Microencapsulation of Saffron (*Crocus sativus* L.) Extract in Copolymer Complexes Using Extrusion Method

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ABSTRACT

This study describes the preparation of alginate-chitosan and alginate-gelatin beads containing saffron components to be incorporated as additives in food products. This study evaluated the influence of incorporating hydrophilic natural polymers, alginate-chitosan and alginate-gelatin on preserving saffron components. The alginate beads were coated with chitosan and gelatin as copolymer by extrusion method with a polyelectrolyte complex reaction between two oppositely charged poly-ions. The beads were formulated, optimized and evaluated to obtain high encapsulation efficiency of crocin, safranal and picrocrocin as the main components of saffron. The encapsulation variables were selected in accordance with Central Composite Design and were further optimized via response surface methodology. Alginate concentration significantly influenced particle size and encapsulation efficiency of alginate-chitosan and alginate-gelatin beads ($p \leq 0.05$). Both chitosan and gelatin positively affected encapsulation efficiency. The optimum condition for preparing alginate-chitosan beads was an alginate concentration of 1.97% and chitosan concentration of 0.925%; this yielded an encapsulation efficiency of 66.3 ± 1.5, 86.2 ± 0.7 and 52.9 ± 3% for picrocrocin, safranal and crocin, respectively. The optimum condition for preparing alginate-gelatin beads was an alginate concentration of 1.95% and gelatin concentration of 3.65%; this yielded encapsulation efficiency of 39.2 ± 2.9, 31.9 ± 1.7 and 18.3 ± 1% for picrocrocin, safranal and crocin, respectively. The results clearly indicated that, in combination with alginate, chitosan was a better copolymer than gelatin for encapsulating saffron components.

Keywords: Saffron, Alginate, Chitosan, Gelatin, Encapsulation

INTRODUCTION

Saffron, a popular spice known for its color, aroma and medicinal properties, belongs to the family *Iridaceae*. It consists of the dried stigmas of *Crocus sativus* L., which is widely cultivated in Iran, Spain, Italy, France and India. Saffron is also known as the most expensive spice in the world (Fernandez, 2004; Khan et al., 2011). Saffron is a valuable and important export product in Iran, playing a significant role in Iran's agricultural economy. The total annual worldwide pro-

duction of saffron is an estimated 190 tons, with Iran accounting for 90% (Negbi, 1999; Fernandez, 2004). With its powerful coloring and flavoring properties due to its glycosidic constituents, saffron has been used since ancient times as a food additive and dye (Ríos et al., 1996; Caballero-Ortega et al., 2004). It has also been used medicinally, with recent research demonstrating its antidepressant and antitumoral properties (Karimi et al., 2001; Srivastava et al., 2010).

The most important compounds in saffron are crocin, picrocrocin and safranal, which are responsible for saffron's color, flavor and aroma, respectively (Fernandez, 2004). Their quantity is used to express the quality of saffron – the higher their quantity, the better the saffron (Tarvand, 2005). Saffron is also very hygroscopic; exposure to moisture risks spoilage. In addition to humidity, it is also instable in the presence of light, air and high temperature. As a result, microencapsulation has been used to protect the product.

Encapsulation is the technique by which one material or a mixture of materials is coated with or entrapped within another material. The coated material is called the core material; the surrounding material forms the shell – called carrier, encapsulant or supporting material. The food industry has used encapsulation technology for more than 60 years to protect liquid and solid ingredients. It is an effective barrier against environmental and chemical interactions until release is desired. In food products, oils and extracts, aroma compounds, vitamins, colorants and enzymes have been encapsulated (Jackson and Lee, 1991; Shahidi and Han, 1993).

Alginate is a natural biopolymer, widely used as supporting material for encapsulation by extrusion. It is obtained from brown algae (Phaeophyta), such as the seaweeds *Laminaria* sp. and *Ascophyllum* sp., that gels in the presence of calcium in the form of egg-box structures. Alginate polymers consist of linear, unbranched polysaccharides with acid residues of 1, 4-linked-β-D-mannuronic acid and α-L-gluronic acid residues. The residues are arranged in blocks along the chain and vary in sequence and composition. An alginate matrix is highly versatile, biocompatible and nontoxic, protecting active components that are sensitive to heat, moisture, light and other factors. It is already used in the food and pharmaceutical industry as a thickener, gelling agent and coating (Moe et al., 1995).

However, an alginate network is highly porous, leading to stability problems. To address this, copolymers are used stabilize the alginate gel matrices and reduce the porosity of the capsule. Alginates are negatively charged polymers and may form strong complexes with polycations, such as chitosan and gelatin. Polycations have been used to stabilize the gel and reduce the porosity of Ca-alginate beads, consequently improving the effectiveness of encapsulation.

Chitosan is a linear polysaccharide polymer derived from chitin that is found in a wide range of natural sources such as crustaceans (e.g., crab, shrimp and lobster), fungi and insects (Tolaimate et al., 2000). Chitosan consists of one amino and two free hydroxyl groups in each unit. It possesses a positive charge that allows interact electrostatically with negatively charged polymers, such as alginate. Chitosan can also be used as coating material for special treatment of alginate beads (Krasaekoopt et al., 2003).

