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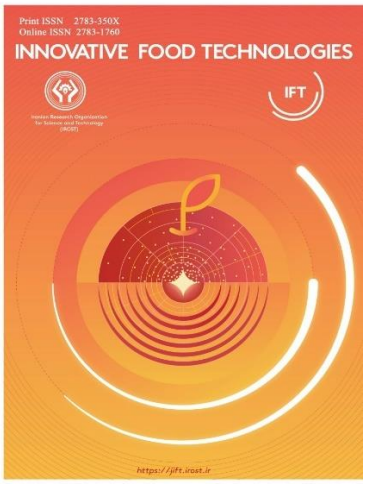

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DOI: https://doi.org/ 10.22104/ift.2025.7604.2213

To appear in: Innovative Food Technologies (IFT)

Received Date: 4 May 2025
Revised Date: 30 June 2025
Accepted Date: 12 July 2025

Please cite this article as: Somayeh Rahimi and Negin Movahedipour, Fabrication of alginate microcapsules carrying saffron extract-loaded by extrusion method, *Innovative Food Technologies (2025)*, doi: https://doi.org/ 10.22104/ift.2025.7604.2213

Fabrication of alginate microcapsules carrying saffron extract-loaded by extrusion method

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Abstract

Saffron is one of the most valuable and expensive spices used to enhance the color, aroma, and flavor of various food products. A considerable portion of the active components of saffron (crocin (color), safranal (aroma), and picrocrocin (taste)) are destroyed during storage due to their volatility or sensitivity to environmental conditions. In this study, saffron aqueous extract was encapsulated via the extrusion method using alginate gum (wall material) at 4 and 6% (w/w), calcium chloride at 0.5, 1, and 1.5 M, and sodium alginate:calcium chloride ratios of 1:1, 1:2, and 1:4 (v/v). Subsequently, encapsulation efficiency, bead size, stiffness, and SEM imaging were evaluated. The effects of all independent variables were statistically significant (p<0.05), and the highest encapsulation efficiency of 99.80±0.14% (for crocin at day 0) was obtained in gel beads prepared with the lowest calcium chloride concentration and the lowest sodium alginate:calcium chloride volume ratio. All beads were spherically shaped with a smooth surface and had a mean size of about 0.11±0.01 to 0.29±0.01 mm. The result of stiffness showed that higher concentrations of sodium alginate (6%) increased the strength of the bead (1.2 N). Therefore, samples prepared with 6% w/w sodium alginate, 0.5 M calcium chloride, and a 1:1 volume ratio of wall were recognized as the best ones.

Keywords: Saffron, Encapsulation, Extrusion, Alginate bead.

1. Introduction

Crocus sativus L., commonly known as saffron, is a perennial stemless plant [1]. Iran is the primary producer, accounting for over 90% of global saffron production [2]. The stigmas of the flower, which are harvested and dried, contain the main bioactive compounds of saffron. The applicable portion of the plant is its stigmas, which are rich in saffron's active components. Saffron is usually used at levels of 1–260 ppm in a wide range of culinary, baked goods, and confectioneries, as well as in alcoholic and non-alcoholic beverages [3]. Moreover, saffron has many health benefits and is used in traditional medicine to treat many illnesses; recent reports have demonstrated various pharmacological properties, including anti-cancer, anti-inflammatory, antidepressant, and antioxidant effects [4, 5]. The main active components of saffron are crocin (soluble carotenoid pigment), picrocrocin (bitter glycoside), and safranal (saffron's aromatic substance) and are responsible for the color, aroma, and taste of saffron, respectively. These components are highly unsaturated and may be affected by temperature, light, oxygen, pH, enzymes, which makes them vulnerable to degradation due to oxidation, isomerization, and other environmental factors, which can lead to losses in their beneficial characteristics and/or nutritive values [3, 6, 7]. To protect saffron's active components against environmental effects, they can be surrounded by a wrap in a process called encapsulation. Encapsulation is a process in which the active agents are entrapped in a wall to preserve the stability of active agents during processing and/or storage [8]. Encapsulation is categorized as macro (>5000 μm), micro (0.2–5000 μm), and nano (<0.2 μm or 2000 Å) according to the size of the finished capsules. Encapsulation has broad application in various industries such as pharmaceutical, food, cosmetic, etc. [9].

