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Microencapsulation of extra virgin olive oil by sequential emulsification and freeze drying processes: Effect of wall materials composition and emulsification method

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ABSTRACT

For a long time, olive oil has been considered for formulation of biopharmaceuticals and received a prestigious place in cuisine for its unique organoleptic and nutritional properties. Nevertheless, oxidation of fatty acids in olive oil provides short shelf-life and undesirable organoleptic properties. Thus, microencapsulation of olive oil is a considerable promising approach to maintain its quality and biological activities. The objective of this investigation was to prepare extra virgin olive oil microcapsule by sequential technologies, such as water emulsification of olive oil with wall material (matrix) and freeze drying of emulsion. The effect of wall material composition was examined to prepare microcapsule of extra virgin olive oil. Different ratios of wall materials such as maltodextrin (MD), carboxymethyl cellulose (CMC), and gum arabic (GA) were used. Furthermore, effects of emulsification technologies, such as homogenisation with

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rotor–stator homogeniser (RSH) and cross-flow membrane emulsification (CFME) were investigated. The stability of emulsion was higher when emulsion was prepared by RSH; however, the droplet mean diameter (D_{32}) was lower in case of RSH compared to CFME. The highest encapsulation efficiency (EE) was found as 68.96 ± 2.6% when CFME was adopted and composition of wall materials was 15 g MD, 15 g GA, and 5 g CMC.

KEYWORDS

microencapsulation of olive oil, rotor–stator homogeniser, membrane emulsification, freeze drying, polymeric carbohydrate, encapsulation efficiency

1. INTRODUCTION

Olive oil has been considered as a culinary for its excellent organoleptic properties due to the presence of ketones, alcohols, aldehydes, hydrocarbons, esters, furans, etc. It also has a great importance for biopharmaceutical formulation, because it prevents the risks of several chronic and acute metabolic disorders (type 2 diabetes, obesity, cancer, rheumatoid arthritis, Alzheimer's disease, and cardiovascular diseases). Olive oil is enriched with monounsaturated fatty acids (ω-6 and ω-3 fatty acids), phenolic antioxidants (hydroxytyrosol and oleuropein), vitamin E, and vitamin K (Chaabane et al., 2022). Unfortunately, oxidative deterioration of fatty acids in olive oil provides short shelf-life, responsible for undesirable organoleptic properties and reduced biological activities. Microencapsulation of olive oil is considered as a promising approach to preserve its quality and biological activities (Koç et al., 2015).

Microencapsulation is an emerging technology, which has attracted interest in food, biopharmaceutical, and cosmetic industries. It is used to protect bioactive compounds within a surrounding layer (coating) and control their release into the environment. In this process, a small droplet of liquid or solid particle is surrounded by a thin film, known as a wall material or matrix (Bakry et al., 2016). For microencapsulation of vegetable oil, several emulsification technologies have been used. Presently, membrane emulsification has come to the fore and is considered an emerging technology. In the platform of "process intensification", membrane emulsification is a low energy consuming technique with lower equipment footprint. In membrane emulsification technology, the dispersed phase (olive oil) is pressed through the membrane pores into a continuous phase (wall materials and water) and form the emulsion (Charcosset, 2009).

The objective of this study was to encapsulate extra virgin olive oil by sequential technologies, such as preparation of emulsion (oil in water with polymeric carbohydrate) and, subsequently, freeze drying of the emulsion. In this investigation, polymeric carbohydrates such as maltodextrin (MD), carboxymethyl cellulose (CMC), and gum arabic (GA) were used as a matrix. Concentrations of CMC and MD with dextrose equivalent (DE) 5 were varied; however, the concentration of GA was constant. CMC and MD with DE 5 are considerably hydrophobic (Lee et al., 2018; Liu et al., 2021), which influence the detachment of oil droplets (hydrophobic nature) from the membrane pores due to surface tension (hydrophobic-hydrophobic interaction). Furthermore, effects of emulsification technologies, such as homogenisation with rotor–stator homogeniser (RSH) and cross-flow membrane emulsification (CFME) were investigated.

2. MATERIALS AND METHODS

2.1. Materials

Extra virgin olive oil was purchased from a local supermarket in Budapest, Hungary. MD having DE 5 was procured from AppliChem GmbH, Germany. GA was purchased from Bi-Bor Kft., Hungary. CMC (E466) was procured from Gréta-tortadekoracio, Hungary. Tween 80 and hexane were purchased from Sigma Aldrich, USA. Milli-Q ultrapure deionised (DI) water (18.2 MΩ cm) was obtained from Milli-Q Synergy/Elix water purification system (Merck-Millipore, France) and used in all experiments.

