solution after reaching a suitable own weight, independently from the oil-polymer ratio (Table 2) Therefore, increase of the chitosan solution flow rate causes increase of the ratio of shell in the capsule (relative to the core) detached from the needles. According to Table 2, encapsulation of about 35 g requires 1 g of polymer under optimum conditions.

#### **4.2 Effect of gas flow rate on diameter and oil content**

In contrast to the above results, the capsules described in this section were collected not only by a gravity fall to a hardening solution but also thanks to nitrogen cutting force. In order to investigate this association as constant parameters, optimal oil and chitosan solution flow rates were used, for which the highest oil content, and the most spherical shape were achieved (Table 2, Figure 3). It was found that the mean diameter of the microcapsules decreased with increasing gas flow rate (Table 3).

On the other hand, this dependence is not significant for the nitrogen flow rate lower than 200 l/h, because gas cannot provide sufficient driving force to detach the drop in drop structure to hardening solution. Gas velocities in excess of 600 l/h resulted in significantly lower encapsulation efficiency, which in Table 3 was shown only as a qualitative result. As shown in the Figure 4, obtained capsules were characterized by a more spherical shape compared to the gravitationally collected capsules (Figure 3). (place of Figure 4)

The oil content in the capsules collected with a stream of nitrogen in the range from 0 to 400 l/h varied slightly and represented the highest oil content possible to entrap in the described method. The increase of nitrogen flow rate to 600 l/h caused approximately 10% decrease of oil content in the capsules, relative to the values obtained in the range from 0 to 400 l/h. The yield of prepared capsules in the nitrogen flow rate of 800 l/h was so low that the result was considered to be the limit value for which formation of drop in drop structures was impossible.

According to the oil - dry polymer weight calculations (Table 2, Table 3), based on the equations 2 and 3, optimal conditions turned out to be for the capsules created without the



presence of the gas detaching drops to hardening solution and for  $V_s$  and  $V_c$  parameters equal 4.45 g/min and 2.00 g/min, respectively.

### 4.3 Mechanical properties

The evaluation of the capsules mechanical properties was based on their compression susceptibility. It consisted of the measurements of the maximum force under which the external appearance of the sphere or capsule was destroyed. Single microcapsules were compressed to large deformations or ruptured and the force being imposed on them was measured simultaneously. However, the hydrogel capsules bursting under compression could not be clear. Therefore, their mechanical strength was assessed by measuring the force required to cause their deformation to 50%. The studies did not include the relaxation test. As presented by Bartkowiak and Brylak [29] even a few hundred cycles of capsules deformation caused small changes in their susceptibility to compression. This could be connected to a high flexibility of hydrogel capsule wall in which water acts as a plasticizer. In these measurements, it was also determined that for the strain rate conditions higher than 0.6 mm/s, the results of the mechanical tests of hydrogel capsules were independent of their diameter [29].

Conditioning of the capsules in a humidity chamber before the measurements were performed to assure uniform water content of each sample. In the next step 10 capsules from each batch were randomly selected and compressed until breaking down. In this paper, mechanical strengths of capsules obtained according to point 2.8 were compared (Figure 5.A, B, C, D, E,F). The effect of gas detaching the capsules to hardening solution on their mechanical properties was also determined (Figure 5.G). (place of Figure 5)

In the case of capsules, collected by gravity dropping, the increase of shell solution flow rate resulted in the increase of the force ( $F_{max}$ ) needed to destroy the finished capsule in the range of from 0.48 N to 1.51 N (Figure 5.A, B, C, D, E,F). The reason was to strengthen the capsule shell with respect to its core, which was also confirmed by the data in the last column in Table 1. The highest value of the force inducing the shell rupture was obtained for capsules collected to hardening solution with a stream of nitrogen using the following parameters:  $V_s = 4.45$  g/h,  $V_c = 2.00$  g/h  $V_N = 600$  l/h. Taking into consideration that the weight ratio of oil to polymer in this case is 18.56,  $F_{max}$  force gravity collected capsules would be in the range from 0.49 to 0.52 N. Four times higher value of this force obtained for capsules detaching in the above-mentioned conditions was the result of a smaller diameter of the capsule and thereby a smaller core radius, which practically did not put up any resistance during sample compression (Figure 5.G).



