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Preparation of vitamin D3-loaded oil-in-water-in-oil double emulsions using psyllium gum: optimization using response surface methodology

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Abstract

In this study, the encapsulation of vitamin D3 into a double emulsion with psyllium gum in the aqueous phase and lecithin in the oil phase was optimized and modeled. The optimal values of the three independent variables were generated using a faced-centered central composite design (FCCD). The *Z*-average (diameter of the emulsion droplets), polydispersity index (PDI), zeta potential, interfacial tension, creaming index, and encapsulation efficiency are among the quality evaluation metrics. According to the findings, the *Z*-average in the double emulsion was inversely affected by the psyllium gum concentration. The findings indicated that time after production had a significant direct influence on the *Z*-average. All freshly manufactured formulations may be characterized as good stable emulsions, according to the measurement of double emulsions' zeta potential after preparation (negative charge lower than − 40.1 mV). During storage, the zeta potential value exhibited an upward trend. The creaming index was influenced significantly by storage time ($p < 0.05$) and at the end of storage time, the creaming index was 19.2% (in the sample with no gum and containing 0.25% lecithin). Analysis revealed that the interfacial tension was reduced as a result of the inclusion of the psyllium gum. On the other hand, prolonging storage lengthened the interfacial tension's magnitude. According to the findings, gum content and time had a significant impact on the encapsulation efficiency of primary and double emulsions. Finally, the optimal double emulsion preparation parameters based on maximum encapsulation efficiency were 1% psyllium gum, 1.125% lecithin, and a storage time equal to 25 days at 8 °C, with an obtained encapsulation efficiency of the double emulsion of 93.26%.

Keywords: Double emulsion, Psyllium gum, Vitamin D3

Introduction

Double emulsions, such as oil-in-water-in-oil (O/W/O) and water-in-oil-in-water (W/O/W), are complex emulsions composed of other emulsions inside their droplets. Double emulsions are an appropriate vector for microencapsulation in the food and pharmaceutical industries due to their excellent ability to control the release of bioactive components [1].

One of the main obstacles to the industrial use of double emulsions is their lower stability compared to single emulsions. Instability in double emulsions was caused mainly by coalescence, creaming, and Ostwald ripening [2, 3].

The addition of at least two surfactants that distribute at both double emulsion interfaces and increase stability is a different method for lowering the coalescence rate in the double emulsion. A large concentration of surfactants is required to stabilize double emulsions with

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a high-water content, whereas the interaction of surfactants at interfaces is related to a double emulsion's stability [4]. However, these additives have low acceptable thresholds in the food industry. In addition, the thermodynamic stability of surfactant-stabilized double emulsions against coalescence, Ostwald ripening, and flocculation is insufficient. In double emulsions, the multiphasic system permits additional destabilizing mechanisms, such as the coalescence of the inner droplets and diffusion of the inner phase and the outer phase due to gradients in osmotic and Laplace pressure. To solve these issues, pickering particles or a mix of surfactants and gelling agents (to stabilize the inner or outer emulsion or gel the water phase or crystallize the fat phase) may be used [5]. Therefore, most recent research has focused on formulating double emulsions stabilized by biopolymers [6], such as milk protein concentrate [7], sodium caseinate [8], peanut protein isolate, rice bran protein isolate, soybean protein isolate, whey protein isolate [9], sodium caseinate and k-carrageenan [10], alginate [11], chitosan, cellulose, arabinogalactan, guar and their derivatives [12], calcium alginate [13] and xanthan gum [14, 15].

Oil-in-water nano emulsions are individually efficient oral delivery vehicles for hydrophobic bioactive components, such as non-polar antimicrobials, antioxidants, colors, flavors, and vitamins. Oil-in-water nano emulsions are preferably suitable for the encapsulation and delivery of fat-soluble vitamins, due to the small oil droplets being rapidly digested within the human gastrointestinal tract (GIT), thereby fast generating mixed micelles enable solubilizing and transporting them [16]. Some research focused on the application of double emulsions for encapsulation of various components, such as double emulsion encapsulation for *Sambucus nigra* L. coloring systems, [17] pomegranate peel extract (*Punica granatum L.*), [18] anthocyanins, [19] β-carotene [13]. Double emulsions were studied for encapsulation of various vitamins, such as resveratrol or vitamin B12, [20] calcium and vitamin D3, [21] trans-resveratrol, and vitamin D3 [22].

Psyllium seed (*Plantago ovate Forsk*) is known as a source of natural biopolymers with functional properties arising from the presence of polysaccharides in its husk containing 74.65% xylose, 22.6% arabinose, and some other sugars [23]. Psyllium gum also has a high-water binding capacity and produces intense viscosity [24].

The objective of the present research is optimizing fabrication a double emulsion (O/W/O) using psyllium gum loaded with vitamin D3 via response surface methodology (RSM) and assessment the impact of psyllium gum, lecithin content and storage period on the several double emulsion parameters (*Z*-average, PDI, zeta potential, interfacial tension, creaming index and encapsulation efficiency) and identification the optimum conditions for maximizing vitamin D3 encapsulation in double emulsion.

Materials and methods

Materials

Phosphatidylcholine (purity > 99%) was purchased from Sigma (Germany). Psyllium seeds were bought from a local market (Neyshabur, Iran). Refined sunflower oil was purchased from an industrial supplier (Famila. CO). Vitamin D3 was also purchased from Sigma-Aldrich (St Louis, MO, USA). Isooctane, ethyl alcohol, and ethanol were purchased from Merck (Germany).

Extraction of psyllium seed gum

The extraction and purification of psyllium seed gum were performed according to the method outlined by Askari [24]. First, psyllium seeds were washed with ethanol (96% w/v) three times, and then, drying was done. The extraction of gum was carried out by mixing 10 g psyllium seeds with 200 ml of distilled water (70 °C) and placed on a heater (at 70 °C) and stirred at 1200 rpm for 90 min. The final steps were filtration by cloth and drying (40 °C, 24 h). After milling the dried gum, the powder was kept at room temperature.

Double emulsion preparation

Two primary emulsions (O/W) were produced by dispersing sunflower oil containing vitamin D3 (7 mg/100 g) [7] into the water phase (containing psyllium gum) using a homogenizer (Ultra Turrax T25, IKA, Germany) with the speed of 8500 rpm for 5 min [11] at 70 °C. The formulation of primary emulsions is shown in Table 1. Thereafter, the formation of the double emulsion was done by slow addition of 100 g emulsion A to 100 g emulsion B at the homogenization speed = 5000 rpm, time = 5 min, and temperature = 50 °C. Then, the mixture was cooled to 5 °C with a rate of 10 °C/min and homogenized at 5000 rpm for 15 min which cause the phase inversion and the double emulsion formation occurred [10].

Table 1 Composition of primary emulsions

Ingredient	Primary emulsion A (100 g)	Primary emulsion B (100 g)
Sunflower oil	60	60
Lecithin	0.25–2	0.25–2
Psyllium gum	0–2	0–2
Vitamin D3	7 mg	–
Water	Remaining	Remaining

The ratio between emulsions A and B in the double emulsions is 1:1

Emulsion characterization

Visual inspection

The visual inspection of the emulsion structure was performed using an Olympus BX50 light microscope (Olympus, Japan) with 10–100× magnification.

Creaming index

The stability of emulsion samples was assessed based on the visual inspection of two separate phases: the cream layer (at the top) and the serum layer (at the bottom layer):

$$\text{The creaming index} = {}^{\text{Hl}}/_{\text{He}} \times 100$$

where HL and HE were the height of serum layer and total emulsion height, respectively [8].

Particle size

A Zetasizer Nano ZS90 (Malvern Instruments Ltd., UK) was applied for the determination of the mean diameter of droplets (*Z*-average) in double emulsion and Polydispersity index (PDI).

Zeta potential

Zeta potential determination was performed by a Zetasizer Nano ZS (Malvern Instruments Ltd., UK).

Interfacial tension

Interfacial tension was measured at room temperature using a Sigma 700 tensiometer (KSV Instruments Ltd., Finland) [22].

Encapsulation efficiency

The encapsulation efficiency of vitamin D3 in the emulsions was determined by analyzing the concentration of vitamin D3 in the stable phase of emulsions by UV spectrophotometry. [21] The encapsulation efficiency (EE) in primary and double emulsions was measured for different storage times for 50 days at 8 °C, and it was determined using Eq. (1) as below:

$$\text{EE} = \frac{\text{CUe}}{\text{CUi}} \times 100 \quad (1)$$

where Cue is the concentration of vitamin D3 in the stable phase of emulsions at a specific condition and Cui is the initial concentration of vitamin D3 added [25].

Vitamin D3 determination

For determination of the amount of vitamin D3, the method outlined by Dima and Dima [21] is applied. For the extraction of vitamin D3, over 10 mL mixture of isooctane and ethyl alcohol (1:3 v/v), 2 mL of the emulsion was added. After gentle stirring, the mixture was centrifuged (1700 g, 15 min). The concentration of vitamin D3 in the supernatant phase was determined by spectrophotometry method using UV–VIS spectrophotometer at 265 nm wavelength. A calibration curve (R2 = 0.99) was used for the calculation of vitamin D3 concentration [21].

