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Investigation of the effects of some wall materials and encapsulation techniques on the microencapsulation of olive leaf extract

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ORIGINAL RESEARCH PAPER

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ABSTRACT

In this study, it is aimed to encapsulate some functional components of the olive leaves. Olive leaf extract was encapsulated using solution of sodium alginate, sodium alginate/gelatine, and sodium alginate/agar as wall material by ionic gelation technique. Also, olive leaf extract was encapsulated using solution of gelatine as wall material by cold gelation technique. The viscosities of the coating materials used in the study were investigated. An optimisation process was carried out to determine the injection time to be applied in the ionic gelation technique and the encapsulation efficiencies, particle sizes, swelling ratios, *in vitro* release profiles, and antioxidant activities of the obtained capsules were determined. While the encapsulation efficiency of the capsules obtained by the cold gelation technique was determined as the highest ($98.2 \pm 0.99\%$), it was revealed that the viscosity of the wall material used in the ionic gelation technique was important in the encapsulation efficiency. The particle size and swelling rate of the capsules obtained using the cold gelation technique were the highest. The release rate of oleuropein was generally higher at gastric pH than at intestinal pH. A correlation was found between antioxidant activities and the encapsulation efficiency of capsules.

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1. INTRODUCTION

Olive is one of the most important fruits in Mediterranean countries. Olive leaves, one of the by-products of the olive tree, are present on the olive tree throughout the year and become available during the pruning of the olive tree, the harvesting of the olives and their conversion into the oil. During the harvesting of the olives, 25 kg of branches and olive leaves are obtained per year for each tree, which is 10% of the total olive weight during processing (Abaza et al., 2015; Dobrinčić et al., 2020; Goncalves et al., 2020). Studies show that olive leaves have antioxidant, antimicrobial, antiviral, hypotensive, hypouricaemic, and hypoglycaemic activities (Silva et al., 2006; Lafka et al., 2013). Flavones, flavan-3-ols (catechin), substituted phenols, and oleuropein are the major phenolic compounds present in olive leaves (Abaza et al., 2015). Oleuropein, which is abundant in olive leaves, is the main component of olive leaf extract and is responsible for the bitter taste and pungent aroma of olive oil (Ahamad et al., 2019; Essafi et al., 2019). Oleuropein is used as a commercial food supplement in Mediterranean countries due to its hypocholesterolemic, hypo-glycaemic activities and pharmacological properties such as antioxidant, anti-inflammatory, antiatherogenic, anti-cancer, antimicrobial, and antiviral (Silva et al., 2006; Omar, 2010). Due to the low stability of phenolic compounds, their activities during processing, distribution, storage and consumption and their bioavailabilities in the gastrointestinal digestion (pH, enzymes, other components) decrease. For this purpose, today, various encapsulation techniques provide stabilisation of phenolic compounds (Munin and Edwards-Levy, 2011).

In this study, microencapsulation of olive leaf extract was aimed using different wall materials and different encapsulation techniques. In this context, it was aimed to determine the effect of the viscosities of the coating materials used in the study, the injection time to be applied in the ionic gelation technique, and some physicochemical properties such as encapsulation efficiency, particle size, swelling rate, *in vitro* release profile, and antioxidant capacity.

2. MATERIALS AND METHODS

2.1. Materials

Olive leaf extract (OLE), obtained in the study of Oral (2017), stored at 4 °C and having a 13.58% oleuropein content, was used as the raw material in the study. Sodium alginate, agar, 2,2-diphenyl-1-picrylhydrazyl (DPPH), methanol, and calcium chloride were obtained from Sigma-Aldrich (St Louis, USA), gelatine was obtained from Smart Chemistry Company (İzmir, Turkey). Sunflower oil from a commercial brand (Yudum, Turkey) was used.

2.2. Microencapsulation

OLE was encapsulated using two different encapsulation techniques (ionic gelation and cold gelation) Figure 1.

