

Optimization low-fat and low cholesterol mayonnaise production by central composite design

H. R. Mozafari¹ · E. Hosseini² · M. Hojjatoleslamy³ · G. Hossein Mohebbi⁴ · N. Jannati¹

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Abstract In this study, the optimized process variables for mayonnaise low in cholesterol and fat, which contained soy milk as a yolk substitute with different levels of Xanthan gum, Zodo gum, and oil, were determined by response surface methodology using a central composite design. Polynomial equation was fitted with an insignificant lack of fit factor in order to study the relationship between variables and responses including apparent viscosity, consistency coefficient, flow index, firmness, and stability of mayonnaise sauces. Results showed that increased amounts of Xanthan gum, Zodo gum and oil led to an increase in the apparent viscosity, the consistency coefficient, the firmness/emulsion stability of the mayonnaise, while the mayonnaise flow index was reduced. The interaction effects between Xanthan gum and Zodo gum, and between Xanthan gum and oil were significant on apparent viscosity. Optimum conditions of variables were obtained due to response ranges of commercial mayonnaise as following ingredients: 0.25% Xanthan gum, 3.84% Zodo gum, 37.50% oil, and with the replacement of 63.61% soy milk. Yolk, however, was replaced with soy milk without

emulsion fracture up to 100%. This study showed good potential for Zodo gum native mixed with Xanthan gum and soy milk to be used as a fat and yolk substitute in mayonnaise, respectively.

Keywords Zodo · Xanthan · Soy milk · Response surface methodology · Viscosity

Introduction

Mayonnaise sauce is a semi-solid, oil-in-water emulsion prepared through the mixture of yolk, vinegar, oil, and spices (in particular mustard). It also consists of 70–80% oil (Shen et al. 2011). The obvious relationship of food-item fats with increased cardiovascular diseases, high blood pressure, and obesity has led to the tendency toward low-fat products (Worrasinchai et al. 2005). Creaming, flocculation, and coalescence mechanisms are influenced by some factors such as oil volume, amount of yolk, viscosity, oil/water ratio, mixture method, water quality, and temperature which lead to mayonnaise instability (Rahmati et al. 2012; Thomareis and Chatziantoniou 2011). On the other hand, food manufacturers are faced with major challenges such as products with higher levels of plant foods and fewer calories in order to manufacture novel types of salad dressings and mayonnaise. Emulsifiers or thickening agents can be used to solve this problem to overcome system activation energy and form stable emulsion (Dickinson and Golding 1997). Xanthan gum, propylene glycol alginate, tragacanth, microcrystalline cellulose, pectin, starches, carrageenan, guar gum, locust bean gum, gellan, carboxy methylcellulose, gelatin, agar, and methylcellulose are some important examples of gums used in food industry. Xanthan gum is used in a wide range

✉ H. R. Mozafari
hamidreza.mo87@yahoo.com

¹ Young Researchers and Elite Club, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran
² Department of Food Science and Technology, Faculty of Agriculture, Kazeroon Branch, Islamic Azad University, Kazeroon, Iran
³ Department of Food Science and Technology, Faculty of Agriculture, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran
⁴ Department of Marine Toxinology, Persian Gulf Marine Biotechnology Research Center, Bushehr University of Medical Science, Bushehr, Iran

of food items due to some important reasons including emulsion stability, heat stability, adaptability with food components, and shear-thinning rheology features (Naji et al. 2012). Nowadays, tendency to use new and native polysaccharides has considerably increased due to high price and sometimes lack of easy accessibility to common stabilizers. Zodo gum is a transparent leakage gum taken from *Amygdalus Scoparia Spach*. This gum, like other ones, produces viscose and sticky solution and it is used in various industries such as pharmaceutical industry as suspensor agents and emulsifier along with Arabic gum and tragacanth (Mohammadi et al. 2011). Zodo gum comes in three colors: white, red, and yellow with chemical composition including 0.21% protein, 12.08% moisture, 98.4% carbohydrate content (Dry weight basis), and 1.67% ash. White types of Zodo gum have higher level of carbohydrate and molecular weight content (4.74×10^6 Da) and show more viscosity compared to arabic gum (Fadavi et al. 2014). Zodo gum has promisingly shown to be considered as a potential novel food thickening agent (Jafari et al. 2012). Another important factor of mayonnaise structure stability is yolk; however, one of yolk problems is its high cholesterol (Abu Ghoush et al. 2008). Complete or partial replacement of yolk with other emulsifiers leads to reduction in fat and cholesterol, microbe pollution, and in some cases in costs (Nikzade et al. 2012). Soy flour, compared to other protein products, yields the minimum number of production processes. Thus, by using it as a replacement for yolk, the cost falls noticeably (Liu 2004).

The application of response surface methodology for predicting and modeling is a novel approach of which both mathematical and statistical techniques were used for the optimization of multiple variables in order to predict the best performance conditions with a minimum number of experiments (Huang et al. 2006). Therefore, according to the importance of low cholesterol and low fat products as well as reduced production expenses, this article aims to model and evaluate the combined effects of Zodo gum, Xanthan, oil, and soy milk level on texture characteristics and stability of mayonnaise with similar characteristics of mayonnaise with complete yolk and fat, applying the response surface methodology in order to determine the optimal formulation of novel low-fat and low-cholesterol mayonnaise.

Materials and methods

Materials

Sunflower oil and other ingredients used in the preparation of mayonnaise were purchased from local market. The distilled vinegar (acidity 5%) was used. Xanthan gum was

purchased from Sigma Aldrich Chem. Co., England. Zodo gum was taken from *A. scoparia spach* trees in Kazeroun, Fars province, Iran.

Methods

Preparing emulsifiers

Soybean's composition was characterized according to AOAC official methods that revealed as follows: 38% protein, 18% oil, 5.5% ash, and 4% fiber. Soybeans were soaked in water for 10 min to achieve 23% moisture. After 1 h resting, it was heated at 110 °C oven for 15 min to blanch. Then, blanched beans were dried and ground into powder by colloid mill and passed through 45-mesh sieve (Liu 2004). Next, soy flour was mixed for 20 min with 80 °C water (3:1 ratios) using mixer at 700 rpm. Washed and fresh eggs were broken by hand and their yolks were separated from albumen. Finally, they were kept at 4 °C until mayonnaise production (Nikzade et al. 2012).