Gelatin is also of interest as a copolymer. Gelatin is a natural and biodegradable polymer derived from collagen, which is colorless or slightly yellow, almost tasteless, and odorless. Gelatin is widely known for its property of gelling with heating and subsequent cooling. The hydrocolloidal feature of gelatin yields many applications in the food industry, including: confectionery and jelly deserts, dairy products, meat products and hydrolyzed gelatin applications (Nishimoto et al., 2005).

The microencapsulation of saffron extract using a copolymer has not been reported elsewhere.

This research aimed to optimize the microencapsulation of saffron extract by using chitosan and gelatin as copolymers and the extrusion method.

MATERIALS AND METHODS

Saffron extraction

Four grams of saffron stigma powders (Novin Saffron, Iran) were suspended in 25 mL of ethanol-water (1:1, v/v) and mixed for 2 min. The mixture was then centrifuged at 4000 rpm (2600 x g) for 10 min to eliminate plant residues and the supernatant was separated. Twenty five milliliters of ethanol-water solution was added to the sediment and the extraction was repeated. This process was repeated six times. The total volume of solvent consumption for 4 g saffron stigmas in extraction process was 200 mL (8×25 mL). The collected supernatant was then kept in a dark container at 4°C (adapted from Hadizadeh et al., 2010).

Optimization of encapsulation by copolymers (experimental design and statistical analysis)

A Central Composite Design and Response Surface Methodology were used to study the effects of two factors on the encapsulation efficiency (EE) of encapsulating saffron in gel matrices. The two investigated factors (independent variables) were alginate concentrations (X_1, 0.8, 1.2 and 1.6% w/v) and copolymer concentrations (X_2, 0.4, 0.6 and 0.8% w/v for chitosan; 1.5, 2.25 and 3.0% w/v for gelatin). The analyzed dependent variables were the encapsulation efficiency of picrocrocin (Y_1), safranal (Y_2) and crocin (Y_3). Each independent variable was studied at three levels. Coded working levels were: a two-level factorial design (coded ±1), star points (coded ±α) and central level (coded 0) (Table 1), where the central point was repeated six times, obtaining 14 experimental trials (Table 2 and 3). All data were statistically analyzed using SPSS version 18.0 (Trial version).

Table 1. Variable and coded level of alginate, chitosan and gelatin concentrations.

Variables	**code**	**Variation levels**				
		-α	**-1**	**0**	**1**	**α**
Alginate %	X1	0.63	0.8	1.2	1.6	1.76
Chitosan %	X2	0.32	0.4	0.6	0.8	0.88
Variables	code	**Variation levels**				
		-α	-1	0	1	α
Alginate %	X1	0.63	0.8	1.2	1.6	1.76
Gelatin %	X2	1.19	1.5	2.25	3	3.31

Table 2. Central composite design with two factors for alginate/chitosan.

Trial	**Alginate (%)**	**Chitosan (%)**
1	-1 (0.8%)	-1 (0.4%)
2	1 (1.6%)	-1 (0.4%)
3	-1 (0.8%)	1 (0.8%)
4	1 (1.6%)	1 (0.8%)
5	0 (1.2%)	0 (0.6%)
6	0 (1.2%)	0 (0.6%)
7	-1.414 (0.63%)	0 (0.6%)
8	1.414 (1.76%)	0 (0.6%)
9	0 (1.2%)	-1.414 (0.32%)
10	0 (1.2%)	1.414 (0.88%)
11	0 (1.2%)	0 (0.6%)
12	0 (1.2%)	0 (0.6%)
13	0 (1.2%)	0 (0.6%)
14	0 (1.2%)	0 (0.6%)

Table 3. Central composite design with two factors for alginate/gelatin.

Trial	Alginate (%)	Gelatin (%)
1	-1 (0.8%)	-1 (1.5%)
2	1 (1.6%)	-1 (1.5%)
3	-1 (0.8%)	1 (3%)
4	1 (1.6%)	1 (3%)
5	0 (1.2%)	0 (2.25%)
6	0 (1.2%)	0 (2.25%)
7	-1.414 (0.63%)	0 (2.25%)
8	1.414 (1.76%)	0 (2.25%)
9	0 (1.2%)	-1.414 (1.19%)
10	0 (1.2%)	1.414 (3.31%)
11	0 (1.2%)	0 (2.25%)
12	0 (1.2%)	0 (2.25%)
13	0 (1.2%)	0 (2.25%)
14	0 (1.2%)	0 (2.25%)

The Response Surface Methodology for the optimization study was applied to the experimental data using STATISTICA version 7.1 (Trial version). The use of a response surface method experimental design permitted the construction of second-order polynomial models that could describe quantitatively the linear, quadratic and interaction effects of the selected factors on the studied response variables. For two factors, the general model corresponded to the following equation:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{11}X_12 + b_{22}X_2^2 + b_3X_1X_2 \quad (1)$$

In this equation, X_1 and X_2 were the independent variables (factors) and Y was the investigated dependent variable (response). b_0 represented the arithmetic average of all quantitative outcomes of all the runs, b_1 and b_2 were related with the independent variables effect on the response, b_{11} and b_{22} were two quadratic relationships and b_{12} represented the interaction effect between the two variables. A coefficient with a positive sign signified a synergistic effect and a negative sign an antagonistic effect.

Preparation of alginate

Alginate solutions (0.8, 1.2 and 1.6% w/v) were prepared by dissolving sodium alginate (Srichem, India) in distilled water at 70ºC. The solution volume was then adjusted to 100 mL.