2.2. Preparation of emulsion

The emulsions were prepared according to Table 1. Different polymeric carbohydrates, such as MD, GA, CMC, and emulsifier Tween 80 were dissolved in DI water with constant stirring at temperature 50 °C.

Subsequently, the emulsion was prepared by homogenisation with RSH or CFME. In homogenisation process, olive oil was added dropwise to aqueous polymeric carbohydrate solution, and RSH (IKA, T25, Germany) was operated at 15,000 r.p.m. for 5 min at room temperature (~25 °C) (Koç et al., 2015). Emulsion was also prepared by membrane emulsification technology. Tubular ceramic membrane with pore size l.4 μm was placed in a stainless steel membrane house. The detailed description of membrane emulsification method is mentioned elsewhere (Albert et al., 2018). Into the membrane tube, a stainless steel mechanical device, known as static turbulence promoter, was inserted. The detailed geometry of static turbulence promoter was mentioned before (Koris et al., 2011). Experimental setup of membrane emulsification process and fluid flow within membrane tube are presented in Fig. 1. In the subsequent step, the emulsion was freeze dried.

2.3. Freeze drying

Emulsions were kept in the freezer at temperature −20 °C for 24 h, and freeze drying was performed using a ScanVac coolsafe 110-4 freeze-dryer (Labogene, Denmark). In the ice condenser, operating conditions were set to temperature −109 °C, vacuum pressure ~0.120

Table 1. Composition of emulsions

	Emulsion 1	Emulsion 2
MD (g)	10	15
GA (g)	15	15
CMC (g)	10	5
Olive oil (g)	30	30
Tween 80 (g)	5	5
De-ionized water (g)	700	700
Wall material/Oil ratio (g g^{-1})	1.16	1.16
Total solids % (w/v)	9.39	9.39

MD: Maltodextrin; GA: gum arabic; CMC: carboxymethyl cellulose.

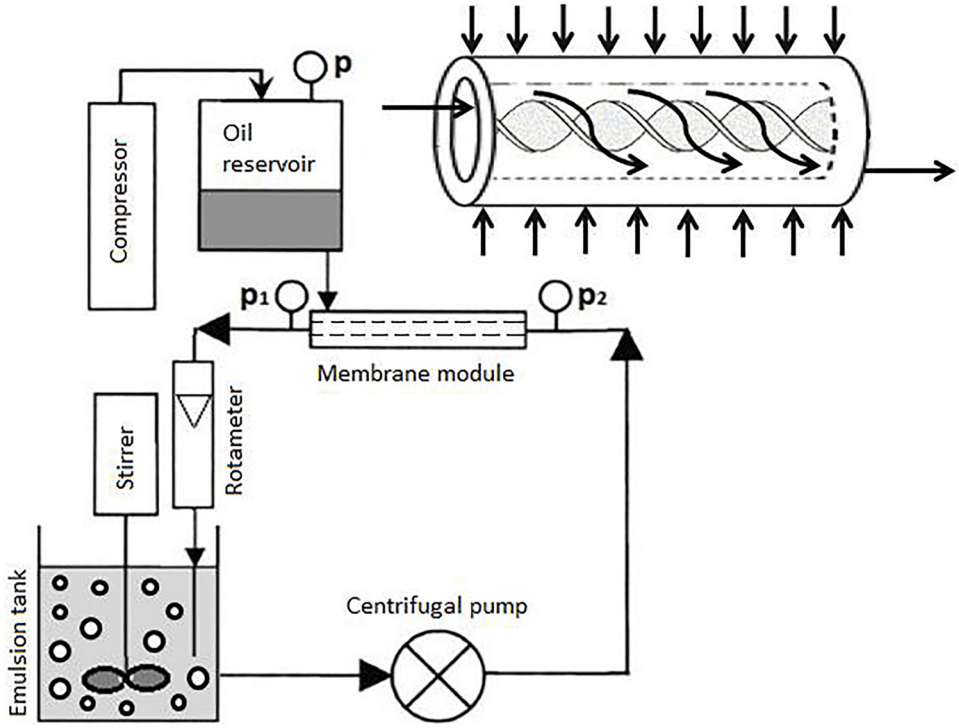

Fig. 1. Membrane emulsification process; inset: fluid flow within membrane tube

mbar, and freeze-drying time 24 h. Following freeze-drying, the samples were manually ground to obtain fine particles.

