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# Influence of different parameters on the preparation of Olive leaves extract beads

**Zam Wissam[1*], Ali Ali[2], Hasan Rama[3]**
[1]Al-Andalus University for Medical University, Faculty of Pharmacy,
Department of Analytical and Food Chemistry
[2]Al-Andalus University for Medical Sciences, Faculty of Pharmacy, Department of Basic Sciences
[3]Faculty of Technical Engineering, Tartous University, Department of Food Technology
***Corresponding author: E-Mail: w.zam@au.edu.sy, Tel: 0963932724703**

**ABSTRACT**

Olive leaves contain a wide range of phenolic compounds which exhibit a variety of physiological properties. They were usually discarded although they can form interesting new compounds for food industry. Microencapsulation of olive leaves extract using different polymers for the protection of polyphenols should be the first step in the preparation of functional foods. Ionotropic gelation method was used in order in order to form calcium alginate/salep beads for the improvement of polyphenols' stability. The effect of sodium alginate, salep and calcium chloride concentrations as the major factors affecting polyphenols encapsulation efficiency and polyphenols release were optimized and analyzed. The formulation containing 100 ml of olive leaves polyphenol extract encapsulated with 3 % of sodium alginate, 1.2% salep cured in 0.3 M calcium chloride for 30 minutes were selected as the best parameters with loading efficiency 53.80±2.59%. The optimized beads released 94.36% of encapsulated after 120 min in SGF 1.2. FT-IR spectroscopy was used to scan the existence of salep within the formed beads besides determining the interaction between sodium alginate and salep. The developed calcium alginate/salep beads of olive leaves polyphenol extract could be a promising technique for pharmaceutical and food supplementation with natural antioxidants.

**KEY WORDS:** olive leaves, phenolic compounds, microencapsulation, ionotropic gelation, calcium alginate/salep beads.

## 1. INTRODUCTION

Phenolic compounds, secondary metabolites in many plants, have been shown to exhibit a wide range of physiological properties (Balasundram, 2006). Nowadays, consumers' interest in healthy and minimally processed food increases widely which encourages researchers from different fields to extract these interesting components from natural matrices especially the food industry byproducts, which are usually discarded or employed to produce animal feed (Herrero, 2011).

Olive (Olea europaea L.) is one of the most important crops in Syrian agriculture. Their leaves extracts are polyphenols rich sources, which would be used in medicinal products and functional foods for their health benefits such as antioxidant, anti-hypertensive, hypoglycemic, hypo-cholesterol, cardio-protective and anti-inflammatory activity (Vogel, 2015; Zam, 2016). However, these polyphenol compounds are unstable and the boiling extract has a short shelf life. In this context, their stabilization for use in industrial purposes could be aided using microencapsulation technologies (Desai, 2005).

Microencapsulation is a technique used in a wide range of industries. A biopolymer is for the encapsulation of a bioactive compound thereby protecting it from environmental conditions such as water, light and oxygen and so improving its stability. This technique is also used to change liquid solutions to powders for easier handling (Gharsallaoui, 2007).

In the food industry, encapsulation is being used for many purposes such as the preparation of several fortified foods and functional foods. During the recent years, nutraceuticals are considered as bioactive compounds with health-promoting properties; thus, encapsulation can provide them with necessary protection against different hard conditions (Ioannis, 2007).

Many physical and chemical methods are used for microencapsulation, the ionic gelation process, is one of the physicochemical methods. It consists of dissolving or dispersing the active material into an aqueous solution of polymer and extruding it through a syringe needle or a nozzle into a dispersant phase. Droplets are transformed, after reaction, into spherical gel particles (Zam, 2014).

Sodium alginate is a polymer used in ionic gelation by the addition of divalent cations in aqueous liquid. It consists of polyanionic copolymer of guluronic and manuronic acids that can form hydrogel beads with divalent cations (Hajaratul, 2016). However, the porosity of alginate is a major problem during alginate beads preparation. In addition alginate is unstable in acidic environment which can cause the decarboxylation of alginate. Therefore, many modifications based on the combination of alginate with other polymers have been investigated (Hajaratul, 2016).

Salep is a hydrocolloid powder obtained from dried roots of Orchis morio var mascula (Razavi, 2014), from the Orchidaceae family (Razavi, 2015). Currently, it is a natural, neutral polysaccharide that is used in food industry

as a thinking agent, as well as for its pleasant aroma, smell, and taste and as a blood sugar manager (Razavi, 2014). It was used in pharmaceutical industry for the development of hydrophilic, gastro-retentive matrix tablets of famotidine (Razavi, 2014) and the formulation of a sustained-release metformin tablets (Karaman, 2010). Salep powder contains glucomannan and starch as well as some minerals and water (Razavi, 2015). Glucomannan showed that it has many applications in many fields due to its biogradablility, gel-forming and very good film-forming abilities (An, 2016).