Preparation of chitosan

Low molecular weight chitosan (0.4 g; low viscosity 14 mPas in 1% w/v solution; Fluka, Australia) (0.8, 1.2 and 1.6 g) was dissolved in 190 mL of distilled water acidified with 0.4 mL glacial acetic acid. NaOH (1 M) was added to adjust the pH of the chitosan aqueous solutions to approximately 5.7-6.0. The mixture was then filtered through Whatman #4 filter paper and the volume was adjusted to 200 mL. The final concentration of chitosan solutions were 0.4, 0.6 and 0.8% (w/v), respectively.

Preparation of gelatin

Gelatin powder (McGarrett, Thailand) (3, 4.5 and 6 g) were soaked and hydrated in distilled water followed by heating until they were completely dissolved. The volume was then adjusted to 200 mL. The final concentrations were 1.50, 2.25 and 3.00 % (w/v), respectively.

Microencapsulation of saffron using extrusion method

Beads were obtained by mixing 7 mL of saffron extract with 100 mL alginate solution as the main supporting material. The mixture was then extruded through a syringe needle (0.80 × 25 mm) in the form of droplets into a hardening solution containing 1% (w/v) $CaCl_2$ (Carlo Erba, Italy) and a copolymer – either chitosan or gelatin. The extrusion distance, the distance between the needle and the surface of the hardening solution, was fixed at 10 cm. The hardening solution was stirred at 150 rpm during extrusion. The beads were then left to stand for 15 min to complete gelation. The microcapsules were washed twice with distilled water (200 mL) and dried at 40°C in an air drying oven. The powder was stored in plastic bags for further analysis.

Encapsulation Efficiency

The values of the three main components of saffron – crocin, safranal and picrocrocin – were used to determine the encapsulation efficiency using the following equation (2):

$$\text{EE (\%)} = \frac{(\text{Value of main components in beads} \times \text{mass of beads (dry basis)})}{(\text{Value of main components in saffron extract} \times \text{weight of saffron used})} \times 100 \qquad (2)$$

Bead yield

The bead yield was calculated using equation (3):

$$\text{Yield (\%)} = \frac{(\text{Weight of the dried beads})}{(\text{Total weight of all ingrediends})} \times 100 \qquad (3)$$

Determination of chemical and physical properties of microencapsulated saffron powder

Crocin, safranal and picrocrocin content. The chemical properties of saffron – coloring strength, aroma and bitterness – are primarily related to the amount of crocin, safranal and picrocrocin present, respectively. There are many methods for saffron component analysis (Tarantilis et al., 1995). According to ISO/TS 3632 (2003), the analytical identification and commercial classification for saffron powder is based on UV-vis spectrophotometry. This study used the standard ISO method to determine the saffron components. Higher quantities of these constituent compounds indicate higher quality saffron. Crocin, safranal and picrocrocin were determined by direct reading of the absorbance of 1% aqueous solution of saffron at the wavelengths of 440, 330 and 257 nm, respectively, by using a UV-visible spectrophotometer (Spectronics Genesys 5 UV/Visible Spectrophotometer, USA) and a 1 cm pathway quartz cell after preparation.

The concentration of crocin ($E^{1\%}_{1cm}$ *440 nm*), safranal ($E^{1\%}_{1cm}$ *330 nm*) and picrocrocin ($E^{1\%}_{1cm}$ *257 nm*) were calculated by using the following formula as equation 4 (ISO, 2003):

$$E^{1\%}_{1cm} = [D \times 10000] / [m \times (100\text{-H})] \quad (4)$$

where D is the specific absorbance; m is the mass of the saffron sample, in grams; and H is the moisture and volatile content of the sample, expressed as a mass fraction.

One milliliter of saffron extract (2%) was added into 1 mL of ethanol-water (1:1, v/v) to obtain 1% saffron extract. Direct reading of saffron solution (1%) was determined by using UV-Vis spectrophotometer.

For microencapsulated saffron, 0.1 g of beads was immersed and dissolved in 50 mL of sodium citrate aqueous solution (5%) to break the capsules and liberate the entrapped saffron. Subsequently, the suspension was centrifuged at 4000 rpm for 5 min and the supernatant was filtered using a 0.22 μm Millipore filter to eliminate impurities. The clear supernatant was collected and analyzed using spectrophotometer at 440, 330 and 257 nm for crocin, safranal and picrocrocin, respectively. Sodium citrate solution (5%) was used as the blank and the analysis was done in three replicates.

Measurement of bead size. The particle sizes of both alginate-chitosan and alginate-gelatin beads were measured with a Vernier caliper. For all measurements, 120 beads were randomly examined and the mean particle size was calculated.

Color measurement. The color of the samples was measured with a Mini Scan EZ (HunterLab, USA). The light source of D65 with observer angle of 10° was used. Color parameters of L*, a* and b* were taken in the Hunter Lab system. Saffron microcapsules were measured to obtain L*, a* and b* parameters. The Hunter Lab color space was organized in a cube form. The L axis runs from top to bottom. The value of lightness (L*) varies from 0 to 100, where 0 is black

and 100 is white. The a* and b* axes have no specific numerical limits. Positive a* is red, while a negative a* is green. Positive b* is yellow, while a negative b* is blue. Higher values for parameter a* indicate a higher concentration of red pigments in the sample.

RESULTS

Saffron extract was encapsulated with alginate as the main supporting material and either chitosan or gelatin as a copolymer using an extrusion technique.