Three parameters to consider when choosing an encapsulation wall or process are the application, economics, and safety [10]. Alginate is most commonly used as a wall for encapsulation because it satisfies all three parameters. Alginate is a linear anionic polysaccharide composed of (1→4) linked α-L-guluronic acid and β-D-mannuronic acid residues, which form ionotropic gels in the presence of divalent cations [11]. Researchers explain the gelling mechanism by the "egg-box" model in which divalent cations bind in the interchain cavities to create rod-like cross-linked complexes [12]. This encapsulation technique is called extrusion, and sodium alginate and calcium-chloride are the most commonly used wall material and divalent cation. This technique does not need high temperatures or the use of organic solvents. The loaded alginate beads containing the active agents can be used for a controlled release of the core [1].

The extrusion method is currently used to encapsulate many agents such as probiotics [13], D-limonene [14], whey protein [15], β-carotene [16], gallic acid [17], lactoferrin [18], lycopene [19], etc.; however, to the best of our knowledge, there are no published studies on the encapsulation of saffron extract using this method. Hence, this study aimed to encapsulate the active components of saffron extract in alginate gel beads in order to increase shelf life, facilitate storage, and improve quality. The effect of sodium alginate (4 and 6% w/w), calcium chloride (0.5, 1, 1.5 M), and the wall:salt volume ratio of 1:1, 1:2, and 1:4 v/v were studied for encapsulation efficiency, size, stiffness, and shape of the gel beads.

2. Materials and methods

Dried saffron stigmas were purchased from a certified supplier in Mashhad, Iran. Sodium alginate (MW ~ 40 kDa) and calcium chloride were obtained from Sigma Aldrich (USA) and Merck (Germany), respectively.

2.1. Preparation of saffron aqueous extract

First, the saffron stigmas were milled to obtain a fine powder, and then 1 g was mixed with 100 ml of distilled water. They were placed in a beaker wrapped in aluminum foil to prevent light exposure. The mixture was stirred for at least 3 hours at 250 rpm at room temperature. When the saffron powder became completely colorless, it was filtered, and the pH and moisture of saffron aqueous extract were measured immediately. Finally, the aqueous extract was stored in the dark in a refrigerator [3]. The main active components of saffron, i.e., the crocin, safranal, and picrocrocin, were measured based on the changes in optical density at 200–700 nm. For this purpose, 500 mg of the sample was dispersed in 1000 ml distilled water and stirred for 1 hour at 1000 rpm in a dark place. Then, distilled water was added to 20 ml of the solution to reach 200 ml. This solution was filtered, and its absorption was read at the max wavelength of the active components (440 nm for crocin, 330 nm for safranal, and 257 nm for picrocrocin) by a UV–Vis spectrophotometer (Unico, South Korea). The amount of the compounds was calculated using equation (1),

$$A_{1\,cm}^{1\%}\,(\lambda_{max}) = \frac{\mathrm{D}\times 10000}{\mathrm{m}\times(100-\mathrm{H})} \qquad (1)$$

where *D*, *m*, and *H* are the absorption rate, weight, and moisture of the initial sample, respectively. Afterward, the following equation $A_{1\,cm}^{1\%}\,(\lambda_{max})$ was used to determine the amount of the component based on its absorption at its max wavelength in 1% w/w of the sample [2].

2.2. Encapsulation by extrusion method

Sodium alginate was dispersed in distilled water and stirred thoroughly at 600 rpm to prepare it for the wall material at the desired concentrations of 4 and 6% (w/w). The dispersion was left overnight to complete the hydration of the gum [10]. The encapsulation process was done by the extrusion method based on the ionotropic gelation process. For this purpose, the mixed solution containing an equal volume of saffron extract (1% w/v) and sodium alginate solution was pressed through a syringe with an internal diameter of 0.4 mm and dropped in $CaCl_2$ solution (0.5, 1, 1.5 M) that was shaking at 150 rpm. To obtain spherical beads, the distance of the syringe was fixed at 15 cm from the surface layer of the salt solution. To study the effects of the wall or salt on the characteristics of the beads, the ratio of wall:$CaCl_2$ was regarded as 1:1, 1:2, and 1:4 (v/v). The beads remained in the salt solution for at least 15 minutes, and lastly, the beads were filtered, washed in distilled water, and dried (40 °C for 2 h) [12, 14].