Thus, the aim of our study is an optimized formulation of Calcium alginate/salep beads to encapsulate the olive leaves polyphenol extract using ionic gelation process in order to use the final beads in pharmaceutical or food products.

## 2. MATERIAL AND METHODS

**Chemical and Reagents:** Chemical reagents were purchased from different sources: Sodium alginate and Folin-Ciocalteu reagent 2N (Sigma-Aldrich, Switzerland), calcium chloride (Carl Roth, Germany), salpe powder (Kahramanmaras, Turkey), sodium carbonate (Himedia, India), gallic acid (Sigma- Aldrich, China). Analytical grade ethanol was obtained from Sharlau (Spain).

**Sample Preparation:** Olive leaves samples were harvested from sunshine area of Safita/Tartous. Fresh leaves were washed and dried in a microwave oven at 2450 MHZ for 80 sec at maximum power.

The dried samples were then powdered in a blender and stored at -18°C until analysis (Zam, 2016).

**Extraction Procedure:** The dried and ground olive leaves were extracted with ethanol 40% at 60°C for 2 h. The liquid extract was separated from solids by centrifugation at 5000 rpm for 5 minutes according to Zam (2016). The supernatant was transferred to a vacuum rotary evaporator for removing the remaining ethanol.

**Optimization of Loading Efficiency:** The formulation of the calcium alginate/salep beads is based on concentration of both the sodium alginate and salep and the ability of calcium ions to cross link with sodium alginate/salep.

The optimization of parameters affecting the formulation of beads were based on three different parameters: sodium alginate concentration (1-4%), salep concentration (0.4-1.6%) and calcium chloride concentration (0.2-0.3-0.4 M).

**Capsules Formulation:** The calcium alginate/salep beads were prepared using inotropic gelation method where calcium chloride ($CaCl_2$) was used as cross-linker.

**Sodium alginate beads:** Sodium alginate (1-4%w/v) was allowed to dissolve in 100 ml of prepared polyphenols extract after removing ethanol by rotary evaporator. This solution was homogenized by ultrasonication for 15 min then 2 ml of the resulting solution was injected through a syringe needle into 50 ml of $CaCl_2$ (0.2-0.3-0.4 M) for 30 min. Finally, the beads were filtered and washed at least two times with distilled water. Then, they were allowed to dry overnight at room temperature (Hajaratul, 2015). Dried beads were stored in a refrigerator until determination of polyphenols encapsulation efficiency.

**Sodium alginate/salep beads:** The sodium alginate at the best concentration, with the best polyphenols encapsulation efficiency, was mixed with salep (0.4-1.6% w/v) and added to 100 ml of prepared polyphenols extract to formulate beads following the same procedure described above.

Finally, sodium alginate and salep at the best concentrations were injected into different concentrations of calcium chloride (0.2-0.3-0.4 M) to formulate beads.

**Determination of Polyphenols Encapsulation Efficiency:** The amount of polyphenols loaded in the beads was estimated by using digestion method. Twenty milliliter of 0.1M phosphate buffer saline (PBS) at pH 7.4 was used to dissolve beads for at least 18 hours at 25°C ± 0.5°C. The total phenolic content was determined using Folin-Ciocalteu reagent. The experiment was done in triplicate.

The following equation was applied to determine the percentage of loading efficiency:

$$\text{encapsulation efficiency } \% = L / L_0 * 100.$$

Where L is the amount of polyphenols determined on the solution of beads in phosphate buffer saline and $L_0$ is the initial amount of polyphenols in the prepared extract (El-Kamel, 2003).

***In Vitro* Release Study:** Capsule release study was carried out using the USP-II dissolution test apparatus. Beads were tested for polyphenols release in 150 mL of simulated gastric fluids prepared using 0.1 N HCl at pH 1.2. After 2 hours, the dissolution medium was replaced by simulated intestinal fluid SIF prepared using phosphate buffer at pH 6.8 until the beads were disintegrated. The dissolution rates were measured at 37±0.5°C under 50 rpm speed. At regular time intervals, 1 ml of dissolution medium was analyzed to determine the polyphenols release from beads the Folin-Ciocalteu reagent method.

**Fourier Transform-Infrared (FT-IR) Spectroscopy:** Small amounts of sodium alginate, salep, and the crushed beads powder were mixed with potassium bromide and separately made into small and thin pellets. The pellets were analyzed using an IFS 28/B FT-IR spectrometer (Bruker Analytik). Spectral scanning was done in the wavelength region between 400 and 4000 $cm^{-1}$ at a resolution of 4 $cm^{-1}$ with scan speed of 1$cm^{-1}$.