Mayonnaise production

In this study, 800 g of each mayonnaise sample was using the modified method of Chen (2005). The mayonnaise ingredients included 8.72% vinegar, 1% salt, 0.7% sugar, 0.4% mustard powder, 10% emulsifier, oil, hydrocolloid, and the remaining was water. Production steps were as follows:

Briefly, salt, sugar, and mustard powder was blended in bowl, followed by the addition of vinegar and mixed for 5 min with spatula. Emulsifier was added and blended at medium speed for 10 min by hand blender of Philips (HR1361 model, China). Hydrocolloid and water were added and blended for 10 min at medium speed. Finally, oil was gradually added, while blending at medium speed for 5 min, followed by at high speed for another 10 min.

Texture characteristics

Rheological features: viscosity of samples was measured by programmable viscometer of Brookfield Engineering model LV DV-III (Laboratories. Inc. USA) equipped with spindle No. 64. Rheometer temperature was adjusted at 4 °C before transferring samples to Rheometer. After putting essential amount of sample inside the viscometer, apparent viscosity was measured at rotating speeds of 0.42, 0.084, 0.125, 0.167, 0.209, 0.418, 0.836, 1.254, 1.672, and 2.09 s⁻¹. Power model was used to model the behavioral features. In Eq. (1), τ indicates shear stress (Newton/m²); $\dot{\gamma}$ is shear rate (s⁻¹); K is consistency coefficient (pa sⁿ); and n is the flow index (dimensionless) (Worrasinchai et al. 2005).

$$\tau = k(\dot{\gamma})^n \quad (1)$$

Extrusion test: texture analyzer (Brookfield company, model CT3, USA) was employed to measure the texture features of mayonnaise samples with 4500 g cell loading. The probe used in this study was a 23 mm-diameter cylinder. Samples were carefully transferred into cylinder made from transparent plastics (25 mm internal diameter and 30 mm height) and one operational cycle was implemented. To this end, penetration rate in to sample and penetration depth were selected 1 mm/s and 28 mm, respectively (Worrasinchai et al. 2005). Firmness factor was obtained from force–time graph. Sample firmness is maximum penetration force in to sample in one cycle (Liu et al. 2007).

Stability of samples

15 g of sample was transferred to test tubes and it was kept for 48 h at 50 °C after the closure with caps. After this duration, emulsions were centrifuged for 10 min at 1881g (m/s^2). The weight of the precipitated fraction was measured and emulsion stability was calculated according to Eq. (2) (Mun et al. 2009). In this equation, F0 and F1 are sample weight and weight of the precipitated fraction, respectively after centrifuge.

$$\text{Stability percentage} = (F1/F0) \times 100 \quad (2)$$

Experimental optimization design and statistical analysis

Response surface methodology, RSM, and central composite design (CCD), were used with four factors of Xanthan gum (0–0.5%), Zodo gum (3–4%), oil (37.5–56.25%), and replacement percentage of soy milk (0–100%) in three different levels (–1, 0 and 1) and six replications in central point (Nos. 3, 6, 8, 12, 13, and 20) on responses of mayonnaise (Table 1). In the CCD, the total number of runs was $2^K + 2K + n_0$, where K is the number of independent variables and n_0 is the number of repetitions of the experiments at the central point, indicating that 30 experiments were required for this procedure. All experiments were performed in triplicate, and mean values of the data are reported. Table 1 shows the factors and their levels at which the experiments were carried out, where X_1 , X_2 , X_3 , X_4 are coded values for Xanthan gum (%), Zodo gum (%), Oil (%) and Soy milk (%), respectively. Responses were analyzed by multiple regression and square least method for fitness of Eq. (3). In this formula, β_{ij} , β_{ii} , β_i , β_0 are coefficient of regression equations, i and j are integers, Y is response, then X_i and

X_j are independent variables. Significant terms in the model were found for each response by the analysis of variance (ANOVA) and significance was judged by the F-statistic calculation from the data. A statistical package, Design Expert, version 8.0.7.1 (statease inc., Minneapolis, USA) was used to analyze data, perform RSM and obtain three dimensional (3-D) graphs of response. Numerical optimization of process variables was performed based on the multiple responses using Design-Expert 8.0.7.1. ANOVA for the data obtained through CCD is presented in Table 2. Also significant differences were determined among the samples using SPSS 18.

$$Y = \beta_0 + \sum_{i=1}^4 \beta_i X_i + \sum_{i=1}^4 \beta_{ii} X_i^2 + \sum_{i=1}^3 \sum_{j=i+1}^4 \beta_{ij} X_i X_j \quad (3)$$

Results and discussion

Table 2 lists regression coefficients for variable performances and responses predicting during optimization process. All responses in Table 1 have significant sum of squares ($p < 0.01$), insignificant lack of fit factor ($p > 0.01$), and high determination factor (R^2) within the range of 0.948–0.993 for regression equations shows the fitness of polynomial equation for changes of responses. Lack of fit also implies that it is not significantly related to the pure experimental error, suggesting that the model correlates well with the experimental values.

