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Effect of Polymer Concentration and Acidification Time on Olive Oil Microcapsules Obtained by Complex Coacervation

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Abstract

Encapsulation of olive oil is an effective method to protect it against environmental deteriorative factors. In this research, olive oil microcapsules were produced by complex coacervation method. The objective was to examine the effect of gelatin and Arabic gum as shell materials, lactose as cryprotectant, and different acidification times on microencapsulation efficiency of olive oil. Arabic gum 2-5% (w/w), gelatin 2-5% (w/w), lactose 1-5% (w/w), and different acidification times (0-60 min) were given to Design-Expert software using the Response Surface Method. The surface appearance and morphology of the microcapsules were characterized by an optical microscope and scanning electron microscope. Microencapsulation efficiency ranged from 43.9 ± 0.98% to 90.5 ± 2%. The highest efficiency was obtained in gelatin 2% (w/w), Arabic gum 2% (w/w), lactose 3% (w/w) and acidification time of 60 min. The best model for describing the microencapsulation efficiency was quadratic model. The highest effect in microencapsulation efficiency was related to interaction of gelatin-Arabic gum and lactose-acidification time because they had higher coefficient estimate.

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1. Introduction

Olive oil contains fat soluble vitamins and antioxidants. It can reduce heart diseases. Olive oil has high amount of oleic acid (55-83%); however, since it is too sensitive for oxidation, microencapsulation process can be used to increase stability of this oil [1].

Microencapsulation is a process in which solid, liquid or gas materials are trapped in small capsules to enhance food shelf-life, and to control the release of food components at appropriate time and place [2]. One of the methods of microencapsulation is complex coacervation, which consists of three main steps: 1) formation of complex between two polymers with opposite charges; 2) formation of a film around the lipid core; and 3) hardening of material walls to create a microcapsule shell. Complex coacervation is separation of a concentrated polymer phase from a polymer-deficient solvent phase. The creation of a complex is the result of two polymers with opposite charges. These polymers are usually protein and polysaccharide [3].

Several researches have been done on microencapsulation using Arabic gum and gelatin. Yeo et al. [4] examined the concentration of bio-polymers, homogenization speed and oil release during heating in flavored oil. Liu et al. [5] studied the effect of concentration of biopolymers, and emulsifying parameters in the formation of capsules and on the physicchemical properties of flaxseed oil. Comunian et al. [6] investigated microencapsulation of ascorbic acid using complex coacervation, and studied the physicchemical properties and structures of obtained microcapsules.

One of the important factors in the formation of coacervation is pH. The degree of ionization of active groups depends on the environmental pH. When the pH of the solution reaches to the isoelectric point of the protein, the protein becomes neutral; thus, no coacervation occurs. To achieve this purpose, it is necessary to put pH in a certain range. It is worth noting that, according to previous studies, the normal range of pH is 4-5 for formation of microcapsules [7].

Another factor in complex coacervation is the total concentration of biopolymers. When concentration of biopolymers increases, the wall materials usually become dry more quickly. Concentration of biopolymers also affects the viscosity of the coacervate phase. The viscosity should not increase because it causes the wall thickness to be increased [8]. Thus, the release of core materials is distorted.

Freeze-drying process is used to stabilize the emulsion particles produced through complex coacervation. Adding cryoprotectant can increase the physical stability of microcapsules after freeze drying [9].

This study aims to examine the effect of gelatin and Arabic gum (as biopolymers of wall formation), lactose (as a cryoprotectant) and acidification time on the microencapsulation efficiency (ME) of olive oil microcapsules.

2. Materials and Methods

2.1. Material

Food grade gelatin and Arabic gum were purchased from Aria Industry Company (Iran) and Digong Company (South Korea), respectively. Refined olive oil was obtained from a local market. Lactose was purchased from Milad Company (Iran), and used as a cryoprotectant during freeze drying. All reagents used in this study were of analytical grade.

2.2. Formulation design

Arabic gum 2-5% (w/w), gelatin 2-5% (w/w), lactose 1-5% (w/w) and different acidification times (0-60 min) were given to the Design-Expert 6.0.6 software (Stat-Ease Inc., Minneapolis, MN) using the Response Surface Method. The software proposed 20 formulas plus 5 replications (Table 1).