2.2.1. Microencapsulation by ionic gelation (IG) technique. Within the scope of this technique, OLE was added to aqueous solutions containing sodium alginate (2% w/v) (SA), sodium alginate (2% w/v)/gelatine (5% w/v) (SAG), sodium alginate (2% w/v)/agar (1.5% w/v) (SAA), and sodium alginate (2% w/v)/gelatine (5% w/v)/agar (1.5% w/v) (SAGA) in a proportion
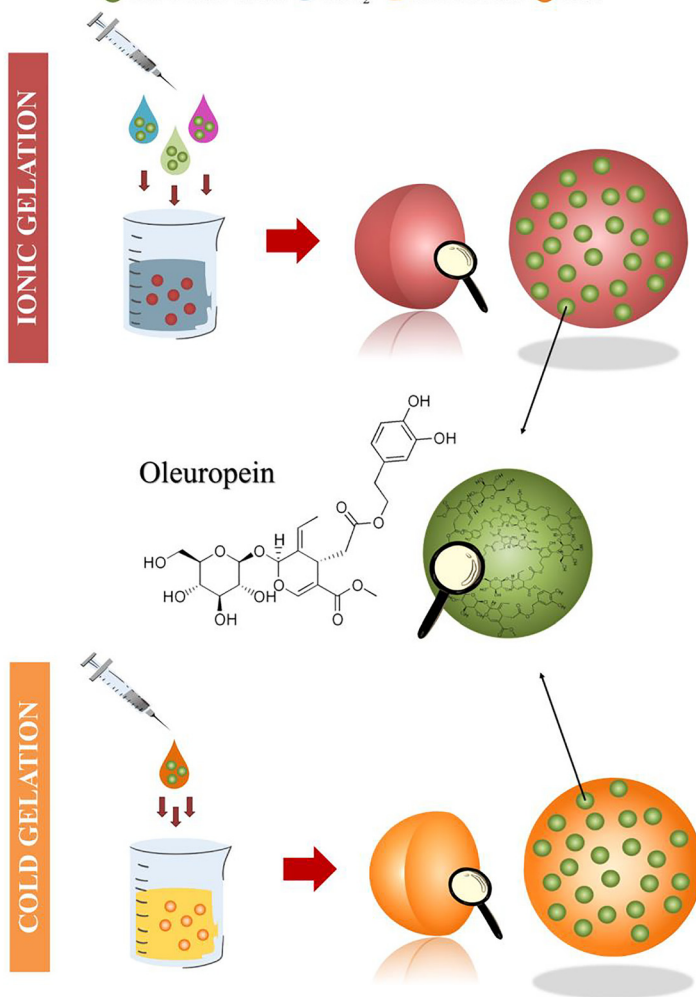
Fig. 1. Illustration of two different encapsulation techniques used in the study

corresponding to 10% of the total dry matter. The microencapsulation process was performed according to Oral's (2017) method with slight modifications. OLE-containing wall material solution was dripped into $CaCl_2$ solution (2% w/v) at 4 °C using a Goldman/AR-03 syringe pump (Biasis, Turkey). The injection times applied in the encapsulation process were determined as 2, 5, and 10 min, with a flow rate of 2 mL. The obtained capsules were dried in a fume hood for 12 h. As a result of the preliminary studies, the injection time with the highest encapsulation efficiency was chosen and applied in all encapsulation processes performed by the IG technique.

2.2.2. Microencapsulation by cold gelation (CG) technique. OLE was added to aqueous solutions containing gelatine (5% w/v) (G), agar (1.5% w/v) (A), and gelatine (5% w/v)/agar (1.5% w/v) (GA) in a proportion corresponding to 10% of the total dry matter. The microencapsulation process was performed according to Oral (2017).

2.3. Rheological measurements

Rheological measurements of the coating materials used in the encapsulation process were carried out using a rheometer (Anton Paar-MCR 302) equipped with a cone plate (CP25-2). The shear rate was increased logarithmically from 1 to 100 s^{-1}. All measurements were carried out at 25 °C. Apparent viscosities of coating solutions at 50 s^{-1} shear rate were recorded.

2.4. Encapsulation efficiency (EE), particle size, swelling rate, and *in vitro* release analysis

These analyses were carried out according to Oral (2017). The oleuropein release of the beads was measured as a function of time. The hyperbolic (Karaca et al., 2021) equation was used in the modelling of oleuropein release kinetics (Equation 1).

Hyperbolic equation:

$$\text{Oleuropein release (\%)} = \frac{(a * t)}{(b + t)} \tag{1}$$

a and b: the model coefficients, t: time (min).

2.5. DPPH antioxidant activity determination

Izli (2017) method was used in the determination of antioxidant activity by DPPH.