As shown by Bartkowiak and Brylak [29]  $F_{\max}$  value required to destroy the hydrogel alginate capsule in the form of spheres with a concentration range from 0.5 to 1.5%, were in the range from 3.2 to 14.2 N. Lower values obtained during our studies seem to be correct, because in case of the capsules with a separate core for the main resistance to compression, the shell of the capsule was responsible. Despite that, the achieved values were sufficient to maintain the formed structure (Figure 5).

Obtaining divergent results of  $F_{\max}$  value even for capsules from the same batch is possible. The deviations from the spherical shape of the capsule or/and asymmetrically positioned core are the reason. This means that the moment of rupture of the capsule is depend on the arrangement of the capsule during its compression. Taking into account that the characteristics shown in Figure 5 have identical course, minimize this error by comparing the forces required for 50% deformation of the samples is possible [29, 30]. The force for the above-mentioned compression strain and for all tested samples was in the range of 0.11-0.19 N (Table 4). Based on the results in Table 4, it can be deduced no association between the mechanical stability of the hydrogel capsules and the flow rate of the shell solution during their formation. On the other hand, for sample C a greater value  $F_{50}$ , compared to the two previous A and B was obtained. Based on values of standard deviations, the increase in strength fits within the margin of error.

## 5. Conclusion

The encapsulation method presented in this paper confirms the possibility of forming capsules with a separate core and coat. It could be an alternative for the spheres production in most of the currently used techniques. Preliminary tests indicate the possibility of closing the majority of liquid compounds regardless of their physicochemical properties, in particular hydrophobic compounds as edible oils. The influential factors such as the varying liquid and gas flow rates, upon the capsules diameter, oil content and the mechanical strength were investigated. The great advantage of the technique is the lack of necessity to transform the oil in the emulsion, greatly reducing the exposure of oil to oxygen. This technique allows the coaxial capsules to be formed in a single step at room temperature, which is particularly important in the case of compounds susceptible to thermal degradation. Moreover, the use of natural polymer like chitosan as the coat of the capsule causes that the described method can be successfully applied in the food and other industries and can be easily scaled to the needs of industrial performance by creating a battery consisting of a plurality of this type encapsulation heads, where the transport media is forced pneumatically. Microencapsulation is the term



used for spherical particles with a size range from a few to even few thousand micrometers. Most reports of scientific literature relate to a maximum few hundred micrometer diameters of the capsules. Therefore, the comparison of our results with the available literature is difficult, but also shows their novelty aspect.

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## 7. References:

- [1] N. Azizia, Y. Chevalier, M. Majdoub, Isosorbide-based microcapsules for cosmeo-textiles, *Ind. Crop. Prod.* 52 (2014) 150–157.
- [2] M. Masuda, Microencapsulation of Pesticides for Controlling Release from Coatings, PhD Thesis, Department of Chemical and Biological Engineering, Chalmers University of Technology, Göteborg, Sweden, 2011.
- [3] F. Nazzaro, P. Orlando, F. Fratianni, R. Coppola, Microencapsulation in food science and biotechnology, *Curr. Opin. Chem. Biol.* 23 (2012) 182–186.
- [4] D. Yang, S. Guo, J. Qiao, J. Nie, Investigation on the preparation and application of chitosan/alginate microcapsules, *J. Controlled Release.* 152 (2011) 71-72.
- [5] Z.E. Sikorski, *Chemia żywności, sacharydy, lipidy i białka*, fifth ed., Warsaw, Poland, 2007.
- [6] E. Choe, D.B. Min, Mechanisms and Factors for Edible Oil Oxidation, *Comp. Rev. Food. Sci. F.* 5 (2006) 169-186.
- [7] E. Domian, I. Wasak, Microencapsulation of rapeseed oil based on spray drying method, *Pol. J. Food Nutr. Sci.* 58 (2008) 477-483.
- [8] G. Gallardo, L. Guida, V. Martinez, M.C. López, D. Bernhardt, R. Blasco, R. Pedroza-Islals, L.G. Hermida, Microencapsulation of linseed oil by spray drying for functional food application, *Food Res. Int.* 52 (2013) 473-482.
- [9] M. A. Przybysz, A. Szterk, M. Zawiślak, E. Dłużewska, Wpływ procesu mikrokapsułkowania i dodatku przeciwutleniaczy na stabilność oleju rybnego, *Żywność: nauka-technologia-jakość.* 2 (2014) 123-138.
- [10] S. Quispe-Condori, M.D.A. Saldaña, F. Temelli, Microencapsulation of flax oil with zein using spray and freeze drying, *LWT-Food Sci. Technol.* 44 (2011) 1880-1887.
- [11] F.Z. Saleeb, V.K. Arora, US5972395, 26.10.1999.
- [12] S. Ghayempour, S.M. Mortazavi, Fabrication of micro-nanocapsules by a new electrospraying method using coaxial jets and examination of effective parameters on their production. *J. Electrostat.* 71 (2013) 717-727.