Table 1. Formula composition of microcapsules (pH=4 was used for all runs.)

Run	Gelatin (w/w %)	Arabic gum (w/w%)	Lactose (w/w %)	Acidification time (min)	Microencapsulation efficiency ± SD (%)
1	3.5	3.5	3	0	72.5 ± 1.32
2	5	2	3	0	60.0 ± 1.05
3	5	3.5	3	30	48.0 ± 0.90
4	5	5	1	0	80.0 ± 1.40
5	2	5	3	0	57.4 ± 0.58
6	2	2	3	60	90.5 ± 2.00
7	5	5	1	0	83.5 ± 1.65
8	2	2	5	30	55.0 ± 1.10
9	5	2	5	60	58.9 ± 0.95
10	5	5	5	0	50.0 ±1.50
11	2	2	1	0	72.0 ± 0.80
12	3.5	2	5	0	45.0 ± 0.70
13	2	5	5	60	61.0 ±1.30
14	2	5	1	60	58.0 ± 0.80
15	2	5	1	30	60.0 ± 1.50
16	3.5	5	1	60	43.9 ± 0.98
17	2	5	3	60	58.9 ± 1.00
18	5	5	5	0	55.0 ± 0.63
19	2	3.5	5	0	52.0 ± 1.25
20	5	5	1	60	48.0 ± 1.28
21	5	2	5	30	63.4 ± 1.90
22	3.5	3.5	3	30	47.0 ± 1.38
23	2	3.5	1	0	56.0 ± 0.90
24	2	2	1	0	70.2 ± 1.80
25	2	2.75	2	45	64.0 ± 0.70

2.3. Preparation of microcapsules

Microcapsules were formed according to Green's triplet principle method [10]. According to the formulation of Design Expert Software output, 10% (w/v) aqueous gelatin solution and 10% (w/v) Arabic gum aqueous solution were prepared at 40°C. Olive oil (2 g) was added to the gelatin solution and mixed for 5 min at 4113 ×g using homogenizer (Ultra Turex T18, Germany) to obtain O/W emulsion.

After forming the emulsion, Arabic gum solution was added to the emulsions and mixed for 2 min at 1028 ×g; next aqueous solution of lactose was added to the mixture. Mixture temperature was adjusted at 50°C and mixed for 5 min; then it was acidified to pH 4.0 using 50% (v/v) aqueous acetic acid solution in accordance with the acidification time. The mixture was mixed at 41 ×g for 15 min and cooled down

slowly to 4°C. Then, microcapsules were separated from the aqueous phase, frozen at -18°C, and freeze dried (Dena, Iran) at -45°C and 0.8 mbar for 24 h. The mixture was grounded by laboratory mill (Moulinex, France) and sieved (Mesh: 100) to obtain a fine and suitable powder.

2.4. Encapsulation efficiency

To measure the efficiency of microencapsulation, we used the method of Westergaard [11] with slight modification. Microcapsules (1 g) were mixed with 10 ml of hexane for 15 min. This action was performed twice at room temperature. Then micro-capsules were separated from hexane through filtration. Oil was separated from hexane by vacuum oven. It was measured in triplicate by using gravimetric method. ME was determined by Eq. 1:

$$ME\% = [(\text{total oil} - \text{surface oil})/\text{total oil}) \times 100 \quad \text{Eq. (1)}$$

2.5. Microcapsule images

Morphological characterization of the micro-capsules was observed using an optical microscope (Nikon ECLIPSE E600, Japan) and a scanning electron microscope (XL 40 Philips, Netherlands). Particle size analyzer (Mastersizer 2000, Malvern, England) was used to determine the size of microcapsules.

2.6 Statistical analysis

Design-Expert 6.0.6 software (Stat-Ease Inc., Minneapolis, MN) was used to analyze the experimental results. Encapsulation efficiency data are presented as Mean ± SD (n=3). An analysis of variance was characterized through the statistical significance of the appropriate models. Differences were significant at $p<0.05$.

3. Results and Discussion

3.1. Formation of microcapsules

Due to gelatin's emulsification property, when oil was added to the aqueous solution of gelatin, O/W emulsion was formed. By adding an aqueous solution of Arabic gum and reducing the pH to 4, i.e. below the isoelectric point of gelatin (7-9), gelatin became positively charged whereas Gum Arabic, due to containing carboxyl groups, was negatively charged. Opposite charges of Arabic gum and gelatin caused the formation of coacervates. Thus, walls were formed around the oil droplets, and microcapsules were produced (Figure 1).

