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Evaluationof physicochemical properties and release of nanoemulsion containing crocin and its application in food model system (chocolate)

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ABSTRACT

The prevalence of depression in our country is an average of 7% of people aged 15 and older. Almost all depressed patients cannot do their tasks easily due to lack of energy. One of the types of medicinal plants for treating depression is saffron, which is used in traditional medicine as an uplifting and reducing sadness. One of the types of medicinal plants for treating depression is saffron, which is used in traditional medicine as a happy and reducing sadness. Crocin, as a bioactive compound effective on depression, is sensitive to temperature, pH and oxygen, and its efficiency decreases. Surfactant ratio (50% to 200%), stirring speed (500 and 1000 rpm) and stirring time (1, 2 and 3 hours) were used as the variables of primary emulsion production. The optimal ratio of surfactant to the aqueous phase was 100% and the viscosity increased with increasing surfactant ratio in all microemulsions. High energy homogenization at high pressure was used to make the secondary emulsion and to compare and increase the stability of biopolymers including Soy protein concentrate, Arabic gum and Pectin at two levels of 5 and 10%. Then the nanoemulsion was added to the chocolate. All treatments were evaluated using Duncan's test at a significance level of 5%. The results showed that both surfactants were able to form nanoemulsions. Emulsions stabilized with pectin showed the highest viscosity and the lowest crocin release in simulated stomach and intestine conditions. Also, the physicochemical and sensory properties of chocolate containing nanoemulsion were compared with the control and it had a higher score than the control.

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1. Introduction

Currently, depression affects 20% of the world's population and is considered one of the ten main causes of death [1]. Today, lithium salts, stimulant drugs, tricyclic antidepressants, selective serotonin inhibitors, monoamine oxidase inhibitors, etc. are used for treatment. depression are used [2]. These drugs show their effects after several weeks to several months of use, and the signs and symptoms of depression are completely resolved in only one-third of the users, and there is still a risk of recurrence of the disease. Recently, atypical antidepressants such as bupropion, nefazdone and mirtazapine are available for the treatment of depression. However, the rate of recovery is low and the risk of disease return or relapse remains high, therefore, agents with greater effectiveness and less toxicity are needed [3]. Some plant extracts are effective sources of new and promising drugs in the treatment of depression. Medicinal plants have attracted the attention of researchers in this field because they have long been used for the treatment of various diseases, including psychological diseases, and compared to synthetic and chemical drugs. They have fewer side effects [3]. Previous studies show that alcoholic and aqueous extracts of saffron stigma and petals have antidepressant effects in mice [4]. During the investigations, it has been determined that 94% of the total amount of crocin in saffron is in the form of glycosylated crocin and 6% is in the form of free crocin. The effective ingredients of saffron (antidepressant compounds) are sensitive to changes in pH, temperature and light, and its quality decreases [5]. Rahai et al. (2015) used the microcoating method using chitosan and sodium alginate to increase the stability of crocin and determined the optimal formula using the response surface method. [6]. Rajabi et al. (2015) used the combination of maltodextrin, gum arabic and gelatin at two levels of 30 and 40% for the microcoating of saffron bioactive compounds by spray drying method. The results showed that the preservation of crocin in the resulting powder was strongly influenced by the amount of dry matter, and the maximum shelf life of dry matter was 40%, and with the increase of maltodextrin, the preservation of crocin inside the capsules increased. [7]. Mehrnia et al. (2017) used whey powder biopolymers, gum arabic and gum persian for crocin nano-coating and showed that double emulsions stabilized with persian gum had the highest viscosity and the lowest release of crocin. [8]. Brett (Rajabi, Ghorbani, Jafari, Mahoonak, & Rajabzadeh, 2015), Rand et al. (2007) used the concept of cellular automata to model drug release from erodible microspheres. In this research, cellular automata was defined in three-dimensional conditions and Moore's neighborhood. Based on the obtained results, a good correlation was obtained between the laboratory data obtained from drug release in two cases where polymer erosion occurs quickly and slowly. [9]. However, the type of crocin extraction can greatly affect the bioavailability of compounds [10]. In recent years, technologies have emerged that lead to the creation of high-quality products with a long shelf life. In recent years, the demand for practical foods has increased with the introduction of ultra-beneficial foods and increasing consumer awareness. As one of the most consumed snacks in the food basket, chocolate is a suitable product for enrichment. Today, consumers are looking for chocolate that preserves health and prevents diseases. The aim of this research was the microcoating of crocin using a double-layer emulsion with the ability to be used in food systems. The low energy method of spontaneous emulsification was used to produce the initial emulsion. Soy protein concentrate biopolymers, gum arabic and pectin were used to produce double-layer emulsion, and nanoemulsion properties and crocin release rate were evaluated. Then the physicochemical, rheological and sensory characteristics of chocolate containing nanoemulsion were evaluated.

