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Formulation and Optimization by Experimental Design of Low-Fat Mayonnaise Based on Soy Lecithin and Whey

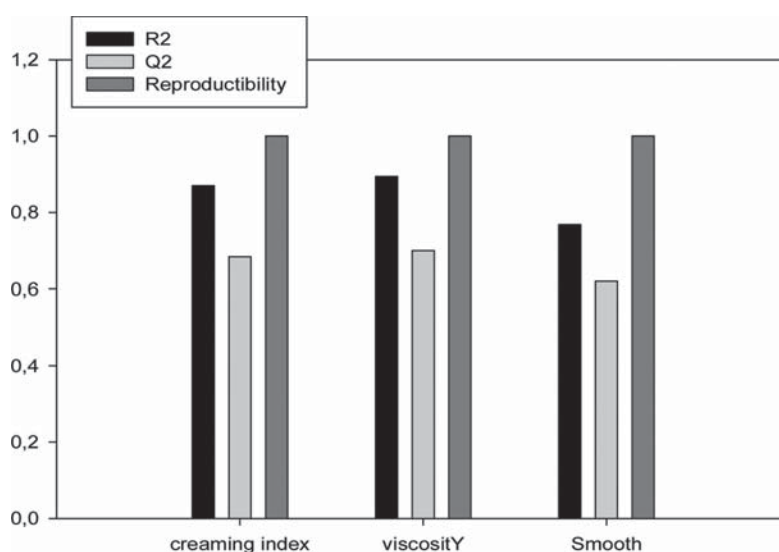
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GRAPHICAL ABSTRACT



The main objective of this study is to develop a new formula for a diet mayonnaise-like sauce without cholesterol. Emulsifying power is provided by the use of soy lecithin and the total fat content was limited to 16%. Droplet size measurement of employed mayonnaise samples at different times show that the largest diameter of fat does not exceed 18.5 μm with a yield stress of 56.1 Pa. Results of stability to centrifugation reveal that the absence of the supernatant oily layer ensures the stability of the emulsion. Using the experimental design method, the number of trials can be limited to a number of 16 experiments, and best formulation of the mayonnaise (without cholesterol) was obtained.

Keywords Dietary sauce, experimental design, low-fat mayonnaise, soy lecithin, whey

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1. INTRODUCTION

It is known that fats are essential for a healthy body and can be a source of energy and transport vital nutrients. Fats also play an important role in food manufacturing and cooking, making our foods taste good. For good health, it is necessary to pay attention to both the total amount and the type of fats in the diet. An excessive consumption of food fats can lead to health problems such as high blood pressure and obesity.

analysis of total cholesterol was performed using Ha and Kim's enzymatic colorimetric method.^[10] Acid titration (AT) was determined by titrations of 10 g mayonnaise to pH 8.1 with 0.1 N NaOH and results were converted to percentage of acetic acid according to the method of AOAC.^[9]

2.3. Determination of the Emulsion Type

Dilution test was sufficient to determine the type of the emulsions obtained: O/W or W/O. For the determination of this parameter, a small amount of emulsion was dispersed into two bottles, one containing the oil phase and the other containing the aqueous phase, an easy dispersion being performed only in the continuous phase of emulsion.^[11]

2.4. Droplet Size Measurement

The mayonnaise microstructures were observed using an optical microscope (Motic BA310). A glass microscope slide was covered with the "mayonnaise" sample and placed on the stage of the microscope to obtain photomicrographs. From microscopic examination and the software tool image, we obtained the diameter of each lipid droplet (droplet size distribution).

2.5. Determination of the Stability to Centrifugation

Emulsions were submitted to centrifugation 1460 g, for 20 minutes to check phase separation or droplet migration, such as creaming or sedimentation.

The creaming index, *IC* which reflects sample stability, is given by the following relationship:

$$IC = (HS/HE) \times 100 \quad [1]$$

where *HS* is the level of the supernatant creamy and *HE* is the emulsion height.

