Encapsulation of Propolis by Complex Coaservation Technique: Preparation and Characterizations

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Abstract

Propolis is a natural antibacterial agent. Encapsulation of propolis has not investigated since now because of its hydrophilic character. This study aims to improve propolis microencapsulation by complex coacervation using both gelatin and gum arabic as encapsulating agents to overcome this problem. To make the coacervation of a hydrophilic core material viable, a water-in-oil emulsion was first prepared using different oils. To determine effect of oil types and stirring velocity, microeccapsulation were fulfilled four oils (thyme, rice, garlic and olive) and three stirring velocity (500, 1000 and 1500 rpm). Twelve microcapsule formulations were prepared containing gelatin, gum arabic and propolis/oil emulsion. The morphology of microcapsules was analyzed by optical microscopy. The hygroscopicity, particle size, Fourier transform infrared spectroscopy and stability of the encapsulated material were also examined. All of the microcapsule formulations were spherical, multi nucleate and only slightly soluble and hygroscopic. It was possible to efficiently encapsulate propolis using the double emulsion method followed by complex coacervation. When olive oil was used, propolis was not encapsulated because of its viscosity and amount of saturated fatty acid. The effective encapsulation process for propolis was obtained with rice oil at 1500 rpm stirring velocity.

Keywords: *Propolis, Oil, Antibacterial, Coacervation*

I. INTRODUCTION

Propolis is a bee product that has been known by its medical properties since ancient times. These properties are included antifungal, antiviral, antibacterial, antioxidant and inflammatory [1]. Propolis has very complex structure. Structure of propolis having a lot of usage area have varied by region (habitat) collected them, and also have affected from collecting time. Chemical composition of propolis includes waxes, resin, water, phenolics and essential oils. Propolis was extracted with many alcohols and fluids, but it has shown that maximum propolis extract can be obtained from ethanol extraction [2, 3]. Note that ethanolic extracts have used in many medical products, hence does not constitute health issues. Propolis extract contains almost wholes hydrophilic compounds such as phenolic acides, aldehydes. In terms of textile, usage propolis is a virgin area. Propolis has not been used before to improve antibacterial properties of textile materials

There are some studies on various microparticulate systems to perform textile functionalities such as making capsules from active ingredients, controlling flammability, bacterial effect or the other technical properties of the textile materials [4, 5, 6, 7, 8, 9]. Biodegredable and biocompatible materials have been investigated on encapsulation for many years [10].

Complex coaservation method is an encapsulation technique for insoluble (or lipid) core materials.To encapsulate water soluble core materials, water/oil and water/oil/water emulsions are used normally. While water/oil emulsion consists of water droplets dispersed in a continuous oil phase, water/oil/water emulsion contains water/oil droplets in a continuous aqueous phase. The properties and encapsulation efficiency of emulsions are influenced by emulsion components, the emulsification processes, and environmental conditions. One of the most important parameter for preparing emulsions is type and concentration of oil phase [11, 12, 13, 14, 15, 16, 17,18, 19, 20].Type of the oil influence emulsion properties because of its specific properties such as viscosity and saturated fatty acid content. When viscosity of oil phase increases, encapsulation efficiency also increases because viscous oil phase retards diffusion into water phase with core materials. Oil containing higher saturated fatty acid has higher hydrophobicity and hence it has lower compatibility with water [11].

In the complex coacervation system, cationic and anionic water soluble polymers interact in water to form polymer rich phase and this reaction is driven mainly by electrostatic interaction. These polymers form shell part of capsules. Core material is pure active ingredient or its solution in oil. Core material has been emulsified in the polymer mixture to complete encapsulation. Complex coacervation starts water solution of shell polymers at generally pH 6-7 above their isoelectric point. Starting temperature can change polymer types depending on their gelling temperatures. At this pH value, both shell polymers are negatively charged. Then, active ingredient or its oil solution has been added drop by drop. To separate two phases (insoluble polymer-rich and aqueous phases), pH of solution has been lowering to below

the isoelectric point of polymers. Thus, electrostatic interactions fulfill between oppositely charged polymers. Coacervate particles have been collected around the droplets of active ingredients dispersed in the solution. Then, to become rigid of coacervat particles, coacervation mixture has cooling below the gelling temperature. To obtained hard microcapsules, crosslinking agent is added in the system [21, 22, 23, 24].Gelatin/gum system is the most widely used pair of biopolymers for the microencapsulation by the complex coacervation process [25, 26, 27]. The gelatine/gum arabic (GE/GA) system are used as positive and negative polyelectrolytes, respectively [28].