- [13] L.S. Kontturi, M. Yliperttula, P. Toivanen, A. Määttä, A.M. Määttä, A. Urtili, A laboratory-scale device for the straightforward production of uniform, small sized cell microcapsules with long-term cell viability, *J. Controlled Release*. 152 (2011) 376-381.
- [14] X.M. Liu, J. He, S.Y. Liu, J.F. Chen, Y. Le, A novel method for the preparation of electrophoretic display microcapsules, *Mater. Sci. Eng. B*. 185 (2014) 94-98.
- [15] M.K. Moghaddam, S.M. Mortazavi, T. Khayamian, Preparation of calcium alginate microcapsules containing n-nonadecane by a melt coaxial electrospray method, *J. Electrostat.* 73 (2015) 56-64.
- [16] J.H. Park, H.E. Jin, D.D. Kim, S.J. Chung, W.S. Shim, C.K. Shim, Chitosan microspheres as an alveolar macrophage delivery system of ofloxacin via pulmonary inhalation. *Int. J. Pharm.* 441 (2013) 562-569.
- [17] Y. Wen, M.R. Gallego, L.F. Nielsen, L. Jorgensen, E.H. Møller, H.M. Nielsen, Design and characterization of core-shell mPEG-PLGA composite microparticles for development of cell-scaffold constructs, *Eur. J. Pharm. Biopharm.* 85 (2013) 87-98.
- [18] V.M. Esquerdo, G.L. Dotto, L.A.A Pinto, Preparation of nanoemulsions containing unsaturated fatty acid concentrate-chitosan capsules, *J. Colloid. Interface. Sci.* 445 (2015) 137- 142.
- [19] Y. Serfert, S. Drusch, K. Schwarz, Chemical stabilisation of microencapsulated oils rich in long chain polyunsaturated fatty acids, *Food Chem.* 113 (2009) 1106 - 1112.
- [20] T. Shao, X. Feng, Y. Jin, Y. Cheng, Controlled production of double emulsions in dual-coaxial capillaries device for millimeter-scale hollow polymer spheres, *Chem. Eng. Sci.* 104 (2013) 55-63.
- [21] I.G. Loscertales, A. Barrero, I. Guerrero, R. Cortijo, M. Marquez, A.M. Gañán-Calvo, Micro/Nano Encapsulation via Electric Field Coaxial Liquid Jets, *Science*. 295 (2002) 1695-1698.
- [22] S. Fuentes, J. Retuert, G. González, Preparation and properties of chitosan hybrid film from microwave irradiated solutions, *J. Chil. Chem. Soc.* 53 (2008) 1511-1514.
- [23] M. Sano, O. Hosoya, S. Taoka, T. Seki, T. Kawaguchi, K. Sugabayashi, K. Juni, Y. Morimoto, Relationship between solubility of chitosan in alcoholic solution and its gelation, *Chem. Pharm. Bull.* 47 (1999) 1044—1046.
- [24] M. Rinaudo, Chitin and chitosan: Properties and applications, *Prog. Polym. Sci.* 31 (2006) 603-632.
- [25] AOAC, 1990 Official method of analysis (15th ed.) Association of Official Analytical Chemists, Virginia (1990).



- [26] M.N.V. Ravi Kumar, A review of chitin and chitosan applications, *React. Funct. Polym.* 46 (200) 1-27.
- [27] M.D. Gades, J.S. Stern, Chitosan Supplementation and Fat Absorption In Men and Woman, *J. Am. Diet. Assoc.* 105 (2004) 72-77.
- [28] M.D. Gades, J.S. Stern, Chitosan supplementation does not affect fat absorption In healthy males fed a high-fat diet, a pilot study, *Int. J. Obes. Metab. Disord.* 26(2002) 119-122.
- [29] A. Bartkowiak, W. Brylak, Hydrogel microcapsules containing natural or chemically modified oligochitosan - mechanical properties and porosity, *Polimery.* 51 (2006) 547-554.
- [30] M. Liu, Understanding the Mechanical Strenght of Microcapsules and Their Adhesion on Fabric Surfaces, PhD Thesis, University of Birmingham, UK, 2010.