The effect on apparent viscosity: as seen in Table 2, the analysis of variance (ANOVA) of regression model demonstrated that the quadratic model was highly significant as witnessed by the Fisher's F test with a very low probability value [(P-model > F) = 0.0001]. Equation (4) has been fitted using second degree polynomial model for independent variable effects including Zodo gum, Xanthan gum, oil, and soy milk on apparent viscosity response. The value of adjusted R^2 is reported 0.9869 indicating the fact that independent variables have been effective in 98.6% of total sum of viscosity changes and only 1.4% of total changes cannot be explained by this model. The value of $R^2 = 0.9932$ also reveals good correlation between experimental values and forecasted responses. Analysis of regression coefficient showed that the linear effect of Xanthan gum (βX_1), Zodo gum (βX_2), oil level (βX_3), interaction effect Xanthan-Zodo (βX_{12}), and Xanthan-oil (βX_{13}) on apparent viscosity are significant ($p < 0.01$) in a way that increased level of these variables led to increased apparent viscosity (Table 2). Among all of these variables, Xanthan gum has the highest effect on apparent viscosity. In addition, in order to gain the better understanding of the effects of the independent variables and their interactions

Table 1 Central composite design of experiments and response variable results

Run no.	Real values (coded)				Viscosity (pa s)	K (pa s ⁿ)	n	Firmness (N)	Stability (%)
	X ₁	X ₂	X ₃	X ₄					
1	0 (-1)	3 (-1)	56.25 (+1)	0 (-1)	80.052 ± 0.001d	0.454 ± 0.007de	0.364 ± 0.006l	1.669 ± 0.132c	95.00 ± 0.17e
2	0.5 (+1)	3.5 (0)	46.88 (0)	50 (0)	236.716 ± 1.240m	1.337 ± 0.009o	0.214 ± 0.005cd	4.422 ± 0.125j	99.55 ± 0.12o
3	0.25 (0)	3.5 (0)	46.88 (0)	50 (0)	158.066 ± 1.500j	0.828 ± 0.006k	0.228 ± 0.002de	3.04 ± 0.109efg	98.50 ± 0.13m
4	0 (-1)	3 (-1)	37.5 (-1)	0 (-1)	14.897 ± 0.754a	0.090 ± 0.004a	0.386 ± 0.008n	0.983 ± 0.088ab	93.00 ± 0.16b
5	0 (-1)	3 (-1)	37.5 (-1)	100 (+1)	15.896 ± 3.131a	0.097 ± 0.004a	0.435 ± 0.010o	0.964 ± 0.099a	90.66 ± 0.10a
6	0.25 (0)	3.5 (0)	46.88 (0)	50 (0)	141.469 ± 4.349i	0.751 ± 0.019j	0.259 ± 0.001gh	2.843 ± 0.093efg	97.50 ± 0.08j
7	0 (-1)	3 (-1)	56.25 (+1)	100 (+1)	291.079 ± 1.009o	1.520 ± 0.006p	0.249 ± 0.006fg	4.901 ± 0.167 k	97.38 ± 0.15j
8	0.25 (0)	3.5 (0)	46.88 (0)	50 (0)	141.069 ± 2.586i	0.754 ± 0.011j	0.254 ± 0.004g	2.814 ± 0.170ef	97.40 ± 0.07j
9	0 (-1)	4 (+1)	56.25 (+1)	100 (+1)	115.175 ± 1.500f	0.658 ± 0.006gh	0.296 ± 0.005j	2.296 ± 0.337d	96.10 ± 0.05 h
10	0.25 (0)	3.5 (0)	37.5 (-1)	50 (0)	88.981 ± 1.417e	0.473 ± 0.006e	0.272 ± 0.002hi	2.070 ± 0.159d	97.50 ± 0.06j
11	0 (-1)	4 (+1)	37.5 (-1)	0 (-1)	26.694 ± 0.300b	0.164 ± 0.002b	0.384 ± 0.022n	1.291 ± 0.172b	94.00 ± 0.13d
12	0.25 (0)	3.5 (0)	46.88 (0)	50 (0)	139.000 ± 1.000hi	0.707 ± 0.013i	0.272 ± 0.006hi	2.876 ± 0.063efg	98.20 ± 0.11 l
13	0.25 (0)	3.5 (0)	46.88 (0)	50 (0)	141.623 ± 4.505i	0.752 ± 0.020j	0.258 ± 0.009gh	2.726 ± 0.243e	97.80 ± 0.11k
14	0.25 (0)	3.5 (0)	56.25 (+1)	50 (0)	238.149 ± 2.099m	1.312 ± 0.008n	0.255 ± 0.004g	3.438 ± 0.261 h	98.91 ± 0.10n
15	0.5 (+1)	3 (-1)	37.5 (-1)	0 (-1)	121.333 ± 5.859g	0.631 ± 0.030fg	0.278 ± 0.003i	3.147 ± 0.085fgh	95.50 ± 0.14f
16	0.25 (0)	3 (-1)	46.88 (0)	50 (0)	120.425 ± 2.503g	0.638 ± 0.014fgh	0.298 ± 0.003j	2.159 ± 0.203d	97.38 ± 0.03j
17	0 (-1)	4 (+1)	37.5 (-1)	100 (+1)	46.390 ± 2.424c	0.268 ± 0.013c	0.377 ± 0.025mn	1.624 ± 0.291c	93.60 ± 0.14c
18	0.5 (+1)	4 (+1)	56.25 (+1)	0 (-1)	350.000 ± 7.184p	1.763 ± 0.006q	0.156 ± 0.000a	6.380 ± 0.171 l	99.90 ± 0.04pq
19	0.25 (0)	3.5 (0)	46.88 (0)	0 (-1)	134.071 ± 1.500h	0.717 ± 0.007i	0.273 ± 0.003hi	2.716 ± 0.054e	98.20 ± 0.19l
20	0.25 (0)	3.5 (0)	46.88 (0)	50 (0)	143.069 ± 0.140i	0.757 ± 0.008j	0.254 ± 0.002g	2.736 ± 0.105e	97.43 ± 0.06j
21	0.25 (0)	4 (+1)	46.88 (0)	50 (0)	171.821 ± 5.400k	0.897 ± 0.019l	0.261 ± 0.007gh	3.093 ± 0.177fgh	97.45 ± 0.10j
22	0.5 (+1)	4 (+1)	56.25 (+1)	100 (+1)	352.000 ± 5.729p	1.769 ± 0.003q	0.175 ± 0.009b	6.179 ± 0.153 l	99.80 ± 0.05pq
23	0 (-1)	4 (+1)	56.25 (+1)	0 (-1)	110.025 ± 1.000f	0.628 ± 0.009f	0.310 ± 0.011j	2.353 ± 0.077d	95.35 ± 0.04f
24	0.25 (0)	3.5 (0)	46.88 (0)	100 (+1)	161.965 ± 1.645j	0.844 ± 0.007k	0.272 ± 0.008hi	2.956 ± 0.048efg	97.39 ± 0.09j
25	0.5 (+1)	4 (+1)	37.5 (-1)	0 (-1)	190.667 ± 3.511l	0.977 ± 0.006m	0.201 ± 0.003c	3.183 ± 0.133gh	99.60 ± 0.10op
26	0.5 (+1)	4 (+1)	37.5 (-1)	100 (+1)	192.333 ± 2.254l	0.959 ± 0.049m	0.213 ± 0.006cd	3.930 ± 0.129i	98.50 ± 0.10mm
27	0.5 (+1)	3 (-1)	37.5 (-1)	100 (+1)	125.073 ± 1.452g	0.662 ± 0.022h	0.236 ± 0.003ef	3.133 ± 0.268fgh	96.52 ± 0.04i
28	0 (-1)	3.5 (0)	46.88 (0)	50 (0)	75.584 ± 1.081d	0.438 ± 0.001d	0.339 ± 0.004 k	2.108 ± 0.042d	95.90 ± 0.09g
29	0 (-1)	3 (-1)	56.25 (+1)	100 (+1)	85.882 ± 0.755e	0.478 ± 0.002e	0.341 ± 0.003 k	1.716 ± .097c	94.00 ± 0.26d
30	0.5 (+1)	3 (-1)	56.25 (+1)	0 (-1)	274.447 ± 6.751n	1.506 ± 0.035p	0.237 ± 0.011ef	5.189 ± 0.545 k	97.37 ± 0.07j

