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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. However, a significant portion of its active compounds—crocin (color), safranal (aroma), and picrocrocin (taste)—are prone to degradation during storage due to their volatility and 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 on 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 diameter of about 0.11±0.01 to 0.29±0.01 mm. The result of stiffness showed that a higher sodium alginate concentration (6%) increased bead strength, reaching up to 1.2 N. Accordingly, the optimal formulation was identified as beads prepared with 6% (w/w) sodium alginate, 0.5 M calcium chloride, and a 1:1 volume ratio of wall materials.

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1. Introduction

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

When selecting an encapsulation wall material or process, three key parameters to consider are the intended application, economic feasibility, and safety [10]. Alginate is one of the most commonly used wall materials 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 using 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 known as extrusion, with sodium alginate serving as the wall material and calcium chloride as the most commonly used 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 investigated in relation to 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 of the powder was mixed with 100 ml of distilled water. They were placed in a beaker wrapped in aluminum foil to prevent light exposure and stirred at 250 rpm for at least 3 hours at room temperature. When the saffron powder became completely colorless, it was filtered, and the pH and moisture of aqueous saffron extract were measured immediately. Finally, the aqueous extract was stored in the dark at refrigerated conditions [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 of distilled water and stirred for 1 hour at 1000 rpm in the dark. Then, distilled water was added to 20 ml of the solution to reach 200 ml. This solution was filtered, and its absorption was measured at the maximum 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 concentrations of the compounds were calculated using equation (1),

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

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 the max wavelength for a 1% (w/w) sample [2].

2.2. Encapsulation by extrusion method

Sodium alginate was dispersed in distilled water and stirred thoroughly at 600 rpm to prepare the wall materials 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 conducted 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 syringe was positioned at a fixed distance of 15 cm above the surface of the calcium chloride solution. To study the effects of the wall and calcium chloride on bead characteristics, 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. Subsequently, they were filtered, washed with distilled water, and dried at 40 °C for 2 hours [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,

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

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 using a mortar and a pestle, and the amount of active saffron components in the beads was calculated according to the previously described method for saffron aqueous extract. It should be noted that the alginate did not interfere with the component absorption. The encapsulation efficiency was evaluated on 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 at room temperature using a micrometer (Ukrmetiz, Russia) from at least three angles, 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 examine the surface morphology of the beads [3]. Moreover, optical images of the beads were taken for further evaluation.

2.7. Statistical analysis

Statistical analysis was performed using SPSS software (version 20) with one-way ANOVA, followed by Duncan's multiple range test to determine significant differences between means (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- responsible for saffron's color, aroma, and taste- serve as key indicators of 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 during stigmas drying [22].

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

The results of the encapsulation efficiency of saffron's active components, i.e., crocin, safranal, and picrocrocin, are reported in Table 2-4. As shown, the effect of the variables was statistically 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, were observed in the gel beads prepared using the lowest calcium chloride concentration (0.5 M) and the lowest sodium alginate to calcium chloride ratio (i.e., 1:1 v/v). In contrast, an increased wall concentration causes thicker walls and, consequently, less encapsulation efficiency. Some researchers used freeze drying and spray drying methods for saffron extract encapsulation; their results are consistent with those of the present study, indicating that the type and concentration of wall material directly affect the retention of saffron's active components in the beads [23,24]. In this study, the most influential parameters affecting the gel beads were the concentration of calcium chloride and the volume ratio of wall material to salt solution. The highest crocin encapsulation efficiency (99.80±0.14%) was observed on day 0, which is remarkable and shows the effectiveness of the selected method for saffron encapsulation. Sharayei et al. (2014) reported a maximum crocin encapsulation efficiency of 78.70% using polyvinylpyrrolidone as the wall material [23].

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}

Na-Alginate [%]	$CaCl_2$ [M]	Na-Alginate: $CaCl_2$ [v/v]	Safranal EE [%]		
			0 (d)	15 (d)	30 (d)
		1:4	29.11±0.45^{c}	24.17±0.78^{b}	24.43±0.45^{d}
		1:1	83.74±0.44^{m}	76.20±0.44^{k}	68.40±0.44^{n}
	0.5	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	48.89±0.45^{h}	46.29±0.90^{f}	38.22±0.78^{i}
6	1	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}
		1:1	44.72±0.45^{g}	43.42±0.45^{e}	35.62±0.45^{h}
	1.5	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)
		1:1	86.37±0.24^{m}	75.33±0.24^{o}	67.83±0.25^{o}
	0.5	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	70.81±1.54^{l}	54.14±0.67^{m}	47.08±0.67^{l}
4	1	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}
		1:1	40.75±0.25^{h}	36.19±0.44^{j}	34.41±0.03^{j}
	1.5	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}
		1:1	89.32±0.25^{n}	78.86±0.24^{p}	66.65±0.44^{n}
	0.5	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	42.66±1.55^{i}	38.54±0.25^{k}	31.04±0.25^{i}
6	1	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}
		1:1	35.75±0.44^{g}	35.75±0.44^{j}	26.92±0.44^{q}
	1.5	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).

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. An increase in sodium alginate and calcium chloride concentrations, as well as in the volume ratio, resulted in larger bead sizes. This can be attributed to the higher availability of alginate polymer chains, leading to more active sites for binding with calcium ions. Laos et al. (2007) used the extrusion method with a 2mm diameter syringe to encapsulate β –carotene within furcellaran walls, resulting in a bead size ranging from 4.49±0.2 to 4.65±0.2 mm [21].