2.3. Determination of encapsulation efficiency

The encapsulation efficiency (EE%) was calculated for the three main active components of saffron (crocin, safranal, and picrocrocin) using the equation below,

$$EE(\%) = \frac{C_1}{C_2} \times 100 \quad (2)$$

where C_1 and C_2 are the amounts of active components in beads and the initial saffron extract, respectively [20]. For this purpose, the beads were crushed in a mortar and a pestle, and the amount of active saffron components in the beads was calculated according to the previous method for saffron aqueous extract. It should be noted that the alginate did not interfere with the component absorption. The encapsulation efficiency was evaluated at days 0, 15, and 30 to determine the saffron component stability in beads during storage.

2.4. Measurement of bead size

The diameter of the beads was measured using a micrometer (Ukrmetiz, Russia) from at least three angles at room temperature, and finally, the average of particle size was calculated [20].

2.5. Determination of the stiffness

The stiffness of beads was evaluated by a compression test with a texture analyzer (Surrey, UK). A 12 mm cylinder probe was used with a constant speed of 0.2 mm/s [21]. This test was performed for beads with the highest EE%.

2.6. Scanning electron microscopy (SEM)

After coating the samples with a thin layer of gold-palladium, a scanning electron microscope (SEM, Vega2 Tescan, Czech Republic) was used to study the morphological properties of the surface of the beads [3]; also, optical pictures were taken of the beads for more evaluation.

2.7. Statistical analysis

Statistical analysis of the results was carried out by SPSS (version 20) software using one-way ANOVA, and then the significant differences between the means were calculated by Duncan's test (p<0.05). All the experiments were repeated at least 3 times.

3. Results and discussion

The physicochemical properties of the saffron aqueous extract used in this study are presented in Table 1. The amounts of crocin, safranal, and picrocrocin relate to the color, aroma, and taste of saffron, respectively, and are used to evaluate the saffron quality. The quality of saffron, such as its chemical and sensory properties, is strongly influenced by pre and post-harvesting conditions, especially the drying method and temperature used for stigmas drying [22].

Table 1.

The results of the encapsulation efficiency of saffron's active components, i.e., crocin, safranal, and picrocrocin, are reported in Table 2-4, and as can be seen, the effect of the variables was significant for all samples (p<0.05). The highest amount of crocin, safranal, and picrocrocin in the beads, as well as the highest encapsulation efficiency, belonged to the gel beads prepared with the

lowest concentration of calcium chloride (i.e., 0.5 M) and the lowest ratio of sodium alginate calcium chloride (i.e., 1:1 v/v). In contrast, increased wall concentration causes thicker walls, and consequently, less encapsulation efficiency. Some researchers used freeze drying and spray drying methods for saffron extract encapsulation, respectively; their results agree with this study, and they also found that the type of wall material and its concentration has a direct effect on the saffron active components in the beads [23, 24]. In this study, the parameters that affected the gel beads the most were the concentration of calcium chloride and the volume ratio of wall to salt. The highest crocin encapsulation efficiency (99.80±0.14%) was observed on day 0, which is remarkable and shows we selected the correct method for saffron encapsulation. The maximum amount of crocin encapsulation efficiency reported by Sharayei et al. (2014) was 78.70%, using Polyvinylpyrrolidone as the wall material [23].

Table 2.

Table 3.

Table 4.

The encapsulation efficiency was slightly diminished in storage up to 30 days due to the gradual degradation of the active components. This result is similar to those of Jafari et al. (2018), which illustrated that crocin content decreased in storage up to 54 days because crocin decomposes to free crocetin and monoesters over time [2].

According to Table 5, the bead size ranged from 0.11±0.01 to 0.29±0.01 mm. It was demonstrated that by increasing the concentration of sodium alginate and calcium chloride, as well as volume ratio, the beads became bigger, which can be attributed to the increase of alginate polymeric chains, and consequently, an increase in active sites that can bind to the calcium ions. Laos et al. (2007) used the extrusion method and a 2mm diameter syringe to encapsulate β –carotene in furcellaran walls resulting in a bead size ranging from 4.49±0.2 to 4.65±0.2 mm [21].

