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The Improvement of Crocin Stability in Rock Candy (Nabat) By Microencapsulation

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ABSTRACT

In the present study, the microencapsulation technique was utilized to improve the stability of crocin in Nabat (rock candy), which is a popular sweet in Iran. For this purpose, crocin was extracted from saffron, and its microcapsules were prepared through the spray drying process. Gelatin solutions with various concentrations (3%, 5%, 7%, and 10% w/v) were used as the wall material. The encapsulated crocin was added to Nabat, and the physicochemical and organoleptic properties of the samples were compared to Nabat containing pure crocin in different storage conditions. The obtained results indicated that increasing the concentration of the wall materials from 3% to 10% significantly increased the particle size of the microcapsules. The optimal efficiency of crocin microencapsulation was observed at the gelatin concentration of 5%. In addition, a significant difference was observed in the color properties of Nabat containing pure and encapsulated crocin during storage. Therefore, crocin microencapsulation could preserve the sensory properties of the samples. Considering the significant effect of light on the stability of crocin, Nabat containing this substance should be protected from direct light by hermetic packaging.

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Introduction

Nabat, also known as rock candy, is a popular sweet in Iran often consumed with tea as a sweetener. Rock candy is resulted from the crystallization of the supersaturated solution of sucrose on a proper surface to nucleate, such as a string or plain granulated sugar [1].Color additives could be used in the mixture to produce colored Nabat. The most popular Nabat is made of saffron, which gives the product both color and flavor [2, 3].

Saffron is collected from the flowers of *Crocus sativus* L. It is an important permitted natural colorant used in foods, as well as other nutritious and pharmaceutical preparations. Crocin is the main pigment of saffron, which is a carotenoid pigment susceptible to oxygen, light, pH, temperature, metallic ions, and enzymes; exposure to these factors could easily deteriorate crocin [4].

A common challenge in the food industry is that coloring agents tend to migrate to the environment of the product [5]. Microencapsulation has been widely used for the stabilization of sensitive compounds in order to extend their shelf life and protect them against environmental condition. In this technique, particles are capsulated in miniscule capsules by various materials that are released after a specific period in specific conditions in terms of temperature and enzymes [6, 7]. The wall material acts as physical and permeability barriers to oxygen and other molecule diffusion, thereby improving the stability of the product, preventing decay during storage, and protecting the functional compounds [6, 8].

Spray drying is commonly used for microencapsulation in the food industry [9]. Spray drying has been used in several studies to encapsulate natural pigments such as curcumin [10], anthocyanin [7], zinc chlorophyll [11], lycopene [12], and carotenoid pigments [13].

An important issue in the production process of Nabat is the color destruction of saffron during marketing and sales. The present study aimed to extract the crocin pigment from saffron, develop a spray drying method to prepare crocin microcapsules using gelatin as the wall material, and compare the stability of the final product during storage.

Materials and Methods

Experimental Materials

High-quality saffron was provided from Sargol Company (Mashhad, Iran). Gelatin and sugar

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were purchased from a local market, and silicon oxide was obtained from Merck (Darmstadt, Germany).

Preparation of Crocin Microcapsules

Crocin was extracted from saffron in an aqueous solution at the temperature of 25ºC for 16 hours (Selim et al., 2000; Barbosa, 2005). In addition, gelatin solutions were prepared at the concentrations of 3%, 5%, 7%, and 10% w/v. One gram of pure crocin was dissolved in each solution by continuous stirring for 20 minutes. Afterwards, 0.5 gram of silicon oxide was added to the solutions as anti-clogging agent, and the mixtures were homogenized using a laboratory homogenizer (model: DIAX, Heidolph) at 2,400 rpm for 10 minutes (Yu and Huang, 2009). Finally, the mixtures were fed into a spray drier (Mini Spray Dryer 190, Buchi B 290, Switzerland) to prepare the crocin microcapsules at the feed flow rate of 10 ml/min, inlet gas temperature of 180±10ºC, outlet gas temperature of 90±10ºC, and drying air flow of 600 l/h [14].