Gelatin obtaining by collagen hydrolysis is a natural and water soluble polymer and its attractive properties are biocompatibility, non-toxic and edible. It is positively charged below its isoelectric point and complexation occurs with negatively charged polymers at lower pH values [29].

Gum arabic is an effective agent in the encapsulation processes because of its colloid functionality. It produces the most stable emulsions with oils over a wide pH range and is compatible with most gums, starches, carbohydrates and proteins [30].

As some studies emphasized, complex coaservation reaction has depended on processing parameters such as pH, mixing ratio, amount of polymers, core material, ionic strength, ion type and agititation speed and oil type [31]. One of the most important parameters for coacervation is also dispersion process to produce droplets of one liquid phase (e.g. oil) in a second immiscible liquid phase (e.g. water). A lot of papers have been published about different polymer couples in the complex coaservation of several core materials and usage properties of the materials that microcapsules have been applied. But there are very limited number papers about effect of processing parameters on the microcapsules characteristics [29]. There is not any paper to explain how oil type affects microparticules yield of hydrophil core materials during complex coacervation. For this reason, the aim of present work has investigated effect of stirring speed and oil type for capsulation efficiency.

II. MATERIALS AND METHODS

A. Materials

All chemicals using in this study are given in Table 1. Commercially available food grade olive oil, garlic oil, rice oil and thyme oil were used without further purification. Others materials are purchased from firms given in the Table 1.

TABLE 1
Chemical reagent used in this study

	Chemicals	CAS Number	Firm/Brand
Exs. Mat.	***Ethanol***	64-17-5	Merck
ComplexsCoa. (Wall Mat.)	***Gumarabic***	9000-01-5	Merck
	Gelatine (Type B)	9000-70-8	Sigma
	Glutaraldehyde	111-30-8	Sigma
	Sodiumhydroxide	1310-73-2	Merck
	Aceticacid	64-19-7	Merck
ComplexsCoa. (Core Mat)	***Propolis***	-	Yalova Beekeepers Union
	Thyme oil	-	Öziş Spice
	Garlic oil	-	Öziş Spice
	Rice oil	-	Öziş Spice
	Olive oil	-	Öziş Spice
	Sodium deodecyl sulfate	151-21-3	Sigma

Properties of oils using in this study are given Table 2.

TABLE 2
Oils properties

Properties	Olive oil [32]	Thyme oil [33]	Garlic oil[34]	Rice oil [34]
Density (g/cm^3)	0,919	0.916		0.919
Viscosity (mPa*s)	0.084	0.033	0.0529	0.0593
Unsaturated fatty acid (%)	13	21.5	8.4	25
Mw (g/mol)	282.47	742,2	488.9	870

B. Preparation propolis extract and emulsion

Firstly, propolis was extracted from raw propoliswith ethanol. 60 g frozen propolis was extracted with 120 ml ethanol (at 50 °C) for 4 hours at 1500 rpm stirring velocity. To be completed extraction, solution in the dark bottle was waited for one week in the refrigerator. Dark part of extract was used in this study as stock propolis solution. Extracted propolis was calculated with the given formula and 120 ml propolis solution contains 25,86 g propolis.

Extracted propolis amount= G_1-G_2 (G_1:60 g frozen propolis, G_2: residual after extraction)

Propolis emulsions (E1) were prepared with different oils and propolis extract in different stirring velocity for 60 minutes each testing set as given in the Table 3. Amount of propolis in each set approximately is 1.428 g.

