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Multivariate-parameter optimization of aroma compound release from carbohydrate–oil–protein model emulsions

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ABSTRACT

Optimization for retention and partition coefficient of ethyl acetate in emulsion model systems was investigated using response surface methodology in this paper. The effects of emulsion model ingredients, tragacanth gum (TG) (0.5–1 wt%), whey protein isolate (WPI) (2–4 wt%) and oleic acid (5–10%, v/v) on retention and partition coefficient of ethyl acetate were studied using a five-level three-factor central composite rotatable design (CCRD). Results showed that the regression models generated adequately explained the data variation and significantly represented the actual relationships between the independent and response parameters. The results showed that the highest retention (97.20 ± 0.51%) and lowest partition coefficient (4.51 ± 0.13%) of ethyl acetate were reached at the TG concentration 1 wt%, WPI concentration 4 wt% and oleic acid volume fraction 10% (v/v).

1. Introduction

The perception of a flavor is an important aspect of eating and therefore an important parameter of consumer acceptability of a food. Hence, the study of the interactions of flavor with food ingredients and their effects on the release and perception of flavor is an important area of study (Samavati, Emam-Djomeh, Mehdinia, Mohammadifar, & Omid, 2012).

The chemical nature of the aroma compounds, the composition and the structure of the foods are the principal characteristics that influence the transfer of aroma compounds within the food and their release. Generally foods matrices are multiphasic, containing liquid (aqueous or lipidic), solid and vapor phases (Seuvre, Philippe, Rochard, & Voilley, 2006).

The nature of the different non-volatile constituents such as proteins, lipids, carbohydrates, and salts have a great impact on the retention of the aroma compounds by the food matrices (De Roos, 2003; Godshall, 1997; Lubbers, Landy, & Voilley, 1998; Van Ruth, King, & Giannouli, 2002).

Physicochemical interactions between flavor compounds and food components can affect their migration in foods. Lipids generally act as solvents, reducing flavor compound volatility (De Roos, 1997; Druaux, Courthaudon, & Voilley, 1996; Guyot et al., 1996). Milk proteins such as native β-lactoglobulin can also reduce their volatility (Charles, Bernal, & Guichard, 1996; Guichard & Langourieux, 2000; Jouenne & Crouzet, 2000; Landy, Druaux, & Voilley, 1995; Nongonierma, Springett, Que're', Cayot, & Voilley, 2006; Van Ruth & Villeneuve, 2002).

The partition coefficient of an aroma compound between the head space and the matrix is influenced by different factors such as its volatility, polarity, water solubility and the temperature. The composition and the physicochemical properties of the matrix as well as the interactions between its constituents and the volatile compound can change the thermodynamic behavior of the aroma compound molecules. Therefore, they can influence its partition coefficient between the matrix and the gaseous phase as only the free dissolved molecules exert a vapor pressure.

Response surface methodology (RSM) is a collection of mathematical and empirical techniques useful for establishing models, and for optimizing processes even in the presence of complex interactions (Mirhosseini, Tan, & Hamid, 2008a,b). This procedure not only determines the interaction between parameters, but also reduces the number of experimental trials, development time and overall cost (Myers & Montgomery, 2002a,b).

The objectives of this study were (1) to establish the effect of composition on the partition coefficients of ethyl acetate in various dispersions and to improve the understanding of the distribution of these compounds between the headspace and the product, (2) to study the SPME method based on HS-SPME-GC for quantitative and qualitative analyses of headspace volatile compounds released

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from emulsion models and (3) to optimize the retention and partition coefficient of ethyl acetate in emulsion model systems by response surface methodology.

2. Materials and methods

2.1. Materials

Oleic acid ($C_{18}H_{34}O_2$, purity 65–88%, $\rho = 0.889–0.895\ kg/m^3$) were purchased from Merck Company (merck, shuchardt OHG 85662, Hohenbrunn, Germany). WPI (protein, 90% wt) was obtained from Davisco Foods International, Inc. (Eden Praire, MN). Tragacanth Gum was obtained from Shaheed Beheshti Medical University (Tehran, Iran). Then, gums pulverized, sieved and the collected powders (mesh size 200–500) were used. All other chemicals and solvents used were of analytical grade.

2.2. Emulsion preparation

The continuous phase of emulsions were prepared by dissolving suitable amounts of WPI and TG powders separately into distilled water at 40 °C, followed by stirring for 30 min in 10,000 rpm to ensure complete dispersion. Then, solutions were kept 24 h at room temperature to allow full hydration. The O/W emulsions were obtained by slowly mixing oleic acid into WPI solution, and then adding TG solution; the WPI solution/oleic acid/TG Solution mixture was finally emulsified with stirring by Ultra Turrax (IKA T25 Digital, Germany) in 15,000 rpm for 10 min.

2.3. HS-SPME sampling procedure

The different emulsion models were flavoured with the 300 ppm ethyl acetate. The flavour release in the headspace of prepared model samples was measured by headspace SPME with a 40/30 μm DVB/Car/PDMS fiber (Supelco, Bornem, USA).

Flavoring extraction was performed in the headspace above all 10 ml of samples placed in 25 ml vials. The vials containing samples and fibre were incubated at 20 °C for a time period typically 5 min (extraction time). The fibre was then introduced in a GC injector for 5 min, where the analytes rapidly desorbed from the fibre and then were delivered to the head of a GC column for subsequent separation and detection. Controlled experiments testing the stability and repeatability of the SPME fibre were also carried out.

2.4. GC–MS analysis

The analysis was carried out in an Agilent 6890 NGC equipped with a flame ionization detector (FID) and a 5975 inert mass selective detector (MSD) (Agilent Technologies, Palo Alto, CA, USA). Separation of the volatiles was performed on a 50 m × 0.25 mm × 0.25 μm FFAP capillary columns from Quadrex (Wood bridge, USA). Samples were directly injected by a manual SPME. The operating conditions of the GC were as follows: injector temperature of 25 °C; splitless mode; carrier as of helium (with an inlet flow rate of 1.3 ml), The temperature gradient used began at 40 °C for 4 min, then was raised to 230 °C at 5 °C/min and held at this temperature for 30 min. FID temperature was at 250 °C, and MSD was operated in the electron impact (EI) mode at 70 eV. Identification of the flavour compounds was achieved by comparing retention times with those in the MS library. At least two replicates of each measurement were made after different times of storage at 20 °C.