2- Materials and methods

2-1- Materials

Crocin (digenthiobioscrostin ester) and dialysis bag (D0655) from Sigma company, polyglycerol polyresinoleate from Palsgard Denmark company, gum arabic, pectin and soy protein concentrate from Azaran Lotus company.

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2-2- Methods

2-2-1- Preparation of water-in-oil microemulsion

To prepare water-in-oil microemulsion, lipophilic surfactant polyglycerol ricinoleate with[1]HLB 1.5 was used. 10% w/w crocin solution with PGPR surfactant[2] It was mixed on a magnetic stirrer (RET basic, IKA). The aqueous phase containing surfactant was added dropwise to the oil phase of 80% w/w. Then the resulting emulsion was mixed with constant rotation. Surfactant ratio (50-200%) and stirring speed (Ultra-Turrax, IKA) (500 and 1000 rpm) and stirring time (1, 2 and 3 hours) were used as variables for producing emulsions.

2-2-2- Preparation of two-layer emulsion

10% solutions of pectin, gum arabic and soy protein concentrate were prepared by dissolving the samples in distilled water and were kept overnight in the refrigerator for complete dehydration. Water-in-oil microemulsion 10% w/w was added to the aqueous solution of 90% w/w containing biopolymers and mixed for 5 minutes at 1000 rpm by ultrathorax and then processed with a high pressure homogenizer 35-2000 bar (Emulsifex). [8].

2-3- Tests

Nanoemulsion tests including droplet measurement by Dynamic Light Scattering method, viscosity measurement, rheometric properties and release measurement in stomach and intestinal conditions were performed using dialysis bag at 37 temperature and 100 rpm [11]. Chocolate tests including moisture, water activity, particle size, histometry, color, viscosity and sensory evaluation of chocolate enriched with crocin nanoemulsion (extract containing 15 mg of crocin nanoemulsion per 10 grams of chocolate) were evaluated.

2-4- Statistical evaluation

The effect of various variables including the ratio of surfactant to aqueous phase (at levels of 50 to 200%), the type of biopolymer (soy protein concentrate, gum arabic, pectin), the concentration of biopolymers at levels of 5 and 10% in the production of crocin nanoemulsion and also the effect of adding crocin nanoemulsion Antidepressant chocolate was measured on dependent variables in three repetitions and compared and evaluated with Duncan's multi-range test and SPSS 22 software. Excel 2007 was used to draw the graphs. Curve Expert Professional software (2.6.5) was used to model the experimental data of crocin flow behavior and release.

3. Results and Discussion

3-1- Production of microemulsion

3-1-1- Examining the effect of PGPR concentration

To investigate the effect of PGPR concentration on the ability to produce microemulsions by the coacervation method, the ratios of[3]SWR from 50 to 200 was investigated. Other conditions were considered constant. The results showed (Table 1) that the smallest diameter related to the ratio of surfactant to the aqueous phase was 100%, and with the increase of the ratio of surfactant to the aqueous phase, the droplet size increased and reached 66 and 120 nm in ratios of 150 and 200, respectively.