2.6. Rheological Characterization

Rheological measurements were performed with a rheometer (Paar Physica MCR 300 equipped with Rheolab Software-US 200). Yield stress was determined by applying a stress, τ , from 5 to 100 Pa with a constant pitch. Thixotropic behavior was demonstrated by the study of the experimental equilibrium curve via a ramp test in logarithmic strain ranging from 5 to 200 Pa in ascending and descending (charge and discharge) order.

Yield stress, flow behavior index data were obtained by using the Herschel–Bulkley's equation model as follows:

$$\tau = \tau_0 + K \cdot \gamma^n \quad [2]$$

where τ is the shear stress (Pa), τ_0 is the yield stress (Pa), γ is the shear rate (1/s), *K* is the consistency index (Pa s^{*n*}), and *n* is the flow index.

2.7. Sensory Analysis

Sensory evaluation was performed on samples after 1-day storage at room temperature (25°C). Sensory analyses, namely, appearance, aroma, mouth feel, and flavor, were conducted by 20 trained panelists. A rating scale linear structured (0 = dislike extremely to 10 = like extremely) was used to evaluate the intensities of perceptions of each sample.

2.8. Microbiological Analysis

Total bacterial counts (TBC), yeast, mold counts, *E. coli*, *Staphylococcus aureus* (*S. aureus*), and *Salmonella* spp. were determined according to the method given by the American Public Health Association.^[12]

2.9. Experimental Design

In order to optimize the formula corresponding to a diet mayonnaise-like sauce without cholesterol, we used experimental design method. The strategy adopted in this study is based on the response surface methodology (RSM). This strategy is used to determine the values of influencing factors corresponding to a particular response of studied system. The mathematical model adopted (Equation (3)) in our system (given by Equation (3)) is a second degree polynomial model with three factors: whey, soy lecithin, and guar gum. The experimental results were summarized and analyzed with the software MODDE 6, Umetrics, Sweden (2001). Relations between factors and responses were found by fitting a quadratic model with nine terms for each response:

$$y = a_0 + a_1x_1 + a_2x_2 + a_3x_3 + a_4x_1x_2 + a_5x_1x_3 + a_6x_2x_3 + a_7x_1^2 + a_8x_2^2 + a_9x_3^2 \quad [3]$$

where *y* was a response, *x*₁ and *x*₂ were input variables, *a*₀ was a constant term, and *a*₁,...*a*₉ were the model parameters.

The goodness of the models, that is the correlation between the input and the response data, was evaluated using a summary of the fit. This method includes the goodness of fit *R*², and the goodness of prediction *Q*² where *R*² is an overestimated measure and *Q*² is an underestimated measure of the goodness of fit of the model.^[13]

3. RESULTS AND DISCUSSION

3.1. Chemical Composition

Chemical compositions of the 16 formulas and RP are reported in Table 2. According to Hou-Pin et al.,^[18] the solids content is 834.4 g kg⁻¹ for a full-fat mayonnaise (73% fat). However, a value of 477 g kg⁻¹ was reported for a preparation of low-fat mayonnaise (36.5%)

TABLE 2
Chemical composition analysis of the 16 tests formulated (g kg^{-1})