Peak areas obtained by static head space analysis were converted to concentrations using calibration curves. Linear correlation coefficients were found to range from 0.994 to 0.999 for both analytes. The mass partition coefficient between gaseous phase and matrix was calculated (0 0 0)

$$K_{mass} = C_g^{\infty}/C_m^{\infty}, \quad (1)$$

where C_g and C_m are, respectively, the flavor mass fractions (w/w) in the gas and in the matrix phase.

Table 1
Some physical–chemical characteristics of ethyl acetate.

Ethyl acetate	
Molecular formula	$C_4H_8O_2$
Molar mass	88.105 g/mol
Appearance	Colorless liquid
Odor	Ether smell
Density	0.897 g/cm^3, liquid
Melting point	−83.6 °C
Boiling point	77.1 °C
Solubility in water	8.3 g/100 mL (20 °C)
log *P* (Hydrophobicity)	0.70
Solubility in ethanol, acetone, diethyl ether, benzene	Miscible

2.5. Retention of ethyl acetate

Modifications of flavor compound volatility in different matrices compared to water were attributed to physicochemical interactions with the matrices components (Landy et al., 1995). Percentages of retention, relative to water, were defined as the percentage decrease of their volatility in the matrix as compared to water at the same temperature. The higher the volatility decreases in the matrix, the higher the retention (Nongonierma et al., 2006). Percentages of retention relative to water were calculated in the different matrices using Eq. (3):

$$\%\text{Retention} = \left(1 - \frac{K_{matrix}}{K_{water}}\right) \quad (2)$$

where K_{matrix}, the vapour/matrix partition coefficient (expressed in mass fractions) and K_{water}, the vapour/water partition coefficient (expressed in mass fractions). A positive percentage value indicates an aroma compound retained by the matrix and a negative value a compound released by the matrix.

2.6. Experimental design and statistical analysis

RSM was used to determine the effect of three emulsion components namely TG (0.5–1% (w/w), X_1), WPI (2–4% (w/w), X_2), and oleic acid as oil phase (5–10% (v/v), X_3) on the partitioning coefficient (Y_1) and retention percentage (Y_2) of ethyl acetate in oil-in-water emulsions model. Twenty treatments were conducted based on the central composite retortable design (CCRD), each at five coded levels −2, −1, 0, 1, and 2 (Table 1). The center point was repeated six times to calculate the repeatability of the method (Myers & Montgomery, 2002a,b).

The applied design was integrated to determine a reasonable relationship between three independent variables and each response and to find the optimum level of the independent variables resulting in the desirable objectives.

Experiments were randomized in order to minimize the effects of unexplained variability in the observed responses due to extraneous factors. The responses functions (y) were related to the coded variables (x_i, $i = 1$, 2 and 3) by a quadratic model using equation below:

$$Y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_{11}x_1^2 + b_{22}x_2^2 + b_{33}x_2^2 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3 \quad (3)$$

The uncoded independent variables ($X_1, X_2, \ldots, X_k$) are coded in accordance with the transfer equation given below:

$$x_i = \frac{X_i - X_0}{\Delta X_i} \quad 1, 2, 3, \ldots, k \tag{4}$$

where x_i, is the dimensionless value of an independent variable; X_i is the real value of an independent variable; X_0 is the real value of an independent variable at the center point; and ΔX_i is the step change of the real value of the variable i corresponding to a variation of a unit for the dimensionless value of the variable i.

The coefficients of the polynomial were represented by b_0 (intercept), b_1 and b_2, b_3 (linear effects), b_{11} and b_{22} and b_{33} (quadratic effects), and b_{12}, b_{13} and b_{23} (interaction effects). The quality of the fit of polynomial model was expressed by the coefficient of determination R^2 and R^2_{adj} in Eqs. (3) and (4), respectively.

$$R^2 = 1 - \frac{SS_{Residual}}{SS_{Residual} + SS_{Model}} \tag{5}$$

$$R^2_{adj} = 1 - \frac{SS_{Residual}/DF_{Residual}}{(SS_{Residual} + SS_{Model})/(DF_{Residual} + DF_{Model})} \tag{6}$$

$$PREES = \sqrt{\sum_{I=1}^{N} (Y = y_{Pred,1} - y_{Exp,1})^2} \tag{7}$$

Adequate precision compares the range of the predicted values at the design points to the average prediction error. The definition of adequate precision is in Eqs. (8) and (9):

$$\text{adequate precision} = \frac{\max(\bar{y}) - \min(\bar{y})}{\sqrt{\bar{V}(\bar{y})}} \tag{8}$$

$$\bar{V}(\bar{y}) = \frac{1}{n}\sum_{i=1}^{n} V(\bar{y}) = \frac{p\sigma^2}{n} \tag{9}$$

In Eqs. (5)–(9), SS is the sum of squares, DF is the degrees of freedom, $y_{i,exp}$ is the experimental responses, $y_{i,pred}$ is the predicted responses, $\bar{y}$ is the predicted value, p is the number of model parameters, σ^2 is the residual mean square from ANOVA table, and n is the number of experiments.

Numerical and graphical optimization technique of the Design Expert software was used for simultaneous optimization of the multiple responses. The desired goal for variables and response was chosen. All the independent variables were kept within range while the response was maximized.

2.7. Statistical analysis

All the flavour released data were subjected to multivariate analysis of variance (MANOVA) and least significant difference Tukey test to determine significant differences between the different custards (SAS 9.1.3 software). A significance level of $p < 0.05$ was applied.

3. Results and discussion

3.1. Preliminary investigation

Preliminary tests carried out to (1) determine the effect of TG, WPI and oleic acid contents on the retention and partition coefficient of ethyl acetate in emulsion model systems. Levels for each parameter were based on preliminary single-factor experiments in our laboratory (data not shown).

