

Modeling the Relationship between the Main Emulsion Components and Stability, Viscosity, Fluid Behavior, ζ -Potential, and Electrophoretic Mobility of Orange Beverage Emulsion Using Response Surface Methodology

HAMED MIRHOSSEINI,[†] CHIN PING TAN,^{*†} NAZIMAH SHEIKH ABDUL HAMID,[‡]
 AND SALMAH YUSOF[§]

Departments of Food Technology and Food Science, Faculty of Food Science and Technology, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia, and Faculty of Science and Technology, Islamic Science University of Malaysia, 71800 Nilai, Negeri Sembilan, Malaysia

The possible relationships between the main emulsion components (namely, Arabic gum, xanthan gum, and orange oil) and the physicochemical properties of orange beverage emulsion were evaluated by using response surface methodology. The physicochemical emulsion property variables considered as response variables were emulsion stability, viscosity, fluid behavior, ζ -potential, and electrophoretic mobility. The independent variables had the most and least significant ($p < 0.05$) effect on viscosity and ζ -potential, respectively. The quadratic effect of orange oil and Arabic gum, the interaction effect of Arabic gum and xanthan gum, and the main effect of Arabic gum were the most significant ($p < 0.05$) effects on turbidity loss rate, viscosity, viscosity ratio, and mobility, respectively. The main effect of Arabic gum was found to be significant ($p < 0.05$) in all response variables except for turbidity loss rate. The nonlinear regression equations were significantly ($p < 0.05$) fitted for all response variables with high R^2 values (>0.86), which had no indication of lack of fit. The results indicated that a combined level of 10.78% (w/w) Arabic gum, 0.56% (w/w) xanthan gum, and 15.27% (w/w) orange oil was predicted to provide the overall optimum region in terms of physicochemical properties studied. No significant ($p > 0.05$) difference between the experimental and the predicted values confirmed the adequacy of response surface equations.

KEYWORDS: Stability; viscosity; fluid behavior; ζ -potential; electrophoretic mobility; orange beverage emulsion; response surface methodology; turbidity loss

INTRODUCTION

Emulsions are thermodynamically unstable systems, and so, they have a tendency to break down over time (1, 2). Being thermodynamically unstable systems, food emulsions are stabilized by improvement of their kinetic stability (2, 3), where stability may be defined as the resistance to physical changes (4). The breakdown processes of an emulsion may be distinguished through a number of different physicochemical mechanisms, including creaming or sedimentation (caused by gravity), flocculation, Ostwald ripening, coalescence, and phase inversion (2, 5). The physical stability of the emulsion system is influenced by several factors: fat content, type and ratio of emulsifier to fat,

viscosity of the continuous phase, volume fraction of the dispersed phase, and the droplet size distribution (6–9).

The shelf life of many food emulsions depends on the rheological characteristics of the component phase (e.g., the creaming of oil droplets) (10). Food emulsions exhibit a wide range of rheological behaviors ranging from low-viscosity fluids (such as milk and fruit juice beverages) to fairly hard solids (such as refrigerated margarine or butter). Rheological characterization of a particular system depends on both the type and the concentration of ingredients it contains as well as the processing and storage conditions (2). For instance, the presence of a large number of high molecular weight molecules increases the resistance to flow, which, in turn, increases the apparent viscosity of the emulsion system (11). Taherian, Fustier, and Ramaswamy (12) studied the effect of added oil and modified starch on the rheological properties of beverage cloud emulsions. They observed that an increase in the starch concentration contributed to changes in the flow behavior of the water phase from Newtonian to slightly shear thinning.

* To whom correspondence should be addressed. Tel: +603-89468418. Fax: +603-89423552. E-mail: tancp@putra.upm.edu.my.

[†] Department of Food Technology, Universiti Putra Malaysia.

[‡] Department of Food Science, Universiti Putra Malaysia.

[§] Islamic Science University of Malaysia.

It is well-known that the electrical properties of the oil/water interface affect the emulsion stability, the flow behavior of emulsions, changes in reservoir wettability, and thus oil recovery. The instability of an emulsion usually occurs in the interracial region, which often contains surface-active agents or macromolecules. Surfactants and polymeric substances play an important role in coalescence by modifying the interfacial mobility. Stabilization of dispersions with polymers can occur via two main mechanisms that depend upon the surface association of the polymer and particle (13): The steric stabilization results from macromolecules adsorbed to the particle surface, and the second mechanism consists of the electrostatic stabilization (which is not brought about exclusively by a polymeric system) and arises from the mutual repulsion between both particle's electrical double layer. The electrostatic contribution to this stabilization is quantified by the ζ -potential, calculated from the electrophoretic mobility (14).

The response surface methodology (RSM) is an empirical modeling approach for determining the relationship between various process parameters and responses with the various desired criteria and searching the significance of these process parameters on the coupled responses (15). The RSM can be utilized as a helpful statistical technique to reduce the number of experimental trials needed to evaluate multiple parameters and their interactions. RSM has been successfully used to model lipid oxidation and Maillard reactions in meat emulsions (16), to study the dependence of emulsion ability with time and protein concentration (17), to optimize processing conditions of raw sausage emulsion (18), and to optimize the formulation of sodium caseinate/stearic acid emulsion coating (19).

The main objective of present study was to determine the optimum level of independent variables that led to (i) the highest emulsion stability, electrophoretic mobility, and viscosity simultaneously along with viscosity ratio and (ii) the largest magnitude of ζ -potential for orange beverage emulsion. A three-factor central composite design (CCD) was used to study the effect of Arabic gum (13–20% w/w), xanthan gum content (0.3–0.5% w/w), and orange oil content (10–14% w/w) on the physicochemical emulsion properties (namely, turbidity loss rate, viscosity, viscosity ratio, ζ -potential, and electrophoretic mobility). It should be noted that other critical parameters such as average droplet size, polydispersity index, turbidity, size index, creaming stability, viscosity, flow behavior, pH, density, and flavor release of orange beverage emulsion have also been investigated in our other studies.

MATERIALS AND METHODS

Materials. Arabic gum was provided by Colloides Naturels International Co. (Rouen, France). Xanthan gum was donated by CP Kelco (Chicago, IL). Citric acid, sodium benzoate, and potassium sorbate ($\geq 95\%$) were purchased from Fisher Scientific (Pittsburgh, PA). Valencia cold-pressed orange flavoring oil was provided by Danisco (Cultor, Aarhus, Denmark).