Table 5.

High mechanical strength is desirable for practical application of microcapsules during processing and storage [21]. The stiffness measurement was carried out for the best samples with the highest encapsulation efficiency (i.e., the beads prepared with the lowest concentration of salt and also the lowest volume ratio of wall:salt), which were 1.2 and 0.7 N for the beads prepared with 6 and 4% (w/w) sodium alginate, respectively. It was found that the sodium alginate concentration plays a direct role in increasing the strength of the bead wall, which is in agreement with Laos et al. (2007) [21].

As seen in Fig. 1, the gel beads were spherical and preserved their shape during storage. According to the SEM images, the outer layer of the beads was smooth but had some bumps and dents due to mechanical stresses caused by drying.

Fig. 1

4. Conclusion

In this study, the encapsulation technique was used to protect saffron's active components against environmental conditions. The alginate gel beads were prepared by the extrusion method for the first time, and the stability of saffron's active components was evaluated over 30 days storage. The results

showed that the highest amount of encapsulation efficiency for the crocin, safranal, and picrocrocin was observed at the lowest concentration of calcium chloride (0.5 M) and a volume ratio of sodium alginate:calcium chloride of 1:1 v/v. The gel beads were spherical and smooth with high stiffness, which improves their usability as additive in food products such as ice cream, jelly, drinks, etc., and provides good stability against environmental conditions during storage. The evaluation of the stability of gel beads containing saffron's active components in a real food matrix could be suggested for future research.

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Table 1. Some properties of the prepared aqueous saffron extract (%1 w/w)

Properties	Mean±SD
Moisture [% w/w]	91.86±0.80
pH	5.07±0.01
Crocin [Absorption of solution (1% w/w) at 440 nm based on dry matter]	198.71±0.36
Safranal [Absorption of solution (1% w/w) at 330 nm based on dry matter]	54.47±0.80
Picrocrocin [Absorption of solution (1% w/w) at 257 nm based on dry matter]	96.28±0.57

Table 2. Crocin EE% during 30 days of storage (75% relative humidity and room temperature)

Na-Alginate [%]	$CaCl_2$ [M]	Na-Alginate: $CaCl_2$ [v/v]	Crocin EE [%]		
			0 (d)	15 (d)	30 (d)
4	0.5	1:1	99.80 ± 0.14^{q}	92.76 ± 0.32^{r}	84.20 ± 0.32^{p}
		1:2	82.28 ± 0.12^{n}	75.43 ± 0.12^{p}	70.73 ± 0.24^{n}
		1:4	45.77 ± 0.21^{j}	42.28 ± 0.12^{j}	39.99 ± 0.21^{k}
	1	1:1	83.28 ± 0.24^{o}	73.72 ± 0.12^{o}	58.82 ± 0.21^{m}
		1:2	42.06 ± 0.12^{h}	37.21 ± 0.21^{h}	36.78 ± 0.21^{i}
		1:4	36.07 ± 0.12^{f}	32.01 ± 0.12^{f}	26.87 ± 0.32^{f}
	1.5	1:1	44.42 ± 0.12^{i}	41.42 ± 0.12^{i}	37.00 ± 0.21^{ij}
		1:2	32.15 ± 0.33^{d}	28.44 ± 0.21^{d}	26.59 ± 0.12^{f}
		1:4	22.17 ± 0.12^{c}	20.67 ± 0.12^{c}	19.60 ± 0.12^{c}
6	0.5	1:1	95.47 ± 0.12^{p}	89.48 ± 0.12^{q}	80.07 ± 0.12^{o}
		1:2	62.81 ± 0.12^{m}	59.96 ± 0.12^{n}	49.89 ± 0.09^{l}
		1:4	38.57 ± 0.12^{g}	36.28 ± 0.12^{g}	25.30 ± 0.12^{e}
	1	1:1	45.70 ± 0.12^{j}	44.13 ± 0.12^{l}	36.43 ± 0.12^{h}
		1:2	32.79 ± 0.12^{e}	31.51 ± 0.12^{e}	24.76 ± 0.15^{d}
		1:4	20.88 ± 0.12^{a}	19.74 ± 0.12^{b}	15.39 ± 0.21^{a}
	1.5	1:1	49.12 ± 0.12^{l}	44.56 ± 0.12^{m}	37.29 ± 0.12^{j}
		1:2	46.70 ± 0.12^{k}	42.56 ± 0.21^{k}	31.93 ± 0.12^{g}
		1:4	21.17 ± 0.21^{b}	19.45 ± 0.21^{a}	18.89 ± 0.12^{b}