## 3. RESULTS AND DISCUSSION

**Sodium Alginate Concentration:** Four concentrations of sodium alginate were examined: 1, 2, 3, and 4% (w/v). As shown in Table.1, the loading efficiency raised from approximately 13.19±1.32 to 24.77±1.25% as the concentration of sodium alginate increased from 1% to 3%. As reported by El-Kamel (2003) this may be attributed to the greater degree of cross linking as the quantity of sodium alginate increased (El-Kamel, 2003).

**Table.1. Loading efficiency of olive leaves polyphenols extract using different concentrations of sodium alginate and 0.2M $CaCl_2$**

| Sodium alginate concentration (% w/v) | Polyphenols encapsulation efficiency (%) |
|---|---|
| 1 | 13.19±1.32 |
| 2 | 21.25±1.06 |
| 3 | 24.77±1.25 |
| 4 | 14.37±0.17 |

Higher concentration of sodium alginate is accompanied by a decrease in loading efficiency to 14.37±0.17% which could be explained by an increase in viscosity. The concentration of sodium alginate accompanied with the best loading efficiency in this study was higher than the one used by Deladino (2007) (2% w/v) for the encapsulation of natural antioxidants extracted form *Ilex paraguariensis* and equal to the concentration used by Zam (2014) for the encapsulation of pomegranate peels polyphenols extract.

**Salep Concentration:** The main polysaccharide content of salep extracts is glucomannan (Tang, 2002) which consists of β (1→4) linked glycosyl and mannosyl residues (Georgiadis, 2012). This chemical structure facilitates the gelation of this polymer by calcium ions. Four different concentrations of salep 0.4, 0.8, 1.2, 1.6% w/v were investigated in combination with 3% of sodium alginate solution.

As demonstrated in Table.2, the use of salep for polyphenols encapsulation appears to increase the polyphenols loading within the matrix compared to calcium alginate beads; the highest loading efficiency was obtained with salep 1.2%.

**Table.2. Loading efficiency of olive leaves polyphenols extract using 3% sodium alginate with different concentrations of salep and 0.2M $CaCl_2$**

| Salep concentration (% w/v) | Polyphenols encapsulation efficiency (%) |
|---|---|
| 0.4 | 35.95±3.18 |
| 0.8 | 41.92±1.78 |
| 1.2 | 49.25±2.56 |
| 1.6 | 25.49±3.45 |

This increase in encapsulation efficiency with increase of the amount of salep could be attributable to the high viscosity of polymer solution and through this the polyphenols leaching during beads preparation might be prevented and result in high encapsulation efficiency (Gonzales-Rodriguez, 2002; Hajaratul, 2015) as well as the presence of salep gel was stronger and more stable than alginate gel as it is mentioned by wang (Wang, 2002).

The loading efficiency is enhanced when increasing the concentration of salep in gel to certain limits. Using the concentration of 1.6% salep, the loading efficiency decreases to 25.49±3.45% as higher-viscosity solution is formed which prevents the penetration of calcium ions through this solution (Lee, 2006; Razavi, 2014).

**Calcium Chloride Concentration:** Kuo and Ma (2001), had shown that the use of poorly water-soluble salts such as $CaCO_3$ and $CaSO_4$ for ionic gelation influences the gelation rate and, consequently, mechanical properties of formed beads. It was proved that the use of $CaCl_2$ as a gelling salt with sodium alginate could form a homogeneous and stable gel (Stevens, 2004).

Cross-linking agent concentration as an affecting factor on the loading capacity was studied by using different concentrations of calcium chloride (0.2- 0.3- 0.4 M) with maintaining sodium alginate and salep concentrations at 3% and 1.2% (w/v), respectively.

**Table.3. Loading efficiency of olive leaves polyphenols extract using 3% sodium alginate with 1.2% salep and different concentrations of $CaCl_2$**

| Calcium chloride concentration (M) | Polyphenols encapsulation efficiency (%) |
|---|---|
| 0.2 | 49.25±2.56 |
| 0.3 | 53.80±2.59 |
| 0.4 | 46.42±2.90 |

The results of this study were presented in Table 3 indicating that the increase of calcium chloride concentration from 0.2% to 0.3% leads to increase the loading capacity of the resultant beads from approximately

49.25±2.56% to 53.80±2.59%. These results are in agreement with many previous studies and may be explained by the increase in the gel strength as the calcium ions increased (Stevens, 2004; Mirghani, 2000). Nevertheless, increasing concentration of the calcium chloride over 0.3% led to a decrease in the loading capacity to reach about 46.42±2.90%. This may be due to a damage in microcapsules because of possible saturation of calcium binding sites in the glucoronic acid chain which will prevent further calcium ion entrapment as reported by El-Kamel and Ostberg (Ostberg, 1994; El-Kamel, 2003) or to osmotic stress as reported by Takayuki (2009).