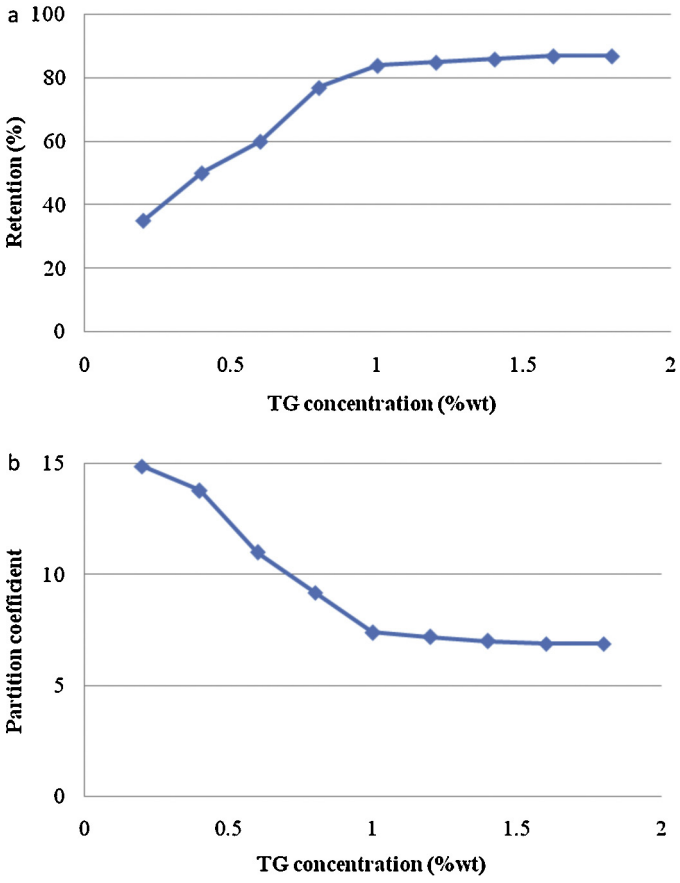

Fig. 1. Effects of different TG concentrations (wt%), on (a), retention and (b), partition coefficient of ethyl acetate (%).

3.2. Effect of TG content

3.2.1. Retention

The effect of TG concentration on retention percentage of ethyl acetate was showed in Fig. 5. The retention of ethyl acetate increased with TG concentration (Fig. 1a). In this work all of the percentages of retention for both aroma compounds were positive values that indicated an aroma compound retention by the matrices. The retention percentage of ethyl acetate was performed at different TG concentration (0.2, 0.4, 0.6, 0.8 1, 1.2, 1.4 and 1.6 wt%) when other components (WPI (3 wt%) and oleic acid volume fraction (7.5%, v/v) were fixed. The retention percentage significantly increased from 35 ± 0.47% to 84 ± 0.49% as TG concentration increased from 0.2 to 1 wt%. The retention percentage of ethyl acetate increases up to its maximum amount at 1 wt%. The Retention percentage no longer increased when the TG concentration continued to rise.

Polysaccharides influence the volatility of the molecules of the aroma compounds and their partitioning between different phases (Baines & Morris, 1987; Wilke & Chang, 1955). This result indicates that TG concentration of 1 wt% is enough in the present work. They observed that the presence of pectin in fat free stirred yoghurts tended to reduce the concentration of aroma compounds in the head space of their samples.

3.2.2. Partition coefficient

Partition coefficients ($K_{a/1}$) of ethyl acetate between gaseous phase and emulsion model systems were determined using Eq. (1) at different TG concentration (0.2, 0.4, 0.6, 0.8 1, 1.2, 1.4 and 1.6 wt%) when other components (WPI (3 wt%) and oleic acid volume

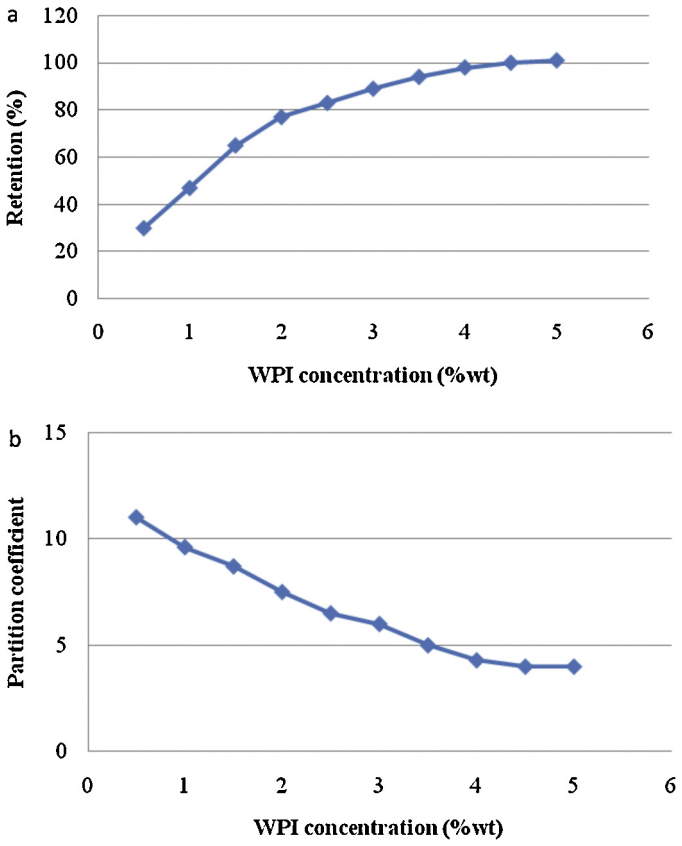

Fig. 2. Effects of different WPI concentrations (wt%), on (a), and retention (b), partition coefficient of ethyl acetate (%).