Analysis of Crocin Microcapsules

Particle Size, Special Surface, and Polydispersity index

The special surface, particle size distribution, and polydispersity index (PDI) of the crocin microcapsules were determined using a particle size analyzer (Malvern Co., model: Etasier nano zs, UK). In addition, the particle size of the samples was described by the surface-weighted mean diameter (D_{32}) using the following equation [14]:

$D32 = \sum n_i d_i^{\,3} / \sum n_i d_i^{\,2}$

$SSA = 6/D_{32}$

where D_{32} is the mean diameter of the samples, z_i shows the number of the particles with the diameter of d_i, and *SSA* represents the special surface of the particles.

Moisture Content

Moisture content was determined using an infrared oven-drying device (Sartorius Co., model: MA35, Germany). To do so, 20 grams of the encapsulated powder was transferred into the plate and dried at the temperature of 135ºC for 10 minutes until achieving a constant weight [15].

Color Properties

Hunterlab (ColorFlex, USA) was used to measure the chromatic characteristics of the encapsulated powders. The colorimeter was applied based on CIELAB values, including L^* (brightness), a^* (red-green), and b^* (yellow-blue). Standard calibration was also performed with black and white references (z=77.25; y=82.09; z=87.27). Additionally, the Chroma parameter (C^*) was obtained using the following equation:

$$C^* = \sqrt{(a^*)^2 + (b^*)^2}$$

To obtain a better correlation between the colorimetric and visual differences, ΔE (total color difference) was measured for each encapsulated samples using the following equation:

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

where $\Delta L^* = L^*_{sample} - L^*_{standard}$, $\Delta a^* = a^*_{sample} - a^*_{standard}$, and $\Delta b^* = b^*_{sample} - b^*_{standard}$.

Solubility

Solubility was determined using the method proposed by Yu and Huang [16]. Initially, 100 milliliters of distilled water was transferred into a blender jar containing 0.1 gram of the encapsulated crocin. Afterwards, solubility was measured as the time within which the samples dissolved completely.

Production Efficiency

The production efficiency of the encapsulated crocin was calculated using the following equation [17]:

$$Production\ Efficiency = \frac{crocin\ (gr)\ in\ microcapsules}{crocin\ (gr) in\ initial\ solution} * 100$$

Preparation of Nabat Containing Pure and Encapsulated Crocin

To prepare Nabat containing pure and encapsulated crocin, 78 milliliters of a supersaturated sugar solution was mixed with 0.06 gram of pure or encapsulated crocin and stirred by a magnetic blender at an ambient temperature for 30 minutes. The solutions were allowed to crystallize onto a stick and dry surface for three days to form Nabat. Finally, the dried Nabat samples were packed in cellophane until further experimentation.

The samples were preserved in direct light and absolute darkness. All the experiments were performed zero, two, four, six, and eight weeks after the production. Nabat samples were analyzed in terms of the physicochemical and sensory properties.