2.4. Analytical methods

2.4.1. Emulsion stability. After the emulsion preparation, 25 mL of emulsion of each sample was transferred to graduated measuring cylinder, sealed with paraffin paper, and stored at room temperature for one day. Volume of the separated upper phase was measured. The stability was assessed according to Equation (1).

$$\% \text{ Separation} = \frac{H_1}{H_0} \times 100 \tag{1}$$

Where, H_0 and H_1 represent the initial volume of emulsion and the volume of upper phase, respectively (Carneiro et al., 2013).

2.4.2. Emulsion droplet size analysis and span. After preparation of emulsion, the values of average droplet size (D_{32}) and span were measured by a laser particle sizer Analysette 22 (Fritsch Nanotec, Germany) (Albert et al., 2018).

2.4.3. Encapsulation efficiency. EE was determined according to the method described by Bae and Lee (2008). According to their hypothesis, total oil was assumed to be equal to the initial oil, since preliminary tests revealed that all initial oil was retained, which was expected, since olive oil is not volatile. EE was calculated according to Equation (2).

$$EE = \frac{(T_o - S_o)}{T_o} \times 100 \quad (2)$$

Where T_o is the total oil in g and S_o is the surface oil in g.

2.4.4. Morphology of microcapsule. The surface morphology of microcapsule was studied by a field emission scanning electron microscope (FESEM) (Model: JSM 5500 LV, Jeol Ltd., Japan). Microcapsules were coated with a combination of gold and platinum (60:40) for 10 min with a 10 mA plasma current, and coated samples were analysed. In FESEM, a working distance of 35 mm and secondary electron flow were used.

2.5. Statistical analysis

All experiments were performed in triplicate. The mean values with standard deviation (S.D.) were calculated. The experimental data were analysed using SPSS 27.0 statistics software (IBM, Armonk NY, USA). Data were subjected to two-way multivariate analysis of variance (MANOVA) with dependent variables, such as stability (% of separation), droplet size (μm), and span, as well as fixed factors, such as emulsification method and composition of emulsion. Droplet size was previously subjected to ln transformation to assure the normality assumption. The normality of the residuals was accepted by the absolute values of their skewness and kurtosis as they were all below 0.7 and 1.2, respectively. The homogeneity of variances was accepted by Levene's test ($F(3; 8) < 2.20$; $P > 0.16$).

3. RESULTS AND DISCUSSIONS

3.1. Emulsion stability

Results of the emulsion stability are presented in Table 2. Olive oil emulsions, prepared by RSH provided a greater stability (no phase separation after 24 h). It might be attributed to the fact that due to high viscosity in presence of CMC, MD, and GA, olive oil droplets cannot move freely inside the emulsion (Tonon et al., 2011). Similar justification was reported by Carneiro et al. (2013) for an emulsion made by flaxseed oil, MD, and GA by RSH. On the other hand, olive oil emulsions prepared by CFME had faster droplet coalescence and phase separation after 24 h. These results might be influenced by the droplet size of the emulsion.

3.2. Emulsion droplet size and span

Results of droplet mean diameter (D_{32}) and span of emulsions are reported in Table 2. D_{32} values of CFME-1 and CFME-2 were significantly higher compared to RSH-1 and RSH-2. It was proven that emulsion were more stable when droplet size and span values were lower (Carneiro et al., 2013). The values of D_{32} for CFME-1 and CFME-2 were determined to be 41.68 ± 2.85 and 20.05 ± 0.49, respectively. Joscelyne and Trägårdh (2000) reported that the size of emulsion

Table 2. Stability (% of separation), droplet size (D_{32} [μm]) and span of emulsions prepared with different emulsification methods and different wall material compositions

	CFME-1	CFME-2	RSH-1	RSH-2
% of separation	20 ± 0.20^{Ba}	24 ± 0.15^{Bb}	–	–
D_{32} (μm)	41.68 ± 2.85^{Ab}	20.05 ± 0.49^{Ba}	5.83 ± 0.60^{Ba}	5.61 ± 0.37^{Aa}
Span (–)	0.81 ± 0.16^{Bb}	0.40 ± 0.09^{Aa}	1.05 ± 0.16^{Ab}	0.33 ± 0.10^{Aa}