X₁ Xanthan gum (%), X₂ Zodo gum (%), X₃ Oil (%) and X₄ Soy milk (%), n flow index, k consistency coefficient
 Letters in the same column show the difference between the groups, ±standard deviation (*p* < 0.05)

Table 2 Regression coefficients and correlation of the fitted model test data in a central composite design

Source	df	Response				df	K (pa s ⁿ)
		Viscosity (pa s)	n	Firmness (N)	Stability (%)		
βX_1	14	86.84**	-0.067**	1.42**	2.03**	10	0.44**
βX_2	1	23.67**	-0.028**	0.36**	0/97**	1	0.11**
βX_3	1	59.70**	-0.019**	0.77**	0.83**	1	0.32**
βX_4	1	4.64	0.0035	0.04	-0/22	1	0.018
βX_{12}	1	10.72**	-0.0021	0.07	0.29	1	0.034
βX_{13}	1	21.93**	0.0062	0.38**	-0.30	1	0.11**
βX_{14}	1	-0.48	-0.0040	-0.003	0.18	1	-0.008
βX_{23}	1	1.050	-0.0021	0.12*	-0.16	1	-0.00
βX_{24}	1	0.082	0.0045	0.069	0.091	1	0.003
βX_{34}	1	0.22	-0.0048	-0.096	0.16	1	-0.003
βX_1^2	1	3.03	0.01	0.46**	-0.56	1	-
βX_2^2	1	-6.99	0.013	-0.19	-0.87	1	-
βX_3^2	1	10.45	-0.0027	-0.060	-0.079	1	-
βX_4^2	1	-5.10	0.0061	0.022	-0.49	1	-
R ²	1	0.9932	0/9521	0.9900	0.9487	1	0.9846
Residual	15	1505.75	5.474E-003	0.53	7.13	19	0.089
Lack of fit	10	1266.52ns	4.429E-003ns	0.47ns	6.10ns	14	0.082ns
Pure error	5	239.22	1.045E-003	0.066	1.04	5	0.0076
Corrected total	29	2.218E+005	0.11	53.12	139.08	29	5.80

βX_1 , Xanthan gum; βX_2 , Zodo gum; βX_3 , oil; βX_4 , soy milk; df, Degrees of freedom; ns, not significant; significant at ** $p \leq 0.01$, * $p \leq 0.05$
n flow index, *k* consistency coefficient

on the dependent variable, 3D response surface plots were formed for the measured responses based on the model equation (Eq. 4) in this study. The interaction of Xanthan gum and Zodo gum (46.88% oil and 50% soy milk) with Xanthan gum and oil (at the 3.5% Zodo gum and 50% soy milk) was shown in Fig. 1. In this contour plot, two component interactions of X_{12} and X_{13} had a significant influence ($p < 0.01$) on the apparent viscosity response of mayonnaise samples. As clearly seen in Fig. 1a, viscosity of mayonnaise samples rose by increased Zodo and Xanthan gums. Therefore, the effect of these variables is associated with the fact that the viscosity of composition number 18 and 22 with 0.5 and 4% Xanthan and Zodo gums had the maximum amount, respectively. Also, at the lowest level of oil, the viscosity of the mayonnaise was found to increase with an increase in Xanthan gum concentration (Fig. 1b). However, the lowest viscosity was observed in the run no. 4, containing 0% Xanthan gums, 3% Zodo gum and 37.5% oil (Table 1). This may be interpreted by the reason that, in the run no. 4, it comprised bigger droplet sizes of fat which resisted less to a shear rate during flow behavior measurement, resulting in low viscosity (Liu et al. 2007). Subsequently, the presence of a large number of particles increased the resistance to the flow. Hence, this phenomenon led to an increase in the

apparent viscosity. Thus, it was concluded that a combination of high content of all main emulsion components resulted in the highest viscosity value for the mayonnaise. The significant effect of hydrocolloids in emulsions was also addressed to the effect of their thickening in the continuous phase (Soleimanpour et al. 2013; Ercelebi and Ibanoglu 2010). Moreover, interactions between gums and proteins are effective in mayonnaise viscosity by forming a protective steric barrier around oil drops.

In general, the apparent viscosity increased proportionally with increases in the disperse-phase volume fraction and the strength of the attractive forces between the oil droplets. However, this effect was attributed by high concentration of Zodo gum and xanthan gum, as fat alternative in the formulation that otherwise strengthens the gel structure of samples. Additionally, Lorenzo et al. (2008) observed that the viscosity of low-in-fat o/w emulsions stabilized with Xanthan/guar mixtures improved by hydrocolloids content.