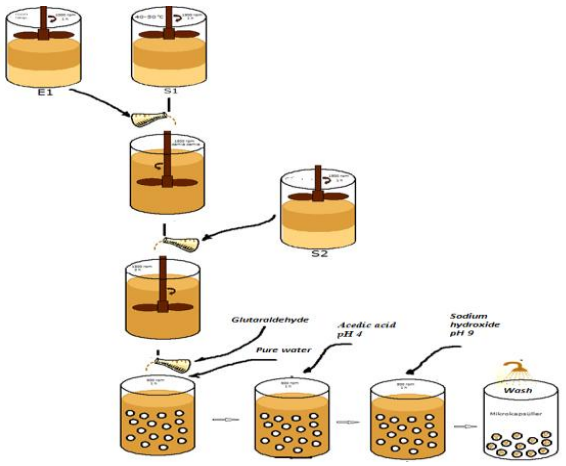
C. Preparation of propolis capsules

As described below, complex coacervation system was prepared and micropartical preparation was carried out according to treatments shown in Table 3. Propolis microcapsules were prepared as schema 1. Different stirring velocity was used each set.

TABLE 3
Formulation and processing conditions during microcapsulationof propolisın gelatin/arabic coacervates (S1: Gelatin solution, S2:Arabic gum solution**)**

code*	S1 (%)	S2 (%)	Prop. (ml)	Oil type (ml)			
				O	Rice	Thyme	Garlic
O500	12	12	20	30	30	30	30
O1000	12	12	20	30	30	30	30
O1500	12	12	20	30	30	30	30
R500	12	12	20	30	30	30	30
R1000	12	12	20	30	30	30	30
R1500	12	12	20	30	30	30	30
G500	12	12	20	30	30	30	30
G1000	12	12	20	30	30	30	30
G1500	12	12	20	30	30	30	30
T500	12	12	20	30	30	30	30
T1000	12	12	20	30	30	30	30
T1500	12	12	20	30	30	30	30

*: O: Olive, R: Rice, T: Thyme, G: Garlic, 500/1000/1500: Stirring speeds



Schema 1: Preparation complex coacervat (E1:propolis+oil, S1:Gelatin solution, S2: Arabic gum solution**)**

In all the experiments mixtures of gelatin and arabic gum were used as a continuous phase. Separate stock solutions of gelatin (12 % w/w) and arabic gum (12 %w/w) were prepared by dispersing the weighed amounts of gelatin and arabic gum in distilled water. The solutions were gently stirred diffirent velocity (500, 1000 and 1500 rpm). Capsulated propolis was washed and dried in the end of process.

D. Microcapsules sizes and morphology

The morphology of microcapsules was investigated by optical microscope (Euromex-FL 100 LED) aided by the Image software to obtain image. To determine diameter of microcapsules, for each formulation, approximately 100 particles diameter were measured and mean of measuring values was calculated.

E. Hygroscopic properties of microcapsules

For each testing test, 1gram microcapsules were taken in Petri dishes at 22 °C. Petri dishes were isolated from ambient conditions. 2 % Na_2SO_4 solution was added, and after one week, the samples were weight and dehumidification of capsules was expressed amount of water absorbed as g/100g.

F. Microcapsules characterization

Thermal behavior of capsules evaluated to determine the decomposition and phase formation from room temperature to 550 °C at a rate of 10 °C/min under nitrogen atmosphere using a DTA/TGA analyzer (Hitachi STA-7300). Al_2O_3 powder was used as a reference material.

FTIR absorption spectra of microcapsules were taken over the range 400-4000 cm^{-1} at room temperature using a Perkin-Elmer FTIR spectrophotometer. Microcapsules were placed directly for measuring.

III. RESULTS AND DISCUSSION

Microcapsules could not be obtained with olive oil at 500 and 1000 rpm velocity. Type of oil phase to be became insoluble core material has been very important because of amount of its saturated fatty acid (Factor affecting the properties of water-in-oil-in-water emulsions for encapsulation of minerals and vitamins [35]. The higher saturated fatty acid containing oil has more hydrophobic characters. For this reason, its compatibility with water is lower. On the other hand, it has been known that using low viscosity oil is necessary to obtain microcapsule [36]. For this reason, as you have seen in Table 2, microcapsule could not be obtained with olive oil having higher saturated fatty acid and viscosity.

A. Assessment of microcapsule appearance and dimension

All samples were viewed with optical microscope and diameter of capsules was measured. Microscope images have been shown in Figure 1.