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## 8. Figure captions

Figure 1. Scheme of microencapsulation set by coaxial method (1-microencapsulation head; 1a-core solution inlet; 1b-shell solution inlet; 1c-inert gas detaching the drops inlet; 2-nitrogen bottle; 3-precision rotameters for adjusting the solutions flow rates; 4-wide measurement range rotameter to control the flow rate of inert gas; 5-core solution vessel; 6-shell solution vessel; 7-beaker with a hardening solution placed on a magnetic stirrer; 8-head view from the bottom; 8d-outlet of inert gas detaching the drops; 8e-outlet of shell solution; 8f-outlet of core solution.

Figure 2. View of capsules obtained directly after falling to a hardening solution (Conditions: Shell solution flow rate  $V_S = 4.45$  g/h, Core (oil) flow rate  $V_C = 2.00$  g/h, Inert gas detaching the drops flow rate  $V_N = 0$  l/h)

Figure 3. Effect of shell flow rate on the capsules diameter and morphology (Gas flow rate forcing shell solution: A-0.6 l/h, B-1.2 l/h, C-1.8 l/h, D-2.4 l/h, E-3.0 l/h, F-3.6 l/h; Measurements made at a constant core flow rate  $V_C = 2.00$  g/h, Inert gas detaching the drops flow rate  $V_N = 0$  l/h)

Figure 4. Effect of inert gas flow rate on the capsules diameter and morphology (Inert gas flow rate detaching the capsules: A-0 l/h, B-200 l/h, C-400 l/h, D-600 l/h. Measurements made at a constant core and shell flow rate:  $V_C = 2.00$  g/h and  $V_S = 4.45$  g/h, respectively. Inert gas detaching the drops flow rate  $V_N = 0$  l/h)

Figure 5. Mechanical properties of obtained capsules based on the force needed to destroy drop in drop structure (Conditions of preparation: A:  $V_N = 0$  l/h,  $V_S = 2.64$  g/h; B:  $V_N = 0$  l/h,  $V_S = 4.45$  g/h; C:  $V_N = 0$  l/h,  $V_S = 13.52$  g/h; D:  $V_N = 0$  l/h,  $V_S = 14.48$  g/h; E:  $V_N = 0$  l/h,  $V_S = 23.21$  g/h; F:  $V_N = 0$  l/h,  $V_S = 27.24$  g/h; G:  $V_N = 600$  l/h,  $V_S = 4.45$  g/h. Measurements made at a constant core flow rate  $V_C = 2.00$  g/h.