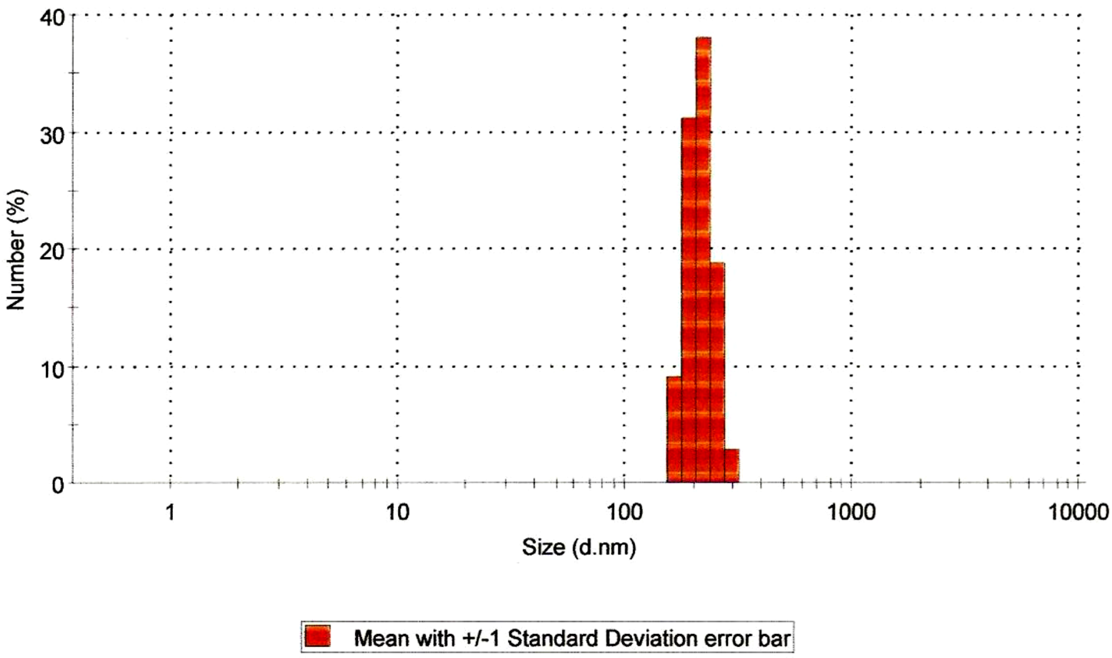
Analysis of Nabat

Physicochemical Properties
Nabat samples were subjected to physicochemical analysis to evaluate the parameters of color, insoluble materials, moisture content, ash content, invert, color in solution, and anhydride sulfur. In addition, color stability was measured according to section 2.3.3, and the other parameters were determined based on the INSO methods [18].

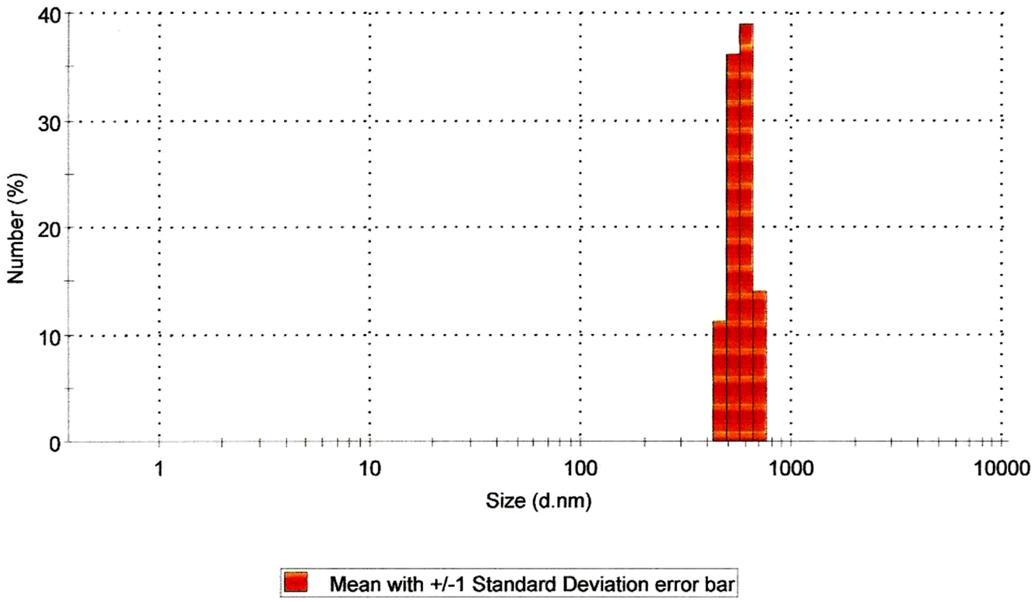
Sensory Properties
The transparency, color, flavor, and total acceptability of Nabat samples were assessed by 18 trained panelists. Additionally, an organoleptic test was designated in a hedonic scale with the sensory rating of 1-5 for each sensory feature.