CFME: Cross-flow membrane emulsification; RSH: Rotor–stator homogeniser.
The overall MANOVA test was significant for both factors emulsification and composition (Wilk's lambda values were below 0.001; $P < 0.001$) as well as for the interaction (Wilk's lambda = 0.01; $P < 0.001$). Results are represented by mean value with standard deviation (±values). In superscript, dissimilar alphabet represents significant difference ($P < 0.05$) between results. Upper case letters are for the comparison of emulsification methods within fixed emulsion compositions, and lower case letters are for comparison of emulsion compositions within fixed emulsification method.

droplets might be 2 to 10 times bigger than the pore size of the membrane. In the present investigation, the average pore size of membrane was 1.4 μm. Therefore, our results are not directly in line with the mentioned principle. Our results can be justified by the fact that non-homogeneous droplets might be formed due to asymmetric size of pores in the membrane surface. After detachment of oil droplets from membrane pores, they can coalesce with each other in presence of polymeric carbohydrates such as GA, MD, and CMC in the continuous phase (Fig. 2).

It is noted that the size of droplet and span value in case of CFME-1 were higher than of CFME-2. Due to higher amount of CMC in case of CFME-1 preparation, it was more viscous, which promoted coalescence among droplets. The larger diameter of droplets in CFME-1 may be related to the higher instability of this emulsion. The smaller droplet size in emulsion, produced by RSH, is related to higher stability of emulsion. Carneiro et al. (2013) reported that emulsion, made by flaxseed oil, MD, and GA by RSH were highly stable with droplet size of ~2 μm. It was noted that span values were significantly influenced by the viscosity of the emulsion. Small droplet size in emulsion represents the monodisperse nature of oil droplets.

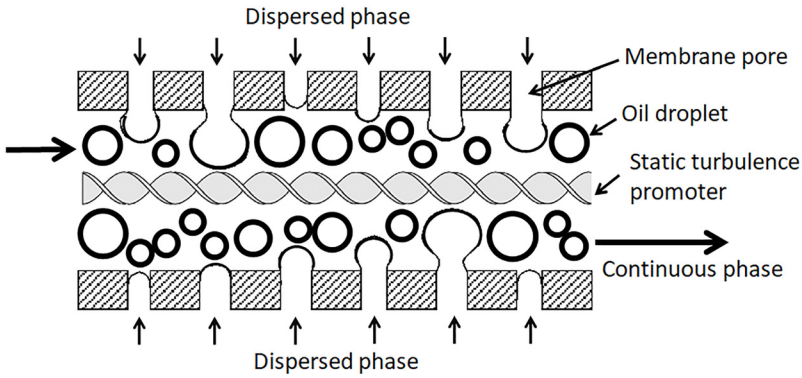

Fig. 2. Formation of emulsion droplets within membrane tube

3.3. Microencapsulation efficiency

EE results of olive oil microcapsule are presented in Fig. 3.

EE was significantly higher for microcapsules produced by CFME than RSH. It can be justified by the fact that when oil droplets were detached from the membrane pores by interfacial force at the droplet base, shear force by continuous fluid cross-flow, and the Young–Laplace force to continuous phase, oil droplets were surrounded by polymeric carbohydrates (Fig. 2). The detailed mechanism is described elsewhere (De Luca et al., 2008). On the other hand, oil droplets agglomerated with polymeric carbohydrates when emulsion was prepared by RSH. In that case, concentration of surface oil was quite higher, so rather oil droplets were encapsulated. Furthermore, it is noted that EE for CFME-2 and RSH-2 were significantly higher than CFME-1 and RSH-1, respectively. It was reported that EE of vegetable oil within polymeric carbohydrate was significantly influenced by the ratio of oil and wall materials and composition of wall materials (Gallardo et al., 2013). They proposed that if this ratio was lower than 2, surface oil might have increased, which might negatively affect the EE. In our investigation, the ratio of wall material to oil was 1.16 (Table 1), which may be the reason for EE being less than 80%. In this study, effect of the polymeric carbohydrates on EE is quite difficult to explain. The degree of the substitute (D.S.) in CMC influences the viscosity of emulsion and it is directly correlated with the concentration of CMC. Furthermore, it has been reported that MD with higher DE-value, such as 5, contained lower amount of oligo-saccharides, which provided higher viscosity (Siemons et al., 2020). Freeze drying process also influences the EE. It was reported that ice crystals formed during the freezing stage prior to encapsulation. It ruptures the emulsion droplets, disintegrates the capsule wall and, subsequently, oil is released to the surface of the microcapsule (Ogrodowska et al., 2020).