Preparation of Orange Beverage Emulsion. In this study, 20 orange beverage emulsions that were composed of gum Arabic (13–20% w/w), xanthan gum (0.3–0.5% w/w), orange oil (10–14% w/w), sodium benzoate (0.1% w/w), potassium sorbate (0.1% w/w), citric acid (0.4% w/w), and deionized water were prepared for the optimization procedure based on a three-factor CCD (Table 1). As mentioned in our previous study (20), sodium benzoate, potassium sorbate, and citric acid were dispersed in deionized water (60 °C) using a high-speed blender (Waring blender 32BL80, New Hartford, CO) to prepare the water phase. While mixing the mixture, Arabic gum was gradually added to the deionized water (60 °C) and mixed for 3 min to facilitate hydration. The Arabic gum solution was kept overnight at room temperature to fully hydrate. Xanthan gum solution was prepared separately by dissolving xanthan

Table 1. Levels of Independent Variables Established According to the CCD

variable	level				
	independent variables	low	center	high	axial ($-\alpha$)
arabic gum content (% w/w)	13.00	16.50	20.00	10.78	22.22
xanthan gum content (% w/w)	0.30	0.40	0.50	0.24	0.56
orange oil content (% w/w)	10.00	12.00	14.00	8.734	15.27

gum in deionized water and then mixed with the Arabic gum solution by using a high-speed blender. While mixing the water phase, the cold-pressed orange oil was gradually added into the water phase to provide an initial coarse emulsion (20). Fine emulsification (i.e., an average droplet size of $<1 \mu\text{m}$ with narrow particle size distribution) was achieved by subjecting the pre-emulsions to prehomogenization using the high shear blender (Silverson L4R, Buckinghamshire, United Kingdom) for 1 min and then passed through a high-pressure homogenizer (APV, Crawley, United Kingdom) for three passes (30, 28, and 25 MPa).

Analytical Methods. *Turbidity Loss Rate (Emulsion Stability Index).* Emulsion breakage was monitored by measuring the absorbance (loss rate of turbidity) at 500 nm (11) by means of a UV-visible spectrophotometer (Spectronic Genesys 10, Geneq Inc., Montreal, Canada). The beverage emulsions were diluted to 0.25% (w/w) in a 10% sugar solution and stored in 1 L plastic bottles at room temperature before the absorbance reading. Absorbance vs time data for each sample were fitted to a first-order model (21) as follows:

$$\ln A = \ln A_0 - k_1 t$$

where t is the time, A is the absorbance at time = t , A_0 is the absorbance at time = 0, and k_1 is the first-order rate constant (22). The emulsion stability end point or "time to breakage" was computed from the above fitting equation at $A = 0.1A_0$. In this study, k_1 (first-order rate constant) was calculated as the loss rate of turbidity. The beverage emulsion having a smaller k_1 value indicated the lower loss rate of turbidity, thus indicating the higher stability than the emulsion having a larger k_1 value. The readings were taken twice daily until the absorbance fell to 10% of the initial absorbance value. Daily absorbance values were the averages of three consecutive readings.

Viscosity and Fluid Behavior. Immediately after the preparation of orange beverage emulsions, their viscosities were measured by means of a steady stress Brookfield viscometer (Brookfield DV-II+ Programmable Viscometer, Brookfield Engineering Laboratories, Middleboro, MA) equipped with the LV spindles. The viscosity readings between 0 and 100 scale units were taken at rotational speeds of 2, 10, 20, 30, 50, 60, and 100 rpm. The procedure for the measurement of viscosity was described by previous study (11). For the evaluation of flow behavior, the measurements of viscosity were usually made at speeds that differed by a factor of 10 (for example, 2 and 20 or 10 and 100 rpm), and then, the viscosity ratio was calculated to judge the fluid behavior. In constructing the ratio, the viscosity value at the lower speed should be placed in the numerator, the one at the higher speed in the denominator. It should be noted that for pseudoplastic (shear thinning) fluids, the ratio would exceed 1.0 as the degree of pseudoplastic behavior increases. Conversely, for dilatant (shear thickening) fluids, the ratio will be less than 1.0 as the degree of dilatancy increases. In this study, the viscosity ratio (vis. 10 rpm/vis. 100 rpm) was taken as a possible index for interpreting the fluid behavior of orange beverage emulsion.

ζ -Potential and Electrophoretic Mobility. The orange beverage emulsions were diluted (1:100) for the measurement of ζ -potential and electrophoretic mobility using a Malvern ζ -sizer (Malvern series ZEN 3500, Malvern Instruments Ltd., Malvern, Worcester, United Kingdom). Measurements of the electrophoretic mobility were carried out to evaluate the surface net charge around lipid droplets (14). To avoid multiple scattering effects, the emulsions were diluted with deionized water prior to analysis and then directly placed into the module. The measurements were performed immediately after emulsion preparation and then reported as the average of three individual injections, with four readings made per injection.

Table 2. Matrix of the CCD

treatment runs	blocks	arabic gum	xanthan gum	orange oil
1	1	20.00	0.50	10.00
2	1	13.00	0.50	14.00
3	1	13.00	0.30	10.00
4 (C) ^a	1	16.50	0.40	12.00
5	1	20.00	0.30	14.00
6 (C)	1	16.50	0.40	12.00
7	2	13.00	0.50	10.00
8	2	20.00	0.50	14.00
9	2	20.00	0.30	10.00
10	2	13.00	0.30	14.00
11 (C)	2	16.50	0.40	12.00
12 (C)	2	16.50	0.40	12.00
13 (C)	3	16.50	0.40	12.00
14 (C)	3	16.50	0.40	12.00
15	3	16.50	0.24	12.00
16	3	10.78	0.40	12.00
17	3	16.50	0.40	15.27
18	3	16.50	0.40	8.73
19	3	22.22	0.40	12.00
20	3	16.50	0.56	12.00

^a(C), center point.

Experimental Design. A three-factor CCD was employed to study the effect of three independent variables, namely, Arabic gum content (13–20% w/w, x_1), xanthan gum content (0.3–0.5% w/w, x_2), and orange oil content (10–14% w/w, x_3), on five response variables (Y_1 – Y_5 , namely, turbidity loss rate, viscosity, viscosity ratio, ζ -potential, and electrophoretic mobility). The experimental design was developed to (i) find a relationship between each response and three independent variables and (ii) to determine the optimum level of the independent variables resulting in the desirable goals. Therefore, 20 treatments were prepared based on the CCD with three independent variables at five levels for each variable (**Table 2**). The center point was repeated six times to calculate the repeatability of the method (15). The concentration range of independent variables and matrix of the CCD including the factor levels and treatments is shown in **Tables 1** and **2**.