3-1-2- Examining the effect of stirring speed

In order to check the stirring speed on the formation of microemulsions in standard conditions, i.e., the ratio of surfactant to aqueous phase is 100% and the time is 2 hours, speeds of 500 and 1000 rpm were investigated. The results showed (Table 1) that at low speed, nanoemulsion is not formed, in fact, there is no energy required to separate the dispersed phase along with the surfactant. At a speed of 500 rpm, the average particle size was 12.50 nm, and when the speed increased to 1000 rpm, the droplet size was 24.4 nm. In fact, the increase in energy causes the reunification of the droplets before the movement of the surfactant in the continuous phase and its placement on the surface of the newly formed droplets [12].

3-1-3- Examining the effect of stirring time

To investigate the effect of stirring time on the characteristics of microemulsions, they were subjected to 1, 2 and 3 hours under standard

[1]. HydrophileLypophile balance

[2]. Polyglycerolpolyricinoleate

[3]. Surfactant to water ratio

conditions, i.e. surfactant to aqueous phase ratio of 100% and speed of 1000 rpm. The results showed that (Table 1) that with increasing time, the average droplet size increased from 10.7 to 50 and 65 nm.

Table 1 Effect of SWR, stirring time and particle size of micro-emulsions at standard conditions.

	SWR				Stirring speed (rpm)		Stirring time (h)		
	50	100	150	200	500	1000	1	2	3
particle size (nm)	11.38±0.01[a]	24.4±0.02[b]	66.04±0.23[c]	120.07±0.54[d]	12.52±0.51[a]	24.4±0.02[b]	10.73±0.14[a]	24.4±0.02[b]	65.04±0.02[c]

*Different letters in the same column indicate significant differences between treatments (P<0.05).

3-1-4- The effect of surfactant concentration on the viscosity of microemulsions

The viscosity of emulsions has an effective role in the stability of emulsions and the increase in viscosity reduces the movement of dispersed phase droplets towards each other and prevents the accumulation of droplets. The results showed (Figure 1) that viscosity increased in all microemulsions with increasing surfactant ratio. This increase in viscosity can be caused by an increase in the amount of free surfactant in the continuous phase, which leads to an increase in the viscosity of the emulsion mass [13].

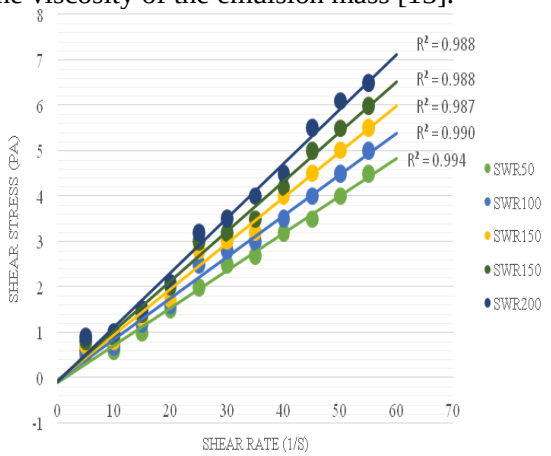

Fig 1 Flow chart of microemulsions.

3-2- Double layer emulsion production

3-2-1- Droplet size and stability of double layer emulsions

As can be seen in Table 2, with increasing concentration of soy protein concentrate and gum arabic, the droplet size decreased, but with increasing pectin concentration, the droplet size increased. The smallest diameter of double-layer emulsions was related to soy protein concentrate, which decreased from 430 to 350 nm with increasing concentration from 5% to 10%. In the case of gum arabic, the particle diameter decreased with increasing concentration, but for pectin, the particle size increased with increasing concentration. The investigation of the droplet size of the emulsions after the storage period showed that the diameter of all the emulsions increased with the increase of the storage time. The largest increase in droplet size was related to Arabic gum with 5% concentration and the lowest was related to 5% pectin. The stability of the emulsion and the lack of growth of globules over time is a function of the complete surface coverage and preventing the droplets from approaching each other. Studies have also shown that the use of polymer compounds with higher molecular weight leads to the formation of more stable double-layer emulsions [14]. The steric hindrance of the surfactant can also prevent the droplets from approaching.

Table 2 Droplet size average and dispersion of two-layer emulsions prepared from biopolymers.