On the other hand, substitution of yolk with soy milk increases the viscosity up to 73% and reduced viscosity; however, generally, this effect was not significant. A study conducted by Abu Ghoush et al. (2008) showed that viscosity decreased significantly by substitution of yolk with wheat protein and carrageenan.

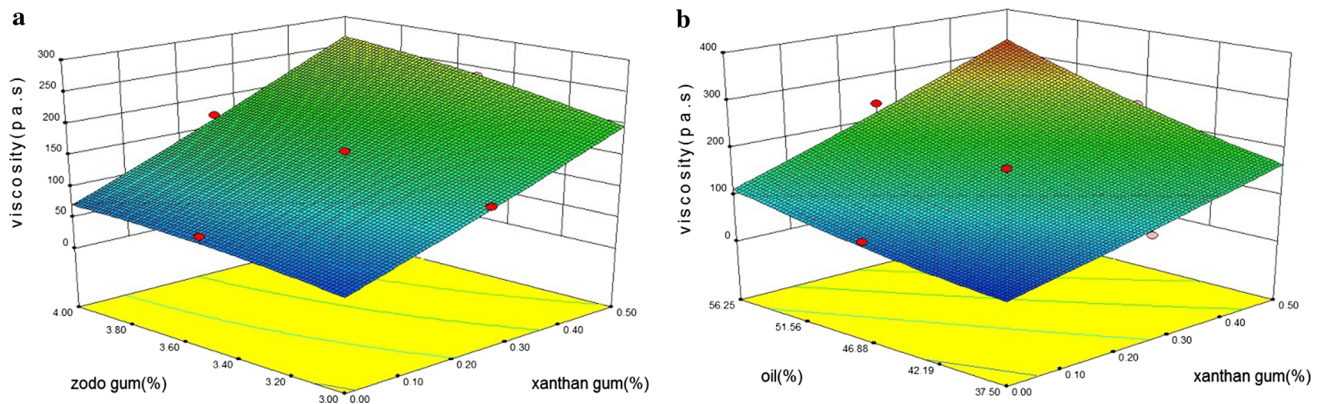


Fig. 1 Response surface plot showing effect of different parameters on viscosity (pa s). **a** Xanthan gum and Zodo gum at 46.88% oil and 50% soy milk. **b** Xanthan gum and oil at 3.5% Zodo gum and 50% soy milk

$$\begin{aligned}
 \text{Viscosity} = & 148.67 + 86.84 X_1 + 23.67 X_2 + 59.70 X_3 \\
 & + 4.64 X_4 + 10.72 X_{12} + 21.93 X_{13} \\
 & - 0.48 X_{14} + 1.05 X_{23} + 0.082 X_{24} \\
 & + 0.22 X_{34} + 3.03 X_1^2 - 6.99 X_2^2 \\
 & + 10.45 X_3^2 - 5.10 X_4^2
 \end{aligned}
 \tag{4}$$

The effect on flow-behavior parameters: Power law model was used to express the relationship between shear stress and shear rate. Note that all samples were fitted by high determination coefficients (0.965–0.999). All produced samples, like previous reports such as Xanthan gum in emulsions, are shear thinning due to less-than-one flow index (n) (range of 0.156–0.435) (Lorenzo et al. 2008). When a high viscosity and good mouth feeling are desired, selecting gum for the mayonnaise needs to have low-flow behavior index (Marcotte et al. 2001). So, the samples are suitable for this purpose according to the observed flow behavior. On the other hand, shear thinning behavior is important for the Power law equation; because it is used to calculate the mouthfeel of food, design of pumps, and mixers (Bourne 2002).

Consistency coefficients (K) of the samples were calculated between 0.090 and 1.769 Pa sⁿ (Table 1) and differences were found to be significant ($p < 0.05$) between the mayonnaise samples with respect to the mentioned power law parameters. The lowest consistency coefficient was observed in run no. 4 and the maximum flow index in run no 5. The common point of these two formulas was absence of Xanthan gum and the lowest level of Zodo gum.

Polynomial Eqs. (5) and (6) have been fitted for the effects of independent variables including Zodo gum, Xanthan gum, oil, and soy milk on K and n in 30 runs, respectively. Regression analysis and coefficients show the linear effect of Xanthan gum (βX_1), Zodo gum (βX_2), oil (βX_3), and interaction effect of oil-Xanthan gum (βX_{13}) on

consistency coefficient ($p < 0.01$) as well as the significant effect of Xanthan gum (βX_1), Zodo gum (βX_2), and oil (βX_3) in linear format ($p < 0.01$) on flow index of mayonnaise samples (Table 2). The interaction of Xanthan gum and oil (at the 3.5% Zodo gum and 50% soy milk) on flow index and consistency coefficient was shown in Fig. 2. In contour plot (b), interaction X_{13} had a significant influence ($p < 0.01$) on the consistency coefficient response of mayonnaise samples where the augmentation of consistency coefficient resulted in an increase oil at the designed range of Xanthan gum from 0 to 0.5%. In other words, consistency coefficient of mayonnaise rose by increased Xanthan gum and oil. The maximum consistency coefficient and lowest flow index were determined under the condition of Xanthan gum 0.5% and oil 56.25%. This may be interpreted that the flocculation in a concentrated emulsion leads to the formation of three dimensional networks of aggregated drops. Increased shear rate causes deformation and disorder in aggregated particles which leads to reduction of emulsion resistance against flow and, consequently, the apparent viscosity rises (Liu et al. 2007). Consistency coefficient is a scale of viscosity and the trend of the viscosity versus shear rate. In other words, increased consistency coefficient in most emulsions is linked to increased nature of their viscosity (Marcotte et al. 2001).

According to these data, it is clear that this effect is more obvious due to intensifying effect of Zodo gum. These results are consistent with those of Sun et al. (2007) and Ibanoglu (2002) concerning increased consistency coefficient by increasing concentration of Xanthan gum and Arabic, respectively (Sun et al. 2007; Ibanoglu 2002). In conclusion, consistency coefficient and flow index of mayonnaise samples rose by increased Zodo gum, Xanthan gum and oil because Zodo and Xanthan gums prevented the release of water with the formation of the compact network structure.