Production yield

The production yield of saffron microcapsules prepared by extrusion technique varied with different concentrations of alginate and copolymers. The yields of all trials were in the range of 40.2 to 81.3% for alginate-chitosan beads (ALG-CS) and 15.6-38.9% for alginate-gelatin beads (ALG-G) (Table 4 and 7). Alginate and copolymer concentrations (chitosan or gelatin) had significant ($p \leq 0.05$) positive effect on the production yield. The highest yield was found in Trial 2 (81.3%) and Trial 9 (38.9%), whereas the lowest was found in Trial 7 (40.2%) and Trial 3 (15.6%) for chitosan and gelatin, respectively. An increase in alginate concentration resulted in an increased production yield. There was also no interaction effect between ALG and CS, while alginate and gelatin concentrations had an interaction effect on the production yield of beads.

Encapsulation efficiency

The encapsulation efficiency of saffron was measured as the encapsulation efficiency of its three main compounds: picrocrocin, safranal and crocin. The independent variables were alginate concentration (X_1) and chitosan/gelatin concentration (X_2), while the analyzed response variables were the encapsulation efficiency of picrocrocin (Y_1), safranal (Y_2) and crocin (Y_3). The results of 14 trials are shown in Table 4 and 7. The polynomial model equations of each response variable were generated from multiple regression analysis. The obtained models to describe the variables were selected at the 95% confidential level.

Chitosan. From 14 trials, the encapsulation efficiency of picrocrocin, safranal and crocin ranged from 8.5-40.0%, 7.4-50% and 3-24%, respectively. After analysis of variance (ANOVA), regression equations were used as a model to predict the encapsulation efficiency obtained. Encapsulation efficiency could be predicted from the model (Table 5).

Table 4. Some physical and chemical properties of microencapsulated saffron using alginate and chitosan (mean±SD).

Trial	ALG (%)	CS (%)	Yield (%)	Size (mm)	Picrocrocin EE (%)	Safranal EE (%)	Crocin EE (%)	Color values[1]		
								L	a	b
1	0.8	0.4	60.4±2.75	2.1±0.07	18.9±0.37	18.8±0.44	7.2±0.83	46.7±0.56	7.7±0.38	19.0±0.67
2	1.6	0.4	81.3±3.00	3.1±0.20	39.6±0.14	44.0±0.56	21.0±0.42	38.1±0.28	10.6±0.50	16.8±0.45
3	0.8	0.8	40.5±2.40	2.6±0.10	16.9±0.05	16.9±0.38	11.3±0.80	43.9±0.42	12.7±0.61	20.6±0.53
4	1.6	0.8	61.4±1.20	2.9±0.14	40.0±0.35	45.0±0.34	24.0±0.71	36.8±0.63	14.1±0.40	15.9±0.66
5	1.2	0.6	65.8±3.11	2.5±0.10	28.8±0.14	33.5±0.65	14.0±0.34	42.7±0.49	13.3±0.61	18.6±0.55
6	1.2	0.6	63.3±2.90	2.5±0.11	24.7±0.42	27.0±0.77	13.7±0.23	47.5±0.70	11.7±0.55	18.3±0.51
7	0.63	0.6	40.2±2.54	2.0±0.08	8.5±0.11	7.4±0.21	3.0±0.30	48.4±0.45	5.6±0.77	18.6±0.33
8	1.76	0.6	68.5±3.90	2.9±0.14	42.5±0.63	50.0±1.16	19.0±0.39	33.1±0.63	11.9±0.47	14.1±0.48
9	1.2	0.32	78.6±2.05	2.6±0.03	19.5±0.26	18.3±0.39	12.1±0.46	48.2±0.42	12.5±0.51	17.8±0.49
10	1.2	0.88	48.5±1.06	2.5±0.08	27.3±0.15	31.0±0.87	16.2±0.48	41.6±0.37	8.4±0.64	17.4±0.63
11	1.2	0.6	60.0±1.17	2.4±0.10	29.0±0.77	30.1±0.72	12.3±0.40	42.9±0.57	13.3±0.52	20.7±0.72
12	1.2	0.6	61.4±2.56	2.5±0.09	25.7±0.56	26.8±0.56	13.6±0.39	45.0±0.66	11.5±0.65	20.4±0.66
13	1.2	0.6	61.6±2.79	2.5±0.07	26.5±0.84	29.0±0.66	13.4±0.28	46.7±0.59	13.8±0.70	18.7±0.55
14	1.2	0.6	61.3±3.06	2.5±0.08	28.1±0.70	29.9±0.70	15.2±0.36	42.7±0.44	13.0±0.49	21.2±0.50

Note: [1]*L* = lightness, *a* = redness, *b* = yellowness; ALG = alginate; CS = chitosan.

Table 5. Equations and R^2 for alginate-chitosan.

No.	Response	Equation model	R^2
1	EE of picrocrocin	$38.46-40.80X_1-33.53X_2+27.20X_1^2+17.978X_2^2+7.5X_1X_2$	0.84
2	EE of safranal	$35.22-49.60X_1-16.47X_2+33.32X_1^2+4.710X_2^2+9.063X_1X_2$	0.84
3	EE of crocin	$11.52-7.55X_1-17.94X_2+10.43X_1^2+17.63X_2^2-3.31X_1X_2$	0.71

Note: EE = encapsulation efficiency.
When X_1 = Concentration of alginate (%)
X_2 = Concentration of chitosan (%)

These obtained results of encapsulation efficiency for picrocrocin (Y_1), safranal (Y_2) and crocin (Y_3) were fitted by a quadratic model from multiple regression using the enter method. Regression coefficients (R^2) were calculated as 0.84, 0.84 and 0.71.