According to Figure 2, the microcapsules had relatively spherical shape but they had depressions on surface, which could be due to incomplete and inhomogeneous entrapment of cores in coacervates. As shown, they adhered to each other because of the interaction of free oil and polymers on the surface of the particles, which had not participated in microe-ncapsulation. The same issue has been reported by Planas et al. and Tamjidi et al. [12-14].

The size of microcapsules ranged from 3µm to 15 µm. There are many factors that interfere with the particle size produced by complex coacervation such as the velocity and time of homogenization [13, 14].

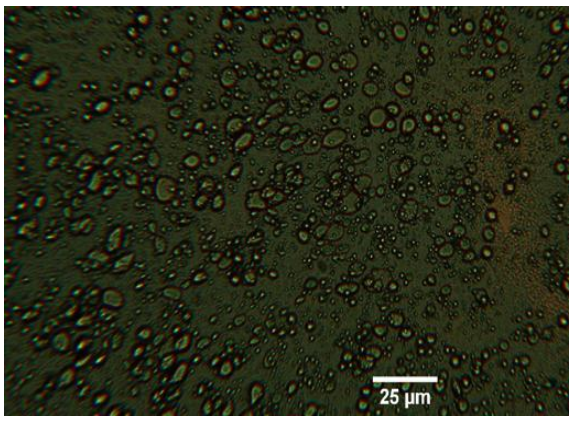

Figure 1. Optical image of microcapsules (×40) for formulas containing: gelatin 2% (w/w), Arabic gum2% (w/w), lactose 3% (w/w) and acidification time (60 min).

3.2. Modeling of microencapsulation efficiency

Microencapsulation efficiency of each formul-ation is presented in Table 1. Quadratic and 2FI model were significant ($p<0.05$). Quadratic model was selected because it had higher R^2 determination coefficient (0.9868) and lower standard deviation. P-value of quadratic model was lower than 0.0001. Analysis of variance results of quadratic model for microencapsulation efficiency and coefficient estim-ate of each term are shown in Table 2. The effects of all components of the formulation on ME were significant ($p<0.05$). Coefficient estimate is a criter-ion for measuring the effect of corresponding term in relation to other terms in the model. The utmost effect in ME is related to interaction of gelatin-Arabic gum and lactose-acidification time because they have higher coefficient estimate.

3.3. The effect of gelatin and Arabic gum on microencapsulation efficiency

Hogan et al. [15] reported that by increasing the ratio of core to wall, ME is reduced. In this study, the amount of olive oil (2%) was unchanged but the gelatin and Arabic gum as wall materials varied from 1% to 5%. The highest ME (90.5 ± 2%) was for the formulation in which the ratio of wall to core was 2:1 and ratio of gelatin to Arabic gum was 1:1. The same result has been reported by Planas et al. [12], Chang et al. [16], and Leclercq et al. [17]. The total concen-tration of biopolymers had direct effect on the viscosity of the coacervation phase. At first, the wall thickness enhanced with increase in the concentration of biopolymers. Thus core materials were protected highly. But further increase in the concentration of biopolymers and increased viscosity, the migration of biopolymers to oil surface decreased and thus the

amount of efficiency was reduced [18]. Also, ME was decreased by increasing the concentration of biopolymers (Figure 3).

Table 2. Estimations of coefficients and probabilities of special quadratic model for ME

Source	Coefficient estimate	p-value[a]
Model	55.52	<0.0001
A[b]	-4.48	<0.0001
B[c]	-3.68	<0.0001
C[d]	2.98	<0.0005
D[e]	-7.06	<0.0001
AB	4.46	<0.0001
AC	-5.19	<0.0001
AD	-1.02	0.1219
BC	-5.14	<0.0001
BD	-2.23	0.005
CD	6.29	<0.0001
A^2	-6.30	0.001
B^2	-1.97	0.151
C^2	17.94	<0.0001
D^2	-3.36	0.0081

[a] P-values less than 0.05 indicate the model terms are significant; p-values greater than 0.05 indicate the model terms are not significant.[b] Gelatin [c] AG [d] Acidification time [e] Lactose

Weinbreck et al. obtained optimum microencapsulation efficiency for encapsulated citrus oil with Arabic gum and whey protein in 1-5% biopolymers concentration [18].