Test	Dry matter content	Fat	Ash	Protein	Minerals			
					K	Ca	P	Na
1	300.8	195.32	17.9	12.18	1.26	0.69	1.33	4.34
2	326	160.62	19.3	12.44	0.99	0.67	0.65	4.26
3	367.2	162.02	11.3	12.64	1.00	0.68	0.66	4.21
4	381.5	182.99	13.9	12.11	1.11	0.67	0.99	4.34
5	383.3	194.16	11.0	12.14	1.18	0.68	1.16	4.36
6	373.1	193.7	11.6	12.14	1.18	0.68	1.16	4.36
7	380.4	187.71	17.8	11.74	1.37	0.71	1.67	4.57
8	442.2	195.31	11.3	11.88	1.24	0.68	1.33	4.43
9	400.4	184.19	11.0	12.14	1.18	0.68	1.16	4.36
10	322.1	160.628	17.4	12.54	0.99	0.68	0.66	4.28
11	388.7	197.75	14.6	11.64	1.37	0.68	1.67	4.39
12	380.1	160.615	12.0	12.37	0.98	0.67	0.65	4.37
13	394.6	194.19	11.2	12.14	1.18	0.68	1.16	4.36
14	437.3	197.7	11.3	11.64	1.38	0.69	1.68	4.34
15	386.5	183	11.5	12.41	1.13	0.69	1.00	4.35
16	407.9	197.7	11.1	11.94	1.39	0.7	1.68	4.36
RP	346.9	100.2	12.0	10.3	0.349	0.103	0.23	4.78

containing 15 g kg^{-1} xanthan gum xanthan gum (XG) and 10 g kg^{-1} GG as a fat substitute.

It can be concluded that the moisture content increases with the addition of fat substitutes (GG), which is a typical characteristic of carbohydrate-based fat replacers.^[14] Fat content lies in the range ($161\text{--}198 \text{ g kg}^{-1}$) which shows the low-fat in our system. This slight variation can be explained by the presence of soybean lecithin at different concentrations in the 16 used formulas. Indeed, our tests contain less oil than a full-fat mayonnaise.

It can be also seen by Table 2 that the ash content, varies between 11 and 19 g kg^{-1} , is not significant relative to the reference mayonnaise (12 g kg^{-1}). However, the values obtained in this study were similar to those obtained by Hou-Pin et al.^[18] (12.4 and 12.5 g kg^{-1}). Compared to the reference product, similar protein content was obtained, and this can be explained by the fact that the protein intake is provided by added whey of our system. Worrasinchai et al.^[15] found a protein content of 12 g kg^{-1} in a conventional (egg yolk-based) mayonnaise (containing 82.19% fat).

Calcium (Ca) content varies between 0.67 and 0.71 g kg^{-1} , phosphorus (P) between 0.65 and 1.68 g kg^{-1} , potassium (K) between 0.98 and 1.39 g kg^{-1} , whereas sodium content of the sodium (Na) varies between 4.21 and 4.60 g kg^{-1} . According to the table of nutrient composition of foods Ciquel, AFSSA 2008 (Agence Française de Sécurité Sanitaire des Aliments), a commercial “mayonnaise” without cholesterol contains 0.07 g kg^{-1} calcium, 0.25 g kg^{-1} phosphorus, 0.14 g kg^{-1} potassium, and 4.86 g kg^{-1} sodium. From these data, we can

conclude that the results found are converging in terms of intake of mineral, compared with the levels of commercial “mayonnaise.” Colorimetric analysis gives null absorbance values (the test is negative for the 16 tests formulated), meaning that our samples contain no cholesterol.

The absorbance value measured for RP is 0.121 , which corresponds to a cholesterol concentration of about 1.175 g kg^{-1} , it is in a good agreement with that reported by the table of nutrient composition of foods (1.16 g kg^{-1}). Obtained results of pH medium varied in the range of 3.20 and 3.82 reveal that there is no significant change in pH for the 16 formulas. pH decreases with increasing whey content, however the pH value does not vary greatly over time. These results agree with those found by Garcia et al.^[16] and Worrasinchai et al.^[17]

In conclusion, the results obtained have a homogeneous profile of variation and a very narrow range, and this demonstrates the good stability. On titratable acidity there are no significant fluctuations between tests and the reference product ($0.50\text{--}0.52\%$ acids). The acidity of the 16 tests is expressed as acetic acid but, in fact, our samples contain three types of acids: acetic acid provided by vinegar (5% acetic acid), citric acid (E330), used as an antioxidant, and lactic acid, provided by whey. GG is a neutral gum and does not affect the pH of food products.