The experimental design for the Central Composite Design and results of encapsulation efficiencies for the prepared beads are shown in Table 4 and 7. Alginate (X_1) and chitosan (X_2) concentrations were found to have no significant effect on encapsulation efficiency of saffron components. It was also observed that the encapsulation efficiency of saffron compounds in ALG-CS beads increased with alginate and chitosan concentrations, whereas encapsulation efficiency was low when either alginate or chitosan concentrations were low.

Although, X_1 and X_2 were not significant ($p > 0.05$) model terms, the obtained regression coefficients showed that encapsulation efficiency had a positive trend. X_1, X_2, X_1^2 and X_2^2 were also kept in the model, as well as the interaction effect between variables (X_1X_2). These variables were not statistically different ($p > 0.05$), and were kept only to support the hierarchy of the polynomial equation. The relationship between encapsulation efficiency and the two independent variables was indicated using a response surface plot in Figure 1. The optimal value of each factor to achieve maximal response levels could be determined.

High concentrations of alginate and chitosan yielded the highest encapsulation efficiency of saffron compounds. The highest encapsulation efficiencies of picrocrocin (42.5%) and safranal (50%) were obtained when 1.76% alginate and 0.6% chitosan were used. For crocin, the highest encapsulation efficiency (24%) was obtained when 1.6% alginate and 0.8% chitosan were used. The results also indicated that increasing chitosan concentrations positively effected encapsulation of crocin, so treatment with 1.6% alginate and 0.8% chitosan concentrations provided the highest encapsulation efficiency (24.0%), whereas treatment with 0.63% alginate and 0.6% chitosan had the lowest encapsulation efficiency (3.0%).

Gelatin. After analysis of variance (ANOVA), regression equations were used as a model to predict encapsulation efficiency. Encapsulation efficiency can be predicted from the model:

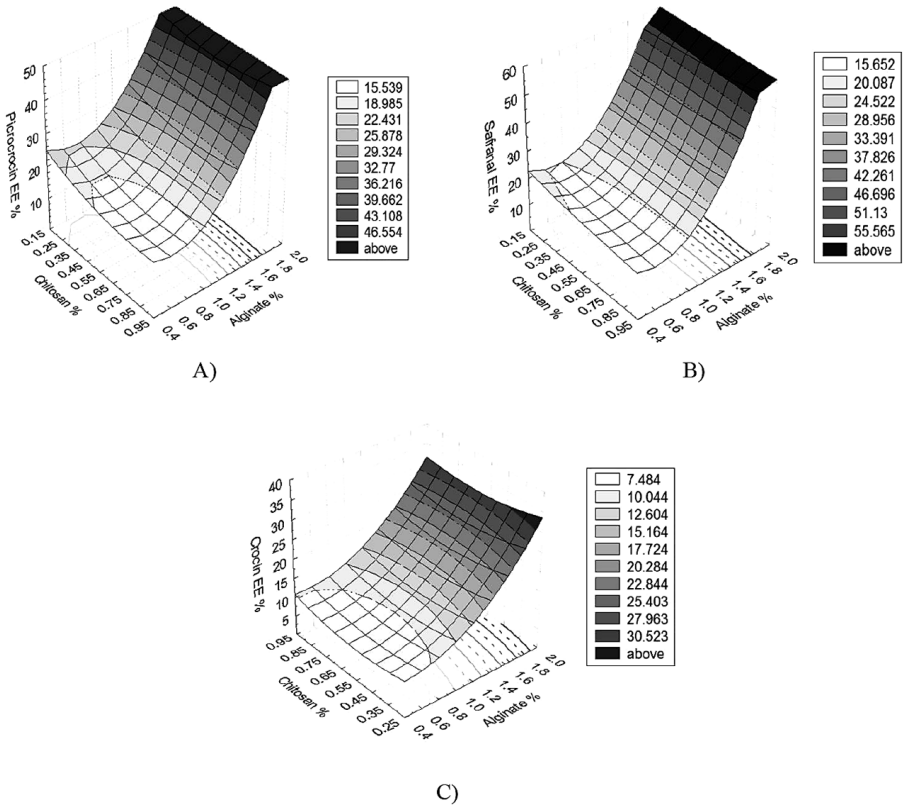

Figure 1. Contour graph and response surface plotted from concentrations of alginate (%), chitosan (%) and encapsulation efficiency (%) of saffron components: picrocrocin (A), safranal (B) and crocin (C).

Table 6. Equations and R^2 for alginate-gelatin.

No.	Response	Equation model	R^2
1	EE of picrocrocin	$44.02-38.98(X_1)-12.51(X_2)+12.53(X_1^2)+1.39(X_2^2)+7.75(X_1X_2)$	0.76
2	EE of safranal	$35.18-30.61(X_1)-10.36(X_2)+10.95(X_1^2)+1.09(X_2^2)+6.00(X_1X_2)$	0.84
3	EE of crocin	$13.46+3.03(X_1)-11.11(X_2)-0.96(X_1^2)+2.28(X_2^2)+1.83(X_1X_2)$	0.73

Note: When X_1 = Concentration of alginate (%)
X_2 = Concentration of gelatin (%)

The coefficient of determination (R^2) for the model was 0.76, 0.84 and 0.73 for picrocrocin, safranal and crocin, respectively, indicating a moderate fit of the model (Table 6).