Experimental design and statistical analysis

A faced-centered central composite design (FCCD) was applied to determine the optimal levels of the three independent variables, namely, gum content (0–2% gum), lecithin content (0.25–2% lecithin) and storage time (0–50 days) coded at three levels (− 1, 0 and + 1) (Table 2). A total of twenty experiments (including six replicates at the center point) were carried out. The data were analyzed using the Design-Expert software (version 13, Stat-Ease Corporation, Minneapolis, MN, the USA) by fitting the second-order polynomial model:

$$\begin{aligned} Y + \beta_0 &+ \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 \\ &+ \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{23} x_2 x_3 \\ &+ \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{33} x_3^2 \end{aligned}$$

where Y is the response calculated by the model (*Z*-average, Zeta potential, Interfacial tension, Creaming Index, Encapsulation Efficiency of Primary and double Emulsion); × 1, × 2, and × 3 denote the independent variables; β_0 is the interception coefficient; β_{11}, β_{22}andβ_{33} are the quadratic terms. β_{12}, β_{13}, and β_{23} are the interaction coefficients. The statistical significance was assumed at a probability level of less than 0.05 ($p < 0.05$). The adequacy of the obtained models was assessed by statistical parameters (R2, adjusted-R2, and coefficient of variation (CV)).

All analytical experiments were carried out in triplicate. Data were analyzed by the analysis of variance

Table 2 Variables and their levels in the central composite design

Factors: independent variables	Level Used	
X1 = Gum content (%)	0	2
X2 = surfactant content (%)	0.25	2
X3 = Storage period (days)	0	50
Responses		
Z-average (nm)	Min.	
PDI	Min.	
Zeta-potential (mV)	Min.	
Creaming index (%)	Min.	
Interfacial tension (mN/m)	Min.	
Encapsulation efficiency of primary emulsion (%)	Max.	
Encapsulation efficiency of double emulsion (%)	Max.	

Table 3 Sequential model sum of squares analyzed for responses (*Z*-average, PDI, zeta potential, creaming index, interfacial tension, and encapsulation efficiency of primary and double emulsions)

***Z*-average (nm)**						**PDI**		
Source	***Df***	**Sum of squares**	***F* value**	**Prob. *F***	***df***	**Sum of squares**	***F* value**	**Prob. *F***
Mean vs total	1	4.646			1	11.77		
Linear vs mean	3	1.033	14.09	< 0.0001	3	1.30	25.49	< 0.0001
2FI vs linear	3	1.718	3.40	0.0506	3	0.1192	3.38	0.0510
Quadratic vs 2FI	3	1.367	5.51	0.0170	3	0.0719	2.96	0.0839
Cubic vs quadratic	4	66,362.59	6.12	0.0260	4	0.0648	6.05	0.0267
residual	6	16,274.01			6	0.0161		
Total	20	6.070			20	13.34		
Zeta potential (mV)						**Interfacial tension (mN/m)**		
Source	***Df***	**Sum of squares**	***F* value**	**Prob. *F***	***df***	**Sum of squares**	***F* value**	**Prob. *F***
Mean vs total	1	25,948.81			1	881.13		
Linear vs mean	3	3428.59	50.95	< 0.0001	3	74.07	8.85	0.0011
2FI vs linear	3	56.48	0.8093	0.5110	3	4.00	0.4265	0.7373
Quadratic vs 2FI	3	132.39	2.60	0.1106	3	36.64	30.60	< 0.0001
Cubic vs quadratic	4	127.44	4.49	0.0511	4	1.43	0.8354	0.5493
residual	6	42.59			6	2.56		
Total	20	29,736.30			20	999.82		
Creaming index (%)						**EE of the primary emulsion (%)**		
Source	***Df***	**Sum of squares**	***F* value**	**Prob. *F***	***df***	**Sum of squares**	***F* value**	**Prob. *F***
Mean vs Total	1	835.92			1	1.268		
Linear vs Mean	3	645.86	40.68	< 0.0001	3	2360.99	31.47	< 0.0001
2FI vs Linear	3	3.37	0.1796	0.9083	3	32.93	0.3887	0.7631
Quadratic vs 2FI	3	62.69	11.23	0.0015	3	343.34	47.97	< 0.0001
Cubic vs Quadratic	4	10.15	1.80	0.2473	4	12.76	1.72	0.2623
residual	6	8.46			6	11.10		
Total	20	1566.45			20	1.296		
EE of double emulsion (%)								
Source	***Df***	**Sum of squares**	***F* value**	**Prob. *F***				
Mean vs Total	1	1.588						
Linear vs Mean	3	1457.60	16.25	< 0.0001				
2FI vs Linear	3	8.94	0.0825	0.9684				
Quadratic vs 2FI	3	446.66	65.36	< 0.0001				
Cubic vs Quadratic	4	8.63	0.9143	0.5123				
residual	6	14.15						
Total	20	1.607						

EE Encapsulation efficiency

(ANOVA), and a p value lower than 0.05 was considered significant in surface response analysis. The three-dimensional response surface analysis was applied for finding the optimal level of parameters.

Results and discussion

Data analysis

To fitting the variation of each response, the sum of the sequential squares, degree of freedom, F value, and p value of the models were assessed using: Mean vs

Table 4 Analysis of variances (ANOVA) of RSM model corresponding to the responses (*Z*-average, PDI, zeta potential, creaming index, interfacial tension, encapsulation efficiency of primary and double emulsions)

Source	Mean	Std. dev	CV	*R2*	Adjusted *R2*
Z-average (nm)	481.95	90.90	18.86	0.9420	0.8898
PDI	0.7670	0.0899	11.72	0.9486	0.9022
Zeta potential (mV)	−36.02	4.74	13.15	0.9052	0.8875
Creaming index (%)	6.47	1.36	21.10	0.9745	0.9516
Interfacial tension (mN/m)	6.64	0.6318	9.52	0.9664	0.9361
Encapsulation efficiency of primary emulsion (%)	79.63	1.54	1.94	0.9914	0.9836
Encapsulation efficiency of double emulsion (%)	89.11	1.51	1.69	0.9882	0.9776

Total, Linear vs Mean, 2FI (two-factor interaction) vs Linear, Quadratic vs 2FI, and Cubic vs Quadratic. The results indicated that the quadratic model was the most desirable one for assessing the responses except for the zeta potential, in which the suitable model was linear (Table 3). According to the ANOVA results, R2 was 0.90–0.99 showing the high precision of the second-order polynomial model (Table 4). In addition, lack of fit was insignificant for all the models at a 5% significance level, which means that the models were good forecasters of the dependent variables (Table 5).

Z-average: ANOVA implies that all the linear, interactive, and quadratic terms had significant impacts on *Z*-average (Table 5). The predicted *Z*-average was determined by the following equation:

$$Z\text{ - average} = 433.42 - 190.41\,x_1 + 292\,x_3 - 144.5\,x_1x_3 + 643.40\,x_3^2$$

Polydispersity index: Considering the data shown in Table 3, the best model fitted with the results of PDI was quadratic model. The linear and interactive terms were significant for the both the gum content and the time of storage and there was no significant interaction between gum and surfactant content and surfactant content and the time of storage. The model for the prediction of PDI is expressed as follows:

$$\text{PDI} = 0.8143 - 0.0959x_1 + 0.3753x_3 - 0.1062x_1x_3$$

Interfacial Tension: As shown in Table 3, the quadratic model suitably fitted the results of interfacial tension. The linear, interactive, and quadratic terms were significant for the independent variables (except lecithin content), while there was no significant interaction between gum and lecithin content and lecithin content and the time of storage. The model for the prediction of Interfacial tension is expressed as follows:

$$\text{Interfacial Tension} = 5.49 - 1.98x_1 + 2.18x_3 + 0.6487x_1x_3 + 5.58x_3^2$$

Creaming Index: The results depicted in Table 5 approve that the effect of time was significant. The equation of the fitted model, after ignoring the non-significant terms, was expressed as follows:

$$\text{Creaming Index} = 4.91 + 8.71\,x_3$$

Zeta potential: Based on the ANOVA results, the linear terms of the model were significant for zeta potential. The model for the prediction of zeta potential would be as follows:

$$\text{Zeta potention} = -36.02 - 4.08\,x_1 - 3.84\,x_2 + 19.29\,x_3$$

Encapsulation efficiency of the primary emulsion

As shown in Table 3, the quadratic model suitably fitted the results of encapsulation efficiency of primary emulsion, the linear, interactive, and quadratic terms have a significant effect on this parameter. The model for the prediction of encapsulation efficiency of the primary emulsion after the sequential ignoring of the non-significant terms would be as follows:

$$\text{Encapsulation efficiency of primary emulsion} = 83.33 + 3.56\,x_1 - 16.28\,x_3 + 1.66\,x_1x_3 - 9.17\,x_3^2$$

Encapsulation efficiency of double emulsion

As shown in Table 3, the quadratic model suitably fitted the results of encapsulation efficiency of double emulsion, the linear and the quadratic terms have a significant effect on this parameter. The model for the prediction of encapsulation efficiency of the double emulsion after the sequential ignoring of the non-significant terms would be as follows:

Table 5 ANOVA and regression coefficients of the response surface models

Response	Source	Sum of square	*df*	Mean square	Value *F*	Prob *F*
Z-average (nm)	Model	1.342	9	1.491	18.04	< 0.0001***
	*x*1	3.082	1	3.082	37.29	0.0001***
	*x*2	444.97	1	444.97	0.0538	0.8212 ns
	*x*3	7.247	1	7.247	87.70	< 0.0001***
	*x*1 *x*2	2738.00	1	2738.00	0.3313	0.5776 ns
	*x*1 *x*3	1.670E + 05	1	1.670E + 05	20.21	0.0012***
	*x*2 *x*3	2048.00	1	2048.00	0.2478	0.6294 ns
	*x*12	40,631.24	1	40,631.24	4.92	0.0509 ns
	*x*22	731.42	1	731.42	0.0885	0.7722 ns
	*x*32	77,253.84	1	77,253.84	9.35	0.0121**
	Lack of fit	67,411.10	5	13,482.22	4.43	0.0641 ns
	Pure error	15,225.50	5	3045.10		
PDI	Model	1.49	9	0.1656	20.49	< 0.0001***
	*x*1	0.0781	1	0.0781	9.67	0.0111**
	*x*2	0.0244	1	0.0244	3.01	0.1132 ns
	*x*3	1.20	1	1.20	148.12	< 0.0001***
	*x*1 *x*2	0.0136	1	0.0136	1.68	0.2235 ns
	*x*1 *x*3	0.0903	1	0.0903	11.17	0.0075***
	*x*2 *x*3	0.0153	1	0.0153	1.89	0.1987 ns
	*x*12	0.0005	1	0.0005	0.0568	0.8165 ns
	*x*22	0.0054	1	0.0054	0.6640	0.4341 ns
	*x*32	0.0204	1	0.0204	2.52	0.1434 ns
	Lack of fit	0.0663	5	0.0133	4.55	0.0608 ns
	Pure error	0.0146	5	0.0029		
Zeta potential (mV)	Model	3428.59	3	1142.86	50.95	< 0.0001***
	*x*1	141.25	1	141.25	6.30	0.0232**
	*x*2	125.03	1	125.03	5.57	0.0313**
	*x*3	3162.31	1	3162.31	140.98	< 0.0001***
	Lack of fit	316.77	11	28.80	3.42	0.0928 ns
	Pure error	42.13	5	8.43		
Creaming index (%)	Model	711.92	9	79.10	42.50	< 0.0001***
	*x*1	0.4944	1	0.4944	0.2657	0.6175 ns
	*x*2	1.13	1	1.13	0.6075	0.4538 ns
	*x*3	644.24	1	644.24	346.18	< 0.0001***
	*x*1 *x*2	2.20	1	2.20	1.18	0.3019 ns
	*x*1 *x*3	0.8450	1	0.8450	0.4541	0.5157 ns
	*x*2 *x*3	0.3200	1	0.3200	0.1720	0.6871 ns
	*x*12	0.3887	1	0.3887	0.2089	0.6574 ns
	*x*22	0.0003	1	0.0003	0.0002	0.9893 ns
	*x*32	4.96	1	4.96	2.67	0.1335 ns
	Lack of fit	11.98	5	2.40	1.81	0.2662 ns
	Pure error	6.63	5	1.33		

Table 5 (continued)

Response	Source	Sum of square	*df*	Mean square	Value *F*	Prob *F*
Interfacial tension (mN/m)	Model	114.71	9	12.75	31.93	< 0.0001***
	*x*1	33.48	1	33.48	83.88	< 0.0001***
	*x*2	0.0136	1	0.0136	0.0341	0.8572 ns
	*x*3	40.57	1	40.57	101.64	< 0.0001***
	*x*1 *x*2	0.5050	1	0.5050	1.27	0.2869 ns
	*x*1 *x*3	3.37	1	3.37	8.44	0.0157**
	*x*2 *x*3	0.1275	1	0.1275	0.3195	0.5844 ns
	*x*12	0.1249	1	0.1249	0.3128	0.5883 ns
	*x*22	0.7904	1	0.7904	1.98	0.1897 ns
	*x*32	5.82	1	5.82	14.57	0.0034***
	Lack of fit	2.28	5	0.4556	1.33	0.3811 ns
	Pure error	1.71	5	0.3427		
Encapsulation efficiency of primary emulsion (%)	Model	2737.27	9	304.14	127.47	< 0.0001***
	*x*1	108.01	1	108.01	45.27	< 0.0001***
	*x*2	1.13	1	1.13	0.4738	0.5069 ns
	*x*3	2251.85	1	2251.85	943.79	< 0.0001***
	*x*1 *x*2	10.81	1	10.81	4.53	0.0592 ns
	*x*1 *x*3	22.11	1	22.11	9.27	0.0124**
	*x*2 *x*3	0.0112	1	0.0112	0.0047	0.9466 ns
	*x*12	0.3488	1	0.3488	0.1462	0.7102 ns
	*x*22	0.6270	1	0.6270	0.2628	0.6193 ns
	*x*32	15.68	1	15.68	6.57	0.0282**
	Lack of fit	15.43	5	3.09	1.83	0.2615 ns
	Pure error	8.43	5	1.69		
Encapsulation efficiency of double emulsion (%)	Model	1913.19	9	212.58	93.32	< 0.0001***
	*x*1	48.48	1	48.48	21.28	0.0010***
	*x*2	25.42	1	25.42	11.16	0.0075***
	*x*3	1383.69	1	1383.69	607.41	< 0.0001***
	*x*1 *x*2	0.4050	1	0.4050	0.1778	0.6822 ns
	*x*1 *x*3	0.1250	1	0.1250	0.0549	0.8195 ns
	*x*2 *x*3	8.41	1	8.41	3.69	0.0837 ns
	*x*12	0.3332	1	0.3332	0.1463	0.7101 ns
	*x*22	1.61	1	1.61	0.7063	0.4203 ns
	*x*32	36.91	1	36.91	16.20	0.0024***
	Lack of fit	15.72	5	3.14	2.23	0.2001 ns
	Pure error	7.06	5	1.41		

$$\text{Encapsulation efficiencyof double emulsion} = 93.27 + 2.39\,x_1 + 1.73\,x_2 - 12.76\,x_3 - 14.06\,x_3^2$$

Response surface plotting

Z-average and (polydispersity index (PDI)

In Fig. 1, the particle size distribution curves of double emulsions after production and after 50 days of storage time were shown. Accordingly, double emulsion just after production, was single-peaked, which indicated uniform droplets in the double emulsion. After 50 days of storage at 8 °C, two peaks appeared that imply more variation in particle size in double emulsion (Fig. 1). The response surfaces in Fig. 2 indicate the profile of the response (*Z*-average) against the independent variables. As shown in Fig. 2, an increase in gum content leads to decreasing

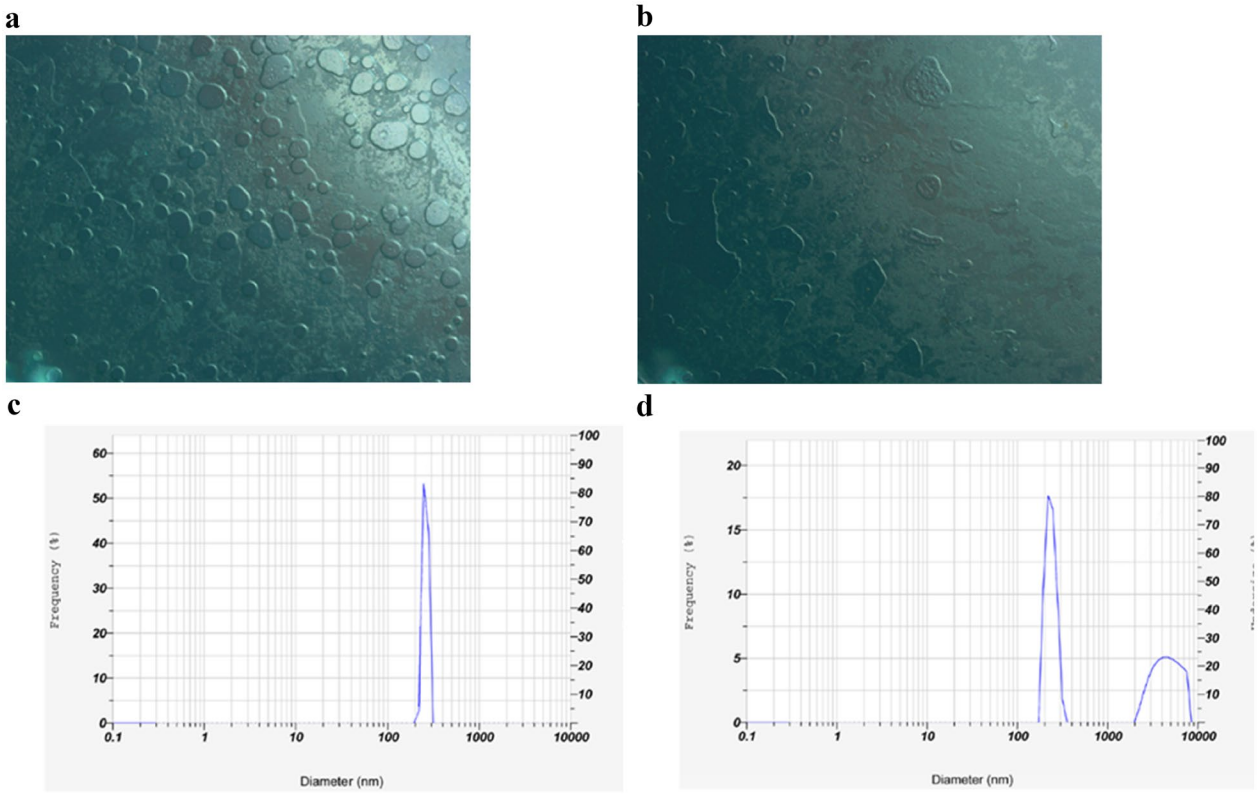

Fig. 1 Visual inspection of double emulsions at 100 × after production (**a**) and after 50 days of storage (**b**). The particle size distribution diagram of freshly produced double emulsion (**c**) and after 50 days of storage (**d**)

the *Z*-average as the lowest *Z*-average belongs to samples containing 2% psyllium gum after production (214–220 nm). Addition the psyllium gum resulted in a smaller particle size in double emulsion (Fig. 2) which is to agree with the report of Guo et al. [13] who reported the average size of dispersed oil droplets in the primary O1/W emulsions reduced slightly with the increase of alginate concentration. Chan et al. [26] stated that smaller oil droplets would be developed if alginate was more concentrated in the aqueous phase and ascribed this to limits the oil droplet's movement due to the increased viscosity of the continuous phase. Wang et al. [27] also pointed out decreasing the average size of emulsions as various polysaccharides (mixtures of *Dendrobium Officinale* polysaccharides and gum Arabic or propylene glycol alginate) were added and attributed to the effectively adsorbed of polysaccharides on the droplet surface and the high viscosity of polysaccharides that caused the limited movement of the droplets, and prevent the re-aggregation.