3.2. Type of Emulsion

Small samples of sauce were dispersed immediately in water after gentle stirring, while a similar amount did not

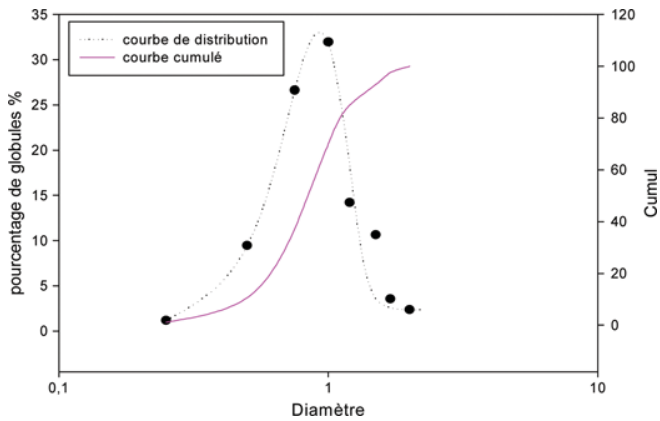


FIG. 1. Particle size distribution of a typical test.

show good dispersion in oil. Therefore, the emulsions belong to the oil-in-water (O/W) type. This is explained as follows: water is the continuous phase of the emulsion, while the oil is the dispersed phase; emulsions are well formulated O/W.

3.3. Particle Analysis

In Figure 1, the differential and cumulative particle size distribution curves for the sample (3), observed and analyzed with the optical microscope, are plotted. From this figure, we can show that the average diameter of the test for the sample (3), containing 5 g kg^{-1} GG stood at $8.5 \mu\text{m}$ representing the smallest diameter, while that of sample (11) containing 20 g kg^{-1} GG is $18.5 \mu\text{m}$ which is the largest diameter (Table 3). According to Canselier and Poux,^[11] full-fat mayonnaise-based egg yolk lecithin has an average diameter of $3\text{--}100 \mu\text{m}$ (this variation depends on the stirring speed) which is similar to our results. In the same context, Hou-Pin et al.^[18] show an average diameter of $7.49 \mu\text{m}$ of control mayonnaise and $12.44 \mu\text{m}$ for low-fat mayonnaise containing 15 g kg^{-1} g of XG and 10 g kg^{-1} GG. A diameter of $27.78 \mu\text{m}$ is given to a low-fat mayonnaise made with 100 g kg^{-1} of citrus fiber (CF) and 5 g kg^{-1} GG.

3.4. Stability to Centrifugation

Due to the difficulty to identify with precision the most stable emulsion after centrifugation, a second round was done to allow a differentiation between the other emulsions. Since all the droplet diameters are almost of the same order

of magnitude, it makes sense that this property is not related to the stability to centrifugation. Therefore, although, according to particle size and distribution, sample (3) was potentially the most stable, samples (4, 5, 6, 7, 8, 9, 11, 12, 13, 14) are the most stable in the absence of the supernatant oily layer due to the presence of the thickening agent in sufficient quantity, which ensures the stability of the emulsion. However, the emulsion of sample (3) was one of the most stable emulsions potentially considering distribution and particle size.

3.5. Rheological Characterization

Characterization of the referent product is designed to take the properties used as responses, and to take these values as targets to be achieved during the step optimization of the formulations.

3.5.1. Yield Stress of Flow of the Reference Product

The obtained rheograms (Figure 2) show two regions of the elastic and plastic, making the value limit of the elastic and early plastic region to be estimated at 58.19 Pa for the reference product and 56.1 Pa for the type tested by software US200.