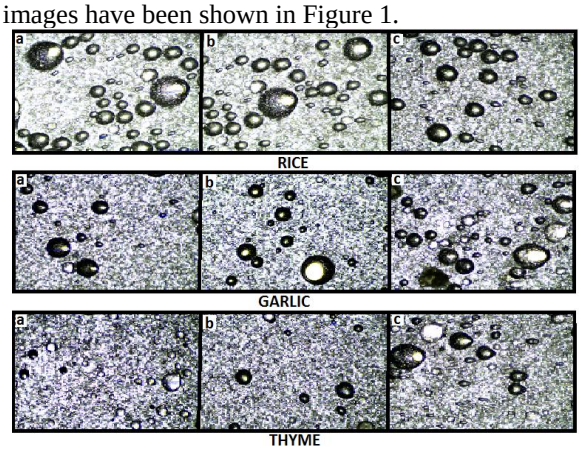


Figure 1: Microcapsule images

As you seen in Figure, capsules were obtained with different stirring velocity and oils. But the most effective capsulation parameters were 1500 rpm stirring velocity when rice oil was used. It was thought that type of fatty acid; oil viscosity and amount of saturated fatty acid have synergistic action on the capsulation process.

Dimension of obtained capsules with different velocity and oils has been different each other because of water/oil emulsion properties and stirring velocity. Capsule size was between 15µm-387µm. The most stable microcapsule dimension was obtained with rice oil at 1500 rpm velocity and it has been approximately 25µm.

B. Assessment of microcapsule hygroscopicity

Hygroscopicity of each sample was given in Table 4. The hygroscopicity values of the microcapsules ranged from 5.63 to 16.27 g water absorbed/100 g sample.

TABLE4
Hygroscopicity of propolis capsules and pure gelatin and gum arabic

	Higroscopicity (g water absorbed/100 g sample)
Gelatin	26.57
Gum	39.15
R500	15.75
R1000	16.27
R1500	14.93
G500	6.75
G1000	5.63
G1500	5.67
T500	11.16
T1000	10.97
T1500	11.89

Gelatin and gum arabic are hydrophilic materials, so their hygroscopic values are high. To encapsulate hydrophilic core materials, oil/propolis emulsions must be formed. Hydrophobic character of oils as parallel of their saturated fatty acid amount has decreased hygroscopicity of microcapsules.

C. Microcapsule characterisation

1. FTIR Analysis

The characteristics of FTIR spectra for microcapsules prepared with rice, thyme and garlic oil and propolis are shown in Figure 2.

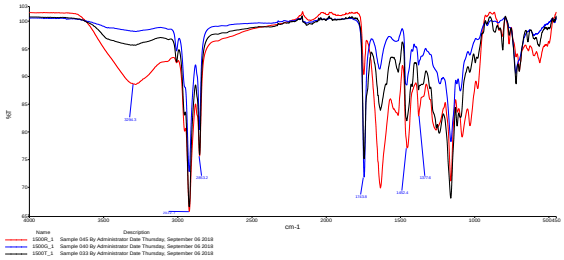


Figure 2: FTIR spectra of microapsules

These spectra look very similar and showed a typical characteristic of absorption peaks for common triglyceride, main component composed oil, gum arabic and gelatin. Strong band absorptions were observed in the region of 3000–2800 cm^{-1} caused by corresponding to C–H stretching vibrations of ring compounds (120-rapor). Bands at 3026 and 3085 cm^{-1} are attributed from the stretching =CH of alkenes and aromatic structures. The wide band at the 3296 cm^{-1}represents –OH groups originating from the structure of the gum arabic or phenolic compounds. On the other hand, OH groups of carboxylic acids also form stretching vibration in the same band [38]. The stretching vibrations of methylene ($–CH_2–$) andmethyl ($–CH_3$) groups can be seen at frequencies of 2922and 2853 cm^{-1}, respectively. Methylene and methyl groupsare also observed at 1465 cm^{-1} and 1377 cm^{-1} due to theirbending vibrations. The peaks shown in the region between 2113-1910 cm^{-1} are attributed from C=C=C asymmetric stretching vibration (121-rapor). The large peak around 1740 cm^{-1} is dueto C=O (from COH group) double bond stretching vibration. Deformation andbending of C–H and stretching vibration of C–O result inpeaks in the 1500–650 cm^{-1} region.

