Effect of different cross-linking agents on encapsulation of Olive Oil

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Abstract
Most edible oils in spite of all proven health benefits are chemically unstable and sensitive to oxygen, light, moisture, and temperature. Hence, microencapsulation of active ingredients and their delivery needs to be employed to stabilize the oil in order to fix the problem. On the other hand, lipophilic drugs could not be encapsulated with water-soluble polymers as shell materials. To overcome this problem, olive oil which contains essential vitamins, fatty acids, and other natural nutrients, is used as a solvent of these hydrophobic drugs.
The present study aims at synthesizing gelatin A–sodium alginate complex coacervates and encapsulation of olive oil in the polymer system. Optimization of different reaction parameters such as pH and ratio between the polymers and cross-linker concentration was carried out to attain higher product yield. The optimum ratio between gelatin and alginate and pH value in this study was set to 3.5 and 3.6, respectively. Microcapsules were crosslinked by glutaraldehyde and sodium tripolyphosphate (TPP). Optical microscopy studies proved the formation of spherical microcapsules of different sizes. It was observed that the effect of using TPP as a crosslinker was acceptable whereas increasing its amount in the case of a constant amount of polymer and oil caused the interruption of the electrostatic attraction between proteins and polysaccharides. Therefore substitution of TPP by glutaraldehyde was studied and led to a desirable result. The optical microscopy analysis showed that the number of microcapsules increased by using glutaraldehyde as the cross-linker and the size of microcapsules was decreased by decreasing the amount of oil, polymer, and crosslinker. Moreover, the encapsulation efficiency was increased by increasing the total amount of oil however it causes more surface oil.

Keywords: Microencapsulation, Olive oil, Complex Coacervation, Gelatin, Sodium alginate, Cross-linker

Introduction
Encapsulation is a technology for trapping sensitive and valuable materials such as plants essential oils with a polymeric layer which is called wall material or shell, in order to save them from degradation and oxidation. Moreover, it is used to control release rate of those materials under specific conditions and speed[1, 2]. Depending on the size, produced particles by encapsulation are called by different names. Particles with an average diameter of more than 5000μm, 1-5000μm and less than 1 μm are called macrocapsules, microcapsules, and nanocapsules, respectively [3].

Complex coacervation is one of the most effective and simple methods for encapsulating active ingredients with high encapsulation efficiency. It usually involves two substances as wall materials which are biopolymers, usually a protein and a polysaccharide, with opposite ionic charges in an aqueous system [4]. Complex coacervation is based on electrostatic interactions between these two biopolymers due to their opposite ionic charges as a result of pH change [5]. Proteins as the cationic biopolymer are charged positively under their isoelectric point and the second substance, anionic biopolymer, charged negatively at that point and in contact with proteins produced coacervates at the pH value between 2-5 depending on biopolymers types [6, 7].
In spite of complex coacervation high efficiency, the microcapsules which are produced by this method are unsteady and have weak mechanical resistance. To overcome this problem, a cross-linking agent should be employed in order to strengthen the stability of the wall structure in order to make it rigid and water-insoluble [8, 9].
Olive oil as a functional food not only contains a high level of monounsaturated fatty acids (MUFA) but also is rich in phenolic compounds and contains other bioactive components. These compounds give olive oil some biological properties. The high MUFA levels lead to decrease the cardiovascular risk factors [10, 11]. On the other hand, lipophilic drugs could not be encapsulated with water-soluble polymers as shell materials. To overcome this problem, olive oil which contains essential vitamins, fatty acids, and other natural nutrients, is used as a solvent of these hydrophobic drugs [12]. Most edible oils in spite of all proven health benefits are chemically unstable and sensitive to oxygen, light, moisture, and temperature. Hence microencapsulation of active ingredients and their delivery needs to be employed to stabilize the oil in order to fix the problem [1].

Experimental

Materials

Gelatin powder, sodium alginate, sodium tripolyphosphate, glutaraldehyde, tween 80 and glacial acetic acid (all from Merck, Germany) were used in the microencapsulation process. Olive oil has been obtained from the local market.

Microencapsulation process

An aqueous solution of gelatin and sodium alginate were prepared separately. A specific amount of olive oil was then added to the gelatin solution which was heated at 60°C under constant stirring by a mechanical stirrer at 350 rpm. After the complete formation of oil in water emulsion, sodium alginate solution was added dropwise and the emulsion was allowed to mix under stirring for another 20 minutes and the PH was then progressively shifted down to 3.6 by addition of glacial acetic acid, to promote complex coacervation. The solution was cooled down to 5-10°C [12].
Two different cross-linking agents were then added in this step. A specific amount of cross-linking agent with respect to biopolymer content was added to the main solution slowly under constant stirring. The temperature was then raised to 40°C and the solution was allowed to react for another 3 h under constant stirring. The solution was then allowed to cool down to room temperature while stirring slowly. Finally, the reticulated microcapsules were collected with centrifuging at 2500 rpm for 5 minutes and oven-dried at 45°C [13].