	SPC5	SPC10	AG5	AG10	P5	P10
Fresh emulsion	430±2.53[a]	350±3.21[b]	700±7.53[c]	574±5.25[d]	1147±10.02[Iti s]	2010±12.34[f]
1 [th] month	541±7.61[a]	429±4.32[b]	898±6.74[c]	680±11.23[d]	1310±19.84[Iti s]	2156±24.45[f]

*Different letters in the same column indicate significant differences between treatments (P<0.05)

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3-2-2- flow behavior

Examining the viscosity of biopolymers at the levels of 5 and 10% showed that in the investigated range of shear rate, all biopolymers showed thinning behavior at all levels. Viscosity increased in all biopolymers with increasing concentration from 5 to 10%. The higher viscosity of pectin led to the formation of larger droplets and more stability of bilayer emulsions in both concentrations (Table 3). The high viscosity of pectin prevented the droplets from getting closer to each other during the storage time and caused the emulsions stabilized with pectin to be more stable, compared to the other two biopolymers.

Table 3 Biopolymers viscosity changes at 5 and 10% levels.

		Shear rate (1/s)						
		10	**20**	**30**	**40**	**50**	**60**	**70**
Viscosity (mpa.s)	**SPC 5%**	8.24±0.21^{a}	9.53±0.05^{b}	9.61±0.14^{b}	11.15±0.22^{c}	11.32±0.25^{c}	10.47±0.03^{c}	9.45±0.32^{b}
	SPC 10%	8.23±0.42^{a}	8.33±0.33^{a}	8.45±0.22^{a}	7.51±0.24^{a}	7.14±0.54^{a}	6.75±0.32^{a}	6.31±0.23^{a}
	GA5%	10.31±0.02^{c}	10.23±0.41^{c}	10.45±0.04^{c}	9.35±0.32^{b}	9.43±0.13^{b}	9.07±0.34^{b}	9.41±0.22^{b}
	GA10%	10.12±0.42^{b}	11.21±0.45^{d}	11.45±0.01^{d}	12.11±0.14^{d}	12.15±0.23$^{lt\ is}$	12.22±0.21^{d}	11.34±0.15^{c}
	P5%	109.21±2.11$^{lt\ is}$	107.32±4.01^{f}	105.34±2.11^{f}	104.21±1.32^{f}	12.14±0.54$^{lt\ is}$	104.41±3.21^{f}	104.33±2.14$^{lt\ is}$
	P10%	85.41±3.13^{d}	86.31±2.01$^{lt\ is}$	86.44±0.41$^{lt\ is}$	86.36±1.05$^{lt\ is}$	11.51±0.36^{d}	85.24±0.74$^{lt\ is}$	85.54±0.76^{d}

*Different letters in the same column indicate significant differences between treatments (P<0.05)

The investigation of the flow behavior (Table 4) based on the power, Herschel-Balkli, Bingham and Cason models shows that all biopolymers have the most compliance with the Herschel-Balkli model and then the power relationship.

Table 4 Flow behavior of two-layer emulsion stabilized with biopolymers at the levels of 5 and 10%.

Emulsion	**SPC**		**AG**		**P**	
	5%	**10%**	**5%**	**10%**	**5%**	**10%**
Power law						
K (Pa^{n})	0.0032^{d}	0.016^{c}	0.009^{cd}	0.013^{cd}	0.059^{b}	0.181^{a}
n	0.991^{a}	0.852^{b}	0.923^{ab}	0.859^{ab}	0.901^{ab}	0.915^{ab}
R^2	99.8	98.6	99.8	99.2	99.6	97.9
Herschel bulkley						
K(Not^{n})	0.002^{c}	0.014^{c}	0.006^{c}	0.025^{c}	0.065^{b}	0.213^{a}
n	0.985^{a}	0.856^{d}	0.956^{ab}	0.881^{cd}	0.936^{abc}	0.871^{bcd}
t_0 (Well)	0.005^{b}	0.231^{a}	0.007^{b}	0.012^{b}	0.231^{a}	0.321^{a}
R^2	99.9	98.2	99.9	99.2	0.251	98.1
Bingham						
K(Not^{n})	0.002^{c}	0.003^{c}	0.005^{c}	0.007^{b}	0.041^{b}	0.123^{a}
t_0 (Well)	0.004^{a}	0.251^{a}	0.026^{b}	0.026^{b}	0.098^{b}	0.125^{bc}
R^2	99.9	98.1	99.6	99.1	99.5	78.8
Casson						
K(Not^{n})	0.004^{d}	0.058^{dc}	0.004^{c}	0.095^{c}	0.394^{b}	1.213^{a}
t_0 (Well)	0.011^{b}	0.015^{b}	0.001^{b}	0.012^{b}	0.016^{b}	0.117^{a}
R^2	92.1	98.1	93.2	99.7	99.9	97.9