The differences among them were clearly small andoccurred only in limited regions of the spectra, especially inpeak intensities at fingerprint regions (1500–650 cm^{-1}) andat 3007 or 3009 cm^{-1}. This difference is because of hydrophobic long chain of different oils and their different fatty acids.

2) Thermal analysis

Thermal investigations are helpful in tracking the phase change behavior of materials, which in turn is helpful for the selection of right area of application. TGA curves of microcapsules are shown in Figure 3, 4 and 5.

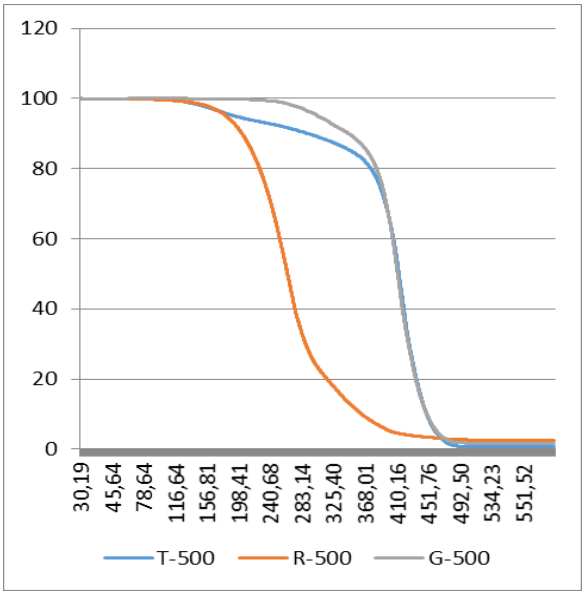


Figure 3: DTA results of microcapsules stirred 500 rpm

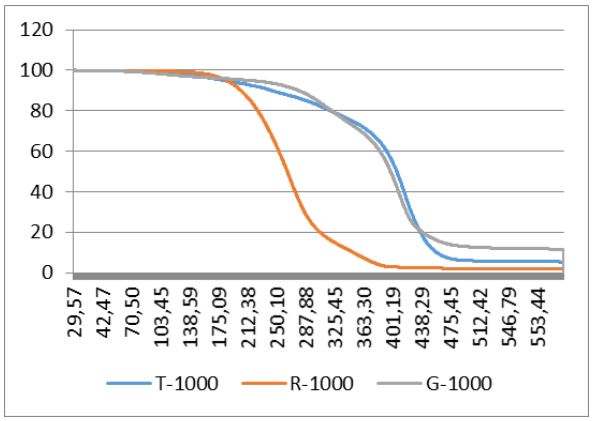


Figure 4: DTA results of microcapsules stirred 1000 rpm

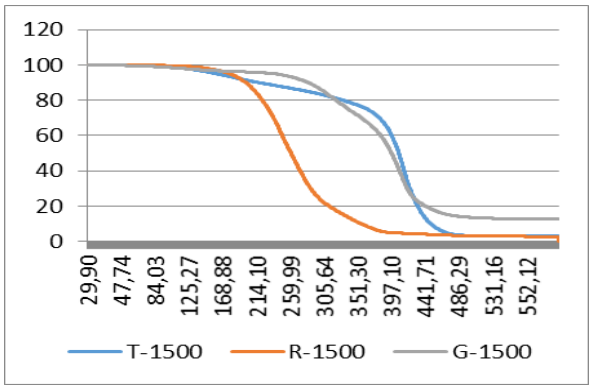


Figure 5: Figure 5. DTA results of microcapsules stirred 1500 rpm

As thermal behavior of microcapsules prepared in the different stirring velocity have been investigated, it is shown that stirring velocity don't have any influence on the TGA. While for microcapsule containing rice oil and thyme oil, weight loss is only up to 5 % until 200 °C, weight loss of microcapsule containing garlic oil is up to 5 % until 300 °C. For this stage, decomposition rate is very low; it is thought that water in the structure have moved away. At the between 200-450 °C, decomposition rate is high. About in the 450-500 °C, almost all of weight of microcapsules loses.

IV. CONCLUSION

Considering the aims and the results of this study, the proposed methodology of double emulsion followed by rice, thyme and garlic oil proved viable to encapsulate propolis. It has been shown that rice oil is the most suitable. Dimension of microcapsule has been affected from stirring velocity and microcapsule sizes close to each other were obtained at 1500 rpm.

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