According to the results, time after production showed a significant impact on the *Z*-average. A longer time led to a higher magnitude of *Z*-average (Fig. 2). Li et al. [9] confirmed the effect of storage time on the stability of food protein-stabilized nano emulsions. Sarheed et al. [11] also reported an increasing average size of droplets over storage time and attributed to the Ostwald ripening (in which droplets of smaller sizes tend to diffuse into larger droplets). Considering data, the PDI of double emulsion were in the range of 0.31–1.47. Accordingly, the freshly double emulsion produced has a narrow PDI (0.31–0.37). It was in agreement of Li et al. [9] that reported the magnitude of PDI of corn oil nano emulsions stabilized by various proteins was < 0.3. Carpenter et al. [25] reported PDI value of secondary emulsion equal to 0.49 ± 0.049. Data indicated that both the content of gum and the storage time have significant effect on the PDI of double emulsions ($p < 0.05$). There was a proportional increment

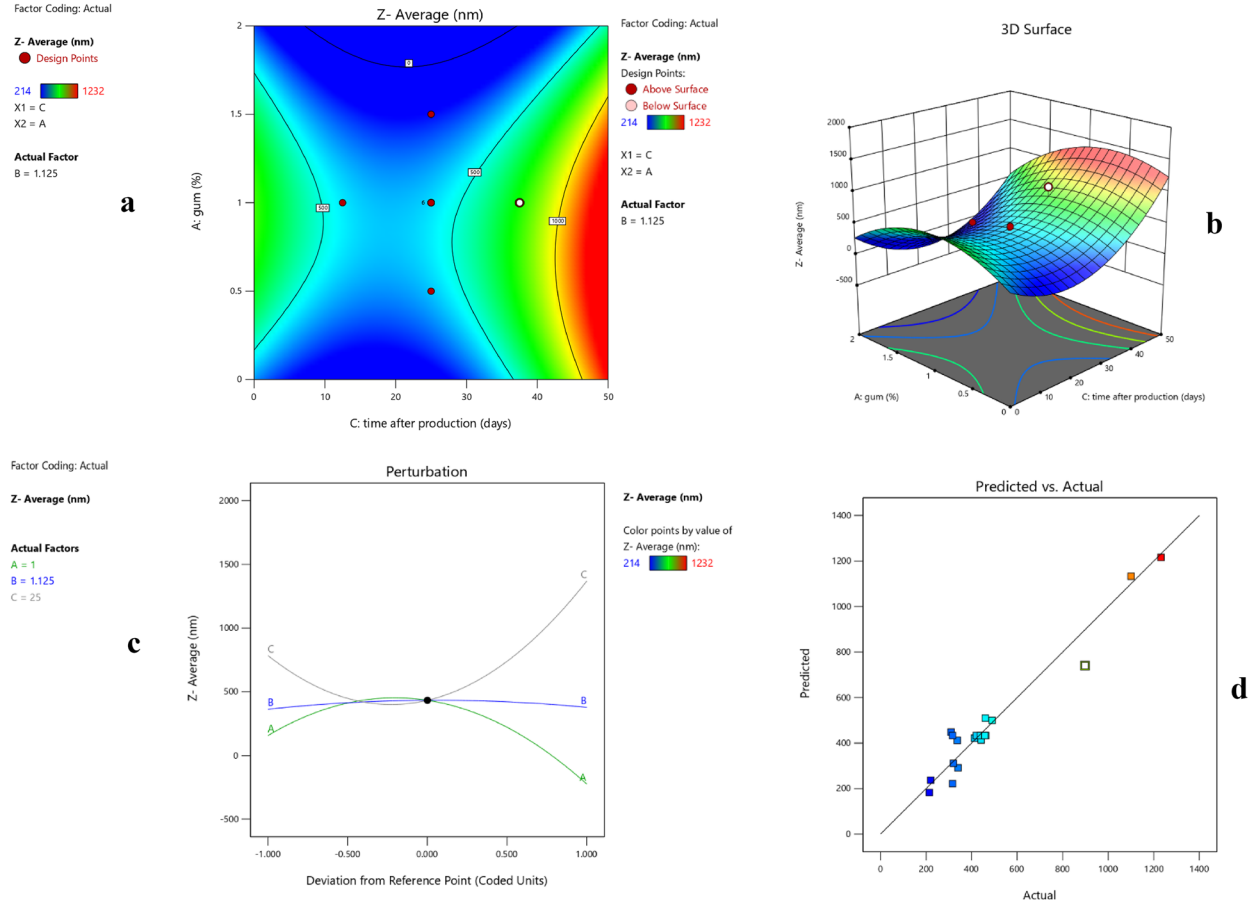

Fig. 2 Contour plot (**a**), three-dimensional response surface plot (**b**), perturbation plot (**c**), and correlation between experimental and approximated values (**d**) according to RSM, showing the impact of variables on *Z*-average of double emulsions

of PDI as the storage time increased (Fig. 3). Inversely, as the content of gum in emulsion increased, the PDI value was decreased ($p < 0.05$).

Zeta potential

All formulations showed a negative charge greater than − 40.1 mV at the time of production. Stability behavior based on the zeta potential is defined as follows: zeta potential equal to 0 to ± 5 mV, Flocculation or coagulation occurred, ± 10 to ± 30 mV, Primitive instability. ± 30 to ± 40 mV, medium stability. ± 40 to ± 60 mV, good stability > ± 60 mV, excellent stability [28]. Accordingly, the freshly prepared double emulsion has good to excellent stability. The stability diminished over time as the zeta potential of double emulsion prepared with or without gum reaches − 20.1 and − 14.6 mV, respectively, which is in the range of incipient instability. The magnitude of measured zeta potential was in the range of zeta potential reported by Chuacharoen et al. [29] for curcumin-loaded nano emulsion containing lecithin and Tween 80 as co-surfactant. The observation showed as the added gum content increased, the zeta potential showed a decreasing trend (Fig. 4). Our result was in agreement with the finding of Sarheed et al. [11] who reported high zeta potential might be due to the chemical structure of alginate and the presence of carboxylate and hydroxyl functional groups that are easily deprotonated at neutral pH. Psyllium gum is also composed of xylose and arabinose and substantial amounts (15%) of uronic acids [30] that might be causative in decreasing the zeta potential as the gum is incorporated in the emulsion.

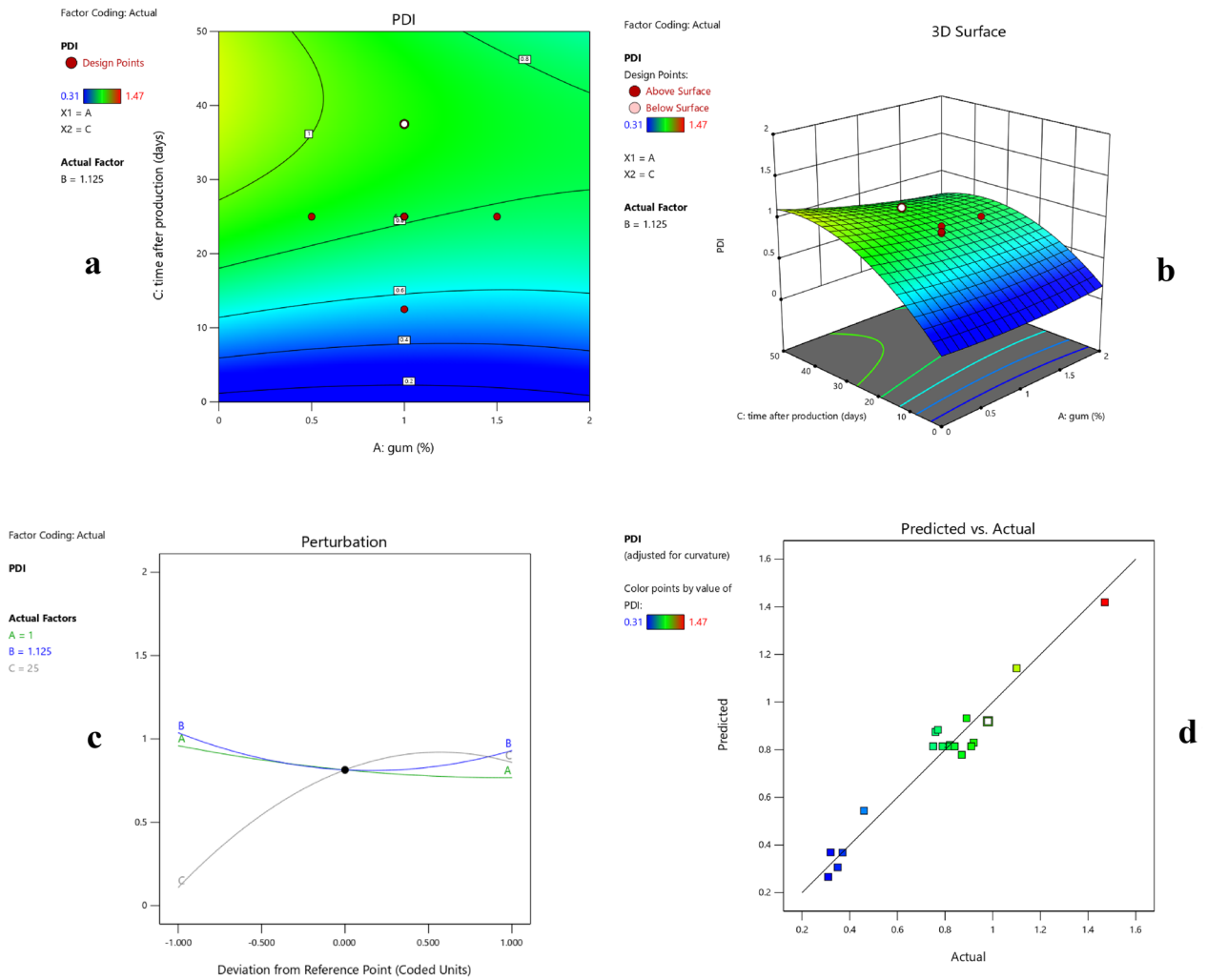

Fig. 3 Contour plot (**a**), three-dimensional response surface plot (**b**), Perturbation plot (**c**), and correlation between experimental and approximated values (**d**) according to RSM, showing the impact of variables on PDI of double emulsions