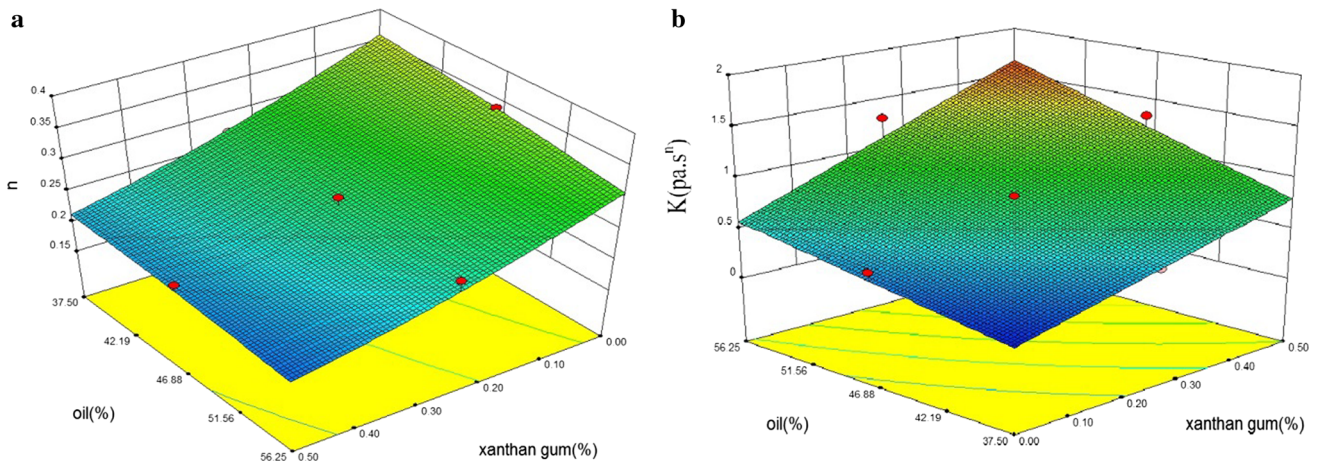


Fig. 2 Response surface plot showing effect of Xanthan gum and oil on **a** flow index (n), **b** consistency coefficient (K) at 3.5% Zodo gum and 50% soy milk

$$K = 0.79 + 0.44 X_1 + 0.11 X_2 + 0.32 X_3 + 0.018 X_4 + 0.034 X_{12} + 0.11 X_{13} - 0.0082 X_{14} - 0.0017 X_{23} + 0.0028 X_{24} - 0.003 X_{34} \tag{5}$$

$$n = 0.26 - 0.067 X_1 - 0.028 X_2 - 0.019 X_3 + 0.0035 X_4 - 0.00216 X_{12} + 0.0062 X_{13} - 0.0040 X_{14} - 0.0021 X_{23} + 0.0045 X_{24} - 0.0048 X_{34} + 0.01 X_1^2 + 0.013 X_2^2 - 0.0027 X_3^2 + 0.006 X_4^2 \tag{6}$$

The effect on firmness: One of important and effective factors in mayonnaise sauce is its level of firmness which is highly important for customers’ satisfaction. Equation (7) has been fitted using second degree polynomial model for the effects of independent variables including Zodo gum, Xanthan gum, oil, and soy milk on firmness in 30 runs. The values of regression coefficients show the significant linear effect of Xanthan (βX_1), Zodo gum (βX_2), oil (βX_3), interaction effect of Xanthan-oil (βX_{13}), Xanthan quadratic (βX_1^2) ($p < 0.01$), and interaction effect of Zodo-oil (βX_{23}) ($p < 0.05$) on mayonnaise firmness. Furthermore, regression coefficients show the minimum effect of soy milk in comparison with other factors on mayonnaise firmness in that the highest firmness was in formula 18 with maximum presence of three factors including Xanthan, Zodo, and oil. On the other hand, the least firmness was in formula 5 with minimum presence of three factors and maximum soy milk (Table 1). According to Fig. 3, two component interaction X_{23} ($p < 0.05$) and X_{13} ($p < 0.01$) had a significant impact on the firmness response. At the highest level of oil, the firmness of the mayonnaise increase rapidly owing to the contribution by the interaction term ($p < 0.01$) of Xanthan gum and oil (Fig. 3b).

For instance, Worrasinchai et al. (2005) reported the effect of adding β -Glucan to the low fat mayonnaise on

textural characteristics which indicated that fat replacer may increase the elasticity of the emulsion as a result of the formation of a strong gel-like structure in the continuous phase. These results may also be interpreted by the reason that the reduction of oil-drop diameter resulting from reduced coagulation process and increased contact between drops and friction leads to increased viscosity. In other words, larger total interfacial area generated by smaller droplets leads to more homogeneous surface. Higher energy of smaller droplets may potentially increase the interactions between the ingredients in the formulation, and hence cause higher viscosity. Viscosity of samples can somehow, not completely, reflect the texture analysis parameters such as firmness (Mun et al. 2009). Thus, it is, in this study, assumed that drop diameters of oil phase in samples have high levels of three variables including smaller Xanthan gum, Zodo gum, and oil.

$$\text{Firmness} = 2.83 + 1.42 X_1 + 0.36 X_2 + 0.77 X_3 + 0.044 X_4 + 0.066 X_{12} + 0.38 X_{13} - 0.004 X_{14} + 0.12 X_{23} + 0.069 X_{24} - 0.096 X_{34} + 0.46 X_1^2 - 0.19 X_2^2 - 0.060 X_3^2 + 0.022 X_4^2 \tag{7}$$

The effect of stability: low-fat and low-cholesterol mayonnaise samples of test show high stability. The highest stability (99.9%) was obtained in formula 18 with maximum presence of Xanthan gum and Zodo gum (Table 1). This is because viscosity of continuous phase increased noticeably by adding Xanthan gum and Zodo gum and, consequently, movement of oil drops decreased effectively. On the other hand, the lowest stability (90.66%) was obtained in formula 5 with minimum presence of Xanthan gum and Zodo gum, suggesting that either bridging or depletion flocculation had occurred.