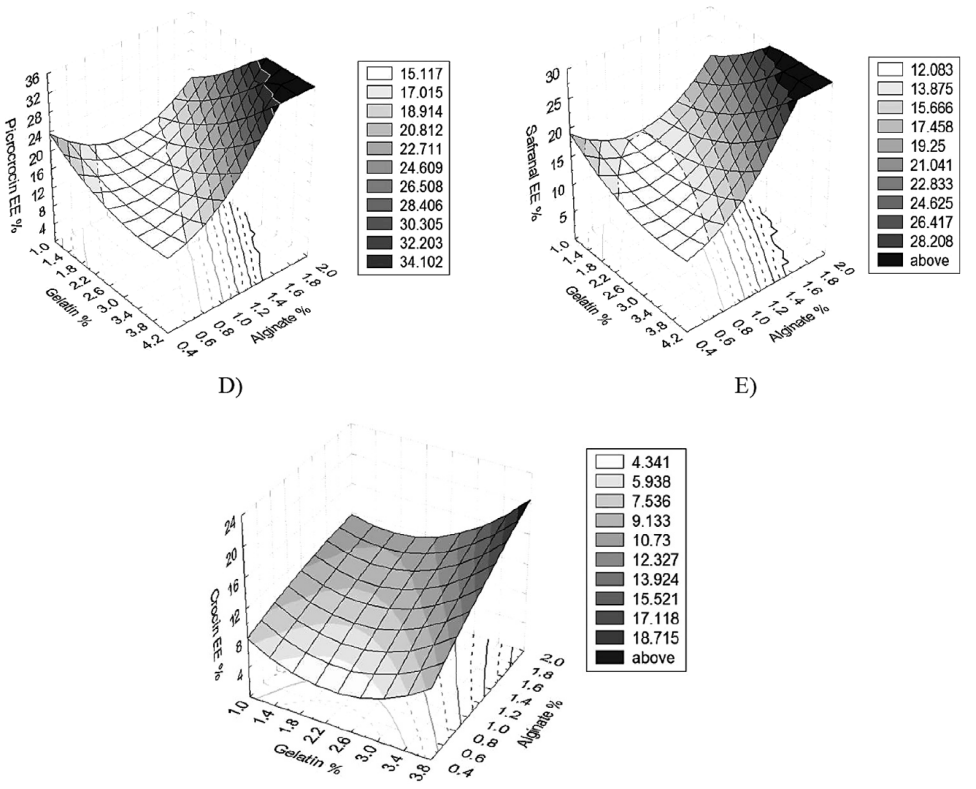

Figure 2. Contour graph and response surface plotted from concentrations of alginate (%), gelatin (%) and encapsulation efficiency (%) of saffron components: picrocrocin (D), safranal (E) and crocin (F).

Figure 2 (D, E and F) indicates the effects of alginate and gelatin concentrations on the encapsulation of the main components of saffron. Alginate and gelatin concentrations positively affected encapsulation efficiency of the saffron components (Figure 2). As the concentration of alginate increased, encapsulation efficiency of the saffron components increased. The results showed that gelatin concentration affected encapsulation efficiency less than chitosan in terms of improving the surface area and pore structure of the beads. For picrocrocin and crocin, the model terms (X_1, X_2, X_1^2, X_2^2 and X_1X_2) had no significant effect on encapsulation efficiency ($p > 0.05$). For safranal, the main effect of alginate (X_1) and X_1^2 were significant model terms ($p \leq 0.05$) and other model terms were insignificant.

The highest encapsulation efficiency of picrocrocin (27.7%) and safranal (22.7%) were obtained with 1.6% alginate and 3.0% gelatin (Table 5). For crocin, the highest encapsulation efficiency (11.4%) was obtained with 1.76% alginate and 2.25% gelatin.

Table 7. Some physical and chemical properties of microencapsulated saffron using alginate and gelatin (mean±SD).