## 9. Figures

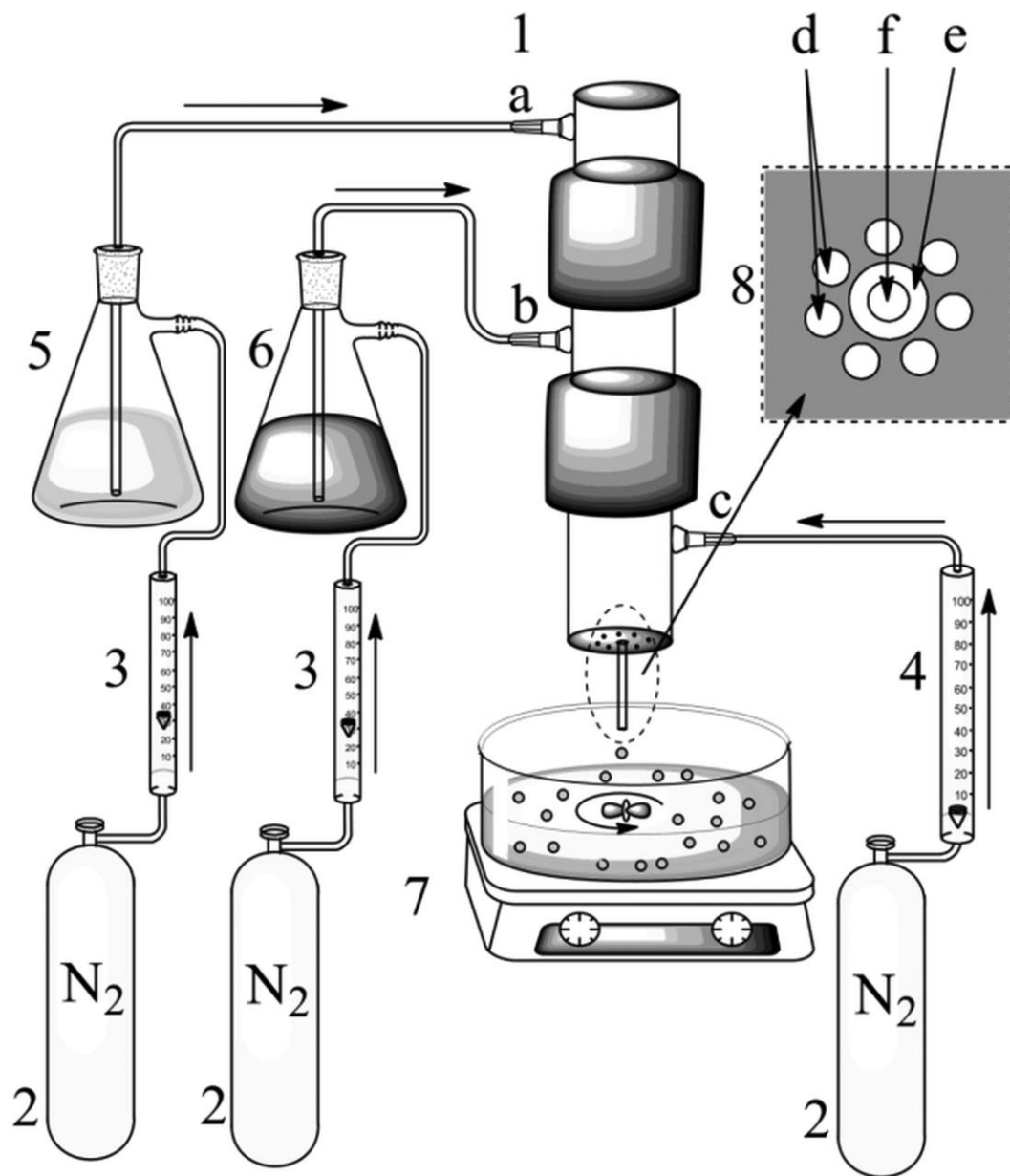


Figure1

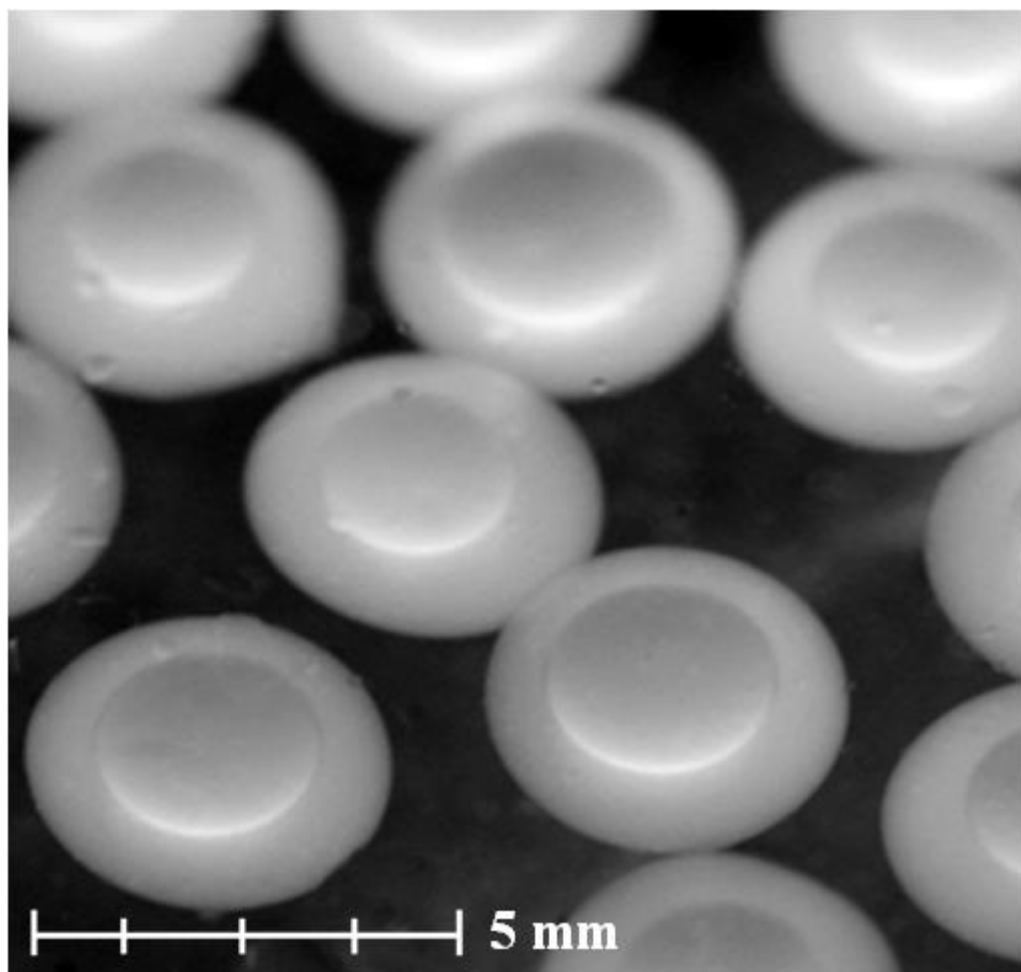