*The components of the model for each biopolymer are compared with each other in columns at a significance level of 0.05

As can be seen, with the increase in concentration, the consistency coefficient (k) increases and the index (n) decreases, which indicates the increase in viscosity and approaching the dilute state (n < 1) with the increase in biopolymer concentration. The highest consistency coefficient and the lowest flow index were related to pectin, gum arabic and soy protein concentrate, respectively.

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3-3- release of crocin

To check the release rate of crocin during the storage time, first the standard curve of crocin was determined based on the absorbance at 440 nm wavelength and in weight/weight concentrations of crocin (Figure 2). The amount of crocin release is shown in Table 5. As can be seen, the highest amount of crocin was related to soy protein concentrate and the lowest amount was related to pectin. It seems that the macromolecule of pectin is a factor to prevent its release [15].

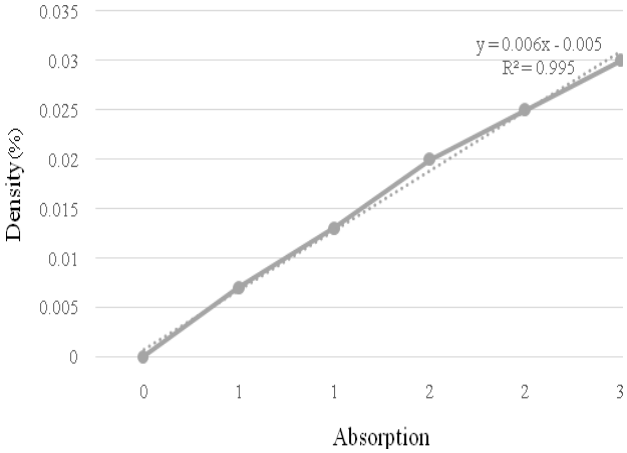

Fig 2 Crocin standard curve.

The obtained detection coefficient (99.56%) showed that there is a high correlation between the concentration of crocin and the amount of absorption obtained.

Table 5 Percentage of crocin release from double-layer emulsion stabilized with biopolymers at 5 and 10% levels

		Time (h)							
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Crocin release	**SPC 5%**	20.21±0.13$^{lt is}$	40.23±0.33^{f}	48.15±0.45^{f}	65.07±0.14^{f}	71.23±0.23^{f}	77.21±0.22^{f}	78.32±0.54^{f}	80.22±0.55^{f}
	SPC 10%	6.44±0.45^{c}	16.21±0.14^{d}	23.11±0.12^{d}	30.33±0.55^{d}	35.22±0.16^{d}	44.27±0.47^{d}	47.28±0.41^{d}	49.54±0.22^{d}
	GA5%	8.74±0.34^{d}	21.01±0.02$^{lt is}$	28.23±0.32$^{lt is}$	40.22±0.35$^{lt is}$	47.36±0.41$^{lt is}$	53.65±0.23$^{lt is}$	56.24±0.25$^{lt is}$	59.32±0.31$^{lt is}$
	GA10 %	4.21±0.54^{b}	11.54±0.07^{c}	16.33±0.46^{c}	22.31±0.24^{c}	27.24±0.37^{c}	35.65±0.08^{c}	39.86±0.38^{c}	41.28±0.19^{c}
	P5%	4.03±0.01^{b}	10.32±0.08^{b}	15.37±0.47^{b}	22.25±0.08^{b}	26.54±0.41^{b}	29.63±0.38^{b}	31.36±0.44^{b}	34.26±0.17^{b}
	P10%	3.31±0.21^{a}	8.34±0.08^{a}	10.87±0.37^{a}	13.24±0.17^{a}	20.25±0.09^{a}	22.71±0.51^{a}	27.33±0.07^{a}	29.81±0.25^{a}

*Different letters in the same column indicate significant differences between treatments (P<0.05).