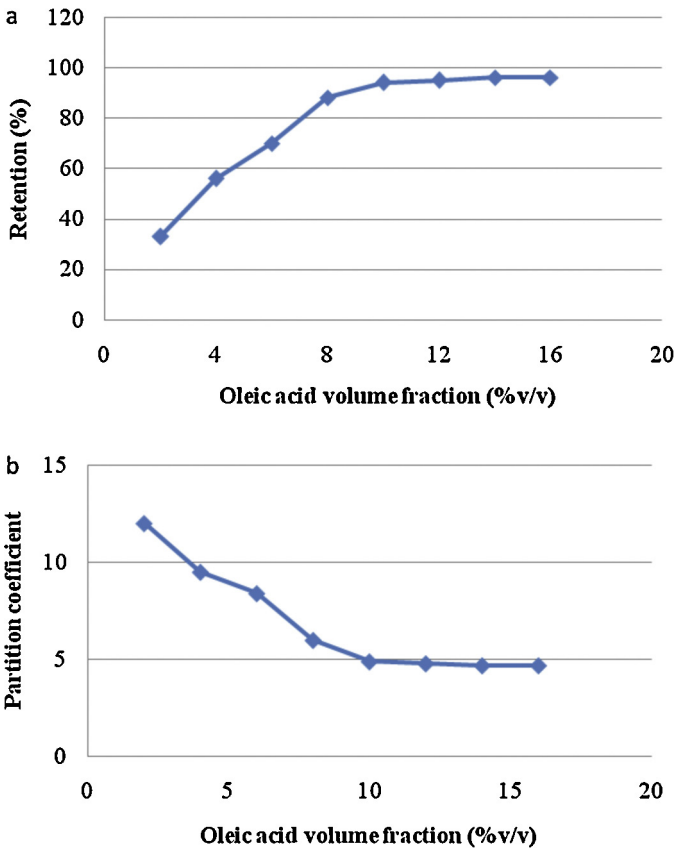

Fig. 3. Effects of different oleic acid volume fractions (%, v/v), on (a), retention and (b), partition coefficient of ethyl acetate (%).

fraction (7.5%, v/v) were fixed (Fig. 1b). The results showed that the partition coefficients of ethyl acetate had an obvious decrease within the TG concentration (0.2–1 wt%). The partition coefficients of ethyl acetate significantly decreased from 14.9 ± 0.18% to 7.4 ± 0.12% (w/w) as concentration of TG increased from 0.2 to 1 wt%, and then the partition coefficients of ethyl acetate no longer obviously changed, when the TG concentration increased. According to Baines and Morris (1987), the main effect of polysaccharides is an increase of the viscosity that hinders the process by which volatile molecules are brought from the interior of the sample to the surface. Roberts, Stephen Elmore, Langley, & Bakker (1996) found that adding thickeners to a system disturbs the balance of the released flavour profile as viscosity increases. This result indicates that TG concentration of 1 wt% is enough in the present work.

3.3. Effect of WPI content

3.3.1. Retention

The effect of WPI concentration (0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5 and 5 wt%) on retention of ethyl acetate (%) was shown in Fig. 2a. The WPI content was set at 0.5–5 (wt%) while other parameters were given as the followings: TG concentration 0.75 wt%, and oleic acid volume fraction 7.5% (v/v). The retention of ethyl acetate (%) significantly increased from 30 ± 0.43% to 98 ± 0.48% as concentration of WPI increased from 0.5 to 5, and then the retention of ethyl acetate (%) no longer obviously changed, when the WPI concentration increased.

The presence of WPI in the aqueous phase influenced the retention of the aroma compounds, with a greater effect in the case of hydrophobic compounds (Seuvre, Espinosa Diaza, & Voilleya, 2001).

Therefore, the WPI concentration of 4 wt% was considered to be optimal in the present experiment.

3.3.2. Partition coefficient

WPI concentration was set at 0.5–5 wt% to examine the effect of different WPI concentration on partition coefficient of ethyl acetate while the other independent variables, TG concentration (wt%) and oleic acid volume fraction (%, v/v) were kept at 0.75 wt% and 7.5% (v/v), respectively. As seen from Fig. 2b, it can be found that the partition coefficient of ethyl acetate significantly decreased as WPI concentration increased from 0.5 to 5 wt%. The minimum partition coefficient of ethyl acetate (4.3 ± 0.12%) was also obtained when WPI concentration was 4 wt% but beyond this WPI concentration, partition coefficient of ethyl acetate reached the plateau region where the yield was minimized and did not further decrease the partition coefficient.

It has been demonstrated that whey proteins interact with and bind flavor compounds causing a decrease in the amounts released (Charles, Lambert, Brondeur, Courthaudon, & Guichard, 2000). As this type of binding is hydrophobic in nature, the effect of the protein interactions is larger on the more hydrophobic compounds, causing them to have a larger retention than the less hydrophobic compounds (Cayot, Fairise, Colas, Lorient, & Brule, 2003).

3.4. Effect of oleic acid volume fraction

3.4.1. Retention

The effect of different oleic acid volume fraction (2, 4, 6, 8, 10, 12, 14 and 16%, v/v) on the retention of ethyl acetate (%) was seen in Fig. 3a, when the other two factors (TG concentration and WPI concentration) were fixed at 0 level (0.75 wt% and 3 wt%). The retention of ethyl acetate (%) significantly increased from 33 ± 0.42% to

Table 2
The optimization of the experimental conditions.

Independent variables	−2	−1	Factor level 0	+1	+2
TG (wt%)	0.33	0.50	0.75	1.00	1.17
WPI (wt%)	1.32	2.00	3.00	4.00	4.86
Oleic acid (wt%)	3.30	5.00	7.50	10.00	11.7

94 ± 0.51% as oleic acid volume fraction increased from 2 to 10% (v/v). The result implied the retention of ethyl acetate (%) was suddenly enhanced to the value (94 ± 0.51%) at the concentration of 10% (v/v), and thereafter there was a little rise when the oleic acid volume fraction continued to increase.

Lipids are the food ingredients that have been shown to have a great impact at the sensorial level because they are an excellent solvent for the aroma compounds: lipids decrease the vapour pressure of numerous volatile compounds and then influence the aromatic perceived profile (Guinard, Wee, McSunas, & Fritter, 2002; Widder & Fischer, 1996). Nevertheless the release of the aroma compounds depends also on their physicochemical characteristics such as polarity, hydrophobicity and solubility (Relkin, Fabre, & Guichard, 2004).