2.6. Statistical analysis

Statistical analysis of the data obtained in the study was carried out using the R Statistics program (version 3.6.0). One-way analysis of variance (ANOVA) was used to compare the encapsulation efficiency and swelling rate of capsules obtained by using different encapsulation techniques and different coating materials. Two-way ANOVA was used to compare release rates. In the ANOVA analysis, the differences between the means were evaluated with Tukey's HSD test at a 0.05 significance level. Kruskal–Wallis test was used to compare capsule volume and antioxidant activities. *t*-tests were used to compare the effects of different injection times used in the IG technique on the EE. The Pearson correlation test was used to evaluate the relationships between antioxidant activity and EE.

3. RESULTS AND DISCUSSION

G had the lowest apparent viscosity (6.67 ± 0.26 mPa s) followed by SA (27.61 ± 0.42 mPa s), SAG (97.74 ± 1.37 mPa s), and SAA (386.01 ± 20.68 mPa s).

In the preliminary analysis, to determine the injection time to be used in the IG technique, capsules were produced using three different injection times. The EEs of the capsules produced

using 2, 5, and 10 min injection times were 52.2 ± 2.01, 47.1 ± 1.20, and 42.6 ± 1.25%, respectively. It was determined that the EE decreases as the injection time increases and the highest EE was achieved with a flow rate of 2 mL min^{-1} and 2 min injection time. The decrease in EE as the injection time increases could be explained by the diffusion of the core material during the curing time in the CaCl$_2$ solution.

Encapsulation of OLE by IG technique was performed at a 2 mL min^{-1} flow rate using SA, SAG, SAA, and SAGA coating solutions. Encapsulation did not occur when the SAGA coating solution was used. This could be explained by the fact that the curing time of the droplets in the CaCl$_2$ solution was insufficient to ensure the stability of the capsule and the sodium alginate concentration remained low compared to the total gelatine and agar concentration (steric hindrance).

Encapsulation of OLE by CG technique was performed using G, A, and GA coating solutions. Capsule formation did not occur when A and GA coating solutions were used. Failure of capsule formation at the use of A coating material could be elucidated by the insufficient agar concentration to ensure capsule stability. When GA coating solution is used, it is thought that no encapsulation occur because the use of two materials together causes clustering (Erge and Zorba, 2018).

The EE of microcapsules obtained by IG and CG techniques was analysed by monitoring the oleuropein (a major phenolic compound in OLE) content. The results of the EE are given in Table 1. The EE analysis shows the encapsulation rate of oleuropein in OLE. Among the encapsulation techniques used in the study, the highest EE with 98.2% was obtained by the CG technique using G as the coating material. Capsules obtained with the IG technique by Gouin (2004) were stated to have a porous structure (Gouin, 2004). It could be interpreted by the fact that oleuropein is a hydrophilic compound, which diffuses in the CaCl$_2$ aqueous solution used in the IG technique and reduces the efficiency of the capsule (Demircan and Oral, 2023). The high efficiency of the CG technique may be due to the very limited penetration of the hydrophilic core material into the oil. In encapsulation processes using the IG technique, the lowest EE was obtained when using a SA coating solution with a ratio of 48.7%. The EE analysis results showed that the use of a second coating material in addition to SA increases the EE. When gelatine was used as the second coating material, the EE was higher than that of the agar. This may be due to the lower dry matter ratio in the coating solutions A. In addition, considering that syneresis, which is defined as the separation of water from the gel structure (Erge and Zorba, 2018), occurs in agar but not in gelatine, the core material may leak out with the aqueous phase and reduce the EE. In a study of IG, it was reported that the EE in the first drop and the last drop falling in the CaCl$_2$ solution (since it was exposed to different curing times) was not the

Table 1. Particle diameters and encapsulation efficiencies of beads

Encapsulation technique	Coating material	PD (mm)	EE (%)
Cold gelation	G	1.52 ± 0.20[a]	98.2 ± 0.99[a]
Ionic gelation	SA	1.03 ± 0.10[d]	48.7 ± 1.26[d]
Ionic gelation	SAG	1.37 ± 0.12[b]	61.5 ± 0.95[b]
Ionic gelation	SAA	1.12 ± 0.11[c]	55.7 ± 0.60[c]

Data are expressed as mean ± standard deviation. G: Gelatine; SA: Sodium alginate; SAG: Sodium alginate/Gelatine; SAA: Sodium alginate/Agar; PD: Particle diameter; EE: Encapsulation efficiency. [a–d]: Means within the same column with different letters are significantly different at $P < 0.05$

same, and the EE of the first drop and waiting in the $CaCl_2$ solution was lower. For this reason, it has been commented that the capsules obtained with this technique are heterogeneous in terms of EE (Oral, 2017).