Creaming index

The creaming index of double emulsions was inspected physically. During 50 days of storage, the creaming index reaches 1% after 12 days of storage and at the end of storage time, it reaches about 19.2% (in the sample without gum and containing 0.25% lecithin).

The structure of the double emulsion is very complicated and easily destroyed during the preparation and storage processes. The favorable encapsulation of bioactive compounds is possible only in a stable double emulsion. In the present study, the suitable double emulsion stability was obvious, and the first signs of instability were observed after 12 days (about 1% creaming index). Keršienė et al. [7] reported creaming stability of 96.0% and 99.2% for loaded emulsion and empty double emulsion, respectively.

Sarheed et al. [11] attributed the high stability of double emulsion to the presence of a double layer of surfactant and polymer surrounding oil droplets which minimize the creaming rate, by controlling the net density of the droplets and keeping it closer to that of the surrounding aqueous phase. The inclusion of hydrocolloids in the water phase could diminish droplet movement and enhance the stability of the dispersed oil droplets against coalescence, resulting in the formation of smaller oil droplets [26].

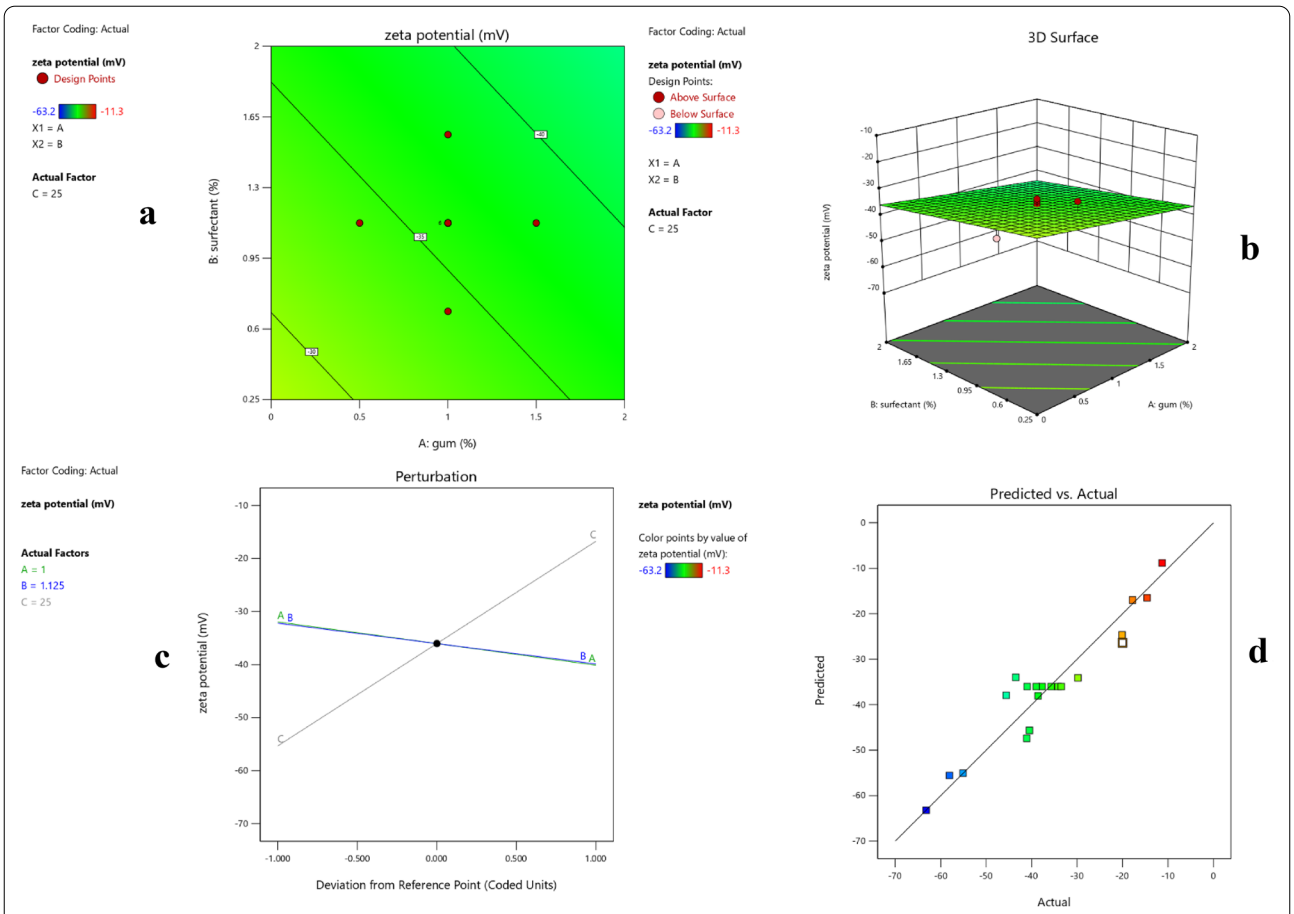

Fig. 4 Contour plot (**a**), three-dimensional response surface plot (**b**), perturbation plot (**c**), and correlation between experimental and approximated values (**d**) according to RSM, showing the impact of variables on zeta potential of double emulsions

There was a direct relationship between storage time and creaming index, as more storage time led to more creaming index in double emulsion (Fig. 5). This observation has good agreement with the results of the *Z*-average and zeta potential of double emulsions during storage time. As previously stated, with increasing the time of storage, the *Z*-average and the zeta potential of particles were increased. Both these phenomena are related to the instability in double emulsions which is obvious after 50 days of storage and reaches about 15.8–19.2% in various double emulsion formulations.

Creaming index (that is a sign of instability in emulsions) was lower in samples including psyllium gum and this observation might be attributed to the high viscosity continuous phase which prevents the aggregation of droplets and the movement of molecules, so the molecular movement resistance would be high, the speed was slow, and macroscopically, the interface movement rate was small [27].

Interfacial tension

The interfacial tension is a sensitive character that gives information about changes at interfaces of emulsion. Analysis showed that two parameters that affecting interfacial tension of double emulsions were gum content and time. The addition of the psyllium gum into the double emulsion formulation caused decreasing interfacial tension. Inversely, increasing the time of storage resulted in increasing the magnitude of interfacial tension (Fig. 6). Mohammadzadeh Milani et al. [31] stated that increasing the concentration of charkhak (*Launaea acanthodes*) gum led to a decrease in surface and interfacial tension