3.4.2. Partition coefficient

The partition coefficient of ethyl acetate (w/w) influenced by different oleic acid volume fraction (2, 4, 6, 8, 10, 12, 14 and 16%, v/v) is shown in Fig. 3b. The measurement was carried out under the following conditions: TG concentration 0.75 wt% and WPI concentration 3 wt%. The partition coefficient of ethyl acetate (w/w) decreased when the oleic acid volume fraction increased from 2 to 10% (v/v). And then there was no decrease when oleic acid volume fraction continued to rise. As shown in Fig. 3b, the minimum partition coefficient was observed when the oleic acid volume fraction was around 10. When the oleic acid volume fraction (%, v/v) was increased more than 10, the partition coefficient of ethyl acetate (%) did not change.

The lipidic medium could induce a decrease of flavor compounds partition coefficient not only because of the affinity of aroma compounds for lipids but also because of the consistency of the lipidic medium (Seuvre, Philippe, Rochard, & Voilley, 2007).

The higher hydrophobic nature of the ethyl acetate could explain its higher retention in the complex matrices with higher oleic acid volume fraction. For many compounds, in fact, the interactions between biopolymers (i.e. protein, gum) and aroma compounds are due to hydrophobic interactions and clearly are favored for the less polar compounds. Moreover, the presence of oleic acid in emulsions could further increase the retention of this ester in the matrix in respect with the more polar one (Diacetyl).

The addition of fat has a significant effect on the retention of flavor compounds (De Roos, 1997). Fat is an organic phase that solubilizes flavor compounds and therefore causes a decrease in the amounts released as previously demonstrated in stirred yoghurts (Brauss, Linforth, Cayeux, Harvey, & Taylor, 1999).

4. Data analysis and evaluation of the model

The effects of two independent variables – TG concentration (wt%) (X_1), WPI concentration (wt%) (X_2) and oleic acid volume fraction (X_3) – on the dependent variables (Y) were considered using RSM, and their interactive relationship was studied. Analysis of variance (ANOVA) was performed to investigate the adequacy of the suggested models and identify the significant factors. The independent and dependent variables were fitted by the second-order polynomial equation to the experimental data. Table 3 gives the statistical significance, the linear and quadratic equations and the interaction of effects calculated for each response. By applying multiple regression analysis on the experimental data, the response

Table 3
Table of ANOVA for the experimental variables as a linear, quadratic and interaction terms of each response variable and corresponding coefficients for the predictive models.

Source	DF	Retention (%)			Partition coefficient		
		Coefficient	Sum of squares	*p*-Value	Coefficient	Sum of squares	*p*-Value
Model	9	82.470	2204.125	<0.0001	7.550	143.209	<0.0001
Linear							
b1	1	89.059	633.919	<0.0001	−33.006	47.970	<0.0001
b2	1	5.416	652.635	<0.0001	−1.166	21.241	<0.0001
b3	1	8.445	471.291	<0.0001	−2.264	36.457	<0.0001
Quadratic							
b11	1	−53.205	159.353	0.0005	17.806	17.849	<0.0001
b22	1	1.624	38.030	0.0337	−0.513	3.799	<0.0001
b33	1	0.034	0.637	0.7567	−0.009	0.042	0.1614 ns
Interaction							
b12	1	8.500	36.125	0.0374 ns	−1.100	0.605	0.0002
b13	1	−1.000	3.125	0.4967 ns	0.280	0.245	0.0044
b23	1	−1.950	190.125	0.0003	0.510	13.005	<0.0001
Residual	10		62.825			0.183	
Lack-of-fit	5		39.325	0.2929 ns		0.068	0.7130 ns
Pure error	5		23.500			0.115	
Total	19		2266.950			143.392	
R^2		0.972			0.999		
Adj-R^2		0.947			0.998		
PRESS		2.150			0.686		
CV		3.077			1.706		
Std. Dev.		0.506			0.135		
Adequate precision		22.956			104.925		

Table 4
Experimental and predicted values for the response variables.

Run	Retention (%) Y_1			Partition coefficient Y_2		
	Y_0[a]	Y_1[b]	(Y_0-Y_1)[c]	Y_0[a]	Y_1[b]	(Y_0-Y_1)[c]
1	59	58.002	0.998	59	58.002	0.998
2	68	68.628	−0.628	68	68.628	−0.628
3	76	77.328	−1.328	76	77.328	−1.328
4	95	96.454	−1.454	95	96.454	−1.454
5	83	80.751	2.249	83	80.751	2.249
6	91	88.877	2.123	91	88.877	2.123
7	82	80.577	1.423	82	80.577	1.423
8	97	97.203	−0.203	97	97.203	−0.203
9	60	61.604	−1.604	60	61.604	−1.604
10	85	84.521	0.479	85	84.521	0.479
11	73	75.436	−2.436	73	75.436	−2.436
12	100	98.689	1.311	100	98.689	1.311
13	75	73.183	1.817	75	73.183	1.817
14	90	92.942	−2.942	90	92.942	−2.942
15	81	82.468	−1.468	81	82.468	−1.468
16	81	82.468	−1.468	81	82.468	−1.468
17	85	82.468	2.532	85	82.468	2.532
18	83	82.468	0.532	83	82.468	0.532
19	85	82.468	2.532	85	82.468	2.532
20	80	82.468	−2.468	80	82.468	−2.468

[a] (Y_0): experimental value.
[b] (Y_1): predicted value.
[c] (Y_0-Y_1): residue.

variables (retention and partition coefficient) and the test variables were related by the following second-order polynomial equations:

$$\text{Retention}(\%) = -20.1236 + 89.05907 \times \text{TG} + 5.416129 \times \text{WPI} + 8.445197 \times \text{oleic acid} + 8.5 \times \text{TG} \times \text{WPI} - 1 \times \text{TG} \times \text{oleic acid} - 1.95 \times \text{WPI} \times \text{oleic acid} - 53.2046 \times \text{TG}^2 + 1.624461 \times \text{WPI}^2 + 0.03364 \times \text{oleic acid}^2 \quad (10)$$

$$\text{Partition coefficient} = +37.29862 - 33.0064 \times \text{TG} - 1.16646 \times \text{WPI} - 2.26437 \times \text{oleic acid} - 1.1 \times \text{TG} \times \text{WPI} + 0.28 \times \text{TG} \times \text{oleic acid} + 0.51 \times \text{WPI} \times \text{oleic acid} + 17.80644 \times \text{TG}^2 - 0.51344 \times \text{WPI}^2 - 0.00861 \times \text{oleic acid}^2 \quad (11)$$