Trial	ALG (%)	Gelatin (%)	Yield (%)	Size (mm)	Picrocrocin EE (%)	Safranal EE (%)	Crocin EE (%)	Color values[1]		
								L	a	b
1	0.8	1.5	25.7±3.11	2.1±0.03	15.7±0.36	13.3±0.27	6.0±0.10	51.8±0.77	8.3±0.47	13.5±0.59
2	1.6	1.5	38.6±2.19	2.7±0.07	16.6±0.09	15.1±0.15	6.8±0.39	33.8±0.44	7.3±0.70	11.4±0.67
3	0.8	3	15.6±1.70	2.3±0.07	17.5±0.07	13.7±1.0	8.1±0.63	46.8±0.65	11.1±0.55	18.4±0.62
4	1.6	3	24.5±3.22	2.9±0.13	27.7±0.13	22.7±0.03	11.1±0.51	49.6±0.58	9.6±0.50	16.5±0.62
5	1.2	2.25	25.2±3.54	2.3±0.08	13.3±0.05	12.2±0.02	7.0±0.10	49.6±0.64	8.0±0.61	18.6±0.54
6	1.2	2.25	25.3±2.99	2.2±0.08	13.2±0.03	11.4±0.52	5.6±0.78	51.0±0.63	7.2±0.58	18.9±0.51
7	0.63	2.25	15.9±2.34	2.2±0.07	11.0±0.20	8.5±0.22	3.1±0.27	45.7±0.47	5.1±0.66	15.3±0.87
8	1.76	2.25	30.1±3.57	2.9±0.14	22.2±0.39	21.4±0.34	11.4±0.73	51.0±0.61	11.0±0.73	17.9±0.55
9	1.2	1.19	38.9±3.09	2.3±0.07	13.1±0.04	11.8±0.17	9.5±0.15	43.2±0.55	12.4±0.49	15.8±0.58
10	1.2	3.31	19.5±2.33	2.2±0.03	15.2±0.23	13.7±0.15	10.7±0.17	46.2±0.38	15.0±0.63	21.1±0.66
11	1.2	2.25	24.8±3.11	2.4±0.09	14.8±0.08	13.7±0.13	8.0±0.05	49.3±0.71	10.6±0.80	20.4±0.70
12	1.2	2.25	26.2±3.00	2.4±0.04	16.7±0.10	14.0±0.27	9.0±0.17	38.4±0.52	11.8±0.67	17.6±0.60
13	1.2	2.25	25.8±2.77	2.3±0.06	15.2±0.15	13.6±0.16	7.5±0.09	48.1±0.49	10.9±0.64	21.9±0.68
14	1.2	2.25	26.4±3.19	2.4±0.06	14.8±0.15	11.1±0.27	6.0±0.16	50.6±0.60	6.9±0.36	24.2±0.72

Note: [1]L = lightness, a = redness, b = yellowness.

Table 8. Encapsulation efficiency of saffron from five selected alginate and copolymer concentrations.

Copolymer	ALG %	Copolymer %	Picrocrocin				Safranal				Crocin			
			Theo.	Exp.	SD	Sig.	Theo.	Exp.	SD	Sig.	Theo.	Exp.	SD	Sig.
Chitosan	1.9	0.95	57.1	48.8b	2.0	**	66.2	63.4b	3.4	ns	27.7	37.9b	2.0	**
	1.925	0.885	49	49.9b	4.9	ns	74.6	64.7b	5.9	ns	25.4	42.3b	7.0	**
	1.97	0.925	58.1	66.3a	1.5	**	67.9	86.2a	0.7	**	26.9	52.9a	3	**
	2	0.885	63.4	51.3b	3.5	**	74.5	66.7b	6.2	ns	30.2	36.9b	6.0	ns
	2	0.95	64.3	47.9b	0.6	**	75.1	59.9b	0.6	**	30.7	45.2ab	3.3	**
Gelatin	1.84	3.8	40.1	34.8a	1.3	**	34.3	29.5b	1.7	**	19.3	11.1c	0.7	**
	1.9	3.6	40	35.8a	2.2	**	34.5	31.1ab	2.3	ns	17.8	12.3c	2.7	**
	1.95	3.65	42.5	39.2a	2.9	ns	36.6	31.9ab	1.7	**	18.6	18.3a	1.0	ns
	2	3.8	46.4	36.2a	1.3	**	39.8	33.1ab	1.9	**	20.3	12.3c	0.9	**
	2	3.3	40.2	38.5a	2.7	ns	35.1	34.1a	1.6	ns	15.9	15.0b	0.8	ns

Note: ns: differences are not significant. Theo. = theoretical; Exp. = experimental; SD = standard deviation; Sig. = significance.

To fit the model with the experimental data, five alginate concentrations and copolymer concentrations in the area that provided the highest encapsulation efficiency were chosen and the results are shown in Table 8.

Moreover, there were some significant differences ($p \leq 0.05$) between the theoretical and experimental data.

The optimum condition for preparing alginate-chitosan beads was 1.97% alginate and 0.925% chitosan with encapsulation efficiencies of 66.3%, 86.2% and 52.9% for picrocrocin, safranal and crocin, respectively. The optimum condition for preparing alginate-gelatin beads was 1.95% alginate and 3.65% gelatin with encapsulation efficiencies of 39.2%, 31.8% and 18.3% for picrocrocin, safranal and crocin, respectively. These optima were selected for further experimentation.

Effect of the type of copolymers on the encapsulation efficiency of saffron was also investigated using the optimal condition of each copolymer (Table 9). Gelatin had a significantly lower encapsulation efficiency for all saffron components than chitosan. The encapsulation efficiencies of chitosan were 66.3%, 86.2% and 52.9% for piccrocrocin, safranal and crocin, respectively.

Table 9. Encapsulation efficiency of saffron in alginate-chitosan and alginate-gelatin beads.

Treatment	Picrocrocin (%)	Safranal (%)	Crocin (%)
ALG 1.97%, CS 0.925%	66.3a	86.2a	52.9a
ALG 1.95%, Gelatin 3.65%	39.2b	31.9b	18.3b

Some physical and chemical properties of microencapsulated saffron

Particle sizes of beads. The mean particle sizes of the obtained beads were in the range of 2.1 to 3.1 mm for ALG-CS beads and from 2.0 to 2.8 mm for ALG-G (Table 4 and 7). An increase in polymer concentrations – either the main polymer or the copolymer – significantly increased the bead diameter ($p \leq 0.05$). Alginate and chitosan had a significant effect on the bead size ($p \leq 0.05$), as well as an interaction effect between them. Increasing alginate led to the formation of bigger beads by the extrusion method, whereas chitosan concentrations had the inverse effect on bead size at a given alginate concentration. For alginate-gelatin beads, the concentrations of both alginate and gelatin also had a significant effect on the particle sizes ($p \leq 0.05$), while there was no interaction between concentrations of alginate and gelatin.