Different letters in each column show significant differences at a 95% confidence level ($p<0.05$).

Table 3. Safranal EE% during 30 days of storage (75% relative humidity and room temperature)

Na-Alginate [%]	$CaCl_2$ [M]	Na-Alginate: $CaCl_2$ [v/v]	Safranal EE [%]		
			0 (d)	15 (d)	30 (d)
4	0.5	1:1	92.58±1.18^{n}	87.12±1.19^{l}	81.66±0.45^{o}
		1:2	73.86±0.90^{k}	64.50±0.45^{i}	61.37±0.45^{l}
		1:4	44.20±0.44^{g}	40.30±1.18^{d}	39.78±0.78^{j}
	1	1:1	81.40±0.45^{l}	67.62±0.45^{j}	62.93±0.45^{m}
		1:2	40.88±1.18^{f}	39.00±0.78^{d}	37.18±0.45^{i}
		1:4	38.48±0.45^{e}	34.06±1.18^{c}	31.46±0.45^{f}
	1.5	1:1	54.87±1.18^{i}	49.93±0.78^{g}	48.37±0.79^{k}
		1:2	39.78±0.78^{f}	33.54±0.78^{c}	32.76±0.78^{g}
		1:4	29.11±0.45^{c}	24.17±0.78^{b}	24.43±0.45^{d}
6	0.5	1:1	83.74±0.44^{m}	76.20±0.44^{k}	68.40±0.44^{n}
		1:2	63.19±0.78^{j}	57.73±0.78^{h}	48.11±1.19^{k}
		1:4	34.58±0.44^{d}	33.02±1.19^{c}	21.57±1.18^{c}
	1	1:1	48.89±0.45^{h}	46.29±0.90^{f}	38.22±0.78^{i}
		1:2	35.14±0.73^{d}	34.32±0.78^{c}	28.33±0.44^{e}
		1:4	19.49±0.78^{a}	16.37±0.78^{a}	16.11±0.44^{a}
	1.5	1:1	44.72±0.45^{g}	43.42±0.45^{e}	35.62±0.45^{h}
		1:2	40.56±0.78^{f}	40.04±0.45^{d}	35.37±0.90^{h}
		1:4	24.69±1.18^{b}	24.17±0.78^{b}	20.27±0.78^{b}

Different letters in each column show significant differences at a 95% confidence level (p<0.05).

Table 4. Picrocrocin EE% during 30 days of storage (75% relative humidity and room temperature)

Na-Alginate [%]	$CaCl_2$ [M]	Na-Alginate: $CaCl_2$ [v/v]	Picrocrocin EE [%]		
			0 (d)	15 (d)	30 (d)
4	0.5	1:1	86.37±0.24^{m}	75.33±0.24^{o}	67.83±0.25^{o}
		1:2	58.41±0.50^{j}	48.70±0.66^{l}	48.13±0.50^{m}
		1:4	34.57±0.25^{fg}	34.41±0.03^{i}	29.28±0.51^{h}
	1	1:1	70.81±1.54^{l}	54.14±0.67^{m}	47.08±0.67^{l}
		1:2	33.81±0.47^{f}	29.58±0.00^{h}	27.51±0.51^{g}
		1:4	32.07±0.25^{e}	26.33±0.25^{e}	25.30±0.51^{f}
	1.5	1:1	40.75±0.25^{h}	36.19±0.44^{j}	34.41±0.03^{j}
		1:2	30.89±0.44^{de}	21.62±0.44^{d}	19.12±0.25^{d}
		1:4	21.77±0.25^{b}	14.85±0.25^{b}	13.09±0.25^{a}
6	0.5	1:1	89.32±0.25^{n}	78.86±0.24^{p}	66.65±0.44^{n}
		1:2	62.39±0.51^{k}	55.17±0.44^{n}	43.40±0.25^{k}
		1:4	28.84±0.91^{c}	27.95±0.25^{g}	16.62±0.25^{c}
	1	1:1	42.66±1.55^{i}	38.54±0.25^{k}	31.04±0.25^{i}
		1:2	29.43±0.25^{cd}	27.06±0.50^{f}	21.92±0.25^{e}
		1:4	16.17±0.24^{a}	13.82±0.25^{a}	12.49±0.25^{a}
	1.5	1:1	35.75±0.44^{g}	35.75±0.44^{j}	26.92±0.44^{q}
		1:2	34.41±0.03^{fg}	28.98±0.67^{h}	25.59±0.43^{f}
		1:4	21.32±0.25^{b}	16.17±0.24^{c}	14.85±0.25^{b}