3-4- Chocolate tests

3-4-1- Physicochemical properties

The results of analysis of variance showed that there is a significant difference between the samples of chocolate containing nanoemulsion in terms of moisture content and water activity. As can be seen, by adding nanoemulsion to chocolate, these factors also increase. The results of analysis of variance showed that between chocolate samples, in terms of particle size index, D_{90} (in the sense that 90% of the particles are smaller than the value of this index) and D_{10} (in the sense that 10% of the particles are smaller than this index) [16]. There is no significant difference (Table 6). As can be seen, with the addition of nanoemulsion, the size of the particles did not increase significantly. The rheological characteristics and final quality of chocolate are significantly affected by the particle size distribution and primary compounds of chocolate, which ultimately plays a significant role in the sensory perception of this product in the mouth. Coarse particles are very effective in mouthfeel due to their gritty nature, but fine particles are more important due to their effect on the flow characteristics of chocolate [17]. Reducing the size of particles causes an increase in viscosity and yield stress. The particle size distribution is important because it can be

optimized to achieve the desired rheological properties without changing the overall chocolate formulation. If the maximum size of particles D_{90} Between 20-33 micrometers and minimum particle size D_{10} If it is less than 6 micrometers, the desired rheological properties will be achieved with the best sensory state in the mouth. d_{90}Larger than 35 µm, it creates a sandy feeling in the mouth, which leads to a decrease in overall acceptance by the consumer. As shown in Table 3, the highest viscosity, hardness in the solid (1) and melted (2) state is related to the enriched treatment, and one of the reasons for this can be the increase in humidity. Skiliman and Beckett (2000) stated that moisture (even in very low amounts) increases the hardness of chocolate [18]. In this connection, Akova et al. stated that various factors such as structure, production conditions, especially chocolate tempering and polymorphism of fat crystals also affect the hardness of chocolate [19]. The results of analysis of variance showed that there is a significant difference between chocolate samples in terms of a, b, and L factor (p 0.05<). As can be seen, by adding nanoemulsion to chocolate, we see a decrease in factor L and an increase in factor a and b..

Table 6 Comparison of mean physicochemical properties of Crocin fortified chocolate

Fortified	Control		
0.36±0.01[b]	0.32±0.04[a]	**Water activity**	
0.73±0.05[b]	0.27±0.01[a]	**Moisture (%)**	
1133.41±10.15[a]	1125.04±11.21[a]	**Viscosity (mpa)**	
23.81±0.12[b]	19.14±0.34[a]	**Hardness1 (N)**	
13.07±0.23[b]	11.61±0.08[a]	**Hardness2 (N)**	
20.47±0.17[a]	19.12±0.12[a]	**D90 (20-33)**	**Particle size (µ)**
1.23±0.12[a]	1.12±0.07[a]	**D10 < 6**	
20.11±0.71[b]	21.31±0.21[a]	**L**	
12.61±0.18[b]	11.71±0.41[a]	**a**	**color**
18.11±0.09[b]	16.91±0.08[a]	**b**	

*Different letters in the same column indicate significant differences between treatments (P<0.05)

3-4-2- Sensory evaluation of chocolate containing nanoemulsion

Ten evaluators were selected after relevant preliminary trainings to perform sensory evaluation and evaluated the produced chocolate samples in terms of flavor, color intensity and mouth melting using the hedonic method (9 points) (Figure 3).