Results and discussion

The encapsulated microcapsules were first washed quickly with hexane to remove the surface oil and then were treated with hexane and allowed to mix under constant stirring for about 3h.

The microcapsules were then filtered out and were dried until their weight becomes constant [14]. The weight difference between each step was measured gravimetrically. The encapsulation efficiency (EE), oil loaded and oil content were calculated using Eqs.1, 2 and 3, respectively:

$$EE = \frac{mass\ value\ of\ total\ oil - mass\ value\ of\ surface\ oil}{mass\ value\ of\ total\ oil} \times 100 \qquad \text{Eq.1}$$

$$oil\ load = \frac{mass\ value\ of\ total\ oil}{mass\ value\ of\ total\ polymer\ used} \times 100 \qquad \text{Eq.2}$$

$$oil\ content = \frac{mass\ value\ of\ encapsulated\ oil\ in\ a\ known\ amount\ of\ microcapsules}{mass\ value\ of\ the\ same\ amount\ of\ microcapsules} \times 100 \qquad \text{Eq.3}$$

Table 1. Effect of variation of olive oil loading, polymer and cross-linker type on behavior of microcapsules.

	Experiments formulation							
	Gelatin A (g)	Sodium Alginate (g)	TPP (g)	Glutaraldehyde (g)	Olive oil (g)	Oil load (%)	Oil Content (%)	Encapsulation efficiency (%)
1	2.8	0.8	0.5	-	7	170	85	90
2	2.8	0.8	-	1	7	170	90	96
3	2.8	0.8	-	0.5	5	138	89	92
4	2.8	0.8	0.5	-	5	138	87	94
5	1.4	0.4	0.3	-	2	95	91	88

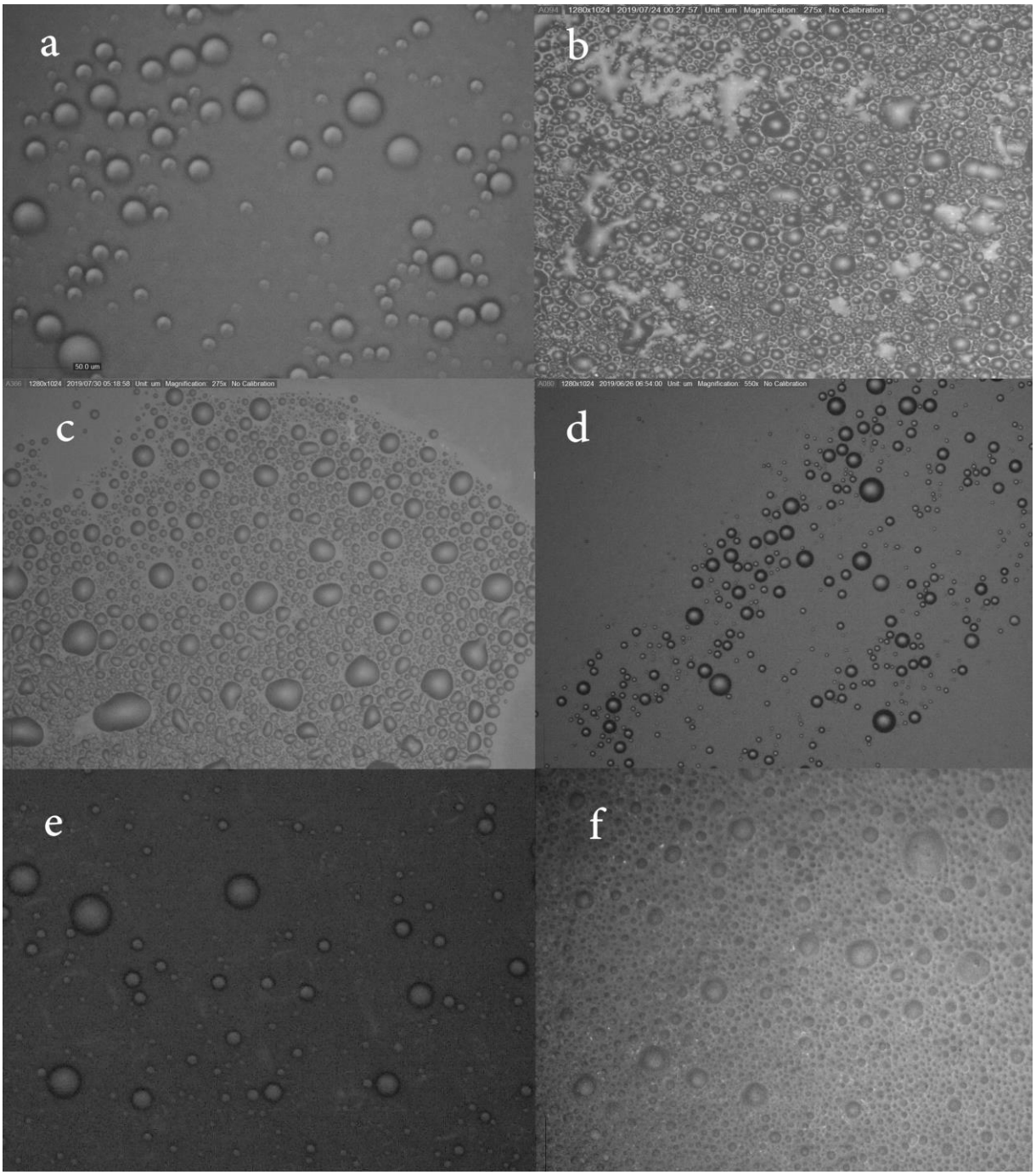
The morphology of the microcapsules was analyzed by optical. An image capture system coupled to a software was employed to measure the size of microcapsules. The results were represented in Figure 1.