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]
		1:1	0.11±0.01^{a}
	0.5	1:2	0.12±0.011^{b}
		1:4	0.14±0.01^{d}
		1:1	0.13±0.01^{c}
4	1	1:2	0.16±0.01^{f}
		1:4	0.17±0.01^{g}
		1:1	0.19±0.01^{i}
	1.5	1:2	0.20±0.01^{j}
		1:4	0.22±0.01^{l}
		1:1	0.15±0.01^{e}
6	0.5	1:2	0.18±0.01^{h}

Na-Alginate [%]	$CaCl_2$ [M]	Na-Alginate: $CaCl_2$ [v/v]	Bead size [mm]
		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).

High mechanical strength is desirable for the practical application of microcapsules during processing and storage [21]. Stiffness was measured for the optimal samples with the highest encapsulation efficiency (i.e., those prepared with the lowest salt concentration and also the lowest volume ratio of wall:salt), yielding values of 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 the findings of 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, with some bumps and dents resulting from mechanical stresses during drying process.

Caption of Figures

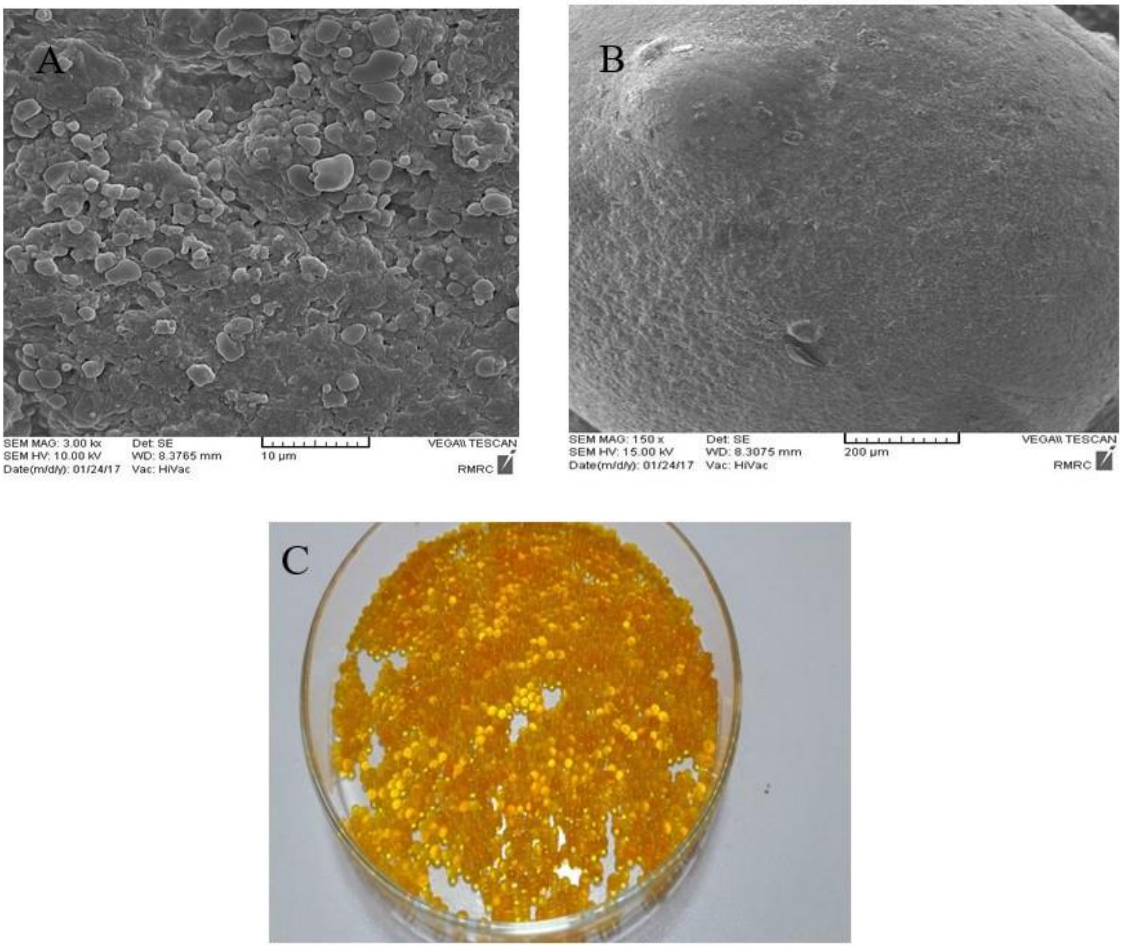

Fig 1. Images of gel beads prepared with 4% [w/w] sodium alginate, 0.5 [M] calcium chloride, and 1:1 [v/v] taken by A) SEM at 3000x magnifying, B) SEM at 150x magnifying, and C) optical camera.

4. Conclusions

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 a 30-day storage period. The findings indicated that the highest encapsulation efficiencies 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, beverages, while providing good stability against environmental conditions during storage. Evaluating the stability of gel beads containing saffron's active components within a real food matrix is recommended for future research.

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فصلنامه فناوری‌های جدید در صنعت غذا

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مقاله پژوهشی

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.

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