Light microscopy images of microcapsules obtained by using different encapsulation techniques and different coating materials are given in Fig. 2. Particle diameter (PD) results of the obtained capsules are given in Table 1. In the study, the PD of the G capsules obtained by the CG technique was determined as the highest. The lowest PD was obtained in SA capsules produced using the IG technique ($P < 0.05$). It has been found that the use of additional coating material in capsules produced by the IG technique increased the particle size. The same result was found in the study of Demircan and Oral (2023). In addition, it has been determined that gelatine, as the second coating material, formed a larger-sized capsule than agar. This situation can be explained by the fact that the aqueous phase in the agar gel matrix leaks out during the drying stage and reduces the particle size, since the syneresis phenomenon is not present in gelatine, but it is highly characteristic of agar, as stated in the study of Erge and Zorba (2018).

The results of the swelling rate analysis of microcapsules obtained by using different encapsulation techniques and different coating materials are shown in Fig. 3. In the study, the highest swelling rate was seen in the microcapsules obtained by the CG technique. The swelling rate in the microcapsules obtained by the IG technique was quite limited. This suggests that the cross-linked calcium-alginate beads formed as a result of a chemical reaction between the
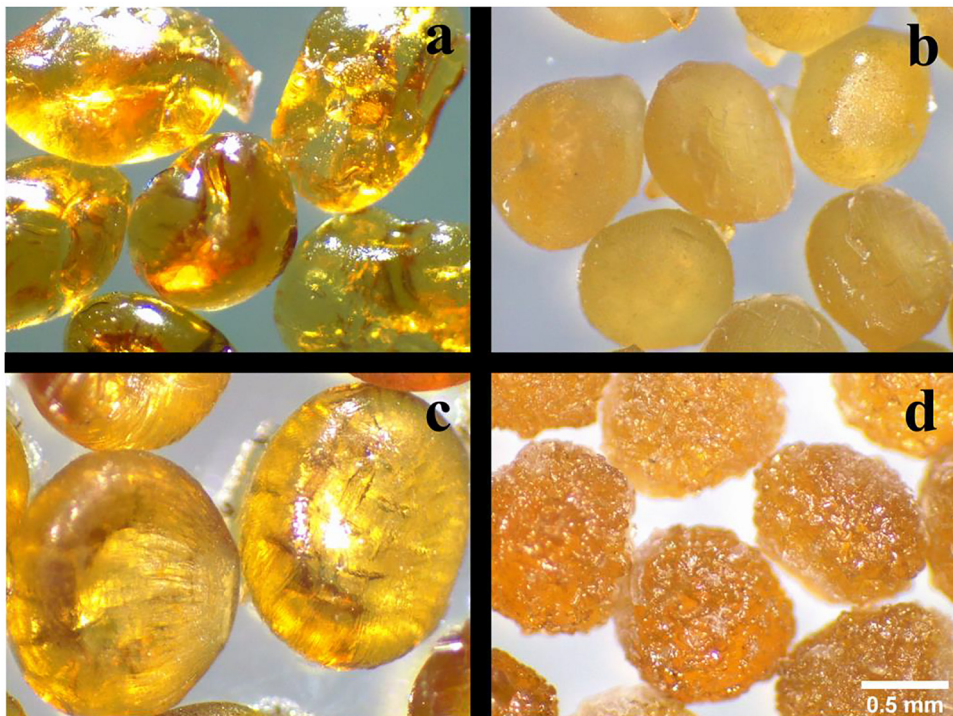

Fig. 2. Microscopic images of beads at 35× magnification: (a) G, (b) SA, (c) SAG, and (d) SAA beads
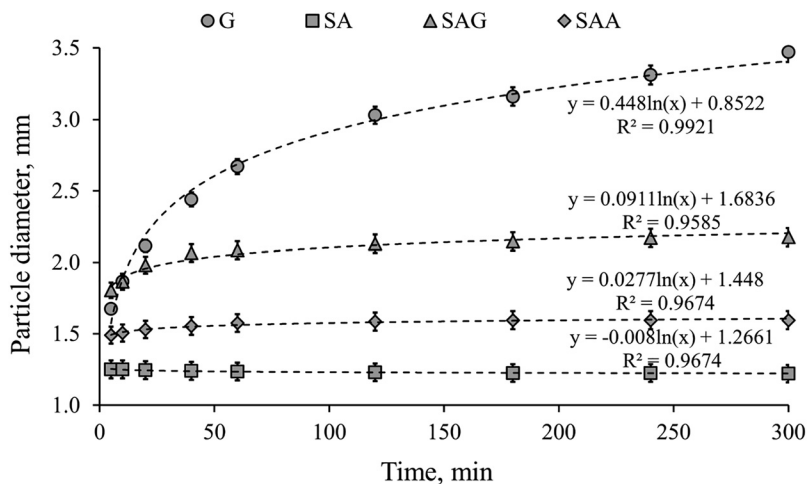
Fig. 3. Swelling rate profile of beads in pure water. G: Gelatine; SA: Sodium alginate; SAG: Sodium alginate/
Gelatine; SAA: Sodium alginate/Agar