Different letters in each column show significant differences at a 95% confidence level (p<0.05).

Table 5. The size of gel beads containing saffron aqueous extract

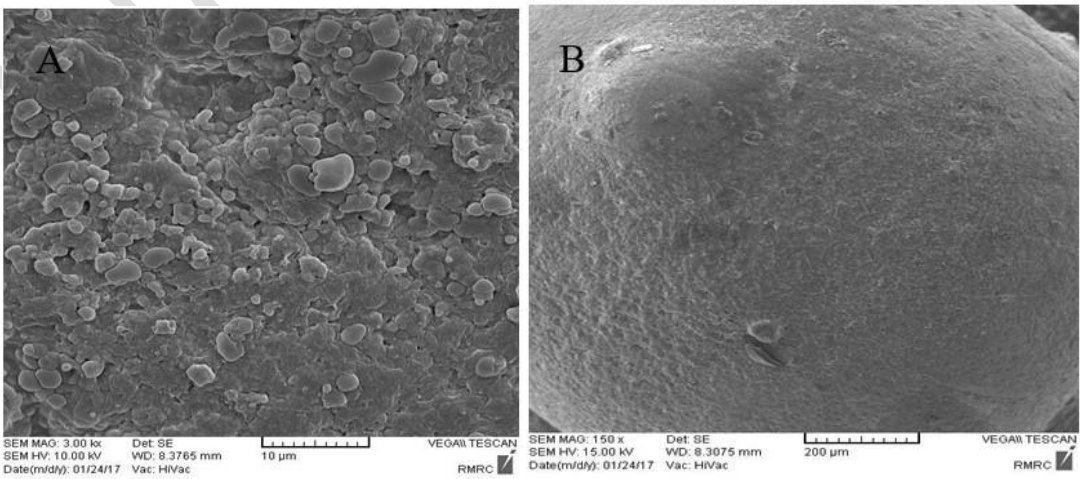
Na-Alginate [%]	$CaCl_2$ [M]	Na-Alginate: $CaCl_2$ [v/v]	Bead size [mm]
4	0.5	1:1	0.11 ± 0.01^{a}
		1:2	0.12 ± 0.011^{b}
		1:4	0.14 ± 0.01^{d}
	1	1:1	0.13 ± 0.01^{c}
		1:2	0.16 ± 0.01^{f}
		1:4	0.17 ± 0.01^{g}
	1.5	1:1	0.19 ± 0.01^{i}
		1:2	0.20 ± 0.01^{j}
		1:4	0.22 ± 0.01^{l}
6	0.5	1:1	0.15 ± 0.01^{e}
		1:2	0.18 ± 0.01^{h}
		1:4	0.21 ± 0.01^{k}
	1	1:1	0.23 ± 0.01^{m}
		1:2	0.24 ± 0.01^{n}
		1:4	0.25 ± 0.01^{o}
	1.5	1:1	0.26 ± 0.01^{p}
		1:2	0.28 ± 0.01^{q}
		1:4	0.29 ± 0.01^{r}