Figure2

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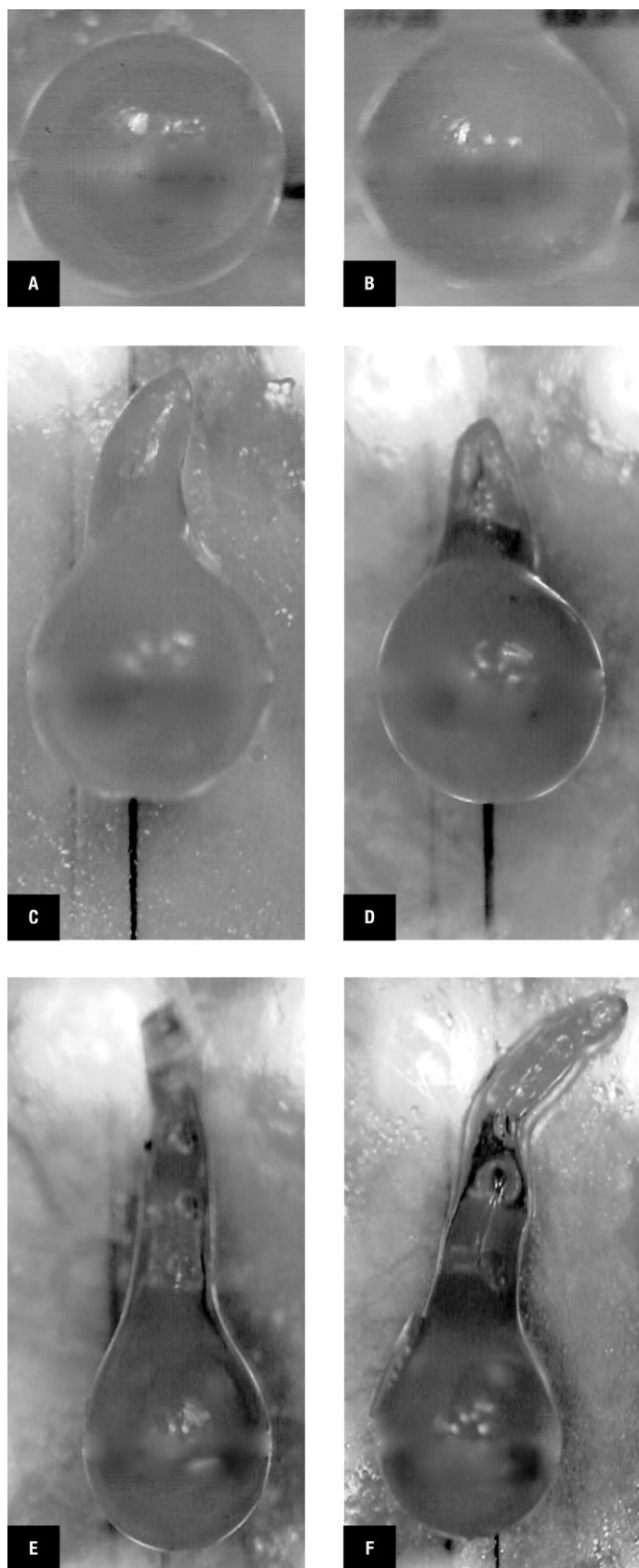