3.5.2. Test Time Dependence of the Reference Product and Typical Test

According to Figure 3b we can see that the viscosity decreases slightly in the first interval of time equivalent to a shear stress ($\tau = 56 \text{ Pa}$). This decrease (relaxation) becomes sharp with increasing stress ($\tau = 100 \text{ Pa}$). Subsequently, a slow regeneration is observed at the third interval when the first returns to the applied stress ($\tau_0 = 56 \text{ Pa}$). By this figure, we can also observe that the reference Benedicta mayonnaise and test type of the sample (11) have some time dependence. However, the viscosity of employed sample decreases versus time, which explain the rapid dispersion of droplets and their relative destruction (restructuring phenomenon) due to the presence of van der Waals as dominates shear forces. This structural condition shows that the resistance of these droplets to flow decreases significantly. This structure is regenerated by reducing the stress to a steady state of viscosity by equality between opposing forces (forces of attraction and repulsion). But the 16 samples of mayonnaise show shear thinning behavior, some are strongly time dependent, while other are not. This difference is due to the change in concentration of the texturizing agent from one test to another.

TABLE 3
Average particle diameter (μm)

Test	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
d (μm)	9.50	13.00	8.50	14.20	11.00	11.32	13.43	15.50	11.40	10.36	18.50	13.70	12.10	11.00	9.10	9.85

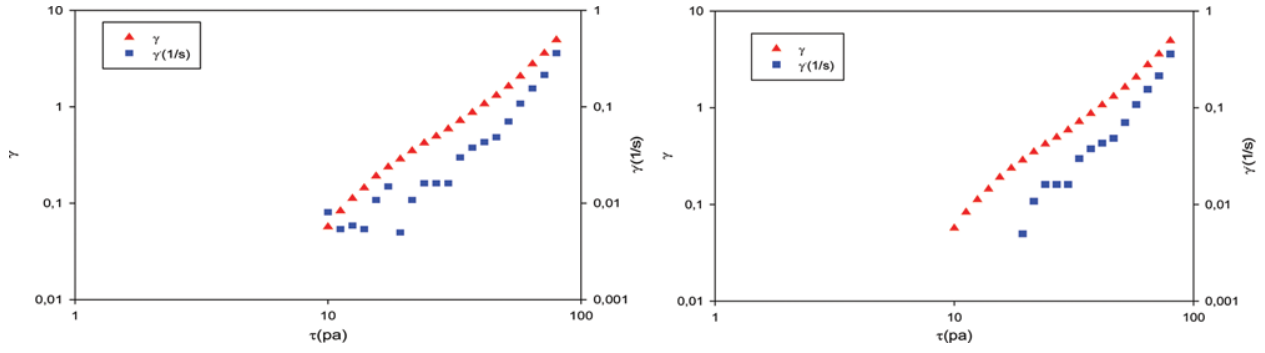


FIG. 2. Evolution of deformation and shear rate as a function of the threshold stress for mayonnaise reference (left) and test type (right).

3.5.3. Rheological Model

Flow curves and the characteristics of samples were fitted to the model of Herschel–Bulkley, as summarized in Table 4.

3.6. Sensory Evaluations

The intensity of these descriptors was noted in a structured rating scale of 0–10. The calculation results are then obtained as averages for each attribute (Table 5). These averages will be introduced as a response at the plane

of experience. Tests of mayonnaise contain balanced proportions of salt, vinegar, and spices (mustard), which has contributed to taste.

3.7. Microbiological Analysis of Mayonnaise

After 12 weeks of storage, our tests contained only a bacterial count lower than the standard.^[19,20] This may be due to low pH, as well as the use of the preservatives sodium benzoate and potassium sorbate. At this pH, most bacteria do not grow. Sodium benzoate has antifungal properties and is active at pH below 4.