Profile of stability changes is illustrated in Fig. 4 in interaction between Xanthan gum and Zodo gum.

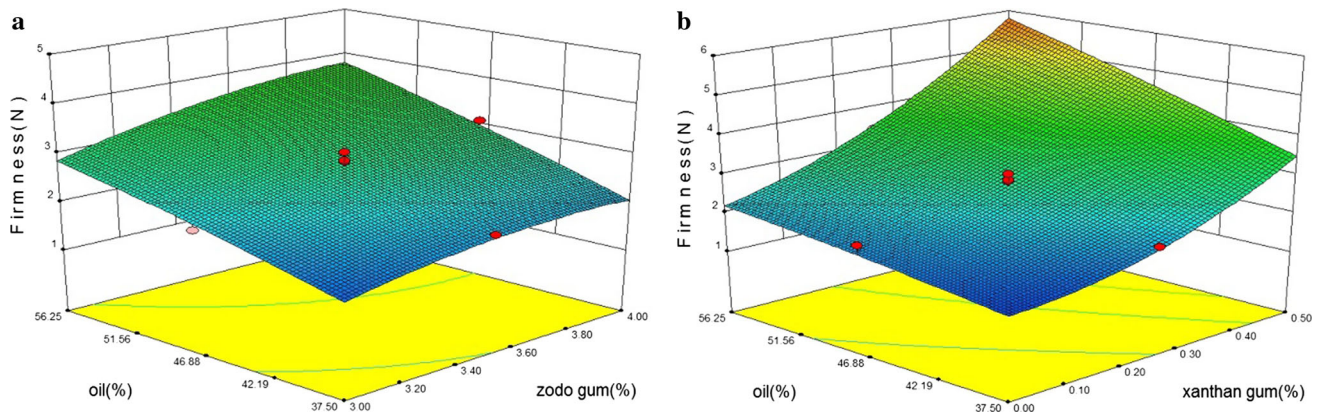
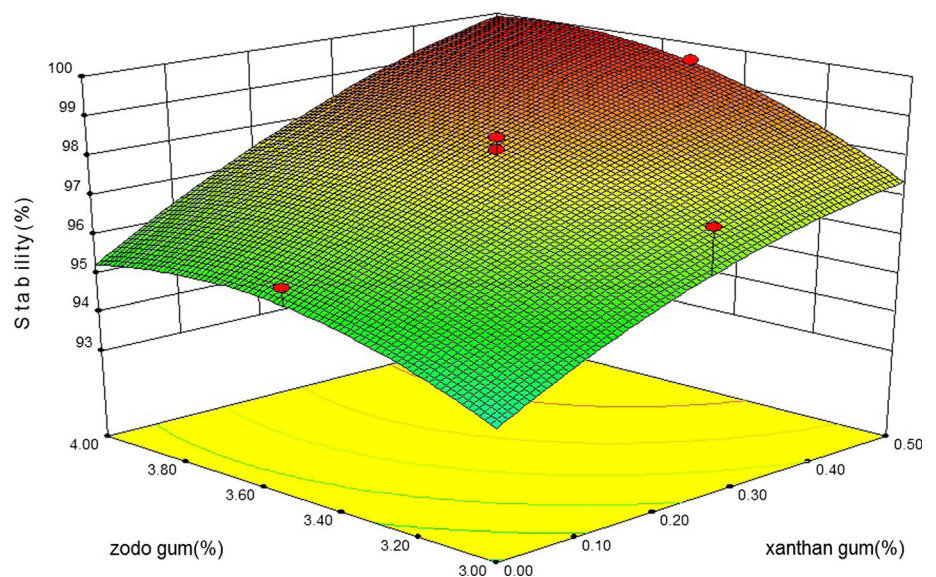


Fig. 3 Response surface plot showing effect of different parameters on firmness (N) (a) and Zodo gum and oil at 0.25% Xanthan gum and 50% soy milk (b) Xanthan gum and oil at 3.5% Zodo gum and 50% soy milk

Fig. 4 Response surface plot showing effect of Xanthan gum and Zodo gum on stability (%) at 46.88% oil and 50% soy milk



Regression coefficients in Eq. 8 show linear effect of Xanthan (βX_1), Zodo gum (βX_2), and oil (βX_3) ($p < 0.01$) on stability (Table 2). Mun et al. (2009) reported the stability of low fat mayonnaise because of increased viscosity of continuous phase as a result of adding Xanthan gum. Jafari et al. (2012) stated increased viscosity of continuous phase and lowering the movement of distributed drops as the significant effect of Zodo gum in emulsion stability. In other words, the situation in which gums form layer around emulsion drops leads to reduced speed of instability like Coalescence, Ostwald ripening, and so on that lead to emulsion stability (Jafari et al. 2012). Therefore, according to results related to emulsion stability of the mayonnaise which contained high levels of Xanthan gum and Zodo gum, it is assumed that these samples were resistant to droplet coalescence which accelerates the creaming process. Also, Stokes equation, reduced density between oil phase and water phase, and increased viscosity can be

introduced in stability of mayonnaise sauce by adding hydrocolloid and reducing creaming due to the viscoelastic characteristics of emulsion droplets that have become flocculated into a gel-like network (McClements 2009).