***In Vitro* Release Study:** The polyphenols release of alginate/salep optimized beads is shown in Figure.1.

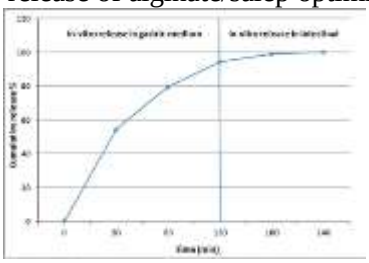

**Figure.1. *In vitro* release study of polyphenols from alginate/salep optimized beads in simulated gastric fluids, SGF (0.1 N HCl, pH 1.2) for 2 h, followed by simulated intestinal fluid SIF phosphate buffer (pH 6.8)**

The polyphenol release from the alginate/salep beads depends on the penetration of the dissolution medium into the beads, swelling and dissolution of alginate/salep matrix, and the dissolution of the polyphenol through the swollen matrix in different dissolution media. The release patterns showed fast dissolution effect in simulated gastric fluids with a cumulative release of 75.41% after 60 min and 94.36% after 120 min. The remaining polyphenols released within 2 hours after replacing the dissolution media with SIF.

At a low pH (pH=1.2), the negatively charged carboxylate groups of sodium alginate began to protonate to form uncharged –COOH groups. This reduced the degree of cross linking due to decreased electrostatic interactions among thae alginate and salep chains within the beads, resulting in increasing fluids uptake and swelling of the beads (Bajpai, 2006). The fast release of polyphenols during the first hour in SGF medium can be explained by the rapid hydration of salep; however, the high swelling and gel-forming ability of salep formed strong and viscous gel gradually which explain the decrease in release during the next hour of dissolution in SGF medium (Karaman, 2010). The slow release pattern at SIF medium could be due to the high viscosity of salep at this pH, compared against pH 1.2 (Razavi, 2014).

**FT-IR Spectroscopy:** FT-IR spectroscopy was used to examine the existence of salep within beads after gelling besides determining the interaction between sodium alginate and salep. The FT-IR spectra of sodium alginate, salep, and calcium alginate/salep beads without polyphenols are shown in Figures.2, 3 and 4. Salep's peaks were obtained at 3380, 1428, and 1069 $cm^{-1}$. Alginate distinct peaks are those of the hydroxyl group at 3305 $cm^{-1}$, carbonyl at 1535 $cm^{-1}$ and stretching of –CH, COO-, -CH and C-O-C between 1040 and 2180 $cm^{-1}$. FT-IR of calcium alginate/salep beads showed in Figure 4 confirmed the complex interaction between salep and sodium alginate polymers during beads formation.

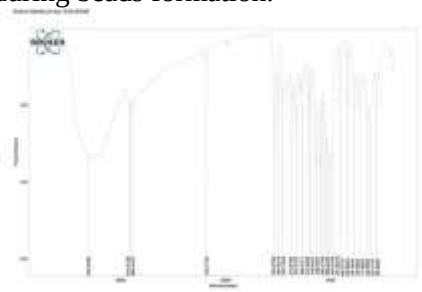

**Figure 2. FT-IR spectra of sodium alginate**

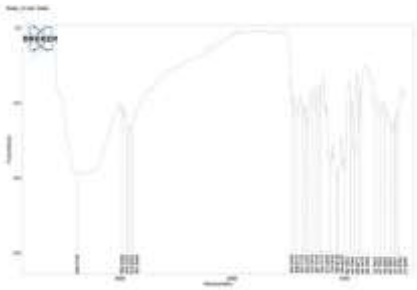

**Figure 3. FT-IR spectra of salep**

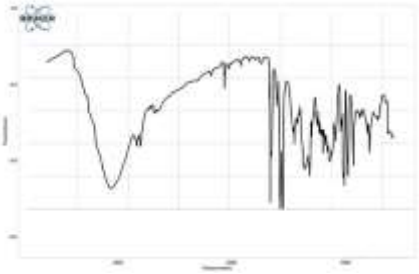

**Figure 4. FT-IR spectra of Calcium alginate/salep beads**

## 4. CONCLUSION

Regarding the results obtained in this study, calcium alginate/salep beads could be a good carrier for polyphenols extracted from olive leaves and have successfully released the encapsulated polyphenols. This method conducted for the preparation of microcapsules containing olive leaves' polyphenol was found to be rapid, simple, and reproducible. The prepared beads containing olive leaves extract will help to produce pharmaceutical and food supplements and can improve the physiological efficiency of polyphenols.

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