3.8. Modeling in Surface Response

Collected data was analyzed by MODDE in order to find a relation between the input variables, x_1, x_2, x_3 , which represents the content of guar gum, soy lecithin, and whey, respectively, and the output variables, that is the responses creaming index, smooth, and viscosity. The mathematical models obtained can therefore be written as follows:

$$\text{Creaming index} = 0.126242 - 0.400903x_1 - 0.168491x_2 + 0.312792x_3 - 0.105288x_2x_3 + 0.160346x_{11}^2 - 0.10947x_{22}^2$$

$$\text{Smooth} = 7.15839 - 0.476875x_1 - 0.350858x_2 + 0.510342x_3 - 0.198217x_1x_2 + 0.163915x_1x_3 - 0.175226x_2x_3 + 0.316916x_{22}^2$$

$$\text{Viscosity} = 6.07558 + 1.50181x_1 - 0.617645x_3 - 0.164598x_1x_2 + 0.346344x_1x_3 - 0.403221x_2x_3 - 0.62459x_{11}^2 + 0.559248x_{22}^2 + 0.143873x_{33}^2$$

where x_1 represents the content of guar gum, x_2 represents the content of soy lecithin, and x_3 represents the content of whey.

The relationships between all factors and all responses could be overviewed by displaying the loading plot named

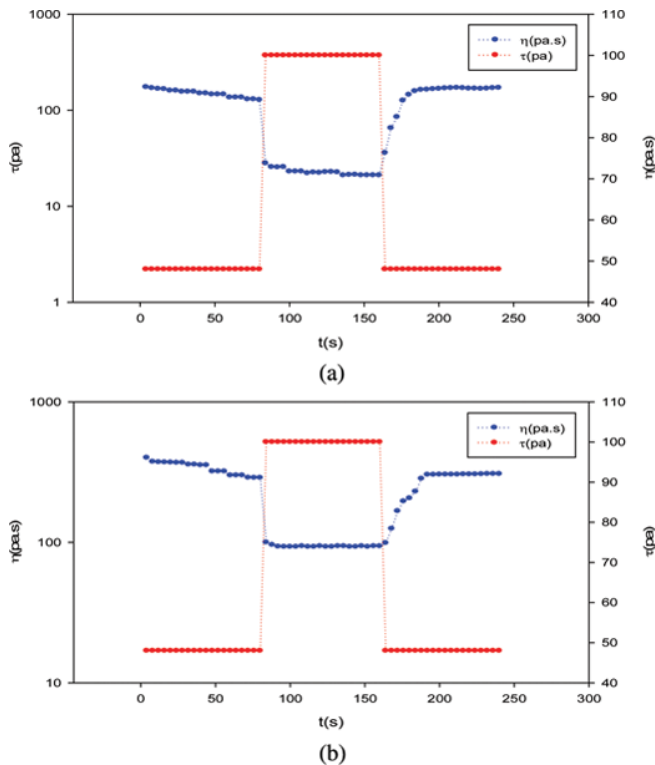


FIG. 3. Evolution of the viscosity versus time at various levels of shear rate of: a) mayonnaise reference and b) test type.

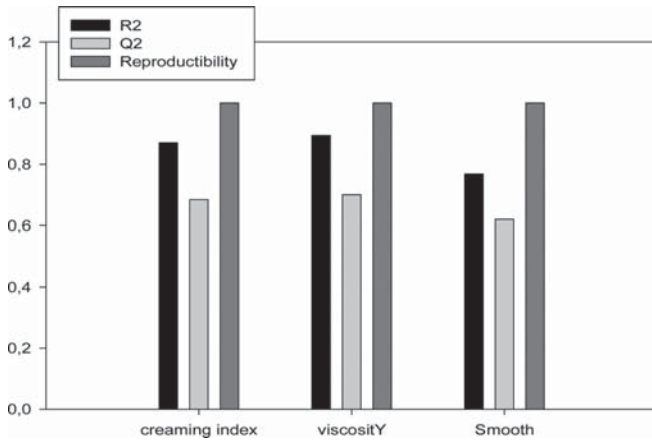


FIG. 4. Histogram of parameters indicative of the quality adjustment and prediction models in response surface.

iso-response curves. From the graphs obtained (Figure 5), the gradual increase in volume of whey increases dramatically up to the smooth maximum values. On the

other hand, when the concentration of guar increases, the smoothness decreases considerably. The negative influence of guar gum on the reply “creaming index” was confirmed. For the viscosity response we can observe a large (negative) influence due to the presence of whey. These results confirmed the growth of these levels with the gradual increase of soybean lecithin and guar gum.