Morphology investigation

The optical microphotograph of oil loaded microcapsules showed that the microcapsules have a spherical shape and the size of microcapsules increases with increasing the amount of polymer. This might happen due to the increase in wall thickness. Also with using tween 80 as a surfactant the number and size of microcapsules were decreased sharply and microcapsules become needle-shaped as shown in Figure 1(c). This happens due to non-ionic nature of tween 80 which leads to reduce surface tension and stabilizes the surface area. As it is shown in Figure 1(b), the number of microcapsules was increased and the size of microcapsules has a neglectable difference. The effect of using TPP as the cross-linking agent instead of glutaraldehyde is represented in Figure 1(a, d). It is interesting to note that substitution of TPP for glutaraldehyde doesn't have the desired effect on number of microcapsules and encapsulation efficiency. Due to the presence of sodium ions in TPP solution, the electrostatic interaction between wall materials was interrupted and led to releasing the previously encapsulated oil. Moreover, the oil content of both samples was decreased as shown in table 1.

Similarly, with decreasing the olive oil loading, the size of the microcapsules becomes relatively smaller as it is represented in Figure 1(d) as well as in Figure 1(e) in compare with Figure 1(a), which is expected due to the increase of oil droplets size with increasing the oil loading in the emulsion. On the other hand with increasing the oil introduced to the process, the surface of the microcapsules become sticky and more surface oil was observed.

The effect of homogenizing speed on the o/w emulsion is represented in Figure 1(f). Due to the high shear strength, the size of oil droplets was reduced and as a result the size of microcapsules



reduced sharply.

Figure 1. Micrographs of olive oil microcapsules loaded with: a) oil=7 g, polymer=3.6 g, TPP=0.5 g (sample 1). b) oil=7 g, polymer= 3.6 g, glutaraldehyde=1 g (sample 2). c) oil= 5 g, polymer= 3.6 g, glutaraldehyde= 0.5 g (sample 3). d) oil=5 g, polymer= 3.6 g, TPP=0.5 g (sample 4). e) oil=2 g, polymer=1.8 g, TPP=0.3 g (sample 5). f) oil= 5 g, polymer= 3.6 g, glutaraldehyde= 0.5 g, homogenized at 10000 rpm for 5 min.

Effect of different oil loading

With increasing the oil loading, the encapsulation efficiency and oil content were increased. Table 1. showed the effect of variation of oil loading on encapsulation efficiency and oil content.

Effect of different polymer concentration

The effect of total polymer concentration is shown in table 1. In spite of encapsulation efficiency which was increased by increasing the polymer concentration, both oil content and oil load were decreased in the case of a similar amount of introduced oil.

Conclusions

The microencapsulation of olive oil with sodium alginate and gelatin by complex coacervation is feasible. Optimizing the gelatin: alginate ratio and also PH value are two important factors, which in this case were set to 3.5 and 3.6, respectively. It was observed that the effect of using sodium tripolyphosphate as a crosslinker was not as desirable as the effect of glutaraldehyde. The encapsulation efficiency was increased by increasing the total amount of oil however it causes more surface oil. Homogenizing the o/w emulsion leads to produce smaller microcapsules with invariable size distribution.

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