Statistical Analyses. Response surface analysis was conducted for (i) determining regression coefficients and statistical significance of model terms and (ii) fitting the regression models to the experimental data aiming at an overall optimal region for all response variables studied. The behavior of the response surface was investigated for the response function (Y_i) using the polynomial regression equation. The generalized model proposed relating the independent variables to the response variables studied is given below:

$$Y = \beta_0 + \sum \beta_i x_i + \sum \beta_{ii} x_i^2 + \sum \beta_{ij} x_i x_j \quad (1)$$

where Y is response calculated by the model; β_0 is a constant; and β_i , β_{ii} , and β_{ij} are main, squared, and interaction coefficients, respectively. Only terms found statistically significant ($p < 0.05$) were included in the reduced model. It should be noted that some variables were kept in the reduced model despite nonsignificance. For example, main terms were also kept in the model if a quadratic or interaction term containing this variable was significant ($p < 0.05$). The experimental design matrix, data analysis, and optimization procedure were performed using the Minitab v. 13.2 statistical package (Minitab Inc., Pennsylvania).

Optimization Procedure. After the response surface models were fitted, the individual and overall response optimizations were carried out to obtain the optimum concentration level of main beverage emulsion components (x_1 , x_2 , and x_3) leading to a desirable orange beverage emulsion. An orange beverage emulsion would be considered an optimum product if the optimized criteria led to (i) maximum emulsion stability, viscosity simultaneously along with viscosity ratio, and electrophoretic mobility and (ii) obtain the largest magnitude of negatively charged ζ -potential for the orange beverage emulsion. For a graphical response optimization, only the significant ($p < 0.05$) interaction variable effects were expressed as three-dimensional response surface plots to visualize the relationship between the response variables and the significance ($p < 0.05$) affecting them. A numerical optimization was also carried out by the response optimizer using the Minitab software for determining the exact value of independent variables resulting in the desirable response goals.

Verification of the Response Surface Models. The adequacy of the regression equations was verified by comparing the experimental data with predicted values obtained from the equations.

RESULTS AND DISCUSSION

Preliminary Study. The objectives of preliminary experiments were to (i) determine the influence of mixing and homogenizing conditions (e.g., speed, time, and pressure) on the particle size and distribution and then (ii) establish the best preparation condition leading to narrow size distribution and small average droplet size ($< 1 \mu\text{m}$). The result indicated that the fine emulsification was achieved by mixing the emulsion for 1 min in a conventional homogenizer and then passed through the high-pressure homogenizer, three passes at 30, 28, and 25 MPa (data not shown).

Response Surface Models. The response surface analysis allowed the development of the polynomial regression relation-

Table 3. Experimental Data Obtained for the Response Variables (Y) (Mean \pm SD)

run	block	response variable				
		turbidity loss rate (Y_1 , $\text{\AA}/\text{day}$)	viscosity (60 rpm) (Y_2 , mPa s)	viscosity ratio (Y_3)	ζ -potential (Y_4 , mV)	mobility (Y_5 , $\mu\text{m cm/Vs}$)
1	1	0.800 \pm 0.205	280 \pm 10	2.220 \pm 0.201	-27.03 \pm 1.08	2.220 \pm 0.085
2	1	0.675 \pm 0.014	370 \pm 6	3.651 \pm 0.104	-29.37 \pm 0.71	2.296 \pm 0.056
3	1	0.618 \pm 0.014	98 \pm 3	2.222 \pm 0.090	-28.80 \pm 2.41	2.257 \pm 0.099
4	1	0.775 \pm 0.021	204 \pm 9	2.530 \pm 0.305	-28.07 \pm 1.05	2.200 \pm 0.082
5	1	0.632 \pm 0.007	304 \pm 13	2.340 \pm 0.073	-27.72 \pm 0.25	2.173 \pm 0.040
6	1	0.773 \pm 0.021	205 \pm 5	2.512 \pm 0.018	-28.31 \pm 0.19	2.219 \pm 0.025
7	2	0.891 \pm 0.021	266 \pm 4	3.016 \pm 0.063	-29.18 \pm 1.22	2.288 \pm 0.051
8	2	0.937 \pm 0.035	463 \pm 11	2.433 \pm 0.056	-29.17 \pm 0.67	2.287 \pm 0.102
9	2	0.619 \pm 0.035	265 \pm 7	2.453 \pm 0.209	-26.82 \pm 1.30	2.103 \pm 0.105
10	2	0.787 \pm 0.050	200 \pm 2	2.292 \pm 0.040	-29.18 \pm 0.95	2.270 \pm 0.065
11	2	0.919 \pm 0.028	250 \pm 5	2.660 \pm 0.085	-27.77 \pm 0.42	2.177 \pm 0.040
12	2	0.867 \pm 0.021	258 \pm 6	2.643 \pm 0.102	-28.29 \pm 0.67	2.218 \pm 0.078
13	3	0.799 \pm 0.028	230 \pm 3	2.545 \pm 0.036	-27.85 \pm 0.45	2.183 \pm 0.035
14	3	0.805 \pm 0.042	250 \pm 8	2.690 \pm 0.079	-28.00 \pm 0.83	2.212 \pm 0.144
15	3	0.646 \pm 0.021	190 \pm 5	2.360 \pm 0.053	-27.43 \pm 0.67	2.151 \pm 0.052
16	3	0.654 \pm 0.050	257 \pm 9	2.920 \pm 0.008	-29.47 \pm 0.72	2.310 \pm 0.049
17	3	0.527 \pm 0.035	320 \pm 8	2.737 \pm 0.400	-27.96 \pm 1.28	2.192 \pm 0.034
18	3	0.589 \pm 0.050	203 \pm 4	2.629 \pm 0.097	-27.75 \pm 0.34	2.175 \pm 0.035
19	3	0.775 \pm 0.007	394 \pm 10	2.138 \pm 0.022	-27.91 \pm 1.08	2.203 \pm 0.084
20	3	0.869 \pm 0.057	365 \pm 5	3.010 \pm 0.066	-29.98 \pm 0.71	2.350 \pm 0.023

Table 4. Regression Coefficients, R^2 , Adjusted R^2 , Probability Values, and Lack of Fit for Five Dependent Variables^a

regression coefficient	turbidity loss rate (Y_1)	viscosity (60 rpm) (Y_{2-1})	viscosity (100 rpm) (Y_{2-2})	viscosity ratio (x_{10x}) (Y_3)	ζ -potential (Y_4)	mobility (Y_5)
b_0	-2.270	792.4	1038.7	-2.442	-47.52	3.469
b_1	-0.028	-52.0	-82.0	0.497	1.48	-0.118
b_2	1.169	-610.2	-925.8	7.564	28.33	-2.031
b_3	0.484	-62.2	-50.5	-0.121	0.33	0.011
b_1^2	-0.002	2.6	2.2	-0.003	-0.02	0.002
b_2^2	-0.462	1349	1125.3	1.964	-23.53	1.907
b_3^2	-0.020	1.9	0.5	0.005	0.02	-0.002
b_{12}	0.116	-58.6	-4.5	-0.819	-0.39	0.062
b_{13}	0.004	0.3	2.3	-0.011	-0.04	0.002
b_{23}	-0.163	91.2	47.2	0.557	-0.65	-0.005
R^2	0.958	0.986	0.991	0.974	0.866	0.937
R^2 (adj)	0.901	0.968	0.978	0.939	0.681	0.851
regression (P value)	0.000 ^b	0.000 ^b	0.000 ^b	0.000 ^b	0.011 ^b	0.001 ^b
lack of fit (F value)	4.36	4.07	2.79	2.76	5.93	1.25
lack of fit (p value)	0.128 ^c	0.139 ^c	0.214 ^c	0.217 ^c	0.087 ^c	0.456 ^c