Statistical Analysis
All the measurements were performed with a completely randomized design and factorial arrangement. Data analysis was carried out using the analysis of variance (ANOVA) in the SAS software, and the mean values were compared using Duncan's multiple range test to determine the significant differences between the samples. The probability level of $P<0.05$ was considered statistically significant, and the curves were plotted in Microsoft Excel. The experiments were carried out in triplicate, and the data were presented as the mean value of each experiment.

Results and Discussion
Analysis of the Encapsulated Crocin
Figure 1 depicts the effects of the wall material concentration (3%, 5%, 7%, and 10%) on the particle size of the crocin microcapsules. According to the findings, the particle size of the encapsulated crocin increased significantly at the higher gelatin concentrations, and smaller particle sizes were formed at low concentrations of gelatin. This could be attributed to the network that was developed by excess wall materials and the increased wall thickness of the microcapsules [14]. The finding is consistent with the previous studies in this regard [17, 19].



(a)


40
30
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10
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Number (%)
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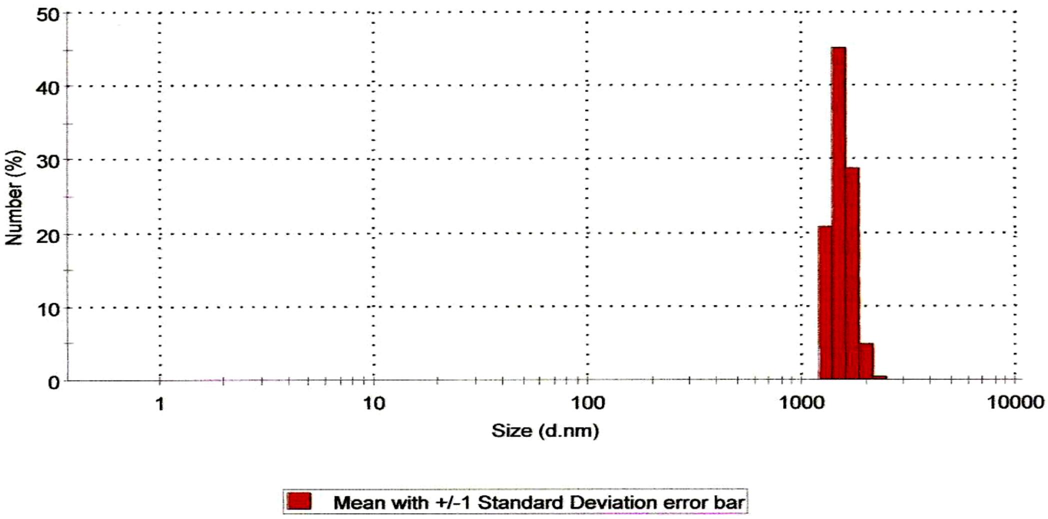
Size (d.nm)
Mean with +/-1 Standard Deviation error bar

(b)