Figure3

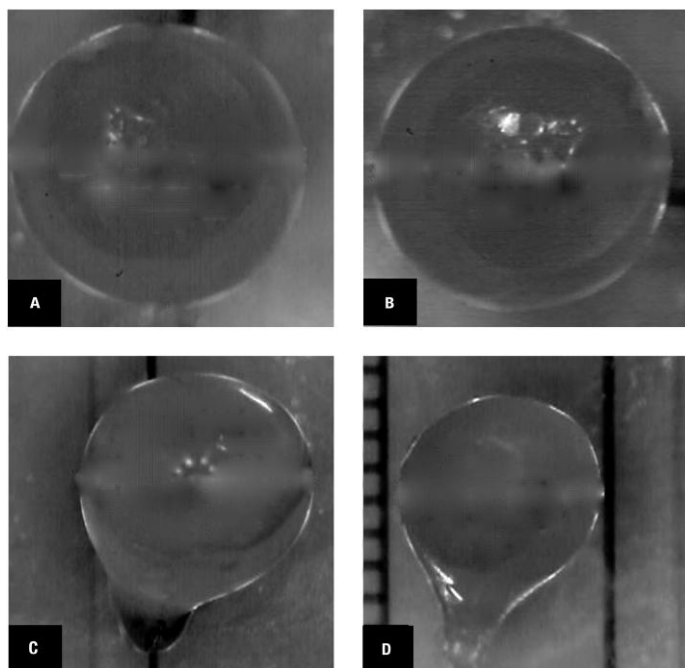


Figure4

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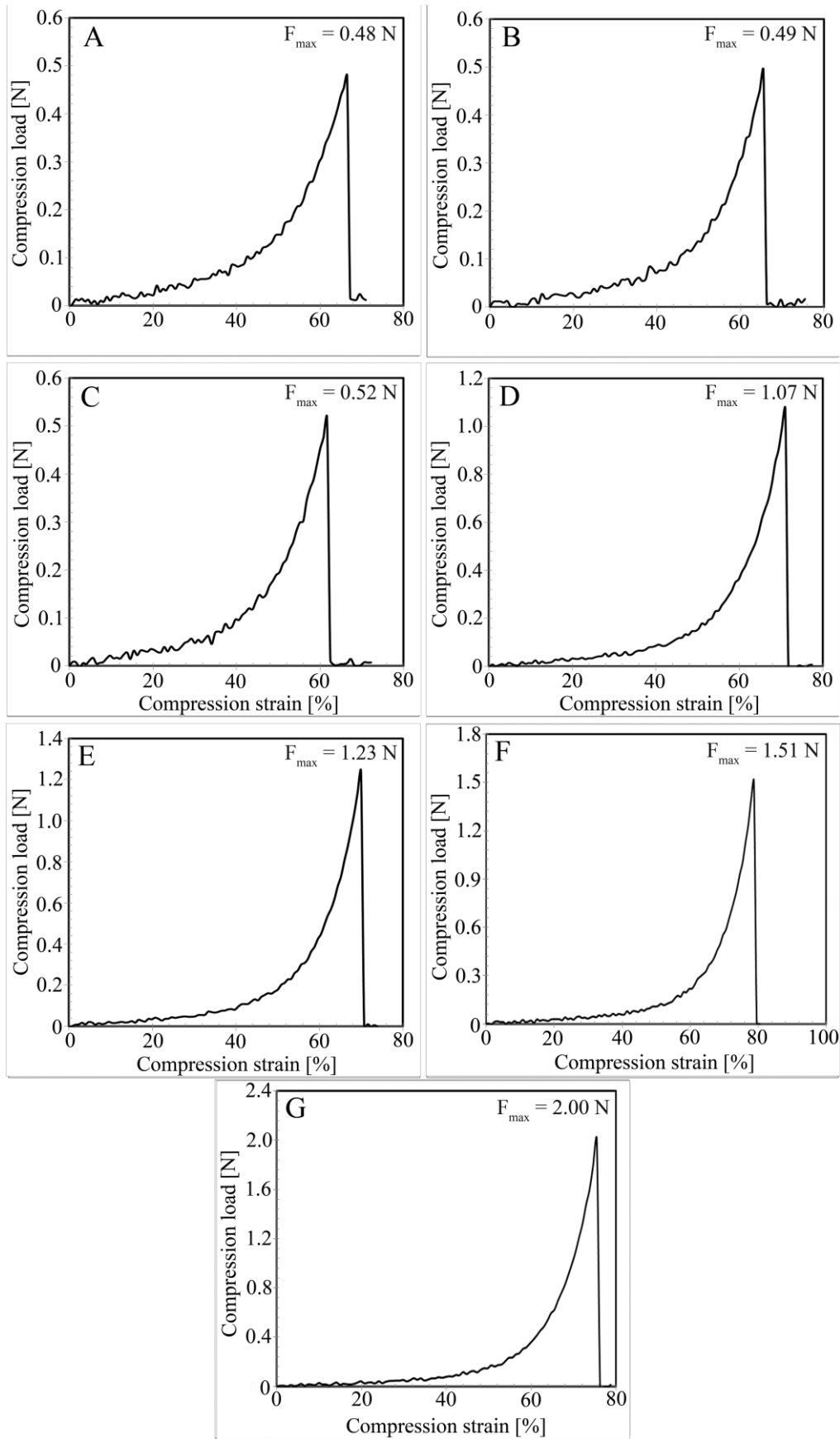