^a Key: b_i , the estimated regression coefficient for the main main effects; b_{ij} , the estimated regression coefficient for the quadratic effects; b_{ij} , the estimated regression coefficient for the interaction effects; and 1, arabic gum; 2, xanthan gum; and 3, orange oil. ^b Significant ($p < 0.05$). ^c Not significant ($p > 0.05$).

Table 5. F Ratio and p Value for Each Independent Variable Effect in the Polynomial Response Surface Models^a

variables		main effects			quadratic effects			interaction effects		
		x_1	x_2	x_3	x_1^2	x_2^2	x_3^2	x_1x_2	x_1x_3	x_2x_3
turbidity loss rate (Y_1)	p value	0.493	0.400	0.000 ^b	0.080	0.668	0.000 ^b	0.016 ^b	0.101	0.040 ^b
	F ratio	0.517	0.790	41.861	4.008	0.198	58.752	9.284	3.437	5.983
viscosity (60 rpm) (Y_2)	p value	0.010 ^b	0.277	0.07	0.000 ^b	0.011 ^b	0.110	0.005 ^b	0.732	0.009 ^b
	F ratio	11.10	1.360	4.38	58.75	10.72	3.240	14.99	0.130	11.88
viscosity (100 rpm) (Y_{2-2})	p value	0.000 ^b	0.047 ^b	0.055	0.000 ^b	0.007 ^b	0.541	0.705	0.004 ^b	0.046 ^b
	F ratio	48.30	5.500	5.000	74.50	13.00	0.400	0.200	15.80	5.600
viscosity ratio (Y_3)	p value	0.001 ^b	0.038 ^b	0.503	0.143	0.436	0.453	0.000 ^b	0.039 ^b	0.007 ^b
	F ratio	30.10	6.200	0.490	2.630	0.670	0.620	86.86	6.050	13.11
ζ -potential (Y_4)	p value	0.019 ^b	0.133	0.740	0.126	0.116	0.541	0.448	0.109	0.467
	F ratio	8.570	2.800	0.120	2.920	3.110	0.410	0.640	3.250	0.580
mobility (Y_5)	p value	0.001 ^b	0.039 ^b	0.818	0.011 ^b	0.019 ^b	0.377	0.031 ^b	0.120	0.907
	F ratio	23.07	6.100	0.060	10.82	8.660	0.870	6.820	3.030	0.010

^a Key: x_1 , x_2 , and x_3 , the main effect of Arabic gum, xanthan gum, and orange oil, respectively. x_1^2 , x_2^2 , and x_3^2 , the quadratic effect of Arabic gum, xanthan gum, and orange oil, respectively. x_1 and x_2 , the interaction effect of Arabic gum and xanthan gum. x_1 and x_3 , the interaction effect of Arabic gum and orange oil. x_2x_3 , the interaction effect of xanthan gum and orange oil. ^b Significant at $p < 0.05$.

ship where each response variable (Y_i) was assessed as a function of Arabic gum content (x_1), xanthan content (x_2), and orange oil content (x_3). The estimated regression coefficients of the response models, along with the corresponding R^2 values and lack of fit tests, are given in **Table 4**. The response surface analysis offered the significant ($p < 0.05$) regression relationships between the independent and the response variables (**Table 4**). The response surface analysis offered highly significant ($p \leq 0.011$) response regression models involving high R^2 values ranging from 0.866 to 0.991 (**Table 4**). Thus, at least 86% of the variation of response variables could be accurately explained by the reliable regression models relating the physicochemical properties to the main emulsion components. The lack of fit tests for all response variables resulted in no significant ($p > 0.05$) F value. This observation exhibited a satisfactory fitness of the response surface models employed for describing the response variations as a function of three independent variables (**Table 4**).

As shown in **Table 5**, at least one interaction effect of the independent variables had a significant ($p < 0.05$) effect on the response variables except for the ζ -potential. The significant ($p < 0.05$) quadratic and interaction effects of independent variables exhibited a nonlinear regression model fitted to the experimental data (**Table 5**). The emulsion viscosity was found to be significantly ($p < 0.05$) influenced by the independent variables more than other response variables, whereas the negatively charged ζ -potential was shown to be significantly ($p < 0.05$) affected only by the main effect of Arabic gum

(**Table 5**). The results indicated that the Arabic gum content had a significant ($p < 0.05$) effect on all response variables except for turbidity loss rate. Therefore, the proportion of Arabic gum should be considered as a primary factor for interpreting the variation of physicochemical properties studied. It should be noted that the polynomial regression equation may be significant ($p < 0.05$) only in the selected ranges. It may not be true beyond the ranges of the factors. Therefore, the model may not be extrapolated beyond these ranges (15).

Turbidity Loss Rate (Emulsion Stability Index). In general, the positive turbidity of loss rate indicated that the turbidity of orange beverage emulsion decreased during storage. Ray et al. (23) and Dłuzewska et al. (23) have also demonstrated that the decrease of turbidity of emulsions and beverages is observed during their storage. Ray et al. (23) described that this phenomenon was due to the aggregation of oil droplets and the changes in refractive index of the oil phase and aqueous phase. Dłuzewska et al. (24) have also explained that the decrease of turbidity of beverage emulsion in its diluted form could be caused by loss of polysaccharides molecules (desorption) for external layers of film formed on the interfacial surface, which led to the change in the refractive index of the disperse phase. As shown in **Table 5**, the quadratic effect of orange oil followed by its main effect had the most significant ($p < 0.05$) effect on turbidity loss rate (Y_1). Thus, these independent variable effects should be considered as critical parameters for evaluating the stability of orange beverage emulsion (**Table 5**). The interaction effects of xanthan gum with Arabic gum as well as its interaction