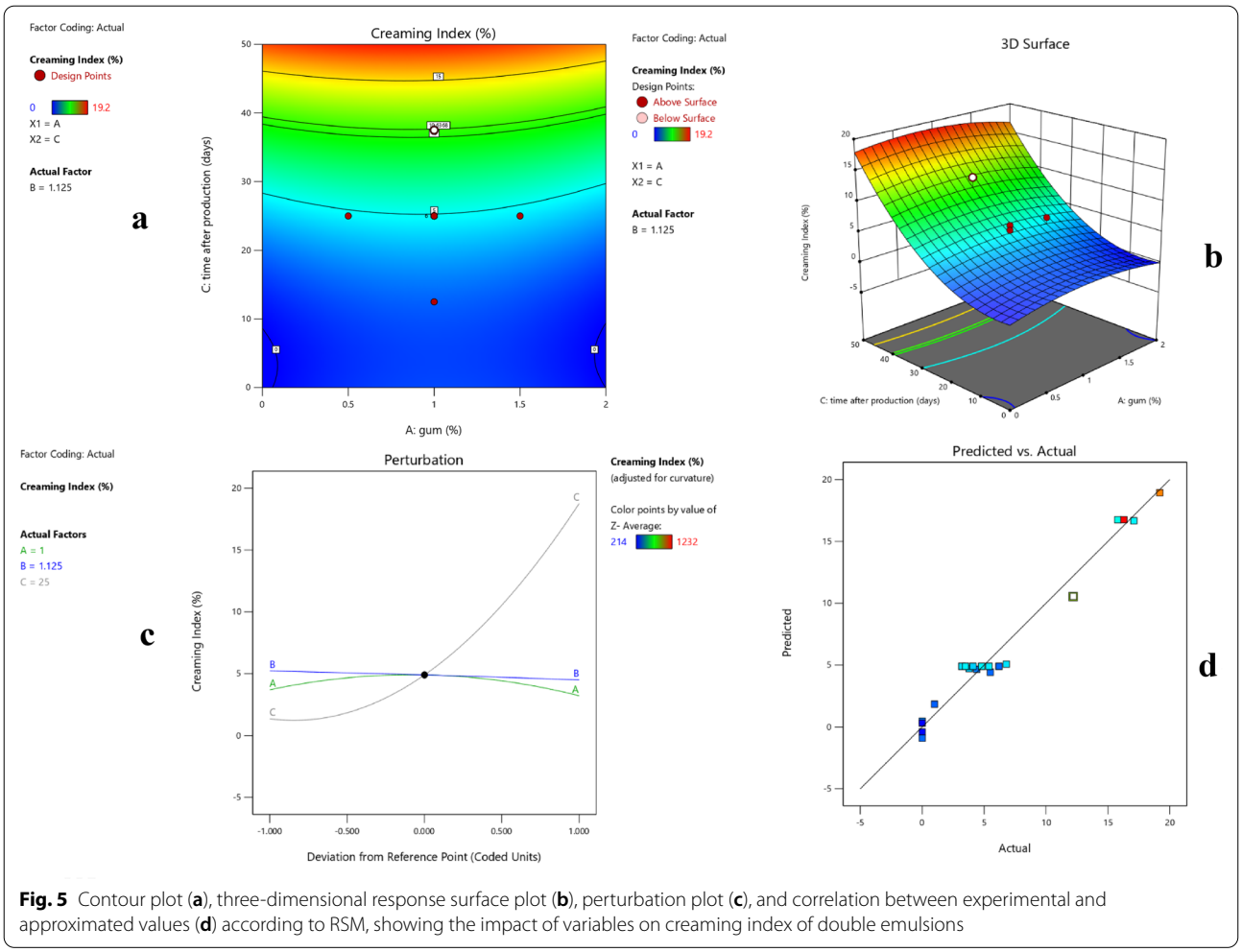

Fig. 5 Contour plot (**a**), three-dimensional response surface plot (**b**), perturbation plot (**c**), and correlation between experimental and approximated values (**d**) according to RSM, showing the impact of variables on creaming index of double emulsions

of emulsion. Many polysaccharides induce decrease in the surface tension since of that they could act as surface-active agents. The ability of some polysaccharides to decrease the surface tension of water follows a general trend: the primary addition of polysaccharides strongly reduces the surface tension until it reaches a saturation concentration. According to Dickinson [32] for reduce surface tension by various components; they should have some specific characteristics: (1) be amphiphilic that is either hydrophilic or hydrophobic, so it can place between two surfaces. (2) generally, could cover the surface, so polysaccharide must be soluble in water to the polymer chains cover the oil particles in the continuous phase.

Encapsulation efficiency of primary emulsion and double emulsion

The encapsulation efficiency, which is also an indication of the stability of vitamin D3 in primary and double emulsion, was analyzed for different concentrations of gum and lecithin as well as during storage time. As shown in Fig. 7, the highest encapsulation efficiency of primary emulsion (93.3%) belonged to the sample containing 2% gum and 2% lecithin after production. In the freshly primary emulsion produced without gum, the determined encapsulation efficiency was equal to 88.9%. According to analysis, gum content and time had a significant effect on encapsulation efficiency of primary emulsion (Table 5). The encapsulation efficiency of primary emulsion, after 50 days of storage, reaches 51.3% in the sample without gum and 65.7% in the sample containing 2% gum and 2% lecithin. The magnitude of encapsulation efficiency in the case of double emulsions were about 98.8% and 94.5% for samples produced with or without psyllium gum, respectively, and reaches 71.2% (without gum) and 75.6% (with 2% gum) after 50 days of storage at 8 °C. Analysis showed that all the parameters included

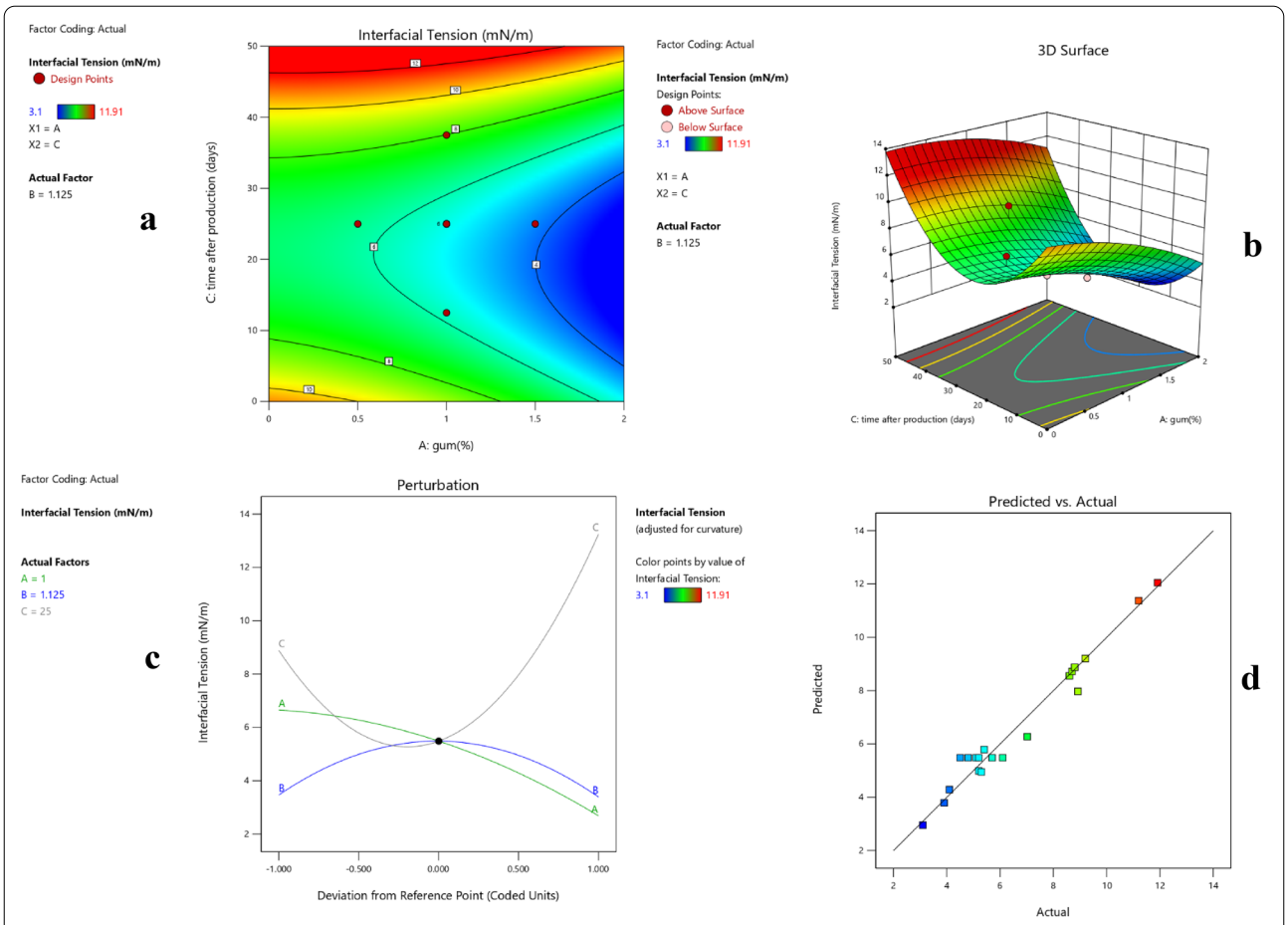

Fig. 6 Contour plot (**a**), three-dimensional response surface plot (**b**), perturbation plot (**c**), and correlation between experimental and approximated values (**d**) according to RSM, showing the impact of variables on interfacial tension of double emulsions

psyllium gum content, lecithin content and storage time had a significant effect on the level of encapsulation efficiency of double emulsions (Fig. 8). These results were in good accordance with Carpenter et al. [25] who evaluated the effects of curcumin encapsulation in multilayer oil-in-water emulsion and reported the encapsulation efficiency of primary emulsion significantly decreased during storage and reaches 56 ± 2.63% after 21 days of storage (from 100 ± 0.78% after production).

Keršienė et al. [7] stated the encapsulation efficiency of vitamin D3 in the double emulsion is equal to 98.52 ± 10.43% after preparation and reaches 78.26 ± 11.90% after 30 days of storage.

The encapsulation efficiency in the present study was higher than the report of Guo et al. [13] in which the encapsulation efficiency was ~50 wt% and attributed the low encapsulation efficiency to the size of droplets in the oil phase (micron size). In the present study the average particle size was significantly lower than their study, so more obtained encapsulation efficiency might be attributed to the smaller particle size which led to better encapsulation efficiency both in primary and double emulsions. With the increase of psyllium gum concentration in the aqueous phase, the encapsulation efficiency of vitamin D3 increased (both in the primary and double emulsion). This correlation has also been reported by Guo et al. [13] that the encapsulation yield of β-carotene-loaded O1/W/O2 emulsions increased with increasing the alginate concentration in the aqueous phase.