Color of beads. The color of the encapsulated beads was evaluated by HunterLab system. The values of L* (lightness), a* (red/green value) and b* (yellow/blue value) are shown in Table 4 and 7.

The ALG-CS beads had the following value ranges: L* = 33.1-48.4, a* = 5.6-14.1 and b* = 14.1-21.2. As the concentration of alginate increased, both a* and b* reduced, while a* increased. On the other hand, increasing the concentration of chitosan increased a*, but did not effect L* and b*. For example, trial 7 (0.63% alginate, 0.6% chitosan) had a maximum L* value (48.4) and a minimum a* value (5.6).

For ALG-G beads, color analysis showed L* values of 33.8- 51.6, a* of 5.1-15.0 and b* of 11.4-24.2. For ALG-G beads, as the concentration of alginate increased, all three values reduced; while all three values elevated, as the concentration of gelatin increased.

The results also demonstrated that b*, which indicates yellowness, had a positive correlation with L* for both ALG-CS and ALG-G beads.

DISCUSSION

The positive effect of alginate and copolymer concentrations on the production yield might be caused by the bead durability improvement at higher alginate concentrations. The production yield was relatively low for all trials due to the high viscosity of the solution used. Furthermore, the chitosan or gelatin was also lost during hardening, because of lack of binding sites within the alginate network structure. Therefore, at the same concentration of alginate, the production yield decreased as the alginate or chitosan concentrations increased.

The regression coefficients for the encapsulation efficiency of chitosan were relatively high, indicating that 84.0%, 84.0% and 71.0% of the data were compatible with the experimental data in the model predictions for picrocrocin, safranal and crocin, respectively. Li and Lu (2005) considered R^2 values higher than 0.9 as having very high correlation. The porosity of alginate beads was responsible for the release of a small amount of the water-soluble components of saffron during encapsulation process. Increasing the alginate concentration, improved encapsulation efficiency; but it was not enough to protect the releasing of core material, due to its macroporous structure (Ribeiro et al., 1999). The porous structure of calcium alginate microspheres allowed chitosan to easily penetrate into the interior of the microspheres and combine with alginate, to prevent saffron extract leakage by changing the macroporous structure to a microporous one. When the concentration of chitosan was low, the chitosan membrane on the interface of alginate microspheres was thin, resulting in low encapsulation efficiency, which was consistent with the study of Sezer and Akbuğa (1999). Moreover, among the three main components, safranal had the highest encapsulation efficiency. This might be due to the smaller size of safranal (molecular weight of 150.21 g/mol), which could be entrapped well by chitosan within the macroporous structure of alginate. Generally, the bigger sized active compounds had low entrapment efficiency within the alginate structure (Yurdasiper and Sevgi, 2010). For gelatin, increasing encapsulation efficiency due to higher gelatin concentration might be caused by an increasing interaction between the functional groups of the gelatin and alginate molecules, enhancing the degree of crosslinking in the network. A negative effect of the copolymer (X_2) on encapsulation efficiency was also found, consistent with the study of Motwari et al. (2008). They reported that higher chitosan concentrations led to the formation of aggregates upon addition of alginate, resulting in low encapsulation efficiency.

In addition, some significant differences ($p \leq 0.05$) between the theoretical and experimental data for model fitting indicated that the chosen points might be

out of the range of the optimal condition or not in the range of concentrations studied for the encapsulation of saffron. However, the R^2 of the Central Composite Design for all saffron components was relatively high.

When gelatin was used as a copolymer, encapsulation efficiency of saffron components was low. This might be due to insufficient cross-linking bonds in the network structure that permitted the saffron to diffuse out during and after gelation. Based on our results, using chitosan as a copolymer resulted in a large increase in encapsulation efficiency. This was probably due to the formation of a rigid network and more firmness in the alginate-chitosan complex during microcapsule preparation, caused by increased ionic interactions between the carboxylate groups in the alginate and the amine groups in the chitosan.

For some physical and chemical properties of microencapsulated beads, the bead size was influenced by the size of the nozzle, the distance between the needle and the gelling bath and the concentration of polymers and opposite ions (Krasaekoopt, 2013). The particle size increased as the f polymer concentration increased. For color, Alonso et al. (2003) indicated that a reduction in a* increased the whiteness value (L*).

CONCLUSION

The application of a 2-factor, 3-level Central Composite Design resulted in a useful tool for the characterization of saffron alginate-chitosan and alginate-gelatin beads. The central composite design was used to estimate the effect of the two independent variables, alginate-chitosan or alginate-gelatin concentrations, on the response factor, encapsulation efficiency. The polymer amount was a major factor affecting encapsulation efficiency of the beads. The second-order polynomial model could be used to optimize the encapsulation efficiency of saffron compounds. With alginate, chitosan was a better copolymer than gelatin for encapsulating saffron components. The optimum condition for preparing alginate-chitosan beads was 1.97% alginate and 0.925% chitosan, yielding encapsulation efficiencies of 66.3%, 86.2% and 52.9% for picrocrocin, safranal and crocin respectively. Further research using other encapsulation methods and copolymers should be carried out to obtain higher encapsulation efficiency. These encapsulated saffron powders show promise for use in confectionary and tea bags, the main goal of our future study.

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