3.8.1. Optimization of the Formula

According to the objective of our study we have proposed to maximize the two first responses and eliminate the index of creaming. The optimum formulation can be obtained by derivation of the model equation to find values of x factor levels. Optimization results gave the following formula composition: whey 64.46%, vegetable oil 16%, vinegar 10%, mustard 3%, guar 1.94%, and sugar 2.5%, salt 1%, preservatives 0.1%, soy lecithin 0.5%, and citric acid 0.5%. This formula thus has a viscous appearance of 8.77/10 and a smoothness of 8.56/10 with a creaming index of 0.

TABLE 4
Parameters of the Herschel–Bulkley’s model

Test	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
τ_0 (Pa)	20.50	34.72	24.99	17.07	32.00	33.74	53.45	52.623	34.24	28.14	71.67	49.83	34.53	45.68	18.03	13.73
R	0.97	1.00	0.99	0.96	0.99	0.99	0.98	0.98	0.99	0.99	0.99	1.00	1.00	0.97	0.99	0.98
K	20.03	28.01	20.56	35.11	20.24	20.41	28.15	29.04	20.1	23.23	21.13	24.52	21.45	21.83	22.35	22.3

TABLE 5
Results of sensory analysis (1 = dislike extremely, 9 = like extremely)

Test	Texture attribute		Flavor attribute		Mouth feel-attribute			Aroma attribute Whey flavor
	Shiny	Creamy	Salty	Acid	Fatty	Smooth	Viscous	
1	8.50	3.00	5.51	8.33	5.50	8.10	3.50	5.00
2	8.20	8.00	5.95	8.35	5.00	9.60	8.00	5.50
3	9.00	2.00	5.97	8.45	6.25	9.12	2.00	6.50
4	2.00	3.50	5.50	8.30	5.25	5.10	7.00	5.00
5	4.40	5.00	5.42	8.20	5.75	6.65	6.00	5.00
6	4.40	5.00	5.42	8.20	5.75	6.65	6.00	5.00
7	7.00	9.20	5.23	8.15	4.70	7.85	8.50	4.00
8	8.00	9.60	5.04	8.11	4.75	8.00	8.00	4.50
9	4.40	5.00	5.42	8.20	5.75	6.65	6.00	5.00
10	9.60	8.50	5.95	8.40	5.00	9.35	7.50	6.00
11	5.10	6.00	5.02	8.10	5.77	4.54	9.60	4.00
12	8.00	9.00	5.85	8.30	5.40	8.53	9.00	5.50
13	4.40	5.00	5.42	8.22	5.75	6.65	6.00	5.00
14	5.40	6.00	5.10	8.13	5.00	7.75	7.80	4.50
15	8.80	7.50	5.97	8.35	5.11	9.25	3.00	5.50
16	9.10	6.00	5.23	8.15	4.83	9.11	4.00	4.50