Figure 5

**10. Tables**

Table.1. Effect of the microwave-assisted modification on rheological properties 2% chitosan solutions.

Sample	Casson model		
	$\sigma_{oc}$ (Pa)	$\eta_c$ (mPas)	$R^2$
Microwaveassisted	$1.76 \pm 0.05$	$723 \pm 3.5$	0.979
Withoutmicrowaveassistance	$2.19 \pm 0.07$	$856 \pm 4.1$	0.981

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Table.2. The influence of the shell solution flow rate on oil content in the capsules and their diameter.

<b>Gas flow rate forcing shell solution [l/h]</b>	<b>**Real shell solution flow rate (<math>V_s</math>) [g/h]</b>	<b>Oil content in hydrogel capsules (OC) [% g/g]</b>	<b>Capsules diameter (CD) [mm]</b>	<b>Oil to the weight of dry polymer ratio in the capsule [g/g]</b>
0.6	2.64	42.13 ± 0.87	4.35 ± 0.07	31.37
1.2	4.45	45.23 ± 1.00	4.41 ± 0.05	35.30
1.8	13.52	14.78 ± 0.63	3.94 ± 0.24	7.82
2.4	14.48	14.52 ± 0.04	4.08 ± 0.12	7.66
3.0	23.21	6.96 ± 0.40	3.61 ± 0.10	3.40
3.6	27.24	8.65 ± 0.54	3.67 ± 0.02	4.30

\* Core flow rate ( $V_c$ ) for all determination was constant and equal 2 g/h

\*\* Value obtained based on gravimetric determination



Table.3. The influence of the inert gas flow rate cutting the drops on oil content in the capsules and their diameter.

<b>Inert gas flow rate detaching the drops (<math>V_N</math>) [l/h]</b>	<b>**Real shell solution flow rate (<math>V_S</math>) [g/h]</b>	<b>Oil content in hydrogel capsules (OC) [%, m/m]</b>	<b>Capsules diameter (CD) [mm]</b>	<b>Oil to the weight of dry polymer ratio in the capsule [g/g]</b>
0		45.23 ± 1.00	4.41 ± 0.05	31.37
200		44.31 ± 1.02	4.32 ± 0.04	34.08
400	4.45	42.12 ± 0.33	3.82 ± 0.02	31.35
600		29.58 ± 1.63	2.81 ± 0.02	18.56
800		-	-	-

\* Core flow rate ( $V_C$ ) for all determination was constant and equal 2 g/h

\*\* Value obtained based on gravimetric determination

Table.4. Mechanical properties of obtained capsules based on the force needed to 50% compression of sample.

*Sample	Flow rate conditions of:		Compression load at 50% sample compression strain $F_{50}$ [N]
	**Real shell solution flow rate ( $V_S$ ) [g/h]	Inert gas flow rate detaching the drops ( $V_N$ ) [l/h]	
A	2.64	0	$0.148 \pm 0.007$
B	4.45	0	$0.137 \pm 0.016$
C	13.52	0	$0.190 \pm 0.041$
D	14.48	0	$0.157 \pm 0.015$
E	23.21	0	$0.182 \pm 0.017$
F	27.24	0	$0.120 \pm 0.009$
G	4.45	600	$0.113 \pm 0.010$

Core flow rate ( $V_C$ ) for all determination was constant and equal 2 g/h. The results of compression load was shown as value  $\pm$  SD (n=3)

\* Accordnig to Figure 5

\*\* Value obtained based on gravimetric determination.