As showed in Table 3, the significant response surface models with high R^2 and adj-R^2 values varied from 0.972 to 0.999, respectively. These values showed a good agreement between the experimental and the predicted values. The low PRESS (0.686–2.150) values suggest for the adequacy of the fitted quadratic models for predictive applications (Table 3). The values of coefficient of variation (CV) were 1.706 and 3.077 for retention and partition coefficient, respectively (Table 2). Adequate precision measures the signal-to-noise ratio. A ratio greater than 4 is desirable (Myers & Montgomery, 2002a,b). For the proposed models, this value was between 22.956 and 104.925 (Table 3), a very good signal-to-noise ratio. All these statistical parameters show the reliability of the models. Comparison between predicted and actual values for the response variables also indicated that the polynomial regression models were suitable to determine optimum formulation for preparing emulsion model with maximum retention and minimum partition coefficient of ethyl acetate (Table 4).

4.1.1. Retention

The retention percentage value of ethyl acetate (Y_1) (Eq. (10)) was positively associated with linear effects of TG, WPI concentrations and oleic acid volume fraction as well as the quadratic effects of TG, WPI concentrations and oleic acid volume fraction (X_1, X_2 and X_3); quadratic effect of WPI concentration ($X_2{}^2$) and oleic acid volume fraction ($X_3{}^2$) and interaction effect between TG and WPI (X_1X_2); while the quadratic effect of TG concentration ($X_1{}^2$), interaction effects between WPI and oleic acid (X_2X_3), and TG and oleic acid (X_1X_3) were found to be significant negative effects ($p < 0.05$) on the retention percentage of ethyl acetate (Table 3).

The importance of the independent variables on retention could be ranked in the following order: TG > WPI > oleic acid. Also, the importance of interaction terms on retention could be ranked in the following order: WPI with oleic acid > TG with WPI > WPI with oleic acid. The results also showed that variables with the largest effect were the linear effects of TG and WPI.

As shown in Table 3, the interaction effects between TG and oleic acid appeared to be a not-significant ($p = 0.4967$) parameter in the regression model fitted for the retention of ethyl acetate.

It is reported that increase in hydrocolloid content influenced the diffusion of volatile molecules in the liquid phase probably through binding and/or physicochemical interactions between them and the hydrocolloids (Terta, Blekas, & Paraskevopoulou, 2006). The foaming properties of whey proteins may have a barrier effect on the transfer of the aroma compounds when the protein is absorbed at the air–water interface (Marin, 1998). The highest response for retention of ethyl acetate in the ranges studied was observed when the emulsion model was produced with 1% (wt%) TG, 4 (wt%) WPI and 10 (%, v/v) oleic acid volume fraction.

4.1.2. Partition coefficient

The regression coefficient values of Eq. (11) were listed in Table 3. The p-values were used as a tool to check the significance of each coefficient, which in turn may indicate the pattern of the