50
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Number (%)
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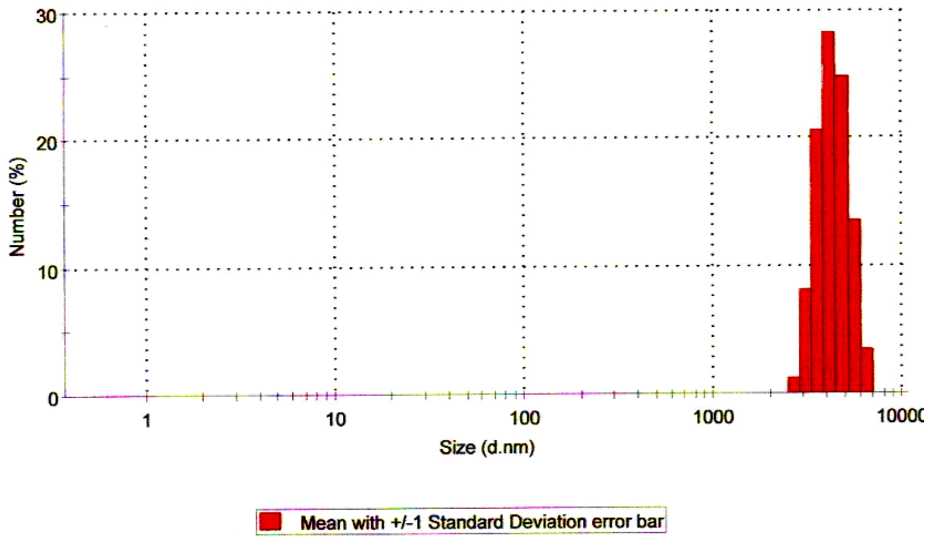
Size (d.nm)
Mean with +/-1 Standard Deviation error bar

(c)



(d)

Figure 1. Diagrams of the particle size analyzer device for size distribution report by number in different gelatin concentrations: a) 3%, b) 5%, c) 7% and d) 10%.

According to the obtained results, the special surface was inversely correlated with the particle size, and the increased particle size led to the smaller special surface (Table 1). Furthermore, the PDI increased significantly with the increased gelatin concentration from 3% to 7%, while it decreased at the concentration of 10% (Table1). The PDI is considered to be an indicator of particle size heterogeneity in a mixture. As the concentration of gelatin increased in the present study, the particle dispersion reduced due to increased viscosity, which in turn increased the PDI. At the gelatin concentration of 10%, the particle size increased uniformly, and the PDI decreased [20].

Table 1. The effect of different gelatin concentration on properties of encapsulated Crosin

Gelatin concentration	Particle size (nm)(mean±SD)	Special surface (m^2/ml)	PDI	%Moisture (mean±SD)	water solubility (min) mean±SD)(	Efficiency (%)
3 %	226.2657±30.48	0.026×10^{-6}	0.379	5.78±0.16	3.33±0.58	17.78
5 %	370.02670±70.5	0.016×10^{-6}	0.543	6.46±0.27	5.67±1.53	18.56
7 %	467.8167±83.38	0.013×10^{-6}	0.939	7.40±0.38	8.33±1.53	17.7
10 %	599.5428±73.91	0.01×10^{-6}	0.297	9.67±0.55	71.67±12.58	15.4

In the current research, the moisture content of the spray-dried powders increased at the higher gelatin concentrations (Table 1). Therefore, it could be concluded that at the higher concentrations of gelatin, the hydrophilic protein compounds absorbed more water, causing the formation of powders with high moisture content and larger sizes [21].

According to the results of the present study, the pure crocin had higher water solubility compared to the encapsulated crocin at an ambient temperature. In addition, the time of water solubility increased significantly at the higher gelatin concentrations (Table 1), which could be attributed to the differences in the wall material. Previous findings have indicated that the water solubility time of olive leaf extract encapsulated in β-cyclodextrin [22] and curcumin encapsulated in hydrophobic modified starch increased at the higher concentrations of the wall material [16]. This was attributed to the fact that hydrocolloids with high concentration prevent the solubility of particles due to their structure [23, 24].

According to the information in Table 1, the optimal efficiency of crocin microencapsulation was observed at the gelatin concentration of 5%. In the present study, the observations also demonstrated that the differences between the encapsulation efficiency could be due to the differences between the polymer matrices formed by each wall material, as well as the film-forming capacity.

Analysis of Nabat Physicochemical Properties

Table 2 shows the physicochemical properties of Nabat samples. Accordingly, the samples containing encapsulated crocin had appropriate physicochemical properties in accordance with the INSO.