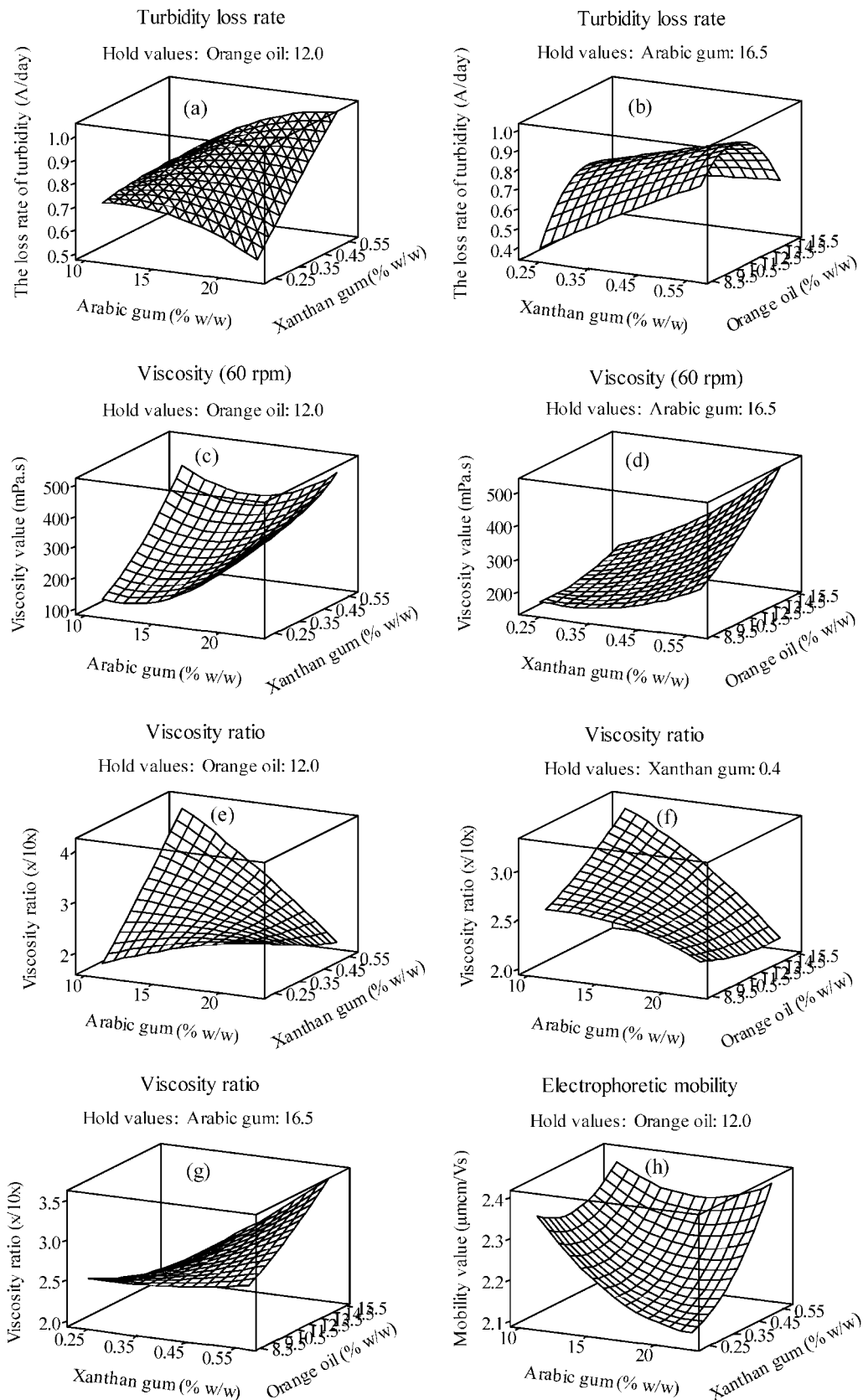


Figure 1. Response surface plots showing the significant ($p < 0.050$) interaction effects on turbidity loss rate (a, b), viscosity (60 RPM) (c, d), viscosity ratio (e-g), and electrophoretic mobility (h).

with orange oil also significantly ($p < 0.05$) affected the turbidity loss rate (Table 5).

As clearly shown in Figure 1a,b, the relationship between the interaction effect of independent variables and the turbidity loss rate was strongly nonlinear in the studied independent

variable ranges. As shown in Table 6, the minimum turbidity loss rate ($Y_1 = 0.184 \text{ \AA/day}$) was estimated to be achieved by a combined level of 22.21% (w/w) Arabic gum, 0.24% (w/w) xanthan gum, and 8.73% (w/w) orange oil. As also shown in the optimization results and Figure 1a,b, the high concentration

Table 6. Desirable Value of Response Variables Established Based on the Optimum Level of Independent Variables

response variable	optimum level of independent variables			optimum value for response variable
	arabic gum (% w/w)	xanthan gum (% w/w)	orange oil (% w/w)	
turbidity loss rate (Y_1)	22.21	0.24	8.73	0.184 (Å/day)
viscosity (60 rpm) (Y_2)	22.22	0.56	15.27	619 mPa.S
viscosity (100 rpm) ($Y_{2,2}$)	22.22	0.56	15.27	619 mPa.S
viscosity ratio ($x/10x$) (Y_3)	10.78	0.56	15.27	4.725
ξ -potential (Y_4)	10.57	0.56	12.09	-30.73 mV
electrophoretic mobility (Y_5)	10.57	0.56	9.88	2.39 $\mu\text{m cm/Vs}$

of Arabic gum led to a decrease in the loss rate of turbidity. Thus, it was concluded that the stability of emulsion was positively influenced by the Arabic gum concentration. The positive effect of Arabic gum on the emulsion stability could be due to its surface activity and ability to form a protective film around emulsion droplets. This functional property may be contributed to the presence of an arabinogalactan attached to a polypeptide backbone (AGP) in the molecular structure of Arabic gum. The polypeptide chain is a hydrophobic fraction; thus, it is believed to anchor the molecules to the droplet surface, while arabinogalactan blocks are the hydrophilic fractions that extend into the solution. The arabinogalactan-protein complex has a coil conformation with a small radius of gyration and equivalent sphere hydrodynamic radius. The presence of these segments in the structure of Arabic gum provides stability against droplet aggregation through steric and electrostatic repulsion. In Arabic gum, the hydrophilic carbohydrate blocks are linked to the protein chain that strongly adsorbs at the o/w interface, promoting emulsion stability (25). In fact, the formation of a thick, sterically stabilizing layer around emulsion droplets induced by Arabic gum enables the flavor oil emulsion to be stabilized both as a concentrate and as a diluted beverage. In contrast with a high concentration of Arabic gum, the low content of xanthan gum was predicted to provide the optimum emulsion stability. In fact, there is a large excess of nonabsorbed polysaccharide in the aqueous phase of emulsions prepared by high concentrations of polysaccharides (26). Under certain circumstances, nonabsorbed biopolymers are most probably responsible for promoting droplet flocculation through a depletion mechanism (2). As also demonstrated in a previous study (27), when sufficient surface-active agents are present to saturate the droplet surface, the increase of surfactant concentrations can be attributed to depletion flocculation caused by excess bulk of two surface-active materials (27–29).