sodium alginate and Ca are resistant to swelling. In addition, it is thought that another reason for the limited swelling in microcapsules may be related to the porous structure of the capsules obtained by the IG technique as stated by Gouin (2004). The highest swelling rate in micro-capsules obtained by using different coating materials by IG technique was obtained in the application, where gelatine was used as the second coating material.

The aim of encapsulation is to protect the active ingredients from the negative effects of environmental factors, thereby keeping the bioavailability of the active ingredients at the highest level (Munin and Edwards-Levy, 2011). For this purpose, it is necessary to increase the bioavailability of the active ingredient by reaching the small intestine without being affected by acidic stomach conditions. In the *in vitro* release study, the release of the capsule contents into fluids that simulated the gastric and intestinal environment without the enzymes was carried out. The change in the oleuropein concentrations of the simulated stomach (pH 1.1) and intestinal (pH 7.4) fluids as a function of time is shown in Fig. 4. It was seen that microcapsules with 50 mg L^{-1} oleuropein content obtained by using G coating solution by CG technique released 73.06% of the capsule contents in 0–12 min under the stomach environment. This showed that the capsules obtained by the CG technique released most of the capsule contents before they reached the intestine as their structure was disrupted under the stomach environment. In the same study, in cases where SA, SAG, and SAA were used as coating materials for the capsules obtained by the IG technique, 38.04, 33.98, and 31.12% of the capsule contents were released, respectively. The release rates indicated that the use of a second coating material for the IG technique reduced release to the stomach environment. When the release rates in the intestinal conditions were examined, the release ratio was 80.82% for the capsules obtained using G coating material by CG technique. For SA, SAG, and SAA beads, release ratios were, 41.00%, 34.62%, and 32.16%, respectively.

Oleuropein release data were fitted with the hyperbolic equations (Equation 1). Table 2 lists the model parameters with standard errors along with the statistical criteria values (adjusted R^2,
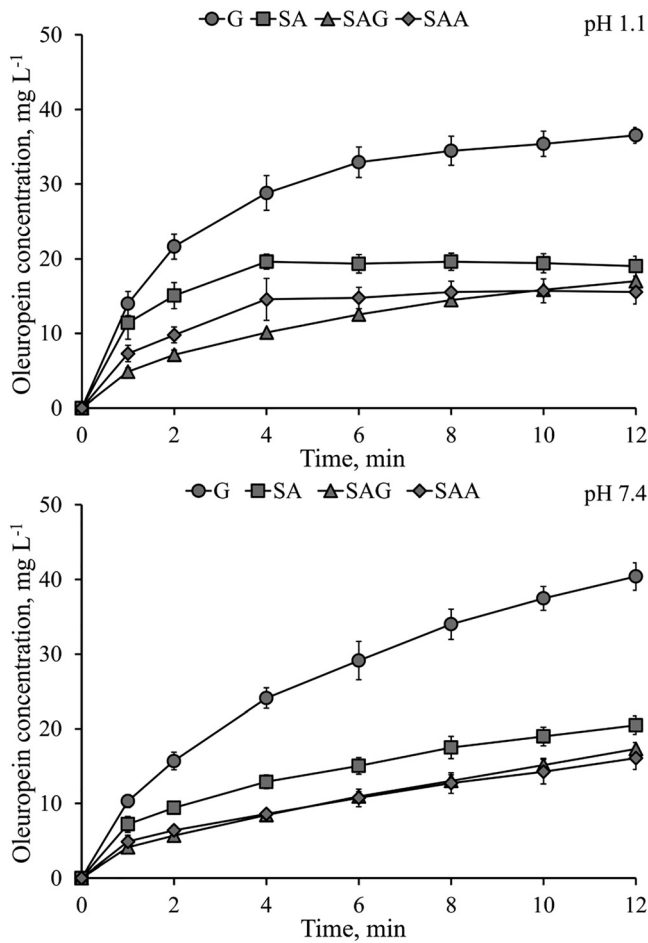
Fig. 4. Oleuropein release rates of beads at different pH values. Data are expressed as mean ± standard deviation. G: Gelatine; SA: Sodium alginate; SAG: Sodium alginate/Gelatine; SAA: Sodium alginate/Agar