3.4. The effect of lactose and acidification time on microencapsulation efficiency

Cryoprotectants are substances with high glass temperature and low hygroscopic property. The majority of these compounds do not have internal hydrogen bonds [19]. In the formulas 9 and 21 (Table 1), in which the ratio of wall to core was 3.5 and the acidification time was 60 min, with the increase of lactose from 1% to 5%, the efficiency reduced from (63.44 ± 1.9%) to (58.9 ± 0.95%) (Figure 4). To remove water during freeze-drying, the pores were created in the surface of the wall. The use of cryoprotectants (compounds with low water absorption) may increase or decrease the number of pores; this depends on the ratio of cryoprotectant to nano- or micro-particle weights [20]. Fonte et al. [20] reported that the best performance after freeze-drying in the nanoparticles of poly-lactic acid ethylene oxide is when the ratio of nanoparticle to trehalose is 1. Perhaps, at low concentration of cryoprotectant that is proportional to the particles, hydrogen bonds between lactose and polar groups are created. These bonds lead to the stability of microcapsule structures and even to closing some pores at the end of the drying phase. In fact, when there is right concentration of cryoprotectant, it can be placed on the surface of microcapsules and cover the pores.

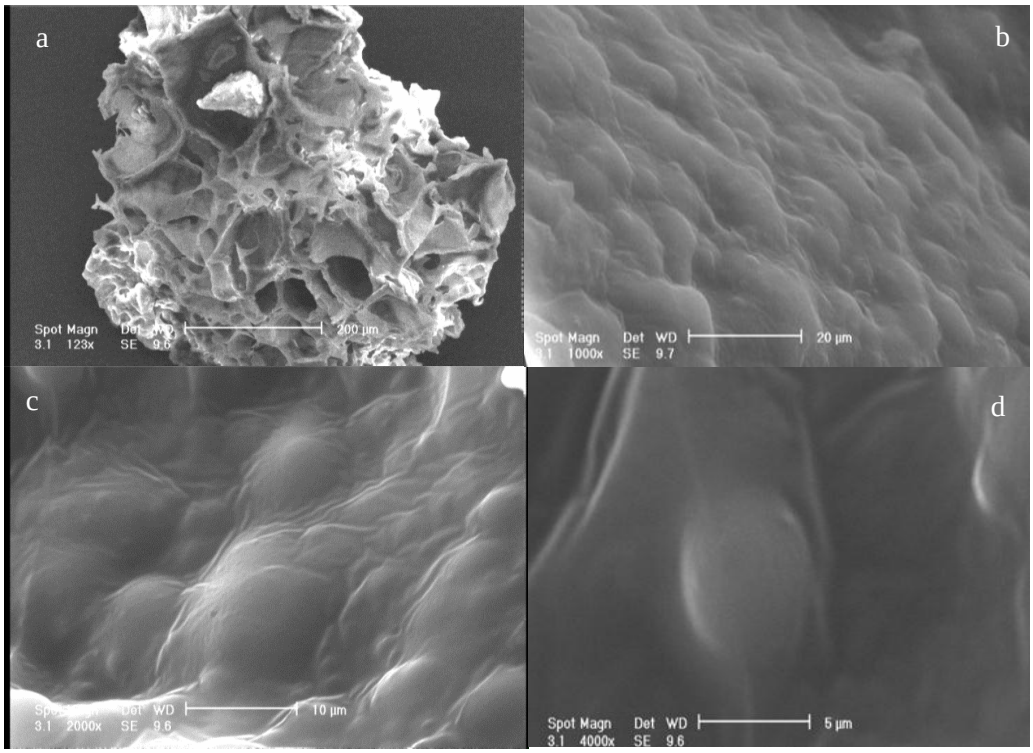

Figure 2. Scanning electron microscopy images of microcapsules: (a) ×123, (b) ×1000, (c) ×2000, and (d) ×4000 magnifications for formulas containing gelatin 2% (w/w), Arabic gum 2% (w/w), lactose 3% (w/w) and acidification time (60 min).