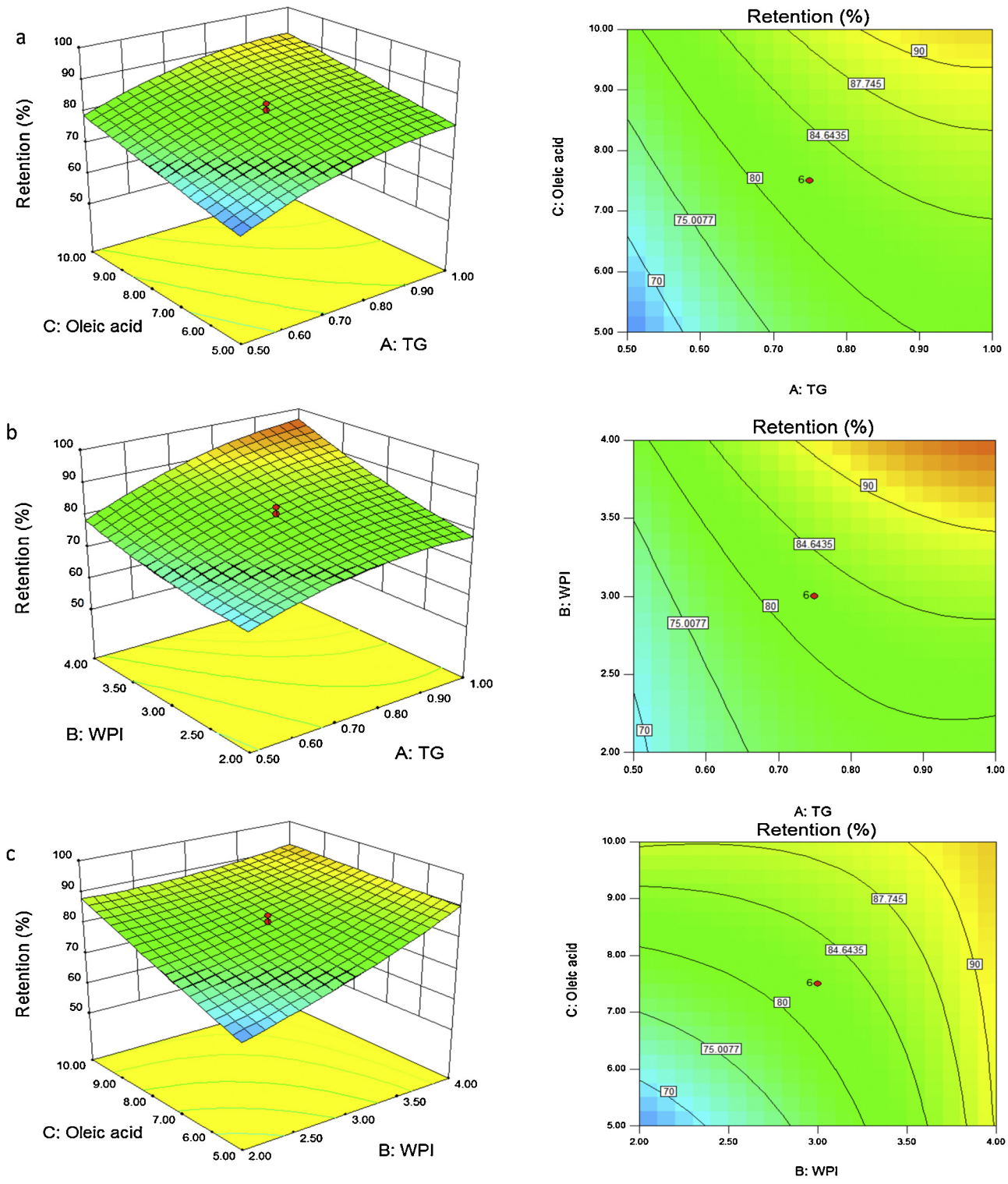

Fig. 4. Response surface (3-D) and contour plots showing the effect of the extraction temperature TG concentration (wt%) (X_1), WPI concentration (wt%) (X_2), and oleic acid volume fraction (%, v/v) (X_3) on the retention of ethyl acetate.

interactions between the variables. The smaller was the value of p, the more significant was the corresponding coefficient. It can be seen from this table that the linear coefficients (X_1, X_2, X_3), a quadratic term coefficient (X_1^2, X_2^2) and mutual interaction coefficients (X_1X_3, X_1X_2, X_1X_3) were significant, with very small p values ($p < 0.05$). The other term coefficients were not significant ($p > 0.05$). The full model filled Eq. (11) was made three-dimensional and contour plots to predict the relationships between the independent and dependent variables.

It can be seen that the variable with the largest effect on partition coefficient was a linear term of TG (X_1) followed by a linear term of oleic acid (X_3), a linear term of WPI (X_2), the quadratic

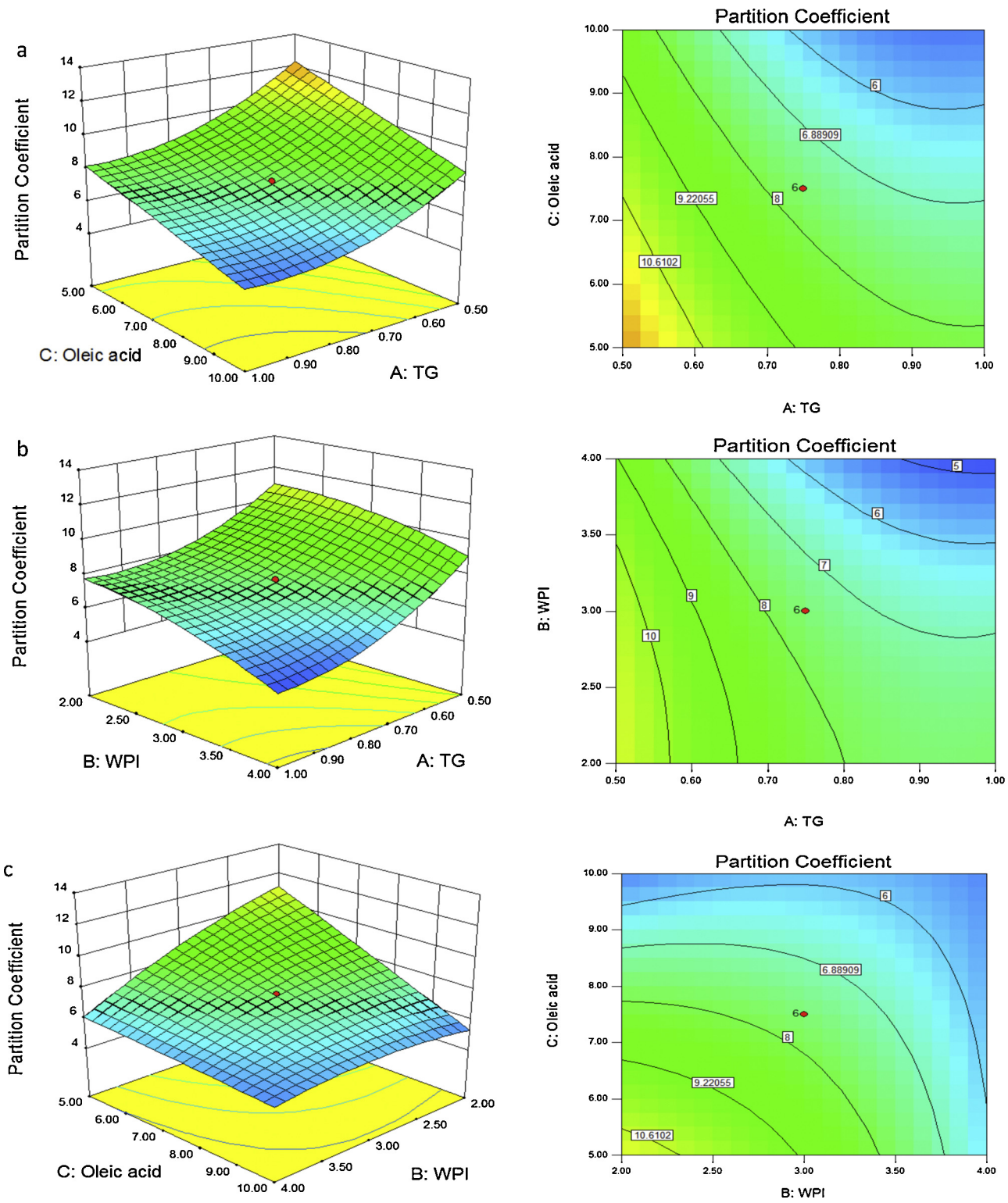

Fig. 5. Response surface (3-D) and contour plots showing the effect of the TG concentration (wt%) (X_1), WPI concentration (wt%) (X_2), and oleic acid volume fraction (%, v/v) (X_3) on the partition coefficient of ethyl acetate.

term of TG (X_1^2), the quadratic term of WPI (X_2^2), the quadratic term of oleic acid (X_4^2) and interaction term of X_1X_2, X_2X_3 and X_1X_3 (Table 4). However, the quadratic term of oleic acid (X_3^2) was found insignificant ($p = 0.1614$). The coefficient of determination (R^2) of the predicted models in this response was 0.998. This would give a good fit to the mathematic model in Eq. (11).