Color Stability

The color stability of the samples containing the microencapsulated crocin was evaluated in various conditions of light during two month storage and compared with the samples containing pure crocin. The difference between the lightness of Nabat containing pure and encapsulated crocin was considered significant ($P<0.0001$), and lightness value in Nabat with pure crocin was superior. Furthermore, the effect of light on the L^* value during storage was more significant than the other factors. The samples stored in direct light showed higher L^* values compared to the samples preserved in the dark. In addition, the effects of direct light on the color properties of both Nabat samples were considered significant at various storage times (Figure 2).

Table. 2. Physicochemical properties of Nabat

Parameters	Acceptable range	Method	Result
Color	As Saffron	INSO 711	As Saffron
Insoluble materials	Negative	INSO 711	Negative
Moisture content (%)		INSO 739	0.45
Ash (puan)	Maximum 16	INSO 739	0.438
Invert	Maximum 0.4	INSO 739	0.26
Color in solution	Maximum 60	INSO 739	0
Anhydride sulfur (ppm)	Maximum 10	INSO 739	5

Table. 3. L*, a*, b*, C* and ΔE values of rock candy duration time

Samples	L^*(mean±SD)	b^*(mean±SD)	a^*(mean±SD)	C^*(mean±SD)	ΔE	Time (week)
Nabat containing encapsulated Crocin	50.498±1.226	45.33±0.586	12.087±0.549	46.68±0.468	49.47±21.73	0
	56.05±0.162	23.627±0.045	6.518±0.049	24.486±0.059	45.984±22.44	2
	75.188±1.585	10.847±0.497	2.197±0.171	11.056±0.512	46.914±18.9	4
	76.415±0.953	10.727±0.295	2.477±0.243	16.127±0.328	46.653±19.343	6
	77.6±0.468	10.558±0.236	2.77±0.064	19.34±1.43	46.907±19.425	8
Nabat containing pure Crosin	54.605±0.358	45.372±1.252	6.431±0.806	46.172±1.255	-	0
	57.087±0.908	23.638±0.059	6.532±0.054	24.548±0.06	-	2
	66.015±0.125	11.658±0.412	0.422±0.146	13.76±4.891	-	4
	67.675±0.910	11.337±0.078	0.108±0.050	11.305±0.115	-	6
	67.817±0.315	11.25±0.67	0.645±0.134	12.64±1.432	-	8

The findings of the current research indicated significant differences in the a^* and b^* values of both Nabat samples (Table 3). Correspondingly, the mean values of the a^* and b^* values were higher in the samples with encapsulated crocin, which indicated the higher stability of the color properties in these samples. Moreover, the effects of direct light on the color properties were considered significant. According to the information in Table 3, the increased storage time was associated with the higher L^* value and lower b^*, a^*, and C^* values. After eight weeks of storage, the lighter color of the samples indicated the destruction of the crocin structure. According to the results of the present study, the color stability of Nabat with encapsulated crocin was higher compared to the pure crocin. Therefore, encapsulation could be considered an effective technique for increasing the color stability of crocin in Nabat. Furthermore, Nabat containing crocin should be protected against direct light during storage, and hermetic product packaging would be proper in this regard.