Viscosity and Fluid Behavior. In the concentration levels studied, the apparent viscosity of all orange beverage emulsions decreased as the shear rate increased (Table 3). This phenomenon may be explained by the effect of xanthan gum, which induced the pseudoplastic behavior. As the shear rate sufficiently increases to overcome the Brownian motion, the emulsion droplets become more ordered along the flow field. Thus, they offer less resistance to flow and hence the lower viscosity in this condition (2). As shown in Table 5, the main effect of Arabic gum, quadratic effect of Arabic gum and xanthan gum, and the interaction effect of xanthan gum and orange oil showed a significant ($p < 0.05$) effect on the viscosities measured at 60 and 100 rpm. Among independent variable effects, the quadratic effect of Arabic gum had the most significant ($p < 0.05$) effect on the viscosity reported at 60 and 100 rpm (Table 5). The viscosity was observed to be reversely proportional to the main effect of orange oil (Table 4). The negative relationship between viscosity and oil phase has also been reported in a previous study (30). This observation was in contrast with the

positive effect of the oil phase on the viscosity reported in the previous study (2). In addition, the main and quadratic terms of orange oil were found to be not significant ($p > 0.05$) on the viscosities measured at 60 and 100 rpm (Table 5). On the other hand, the main effect of xanthan gum and interaction between Arabic gum and orange oil showed a significant ($p < 0.05$) effect only on the viscosity reported at 100 rpm. Conversely, the main effect of orange oil and interaction effect of Arabic gum and xanthan gum significantly ($p < 0.05$) affected only the viscosity reported at 60 rpm.

As shown in Figure 1c,d, the viscosity (60 rpm) increased with an increase of the xanthan gum concentration. This observation has been reported in previous studies (30, 31). As shown in Table 6, the optimum area for both viscosities (the highest values, $Y_{2,1} = 619$ mPa S and $Y_{2,2} = 600$ mPa S) was predicted to be obtained by an orange beverage emulsion containing 22.22% (w/w) Arabic gum, 0.56% (w/w) xanthan gum, and 15.27% (w/w) orange oil. Thus, it was concluded that a combination of high content of all main emulsion components resulted in the highest viscosity value for the orange beverage emulsion. This may be interpreted by the reason that 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. The predicted optimum region also confirmed that the high concentration of xanthan gum provided the high apparent viscosity. This may be interpreted by the fact that xanthan gum has a complex structure with a high molecular weight including a large number of free carboxyl groups, which cause great water absorption capacity. When xanthan gum is dispersed in aqueous solutions, xanthan undergoes a conformational transition from a double helix to a complex that aggregates through hydrogen bonds and polymer entanglement. Because of its complicated network and entanglements, a little increase of xanthan gum resulted in increase the apparent viscosity and pseudoplastic behavior of orange beverage emulsion. As stated by Stokes' law, the velocity of creaming or sedimentation is reversely proportional to the viscosity of water phase.

As clearly shown in Table 4, the main and quadratic effect of xanthan gum had a positive effect on the viscosity ratio (Y_3), thus indicating that the degree of pseudoplastic behavior increased with an increase of the xanthan gum content. A previous study (30) has also demonstrated that the emulsion containing more than 0.2 % xanthan gum exhibited a pseudoplastic behavior at high shear rates. Similarly, Sun et al. (31) described that the emulsions containing relatively high xanthan gum concentration ($\geq 0.05\%$ wt) exhibited the shear-thinning or pseudoplastic behavior with low-shear viscosity. The authors reported that the degree of pseudoplastic behavior was directly proportional to the concentration of xanthan. Several studies investigated the effect of type and concentration oil (32) and emulsifier (33) on the rheological properties of o/w emulsions.

Table 7. Experimental and Predicted Values for the Response Variables^a

run	turbidity loss rate ^b (Y_1 , Å/day)			viscosity ^b (Y_2 , 60 rpm, mPa s)			viscosity ratio ^b (Y_3 , x/10x)			ζ -potential ^b (Y_4 , mV)			mobility ^b (Y_5 , $\mu\text{m cm/Vs}$)		
	Y_0	Y_i	$Y_0 - Y_i$	Y_0	Y_i	$Y_0 - Y_i$	Y_0	Y_i	$Y_0 - Y_i$	Y_0	Y_i	$Y_0 - Y_i$	Y_0	Y_i	$Y_0 - Y_i$
1	0.800	0.817	-0.018	280	279	1	2.220	2.188	0.032	-27.030	-27.597	0.567	2.220	2.242	-0.022
2	0.675	0.639	0.036	370	359	11	3.651	3.583	0.068	-29.370	-29.430	0.060	2.296	2.299	-0.003
3	0.618	0.618	0.000	98	110	-11	2.222	2.250	-0.028	-28.798	-28.766	-0.032	2.257	2.252	0.005
4	0.775	0.782	-0.007	204	209	-5	2.530	2.579	-0.049	-28.070	-27.964	-0.106	2.200	2.205	-0.005
5	0.632	0.634	-0.002	304	297	7	2.340	2.296	0.044	-27.718	-27.576	-0.142	2.173	2.163	0.010
6	0.773	0.782	-0.009	205	209	-3	2.512	2.579	-0.067	-28.310	-27.964	-0.346	2.219	2.205	0.014
7	0.891	0.878	0.013	266	274	-7	3.016	3.065	-0.049	-29.184	-29.379	0.195	2.288	2.299	-0.011
8	0.937	0.926	0.011	463	452	11	2.433	2.410	0.023	-29.175	-29.260	0.085	2.287	2.293	-0.006
9	0.619	0.644	-0.025	265	277	-12	2.453	2.526	-0.073	-26.820	-26.812	-0.008	2.103	2.100	0.003
10	0.787	0.758	0.029	200	202	-2	2.292	2.329	-0.037	-29.183	-28.669	-0.514	2.270	2.249	0.021
11	0.919	0.907	0.012	250	249	1	2.660	2.583	0.077	-27.773	-28.152	0.379	2.177	2.201	-0.024
12	0.867	0.907	-0.040	258	249	9	2.643	2.583	0.060	-28.288	-28.152	-0.136	2.218	2.201	0.017
13	0.799	0.778	0.021	230	241	-11	2.545	2.629	-0.084	-27.848	-28.042	0.194	2.183	2.199	-0.016
14	0.805	0.778	0.027	250	241	9	2.690	2.629	0.061	-28.000	-28.042	0.042	2.212	2.199	0.013
15	0.646	0.642	0.004	190	180	11	2.360	2.305	0.055	-27.434	-27.885	0.451	2.151	2.175	-0.024
16	0.654	0.696	-0.042	257	252	6	2.920	2.894	0.026	-29.468	-29.671	0.203	2.310	2.318	-0.008
17	0.527	0.566	-0.039	320	337	-17	2.737	2.799	-0.062	-27.963	-28.301	0.338	2.192	2.206	-0.014
18	0.589	0.566	0.023	203	185	18	2.629	2.559	0.070	-27.746	-27.329	-0.417	2.175	2.160	0.015
19	0.775	0.749	0.026	394	399	-5	2.138	2.156	-0.018	-27.912	-27.630	-0.282	2.203	2.194	0.009
20	0.869	0.889	-0.020	365	375	-10	3.010	3.058	-0.048	-29.984	-29.454	-0.530	2.350	2.325	0.025