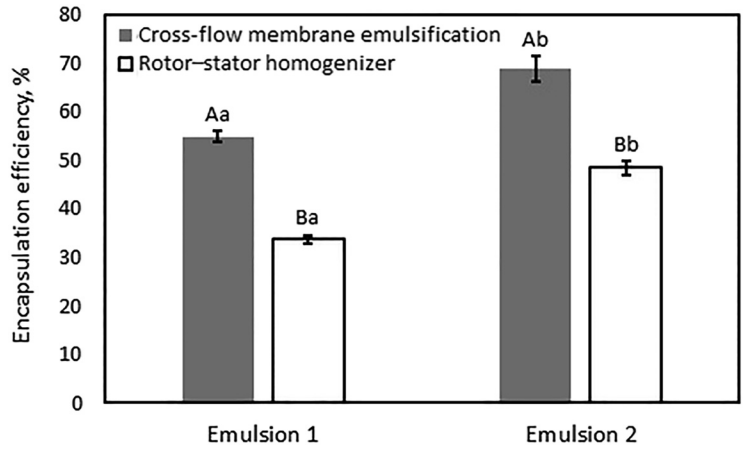

Fig. 3. Encapsulation efficiency of microcapsules, produced by different emulsification methods with different composition of emulsion. Results are represented by mean value with standard deviation (±values). In superscript, dissimilar alphabet represents significant difference ($P < 0.05$) between results. Upper case letters are for the comparison of emulsification methods within fixed emulsion composition, and lower case letters are for comparison of emulsion compositions within fixed emulsification method

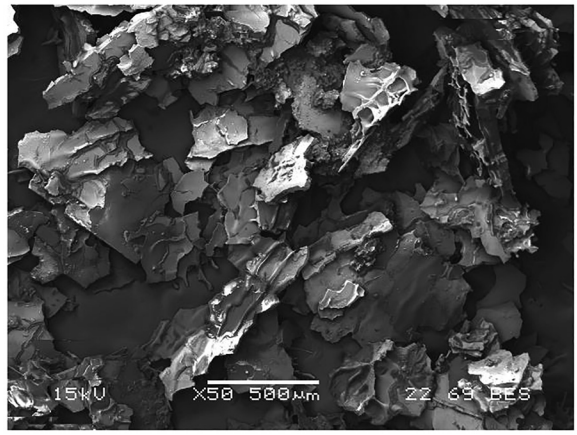

Fig. 4. FE-SEM image of olive oil microcapsule (50 × magnification) prepared by cross-flow membrane emulsification with matrix composition: 15 g maltodextrin, 5 g carboxymethyl cellulose, and 15 g gum arabic

3.4. Morphology of microcapsule

Surface of olive oil microcapsule, prepared by emulsion composition 2 and FD is presented in Fig. 4. Flat porous surface and irregular structure of freeze-dried flakes were observed.

Dehydration by freeze-drying affects the microstructure and integrity of the capsule wall. During the freeze-drying process, a reduction of the surrounding pressure, crystallisation of water in the emulsion, and sublimation of the frozen water at a minimal temperature take place (Ogrodowska et al., 2020). Therefore, flakes with porous skin are produced. CMC is a hydrophobic derivative from cellulose having high glass-transition temperature (T_g) (weak plasticising effect of water) (Liu et al., 2021). MD having DE 5 contains lower molecular weight of saccharides, and it is considerably hydrophobic with high T_g (Lee et al., 2018). Their presence reduces the water activity and agglomeration of microcapsules. Furthermore, due to porous surface of flakes, some oil may permeate to the surface layer of flakes, which offers hydrophobicity and reduces the adsorption of moisture from the environment. Subsequently, it may reduce the agglomeration (Fioramonti et al., 2017). Similar observation was reported by Ogrodowska et al. (2020).

4. CONCLUSIONS

In this investigation, an attempt has been made to prepare microcapsule of olive oil. The effects of emulsification technologies and the composition of wall materials were studied in detail. Two different formulations by changing the concentrations of MD and CMC were used for emulsion preparation; however, the amounts of emulsifiers such as GA and Tween 80 were fixed. The stability of emulsion was higher when the emulsion was prepared by RSH. Values of D_{32} were lower in case of RSH compared to the other one. Higher EE was found by CFME. The most effective wall material composition for the production of olive oil microcapsules was MD 15 g,

CMC 5 g, and GA 15 g. Considering the higher EE, CFME may be more suitable for industrial production of olive oil microcapsule. Further investigation may be performed to understand the effects of the geometry of static turbulent promoter, other formulation of the emulsion, and the quality of encapsulated olive oil during different times of storage.

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