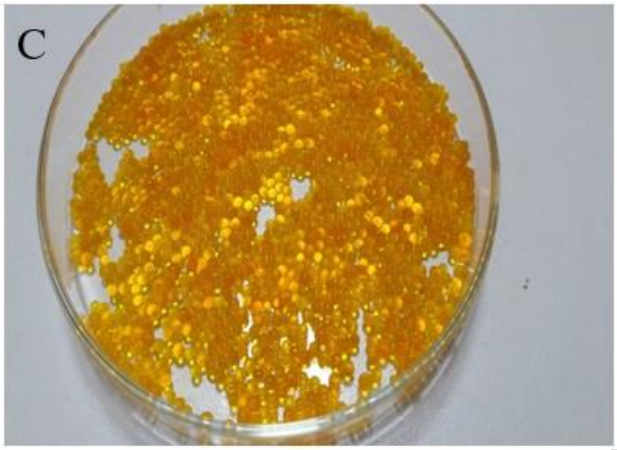
Different letters in each column show significant differences at a 95% confidence level (p<0.05).

Caption of Figures

Fig 1. Images of gel beads prepared with 4% [w/w] sodium alginate, 0.5 [M] calcium chloride, and 1:1 [v/v] taken by A) SEM at 3000x magnifying, B) SEM at 150x magnifying, and C) optical camera.

Figure 1





تهیه میکروکپسول آلژینات حامل عصاره زعفران بارگذاری شده به روش اکستروژن

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چکیده

زعفران یکی از مهم‌ترین و گران‌قیمت‌ترین ادویه‌های مورد مصرف در صنایع غذایی است که برای بهبود رنگ، عطر و طعم بسیاری از محصولات به کار می‌رود. معمولاً، بخش قابل توجهی از ترکیبات مؤثره زعفران (کروسین (رنگ)، سافرانال (عطر) و پیکروکروسین (طعم)) به دلیل فرار بودن و یا حساسیت نسبت به اکسایش، طی مدت زمان نگهداری از بین می‌روند. بدین‌منظور در پژوهش حاضر، ریزپوشانی عصاره آبی زعفران به روش اکستروژن و با کمک صمغ آلژینات (دیواره) در غلظت‌های ۴ و ۶٪، نمک کلرید کلسیم در غلظت‌های ۰/۵، ۱ و ۱/۵ M و هم‌چنین نسبت حجمی آلژینات سدیم: کلرید کلسیم در مقادیر ۱:۱، ۲:۱ و ۴:۱ به منظور حفاظت از ترکیبات مؤثره زعفران انجام شد. در ادامه مقدار ترکیبات مؤثره زعفران، راندمان ریزپوشانی، اندازه ذرات، سفتی بافت و هم‌چنین ساختار میکروسکوپی ریزپوشینه‌ها مورد ارزیابی قرار گرفت. نتایج نشان دادند که اثر تمامی متغیرهای مستقل بر متغیرهای مورد بررسی در سطح اطمینان ۹۵٪ معنی‌دار بوده ($p<۰/۰۵$) و بالاترین میزان راندمان ریزپوشانی برابر با ۹۹/۸۰٪ (برای کروسین در روز صفر) در ریزپوشینه‌های تهیه‌شده با کم‌ترین غلظت کلرید کلسیم و کم‌ترین نسبت حجمی آلژینات سدیم:کلرید کلسیم بدست آمد. تمامی ریزپوشینه‌ها دارای شکل نسبتا کروی و سطحی صاف بوده و میانگین اندازه ذرات آن‌ها در محدوده ۰/۲۹±۰/۰۱ تا ۰/۱۱±۰/۰۱ mm قرار داشت. ارزیابی سفتی بافت نیز نشان داد که غلظت بالاتر آلژینات سدیم (۶٪) می‌تواند استحکام دیواره ریزپوشینه را افزایش دهد (۱/۲ N). بدین‌ترتیب ریزپوشینه تهیه‌شده با آلژینات سدیم با غلظت ۶٪، کلرید کلسیم با غلظت ۰/۵ M و نسبت حجمی آلژینات سدیم:کلرید کلسیم ۱:۱، به عنوان تیمار برتر شناخته شد.

واژه‌های کلیدی: زعفران، انکپسولاسیون، اکستروژن، دانه آلژینات.