Optimal conditions of the double emulsion formation

To achieve the best conditions of the double emulsion formation, the amount of gum content, lecithin content,

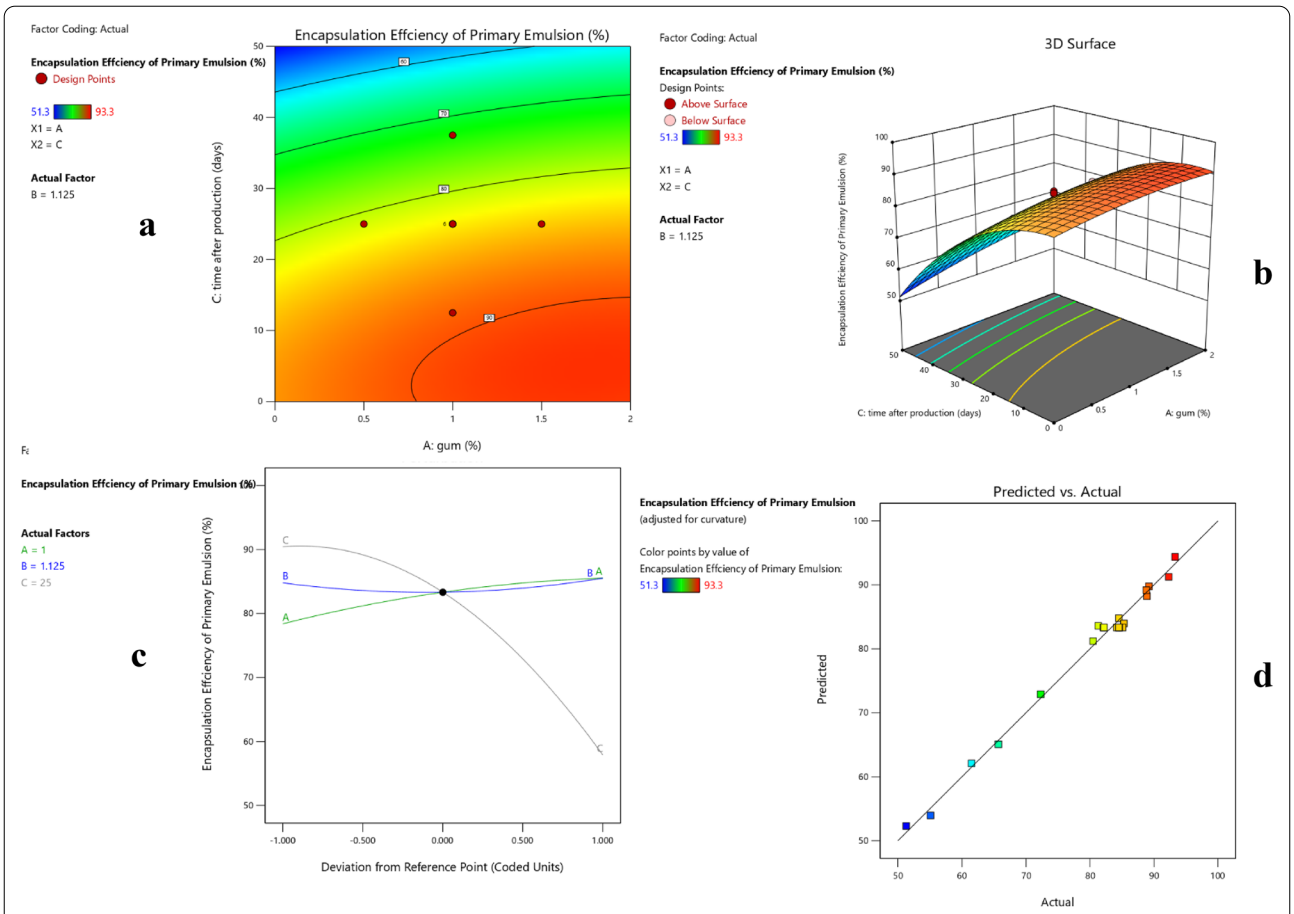

Fig. 7 Contour plot (**a**), three-dimensional response surface plot (**b**), perturbation plot (**c**), and correlation between experimental and approximated values (**d**) according to RSM, showing the impact of variables on encapsulation efficiency of primary emulsion

and storage time were considered in the used range. Accordingly, the optimal condition was as follow: psyllium gum = 1%, lecithin content = 1.125% and storage time = 25 days.

The experimental verification is performed to further illustrate the reliability of the optimization results. With respect to the optimal double emulsion fabrication condition (gum content = 1%, lecithin content = 1.125% and storage time = 25 days), the results achieved from confirmation test for responses are showed in Table 6. As affirmed by comparative analysis, the low deviation between the multi-objective optimization results and the experimental results for the *Z*-average, PDI, interfacial tension, zeta potential, creaming index, and encapsulation efficiency of primary and double emulsion, suggests that the optimization results of this research are acceptable.

Conclusion

The results indicated that the concentration of psyllium gum had an inverse effect on the *Z*-average in double emulsion, and storage time showed a significant direct impact on the *Z*-average. PDI also significantly influenced by gum content and storage time ($p < 0.05$). The zeta potential value showed an increasing trend during storage. During 50 days of storage, the creaming index reaches 1% after 12 days of storage and at the end of storage time, it reaches about 19.2% (in the sample without gum and contains 0.25% lecithin). Analysis showed that the addition of the psyllium gum into the double emulsion formulation caused decreasing the interfacial tension. Inversely, increasing the time of storage resulted in increasing the magnitude of interfacial tension. Gum content and storage time significantly affect the encapsulation efficiency of primary and

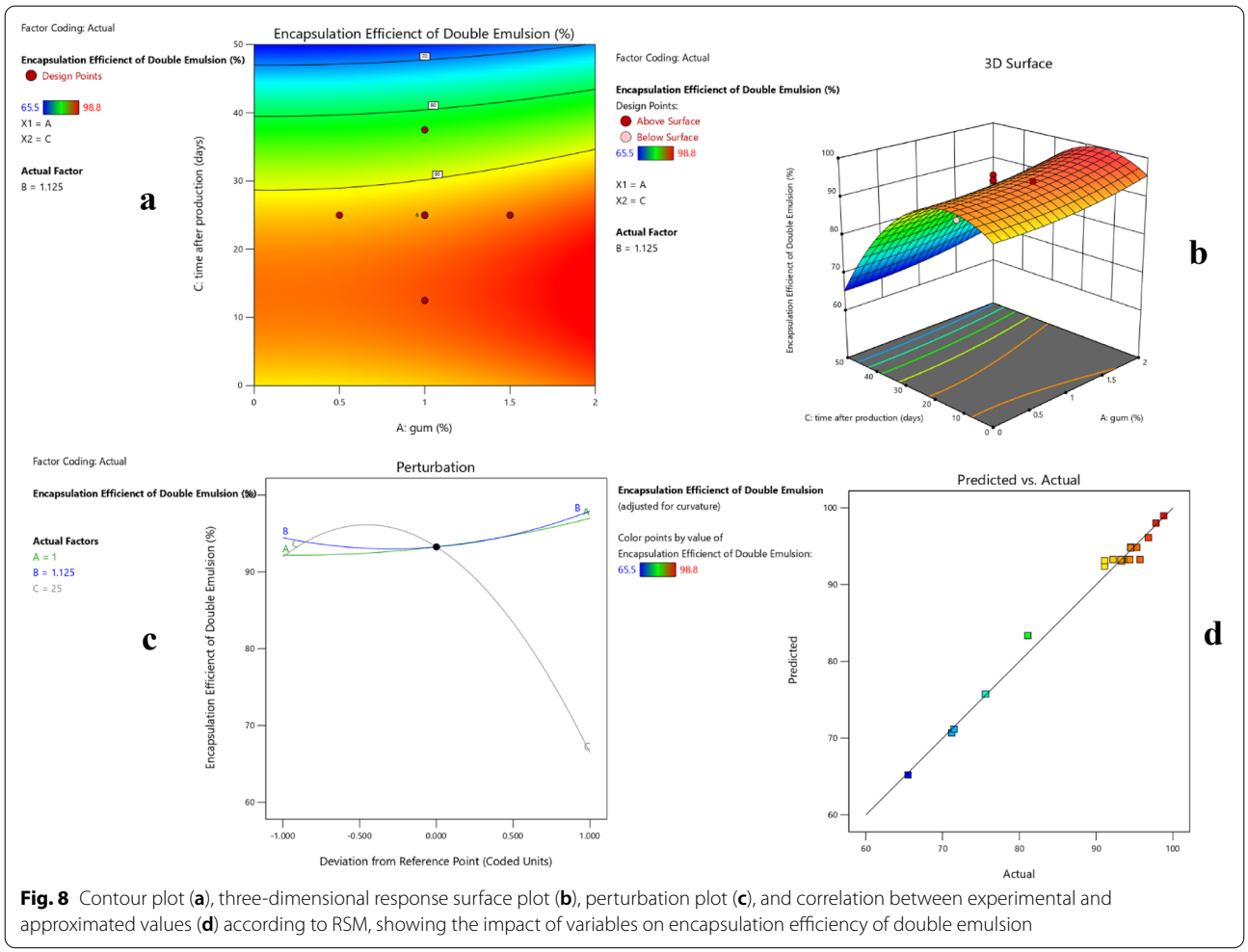

Fig. 8 Contour plot (**a**), three-dimensional response surface plot (**b**), perturbation plot (**c**), and correlation between experimental and approximated values (**d**) according to RSM, showing the impact of variables on encapsulation efficiency of double emulsion

Table 6 Comparison between theoretical and experimental results at the optimum condition predicted by the model

Parameter	Theoretical	Experimental	Goal	Lower limit	Upper limit
Gum concentration (%)	1	1	In range	0	2
Surfactant concentration (%)	1.125	1.125	In range	0.25	2
Storage time (day)	25	25	In range	0	50
Z-average (nm)	433.42	432.27	Min.	214	1232
PDI	0.81	0.82	Min.	0.31	1.47
Zeta potential (mV)	− 36.66	− 36.45	Min.	− 63.2	− 11.3
Creaming index (%)	4.90	5.12	Min.	0	19.20
Interfacial tension (mN/m)	5.48	5.58	Min.	3.1	11.91
EE of primary emulsion (%)	83.33	83.12	Max.	51.31	93.26
EE of double emulsion (%)	93.26	93.28	Max.	65.52	98.81

EE Encapsulation efficiency

double emulsion ($p < 0.05$). Finally, the optimal double emulsion preparation conditions on basis of the maximum encapsulation efficiency were 1% psyllium gum, 1.125% lecithin, and storage time equal to 25 days at 8 °C which the achieved encapsulation efficiency of the double emulsion was 93.26%.