^a Y_0 , experimental value; Y_i , predicted value; and $Y_0 - Y_i$, residue. ^b No significant ($p > 0.05$) difference between experimental (Y_0) and predicted value (Y_i).

They found that the rheological behavior of the studied emulsions ranged between Newtonian and pseudoplastic.

The results indicated that the most significant ($p < 0.05$) effect on viscosity ratio (Y_3) was the interaction effect of Arabic gum and xanthan gum (Table 5). As shown in Figure 1e–g, the variation of viscosity ratio could be significantly ($p < 0.05$) explained as a nonlinear function of the main emulsion components. As shown in Table 6, an orange beverage emulsion prepared by 10.78% (w/w) Arabic gum, 0.56% (w/w) xanthan gum, and 15.27% (w/w) orange oil was predicted to provide the optimum viscosity ratio (the highest value, $Y_3 = 4.725$).

ζ -Potential and Electrophoretic Mobility. As shown in Table 3, ζ -potential values (Y_4) varied from -26.82 to -29.98 (mV). An absolute value, less than or greater than 25 mV, is indicative of flocculated and deflocculated emulsions, respectively (34). Therefore, no flocculation was shown in all prepared orange beverage emulsions. Table 4 showed that all main effects and quadratic effects of orange oil positively affected the negatively charged ζ -potential (Y_4). As clearly shown in Table 5, only the main effect of Arabic gum was found to be significant ($p < 0.05$) on the negatively charged ζ -potential (Y_4). As shown in Table 6, the optimum negatively charged ζ -potential (the largest negative value, -30.73 mV) could be obtained by set level of 10.78% (w/w), 0.56% (w/w), and 12.09% (w/w) for Arabic gum, xanthan gum, and orange oil contents, respectively. As shown in the results, a high concentration of xanthan gum provided the largest negatively charged ζ -potential. It may be explained by the fact that xanthan gum is an anionic polysaccharide composed of a 1-4-linked β -D-glucose backbone. The addition of xanthan gum to the beverage emulsion formulation enhances the negative surface charge of emulsion droplets followed by an increase in the negatively charged ζ -potential.

The results indicated that the mobility (Y_5) was negatively affected by the main effects of Arabic gum and xanthan gum, the quadratic effect of orange oil, and the interaction effect of xanthan gum and orange oil. Conversely, the other terms showed a positive effect on mobility (Y_5) (Table 4). The most significant ($p < 0.05$) effects on mobility (Y_5) were the main and quadratic effects of Arabic gum, respectively (Table 5). The results also showed that the main and quadratic terms of xanthan gum and

interaction between Arabic gum and xanthan gum significantly ($p < 0.05$) influenced the mobility (Y_5) (Table 5). As shown in Figure 1h, the interaction between Arabic gum and xanthan gum had a significant positive ($p < 0.05$) effect on mobility. It was found that the optimum level of independent variables leading to maximum mobility value (the highest value, 2.39 $\mu\text{m cm/Vs}$) was predicted to be achieved by a combined level of 10.78% (w/w) Arabic gum, 0.56% (w/w) xanthan gum, and 9.88% (w/w) orange oil. A close correlation was found between the optimum conditions resulting in the desirable goals for viscosity ratio, negatively charged ζ -potential, and mobility except in terms of the orange oil content. This observation was expected, as the electrophoretic mobility was used for calculating the ζ -potential.

Optimization Procedure. The multiple graphical and numerical optimizations were carried out to determine an optimum set level of independent variables resulting in the desirable response goals. A numerical optimization was also carried out for determining the exact value of individual and simultaneous multiple response optimizations resulting in the desirable goals. The multiple response optimizations exhibited that the overall optimum region was estimated to be achieved by an orange beverage emulsion containing 10.78% (w/w) Arabic gum, 0.56% (w/w) xanthan gum, and 15.27% (w/w) orange oil. Under the optimum condition, the corresponding predicted response values for turbidity loss rate, viscosity, viscosity ratio, negatively charged ζ -potential, and electrophoretic mobility were predicted to be 380, 571, 4.725, -30.51, and 2.323, respectively.

Verification of the Models. The adequacy of the response surface results was verified by the comparison of experimental and predicted values. The experimental and predicted values are given in Table 7. The experimental response values were found to be in agreement with the predicted ones. No significant ($p > 0.05$) difference was reported between the experimental and the predicted values. The closeness between the experimental and the predicted values confirmed the adequacy of response surface relating the experimental data to the independent variables studied. An orange beverage emulsion containing the predicted optimum levels was practically prepared and then evaluated in the viewpoints of the physicochemical properties studied. The results indicated that the corresponding experi-

mental values for turbidity loss rate, viscosity, viscosity ratio, negatively charged ζ -potential, and electrophoretic mobility of the desirable orange beverage emulsion were 0.367, 561, 4.593, -30.139, and 2.284, respectively. From the optimum region, the corresponding response values obtained from the experimental data and those ones predicted by the models were observed to be close together. This observation also verified the accuracy of the response surface models fitted to the experimental data.