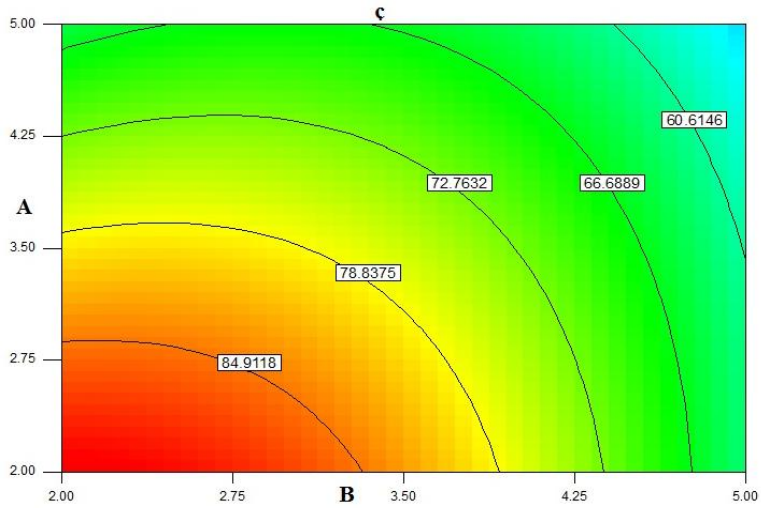

Figure 3. Counter plots of predicted ME%: (A) Gum Arabic, (B) gelatin and (C) ME %.

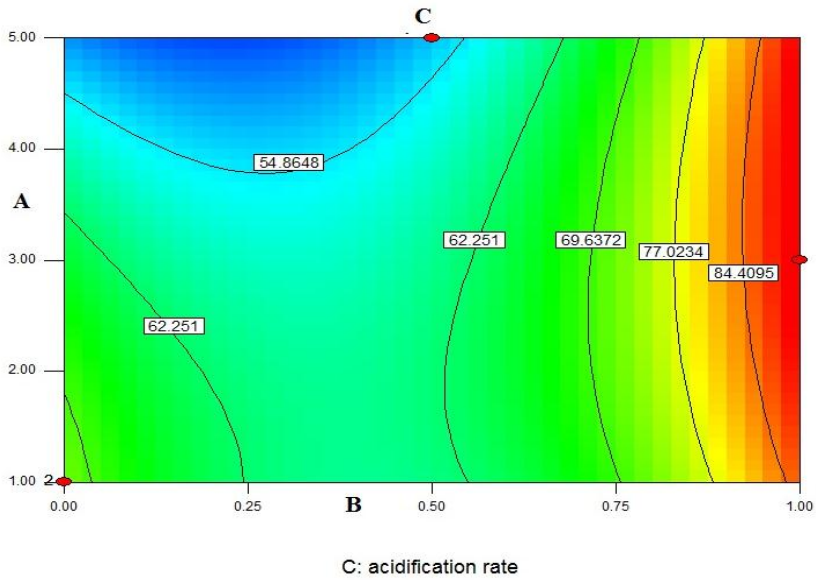

Figure 4. Counter plots of predicted ME%: (A) lactose, (B) acidification time and (C) ME %.

4. Conclusion

The formation of insoluble complexes between Arabic gum and gelatin led to phase separation and formation of coacervate phase. The results of optical microscopy showed that the core was completely surrounded by the wall materials. All microcapsules were spherical. Range of microencapsulation efficiency was 43.9±0.98% to 90.5± 2%. One reason for the low efficiency in some formulas was lack of right proportion of polymers in the microcapsule walls. It damaged the walls and thus the core materials were released. The study findings showed that the best model for describing the microencapsulation efficiency was quadratic model. The highest effect in ME was related to interaction of gelatin-Arabic gum and lactose-acidification time because they had higher coefficient estimate.

First, the wall thickness enhanced and the release of core material was reduced; however, with further increase of wall materials, and consequently, the increase of viscosity, a distortion occurred in the migration of polymers on the surface of oil droplets, and thus ME was reduced. At higher concentrations of lactose, hydrogen bonds were very flexible and could be easily removed from the microcapsules' surface. It caused the increase of core-material release. With increasing the acidification time, the particles became smaller. Thus, more wall polymers covered the surface of particles. Therefore, core-material release was decreased.

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6. Conflict of interest

The authors declare that there is no conflict of interest.

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