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Author contributions
Supervision, funding acquisition, conceptualization, software, methodology, data curation, validation, formal analysis, writing—original draft, ZD; writing—review and editing, MAH. Both authors read and approved the final manuscript.

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Availability of data and materials
The authors confirm that the data supporting the findings of this study are available within the article.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
All authors have read and agreed to the published version of the manuscript. All authors read and approved the final manuscript.

Competing interests
The author declare they have no competing interest.

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References

1. Regan JO, Mulvihill DM. Sodium caseinate-maltodextrin conjugate stabilized double emulsions: encapsulation and stability. Food Res Int. 2010;43:224–31. https://doi.org/10.1016/j.foodres.2009.09.031.
2. McClements DJ. Emulsion design to improve the delivery of functional lipophilic components. Annu Rev Food Sci Technol. 2010;1:241–69. https://doi.org/10.1146/annurev.food.080708.100722.
3. Paez MC, Quezada CM, Bracamontes LI, Ochoa HOG, Lara JLA. Coalescence in double emulsions. Langmuir. 2012;28:5934–9. https://doi.org/10.1021/la205144g.
4. Leister N, Yan C, Kirstein HP. Oil droplet coalescence in W/O/W double emulsions examined in models from micrometer- to millimeter-sized droplets. Coll Interfaces. 2022;6:12. https://doi.org/10.3390/colloids6010012.
5. Tenorio-Garcia E, Araiza-Calahorra A, Simone E, Anwesha Sarkar A. Recent advances in design and stability of double emulsions: trends in pickering stabilization. Food Hydrocoll. 2022;128:107601. https://doi.org/10.1016/j.foodhyd.2022.107601.
6. Schuch A, Helfenritter C, Funck M, Schuchmann HP. Observations on the influence of different biopolymers on coalescence of inner water droplets in w/o/w (water-in-oil-in-water) double emulsions. Coll Surf A. 2015;475:2–8. https://doi.org/10.1016/j.colsurfa.2014.06.012.
7. Keršienė M, Jasutienė I, Eisinaitė V, Venskutonis PR, Leskauskaitė D. Designing multiple bioactives loaded emulsions for the formulations for diets of elderly. Food Funct. 2020. https://doi.org/10.1039/d0fo00021c.
8. Ghasemi H, Darjani S, Mazloomi H, Mozaffari S. Preparation of stable multiple emulsions using food-grade emulsifiers: evaluating the effects of emulsifier concentration, W/O phase ratio, and emulsification process. 4SN Appl Sci. 2020. https://doi.org/10.1007/s42452-020-03879-5.
9. Li Y, Jin H, Sun X, Sun J, Liu C, Liu C, Xu J. Physicochemical properties and storage stability of food protein-stabilized nanoemulsions. Nanomaterials (Basel). 2019;9:25. https://doi.org/10.3390/nano9010025.96+652.
10. Diep TT, Dao TP, Vu HT, Phan BQ, Dao DN, Bui TH, Truong V, Nguyen V. Double emulsion oil-in water-in oil (O/W/O) stabilized by sodium caseinate and k-carrageenan. J Dispersion Sci Technol. 2018;39(12):1752–7. https://doi.org/10.1080/01932691.2018.1462198.
11. Sarheed O, Dibi M, Ramesh KVRNS. Studies on the effect of oil and surfactant on the formation of alginate-based O/W lidocaine. Nanocarriers Nanoemulsion Template Pharm. 2020;12:1223. https://doi.org/10.3390/pharmaceutics,2020,12121223.
12. Demina TS, Kilyashova LA, Popyrina TN, Svidchenko EA, Bhuniya S, Akopova TA, Grandfils C. Polysaccharides as stabilizers for polymeric microcarriers fabrication. Polymers. 2021;13:3045. https://doi.org/10.3390/polym13183045.
13. Guo J, Jiang J, Gu X, Li X, Liu T. Encapsulation of β-carotene in calcium alginate hydrogels templated by oil in- water-in-oil (O/W/O) double emulsions. Coll Surf A. 2021;608:125548. https://doi.org/10.1016/j.colsurfa.2020.125548.
14. Tian HY, Xiang D, Li CF. Tea polyphenols encapsulated in W/O/W emulsions with xanthan gum-locust bean gum mixture: evaluation of their stability and protection. Int J Biol Macromol. 2021;175:40–8.
15. Felix M, Guerrero A, Cecilio Carrera-Sánchez C. Optimization of multiple W1/O/W2 emulsions processing for suitable stability and encapsulation efficiency. Foods. 2022;11:1367. https://doi.org/10.3390/foods11091367.
16. Schoener AL, Zhang R, Lv S, Weiss J, McClements DJ. Fabrication of plant-based vitamin D3-fortified nanoemulsions: influence of carrier oil type on vitamin bioaccessibility. Food Funct. 2019;10(4):1826–35. https://doi.org/10.1039/c9fo00116f.
17. Teixeira LG, Rezende S, Fernandes Â, Fernandes IP, Barros L, Barreira JC, Leimann FV, Ferreira IC, Barreiro M-F. Water-in-oil-in-water double emulsions as protective carriers for *Sambucus nigra L*. Color Syst Mol. 2022;27:552. https://doi.org/10.3390/molecules27020552.
18. Sanhueza L, García P, Giménez B, Benito JM, Matos M, Gutiérrez G. Encapsulation of pomegranate peel extract (*Punica granatum* L.) by double emulsions: effect of the encapsulation method and oil phase. 2022. Foods. https://doi.org/10.3390/foods11030310.
19. Sebben DA, MacWilliams SV, Yu L, Spicer PT, Bulone V, Krasowska M, Beattie DA. Influence of aqueous phase composition on double emulsion stability and colour retention of encapsulated anthocyanins. Foods. 2022;11:34. https://doi.org/10.3390/foods11010034.
20. Matos M, Gutiérrez G, Iglesias O, Coca JC, Pazos C. Enhancing encapsulation efficiency of food-grade double emulsions containing resveratrol or vitamin B12 by membrane emulsification. J Food Eng. 2015;166:212–20. https://doi.org/10.1016/j.jfoodeng.2015.06.002.
21. Dima C, Dima S. Bioaccessibility study of calcium and vitamin D3 co-microencapsulated in water-in-oil-in-water double emulsions. Food Chem. 2020;303:125416. https://doi.org/10.1016/j.foodchem.2019.125416.
22. Díaz-Ruiz R, Valdeón I, Álvarez JR, Matos M, Gutiérrez GJ. Simultaneous encapsulation of trans -resveratrol and vitamin D3 in highly concentrated double emulsions. J Sci Food Agric. 2021;101(9):3654–64. https://doi.org/10.1002/jsfa.10995.
23. Rao MR, Warrier DU, Gaikwad SR, Shevate PM. Phosphorylation of psyllium seed polysaccharide and its characterization. Int J Biol Macromol. 2016;85:317–26. https://doi.org/10.1016/j.ijbiomac.2015.12.043.
24. Askari F, Sadeghi E, Mohammadi R, Rouhi M, Taghizadeh M, Shirgardoun MH, Kariminejad M. The physicochemical and structural properties of psyllium gum/modified starch composite edible film. J Food Process Preserv. 2018;42:e13715. https://doi.org/10.1111/jfpp.13715.
25. Carpenter J, George S, Saharan VK. Curcumin encapsulation in multilayer oil-in-water emulsion: synthesis using ultrasonication and studies on stability and antioxidant and release activities. Langmuir. 2019;35:10866–76. https://doi.org/10.1021/acs.langmuir.9b01523.
26. Chan E. Preparation of Ca-alginate beads containing high oil content: influence of process variables on encapsulation efficiency and bead properties. Carbohydr Polym. 2011;84:1267–75. https://doi.org/10.1016/j.carbpol.2011.01.015.
27. Wang B, Tian H, Xiang D. Stabilizing the oil-in-water emulsions using the mixtures of dendrobium officinale polysaccharides and gum arabic or

propylene glycol alginate. Molecules. 2020;25:759. https://doi.org/10.3390/molecules25030759.
28. Panigrahi D, Sahu PK, Swain S, Verma RK. Quality by design prospects of pharmaceuticals application of double emulsion method for PLGA loaded nanoparticles. SN Appl Sci. 2021;3:638. https://doi.org/10.1007/s42452-021-04609-1.
29. Chuacharoen T, Prasongsuk S, Sabliov CM. Curcumin nanoemulsions under conditions relevant to commercial utilization. Molecules. 2019;24:2744. https://doi.org/10.3390/molecules24152744.
30. Guo Q, Cui SW, Wang Q, Young CJ. Fractionation and physicochemical characterization of psyllium gum. Carbohydr Polym. 2008;73:35–43. https://doi.org/10.1016/j.carbpol.2007.11.001.
31. Mohammadzadeh Milani J, Kheiri Sh, Bagheri R, Maleki G. Evaluation of emulsifying properties of charkhak (*Launaea acanthodes*) gum. J Food Sci Technol (Iran). 2017;62(14):181–9.
32. Dickinson E. Hydrocolloids at interfaces and the influence on the properties of dispersed systems. Food Hydrocoll. 2003;17:25–39. https://doi.org/10.1016/S0268-005X(01)00120-5.

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