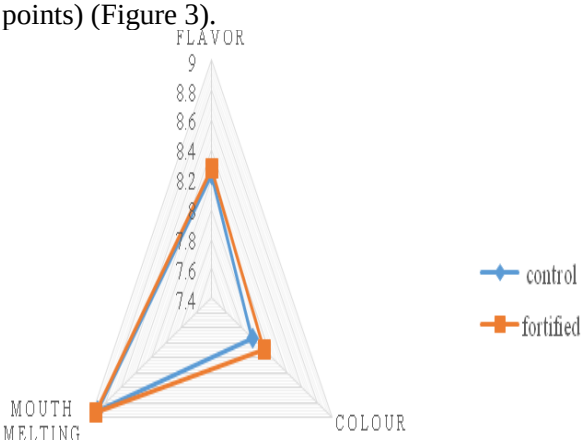

Fig 3 Effect of crocinnanoemulsion addition on sensory properties of chocolate.

The judges gave the highest score to the enriched treatment in terms of aroma and taste, and there was no significant difference between the treatments in terms of mouth melting. In terms of color intensity, the highest score belonged to the enriched treatment.

4 - Conclusion

Crocin is one of the compounds with high solubility in water and its antidepressant effects have been well proven. The results showed that all the two-layer emulsions prepared with biopolymers were still stable after one month and did not become two phases. The investigation of the release of double-layer emulsions in the simulation environment of the stomach and intestine showed that the lowest release rate is related to samples stabilized with pectin biopolymer and the highest rate is related to soy protein concentrate. Moisture assessment, a_{In} And the hardness of the chocolate samples showed that the enriched samples had higher hardness, water activity and moisture than the control sample. The color evaluation showed that the

enriched sample had less L factor and more a and b than the control sample. Comparison of particle size and viscosity of control and enriched samples did not show any significant difference. The sensory evaluation of the samples showed that the enriched sample scored significantly higher.

5- Resources

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مقاله علمی_پژوهشی

بررسی خصوصیات فیزیکوشیمیایی و رهایش نانوامولسیون حاوی کروسین و کاربرد آن در سیستم مدل غذایی (شکلات)

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چکیده

در کشور ما نیز شیوع افسردگی به‌طورمتوسط ۷/۷ درصد جمعیت ۱۵ ساله و بالاتر را در بر می گیرد. تقریباً همه بیمارانافسرده به دلیل کم شدن انرژی، نمی‌توانند وظایف خود را به‌راحتی انجام دهند. یکی از انواع گیاهان دارویی، برای درمان افسردگی زعفران است که در طب سنتی از آن به‌عنوان نشاط‌آور و برطرف‌کننده غم و اندوه یادشده است. کروسین به‌عنوان یک ترکیب زیست فعال مؤثر بر افسردگی، نسبت به دما، PH و اکسیژن، حساس بوده و کارایی آن کاهش می‌یابد. نانوریزپوشانی کروسین با استفاده از امولسیون‌های دولایه روشی برای افزایش پایداری این ترکیب است. نسبت سورفاکتانت (۵۰ تا ۲۰۰ درصد) و دور همزن (۵۰۰ و ۱۰۰۰rpm) و زمان هم زدن (۱، ۲ و ۳ ساعت) به‌عنوان متغیرهای تولید امولسیون اولیه مورد استفاده قرار گرفت. نسبت بهینه سورفاکتانت به فاز آبی ۱۰۰ درصد بود و در تمامی میکروامولسیون‌ها با افزایش نسبت سورفاکتانت، ویسکوزیته افزایش یافت. برای ساخت امولسیون ثانویه از روش پرانرژی هموژنیزاسیون در فشار بالا و برای مقایسه و افزایش پایداری از بیوپلیمرهای کنستانتره پروتئین سویا، صمغ عربی و پکتین در دو سطح ۵ و ۱۰ درصد استفاده شد. سپس نانوامولسیون به شکلات افزوده شد. همه تیمارها با استفاده از آزمون دانکن در سطح معنی‌داری ۵ درصد مورد ارزیابی قرار گرفتند. نتایج نشان داد که هر دو سورفاکتانت قادر به تشکیل نانوامولسیون بودند. امولسیون‌های پایدار شده با پکتین بیشترین ویسکوزیته و کمترین رهایش کروسین در شرایط شبیه‌سازی‌شده معده و روده را نشان دادند. همچنین شکلات حاوی نانوامولسیون ازنظر خواص حسی با نمونه شاهد مقایسه شد و امتیاز بالاتری نسبت به نمونه شاهد داشت.