root mean square error (RMSE)). For each equation, high adjusted R^2 and low RMSE values were obtained. It was determined that the capsules with the highest release rate in the first minute in the gastric (pH 1.1) and intestinal (pH 7.4) mediums were the capsules obtained by using G coating solution by CG technique. It has been revealed that the release was higher at intestinal pH than at gastric pH. Among the microcapsules obtained by the IG technique, the highest oleuropein release was obtained when SAG coating material was used followed by SA and SAA.

The antioxidant activity values of microcapsules determined by the DPPH method are given in Fig. 5. The highest antioxidant activity value (20.26 ± 2.75%) was observed in capsules obtained using G coating solution by CG technique. There was a relatively high correlation between antioxidant activity and EE ($r = 0.984$).

Table 2. Parameters $\pm$ standard errors of the fit of the hyperbolic (Equation 1) equation together with adjusted R^2 and RMSE values

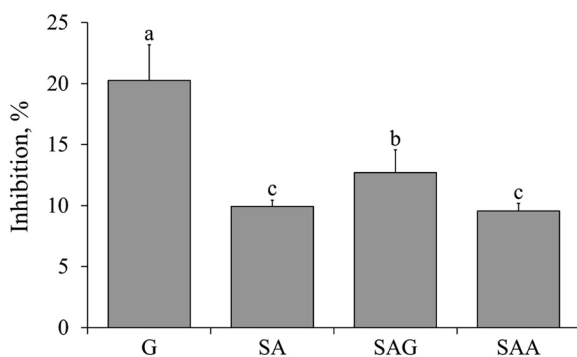| Medium	Model values	Beads			
		G	SA	SAG	SAA
Stomach	a	42.84 $\pm$ 0.46	21.41 $\pm$ 0.70	23.18 $\pm$ 1.12	18.14 $\pm$ 0.65
	b	1.97 $\pm$ 0.08	0.79 $\pm$ 0.15	4.73 $\pm$ 0.56	1.45 $\pm$ 0.22
	Adjusted R^2	0.9991	0.9826	0.9938	0.9865
	RMSE	0.3868	0.9098	0.4647	0.6552
Intestinal	a	57.75 $\pm$ 2.24	25.13 $\pm$ 1.54	29.77 $\pm$ 3.88	22.07 $\pm$ 2.43
	b	5.47 $\pm$ 0.49	3.38 $\pm$ 0.60	9.55 $\pm$ 2.29	5.46 $\pm$ 1.40
	Adjusted R^2	0.9968	0.9838	0.9847	0.9732
	RMSE	0.8014	0.8751	0.7280	0.8732

Fig. 5. DPPH radical scavenging activity of beads. G: Gelatine; SA: Sodium alginate; SAG: Sodium alginate/Gelatine; SAA: Sodium alginate/Agar

4. CONCLUSIONS

The encapsulation technique and coating material selection were found to be effective on encapsulation efficiency, particle size, swelling rate, *in vitro* release profile, and antioxidant capacity. It has been observed that the flow rate and injection time applied in the ionic gelation technique had a significant effect on the encapsulation efficiency. Where the core material is easily soluble in water, capsulation processes with a high flow rate and low injection time can be recommended to obtain high encapsulation efficiency capsules. In the encapsulation of water-soluble core materials, much higher encapsulation efficiency can be obtained by the cold gelation technique compared to the ionic gelation technique. Choosing the right coating material in the ionic gelation technique is important for encapsulation efficiency. The type/combination/viscosities of coating materials directly affect the particle size, swelling rate, release profile, and encapsulation efficiency (hence their antioxidant properties). Within the framework of these data, it is thought that olive leaf extract microcapsules can shed light on pilot and laboratory scale productions and contribute to the development of the product spectrum by using it as a food supplement.

Declaration of competing interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

ACKNOWLEDGMENT

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