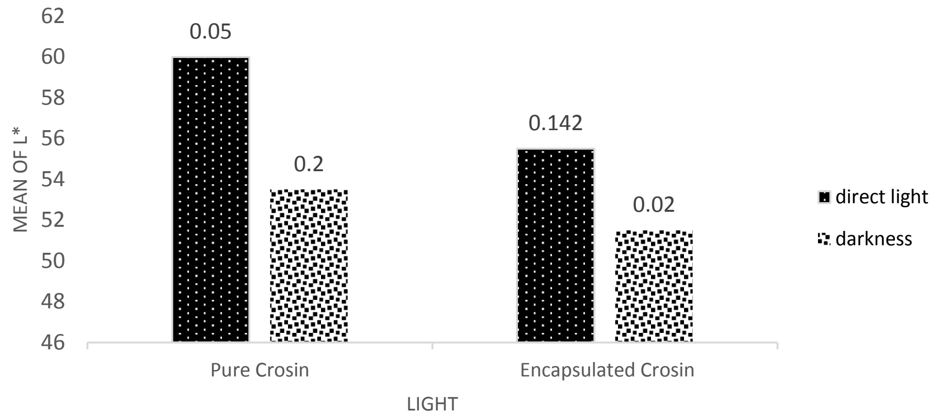


Figure 2. The effect of light on L* duration 60 days

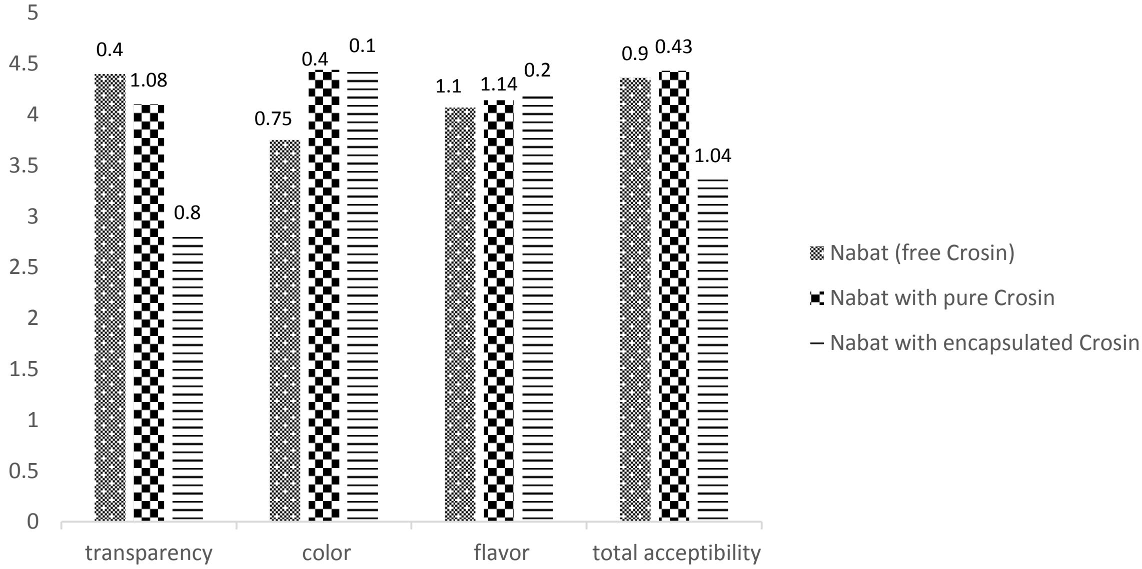


Figure 3. Mean scores for sensory properties of different Nabat sample

Sensory Properties

In the current research, Nabat samples were evaluated in terms of various sensory parameters, including transparency, color, flavor, and total acceptability. Figure 3 depicts the mean scores assigned by the panelists to each parameter. The obtained results showed significant differences in the sensory properties of Nabat samples containing encapsulated crocin and those with pure crocin. Accordingly, the panelists recorded higher scores for the color and flavor of Nabat containing encapsulated crocin. However, the transparency of Nabat with encapsulated crocin was significantly lower compared to the other samples. Notably, all the sensory properties of the samples were acceptable (score limit: 2/5) compared to the controls (without crocin).

Conclusion

In this study, the crocin microcapsules were successfully prepared using the spray drying method and gelatin with various concentrations as the wall material. According to the results, the optimal gelatin concentration to explain efficiency was 5%. Encapsulation could significantly improve the shelf life of crocin in Nabat. The comparison of two types of Nabat containing pure and encapsulated crocin indicated that Nabat containing encapsulated crocin received higher scores of sensory properties (flavor and color). Our findings also demonstrated that direct light reduced the shelf life of the products during storage. Therefore, Nabat containing crocin should be protected against direct light by hermetic packaging.

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