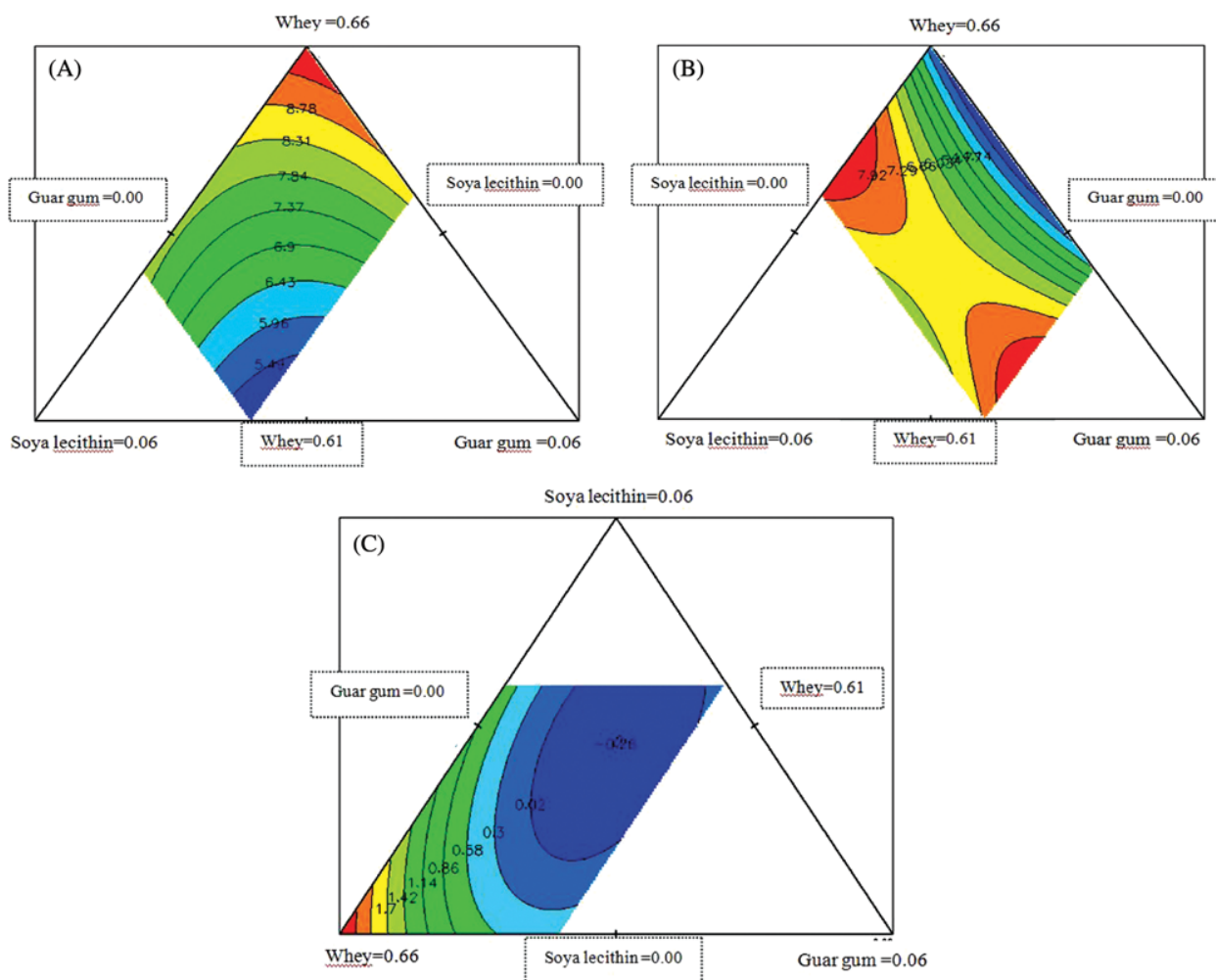


FIG. 5. Contour plots for the responses: a) creaming index, b) viscous appearance, and c) smoothness.

4. CONCLUSION

The objective of this study was to develop a new formula for a diet mayonnaise-like sauce without cholesterol. Treatment of different mayonnaise samples show that the emulsifying power is provided by the use of soy lecithin and the total fat content in our formulations was limited to 16%. Droplet size measurement of employed mayonnaise samples at different times shows that the largest diameter of fat does not exceed 18.5 μm . Results of stability to centrifugation reveal that the absence of the supernatant oily layer ensures the stability of the emulsion. However, the rheological analysis of used mayonnaises gives a yield stress of 56.1 Pa. Using the experimental design method, the number of trials can be limited to a number of design experiments of 16, and best formulation of the mayonnaise (without cholesterol) was obtained.

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