LITERATURE CITED

- (1) Friberg, S. Emulsion stability. In *Food Emulsions*, 3rd ed.; Friberg, S., Larsson, K., Eds.; Marcel Dekker: New York, 1997.
- (2) McClements, D. J. *Food Emulsions: Principles, Practice and Techniques*; CRC Press: Boca Raton, FL, 1999.
- (3) Pittia, P.; Gambib, A.; Lerici, C. R. Hygrometric measurements for the evaluation of the stability of model food emulsions. *Food Res. Int.* **1997**, *30*, 177–184.
- (4) Mulder, H.; Walstra, P. *The Milk Fat Globule Emulsion Science as Applied to Milk Products and Comparable Foods*; Pudoc: Wageningen, Netherlands, 1974.
- (5) Friberg, S. Emulsion stability. In *Food Emulsions*, 3rd ed.; Friberg, S., Larsson, K., Eds.; Marcel Dekker: New York, 1997.
- (6) Bergenstahl, B. A.; Claesson, P. M. In *Surface Forces in Emulsions in Food Emulsions*, 2nd ed.; Friberg, S., Ed.; Marcel Dekker: New York, 1990; pp 41–96.
- (7) Shahidi, F.; Han, X. Q. Encapsulation of food ingredients. *Crit. Rev. Food Sci. Nutr.* **1993**, *33*, 501–547.
- (8) Shaker, R. R.; Jumah, R. Y.; Jdayil, B. A. Rheological properties of plain yogurt during coagulation process: Impact of fat content and preheat treatment of milk. *J. Food Eng.* **2000**, *44*, 175–180.
- (9) Pal, R.; Yan, Y.; Masliyah, J. In *Emulsions: Fundamentals and Applications in the Petroleum Industry*; Schramm, L. L., Ed.; Advances in Chemistry; American Chemistry Society: Washington, DC, 1992; Vol. 231, p 131.
- (10) Buffo, R. A.; Reineccius, G. A. Modeling the rheology of concentrated beverage emulsions. *J. Food Eng.* **2002**, *51*, 267–272.
- (11) İbanoğlu, E. Rheological behaviour of whey protein stabilized emulsions in the presence of gum Arabic. *J. Food Eng.* **2002**, *52*, 273–277.
- (12) Taherian, A. R.; Fustier, P.; Ramaswamy, H. S. Effect of added oil and modified starch on rheological properties, droplet size distribution, opacity and stability of beverage cloud emulsions. *J. Food Eng.* **2006**, *77*, 687–696.
- (13) Fleer, G. J.; Cohen, S. M. A.; Scheutjens, J. M. H. M.; Cosgrove, T.; Vincent, B. *Polymers at Interfaces*; Chapman and Hall: London, 1993.
- (14) Hunter, R. J. *Zeta Potential in Colloid Science*; Academic Press: London, 1981.
- (15) Montgomery, D. C. *Design and Analysis of Experiments*, 5th ed.; Wiley: New York, 2001; pp 455–492.
- (16) Andreo, A.; Garro, O.; Judis, M. Model of lipid oxidation in a meat emulsion related to cooking temperature and time. *Inform. Technol.* **2001**, *12*, 21–24.
- (17) Santiago, L. G.; Bonaldo, A. G.; Gonzalez, R. J. Emulsifying ability of soy protein isolates obtained at pilot plant under simultaneous heat and reducing treatments. *Food Sci. Technol. Int.* **2000**, *5*, 463–470.
- (18) Rahkone, S.; Kaitala, S. The combined effect of cooking and storing temperatures on the quantities of aerobic, coliform and lactic acid bacteria in cooked sausage. *Food Microb.* **1993**, *10*, 501–506.
- (19) Avena-Bustillos, R. J.; Cisneros-Zevallos, L. A.; Krochta, J. M.; Saltveit, M. E. Optimization of edible coatings on minimally processed carrots using response surface methodology. *Trans. ASAE* **1993**, *36*, 801–805.
- (20) Mirhosseini, H.; Yusof, S.; Hamid, N. S. A.; Tan C. P. Solid-phase microextraction for headspace analysis of key volatile compounds in orange beverage emulsion. *Food Chem.* **2007**, *105*, 1659–1670.
- (21) Labuza, T. P.; Nelson, K.; Nelson, G. *Water Analyzer Series II Reaction Kinetics Program Version 2.09*; Department of Food Science and Nutrition, University of Minnesota: St. Paul, MN, 1991.
- (22) Taoukis, P.; Labuza, T. P. Summary: Integrative concepts. In *Food Chemistry*, 3rd ed.; Fennema, O. R., Ed.; Marcel Dekker: New York, 1996; pp 1013–1042.
- (23) Ray, A. K.; Johnson, J. K.; Sollivan, R. J. Refractive index of the disperse phase in oil-water emulsions: Its dependence of the droplet size and aging. *J. Food Sci.* **1983**, *48*, 513–516.
- (24) Dłużewska, E.; Stobiecka, A.; Maszewska, M. Effect of oil phase concentration on rheological properties and stability of beverage emulsions. *Acta Sci. Pol. Technol. Aliment.* **2006**, *5*, 147–156.
- (25) Williams, P. A.; Phillips, G. O.; Randall, R. C. Structure-function relationships of gum Arabic. In *Gums and Stabilizers for the Food Industry*; Phillips, G. O., Wedlock, D. J., Williams, P. A., Eds.; Elsevier: New York, 1990; Vol. 5, pp 25–36.
- (26) Tan, C. T. Beverage emulsions. In *Food Emulsions*, 2nd ed.; Larsson, K., Friberg, S. E., Eds.; Marcel Dekker: New York, 1990; pp 445–478.
- (27) Dickinson, E.; Ritzoulis, C.; Povey, M. J. W. Stability of emulsions containing both sodium caseinate and tween 20. *J. Colloid Interface Sci.* **1999**, *212*, 466–473.
- (28) Cao, Y.; Dickinson, E.; Wedlock, D. J. Creaming and flocculation in emulsions containing polysaccharides. *Food Hydrocolloids* **1990**, *4*, 185–195.
- (29) Bibbette, J. Depletion interactions and fractionated crystallization for polydisperse emulsion purification. *J. Colloid Interface Sci.* **1991**, *147*, 474–478.
- (30) Yemar, Y.; Tamehana, M.; Munro, P. A.; Singh, H. Influence of xanthan gum on the formation and stability of sodium caseinate oil-in water emulsions. *Food Hydrocolloids* **2001**, *15*, 513–519.
- (31) Sun, C.; Gunasekaran, S.; Richards, M. P. Effect of xanthan gum on physicochemical properties of whey protein isolate stabilized oil-in-water emulsions. *Food Hydrocolloids* **2007**, *21*, 555–564.
- (32) Gladwell, N.; Grimson, M. J.; Rahalkar, R. R.; Richmond, P. Rheological behaviour of soya oil-water emulsions: Dependence upon oil concentration. *J. Food Sci.* **1985**, *50*, 440–443.
- (33) Suzuki, K.; Maeda, T.; Matsuoka, K.; Kubota, K. Effect of constituent on rheological properties of corn oil-in-water emulsions. *J. Food Sci.* **1991**, *56*, 796–798.
- (34) Leiberman, H. A.; Reiger, M. M.; Banker, G. S. *Pharmaceutical Dosage Forms: Disperse Systems*; Merck Dekker: New York, NY, 1989; Vol. 2.

Received for review April 11, 2007. Revised manuscript received July 1, 2007. Accepted July 17, 2007.

JF071061K