Fat reduction in salad and mayonnaise sauce can change its rheological behavior and texture, substantially influencing stability during storage time (Ma and Boye 2012). Therefore, the highest and the lowest stability can be observed in formulas 18 and 5 with maximum and minimum presence of fat, respectively (Table 1). Moreover, substitution of soy milk as emulsifier did not have a significant effect ($p > 0.05$) on stability reduction of mayonnaises. Aluko and McIntosh (2005) substituted, in one mayonnaise model, yolk with Canola hydrolyzed protein up to 50% and non-hydrolyzed protein up to 15% without emulsion fracture (Aluko and McIntosh 2005). In this study, yolk was substituted with soy milk up to 100% without emulsion fracture. The molecular structure of

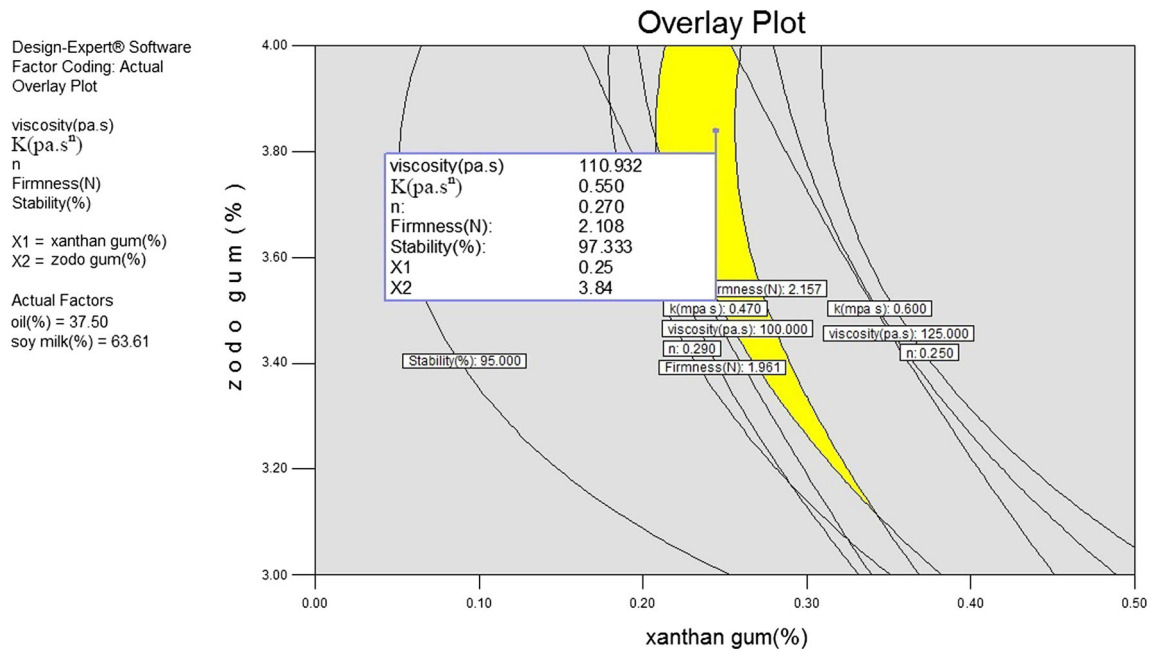


Fig. 5 Overlaid contour plot for the response variables of mayonnaise

Table 3 Optimized level, predicted optimum value and experimental values of viscosity, k, n, firmness and stability

Variables	Optimum value	
Xanthan gum (%)	0.25	
Zodo gum (%)	3.84	
Oil (%)	37.5	
Soy milk (%)	63.61	
Responses	Predicted value	Experimental value
Viscosity (pa s)	110.930	111.717 ± 0.143
K (pa s ⁿ)	0.550	0.572 ± 0.003
n	0.27	0.255 ± 0.004
Firmness (N)	2.108	2.227 ± 0.016
Stability (%)	97.33	98.43 ± 0.347

n flow index, k consistency coefficient

phospholipids in soy milk is partially hydrophobic and hydrophilic which enables it as an effective emulsifier concerning water and oil (Ma and Boye 2012). In other words, molecules of soy milk phospholipids, acting as emulsifiers, are adsorbed at the oil-water interface. When the droplets are close or almost touch each other, strong interactions between phospholipids molecules can be expected. On the other hand, proteins and phospholipids from soy are surface active and reduce interfacial tension between oil and aqueous phases (Abu Ghoush et al. 2008;

Liu 2004). These interparticle interactions were probably responsible for the stability of mayonnaise.

$$\begin{aligned}
 \text{Stability} = & 98.04 + 2.03 X_1 + 0.97 X_2 + 0.83 X_3 \\
 & - 0.22 X_4 + 0.29 X_{12} - 0.30 X_{13} + 0.18 X_{14} \\
 & - 0.16 X_{23} + 0.091 X_{24} + 0.16 X_{34} - 0.56 X_1^2 \\
 & - 0.87 X_2^2 - 0.079 X_3^2 - 0.49 X_4^2
 \end{aligned}
 \tag{8}$$

Optimization and approving optimum composition: Design expert trail version 8.0.7.1 was used in optimization of process variables to create ideal responses by numerical optimization method. Figure 5 shows overlaid contour plot for ideal responses. Desired responses were selected in accordance with certain measurement of commercial mayonnaises in the market. Yellow area shows all appropriate solutions by the software and optimum solutions are illustrated by one light color flag. Optimum values of variables and the prediction of optimum points are listed in Table 3. This prediction was approved by three repetitions in optimum point.

Conclusion

The study successfully optimized the texture characteristics and Stability of low-fat and low-cholesterol mayonnaise by using a CCD and response surface methodology. The results revealed that the polynomial models were well fitted to the experimental data. There was an adequate agreement between observed and predicted values of responses for any combination of independent variables in the

experimental region. ANOVA results revealed that Xanthan gum, Zodo gum and oil had significant linear effects on all responses, whereas the interaction effects between Xanthan gum and Zodo gum, and between Xanthan gum and oil were significant on apparent viscosity. However, the interaction effects of Xanthan gum and oil and Zodo gum and oil had a significant effect on firmness. Yet only the effect of Xanthan gum and oil on the consistency coefficient was found to be significant. Furthermore, the quadratic effect of Xanthan gum on firmness was significant. According to experimental results and contour plots, an increase in Xanthan gum followed by Zodo gum and oil improved the viscosity, consistency coefficient, firmness, and stability; and also declined the flow index. Also, yolk was substituted with soy milk up to 100% without emulsion fracture.

Zodo gum, as a thickening agent and native hydrocolloid, can be used as a mixture with Xanthan gum to improve the texture characteristics and stability when optimum combination levels of gums are taken into account. However, as the analysis of the response surface model suggests, future researches can use the polynomial equation to predict the texture characteristics and stability of mayonnaise in different amounts of selected Xanthan gum, Zodo gum, oil, and soy milk, as independent variables.

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