The negative effect of WPI on the partition coefficient could be due to its surface activity and ability to form a protective film around emulsion droplets. Also, oleic acid with its fatty acid chains could establish interactions with more hydrophobic compounds and retains the aroma compounds (Samavati, Emam-Djomeh, Mohammadifar, Omid, & Mehdinia, 2011).

4.2. Optimization using response surface models

Response surfaces were plotted by using Design expert software (version 8.0) to study the effects of parameters and their interactions on retention and partition coefficient of ethyl acetate. RSM played a key role in identifying the optimum values of the independent variables efficiently, under which dependent variable could reach the maximum or minimum response. The independent variables and maximum and minimum predicted values from Figs. 4 and 5 corresponded with the optimum values of the dependent variables obtained by the equations. The results of retention and partition coefficient of ethyl acetate affected by TG concentration (wt%), WPI concentration (wt%) and oleic acid volume fraction (%, v/v) are presented in Figs. 3 and 4. In the response surface plot and contour plot, the data were generated through keeping three variables at their respective zero level (central value of the testing ranges) and varying the other two within the experimental range. In the two figures, the maximum predicted value for retention and the minimum predicted value for partition coefficient of ethyl acetate indicated by the surface was confined in the smallest ellipse in the contour diagram. Elliptical contours are obtained when there is a perfect interaction between the independent variables (Muralidhar, Chirumamil, Marchant, & Nigam, 2001). The independent variables and maximum retention and minimum partition coefficient of ethyl acetate predicted values from the figures corresponded with the optimum values of the dependent variables obtained by the equations.

The individual optimization procedure showed that the emulsion model containing 1 (wt%) TG, 4 (wt%) WPI and 10 (%, v/v) oleic acid volume fraction would provide the highest retention of ethyl acetate.

The Figs. 4a and 5a illustrated the 3-D response surface plot and the contour plot of retention and partition coefficient of ethyl acetate at varying the TG and WPI concentrations (wt%); at the fixed oleic acid volume fraction 7.5 (%, v/v). The retention of ethyl acetate increased with TG concentration from 0.5 to 1 wt%. The retention of ethyl acetate increased with the increasing the WPI concentration from 2 to 4 wt%. It indicated that the maximum retention of ethyl acetate can be achieved when TG and WPI concentrations (wt%) at the threshold level of 1 and 4 (wt%), respectively.

It can be seen that the minimum partition coefficient of ethyl acetate can be achieved when TG, WPI and oleic acid content were 0.97 (wt%), 4.00 (wt%) and 10.00 (%, v/v), respectively.

Figs. 4b and 5b show the interaction effect of TG concentration (wt%) and oleic acid volume fraction (%, v/v) on retention and partition coefficient of ethyl acetate while the WPI concentration was fixed at 3 (wt%). It can be seen that the retention of ethyl acetate increased with increase of TG concentration (wt%) from 0.5 to 1, then did not further increase with increasing TG concentration (wt%), and reached the maximum value when the oleic acid volume fraction was at 10 (%, v/v), and beyond this level, retention of ethyl acetate did not further increase.

A decrease in the partition coefficient of ethyl acetate could be significantly achieved with the increases of TG and WPI concentrations. It is observed that the partition coefficient of ethyl acetate was increased with the increasing the TG concentration from 0.5 to 1 (wt%).

The Figs. 4c and 5c illustrated the 3-D response surface plot and the contour plot of retention and partition coefficient of ethyl acetate at varying WPI concentration (wt%) and oleic acid volume fraction (%, v/v) at fixed TG concentration 0.75 (wt%).

The retention of ethyl acetate increased with the increasing the WPI concentration (wt%) and oleic acid volume fraction (%, v/v), and reached the peak value at WPI concentration and oleic acid volume fraction, 4 (wt%) and 10 (%, v/v), respectively.

Table 5
Predicted and experimental values of the responses at optimum conditions for ethyl acetate retention (%).

Optimum condition		Oleic acid (% wt)	Retention (%)	
TG (wt%)	WPI (wt%)		Experimental	Predicted
1.00	4.00	10.00	98.04 ± 0.87	97.20 ± 0.51

Table 6
Predicted and experimental values of the responses at optimum conditions for partition coefficient of ethyl acetate.

Optimum condition		Oleic acid (% wt)	Partition coefficient	
TG (wt%)	WPI (wt%)		Experimental	Predicted
0.97	4.00	10.00	4.62 ± 0.14	4.51 ± 0.13

It can be seen that the minimum partition coefficient of ethyl acetate can be achieved when the WPI concentration and oleic acid volume fraction, were 4 (wt%) and 10 (%, v/v), respectively.

Numerical and graphical optimization procedures were carried out for predicting the optimum level of independent variables to obtain the maximum retention and minimum partition coefficient of ethyl acetate. The RSM package's response optimizer determined the overall optimum region to be at 1 (wt%) TG, 4 (wt%) WPI and 10 (%, v/v) oleic acid volume fraction. The corresponding predicted response values under the optimum conditions for retention and partition coefficient of ethyl acetate were 97.20% and 4.51%, respectively.

4.3. Verification of the models

A confirmation of the results using the optimum point (1 (wt%) TG, 4 (wt%) WPI and 10 (%, v/v) oleic acid volume fraction) was accomplished by repeating five additional experiments. The physical characteristics of the emulsions in term of investigate d parameters were evaluated. The results showed that the corresponding experimental values for retention and partition coefficient of ethyl acetate were 97.20 ± 0.51% and 4.51 ± 0.13% respectively (Tables 5 and 6). There was no significant difference ($p > 0.05$) between experimental and predicted values, suggesting that the response surface equations had high capability to determine the formulation parameters for the highest retention and the lowest partition coefficient of ethyl acetate in emulsion model systems.

5. Conclusion

Response surface methodology was used to determine the optimum formulation that gives maximum retention and partition coefficient of ethyl acetate from food emulsion model systems. Based on the single-factor experiments, the RSM was used to estimate and optimize the experimental variables: tragacanth gum (wt%), whey protein isolate (wt%) and acid oleic volume fraction (%, v/v). The overall optimum regions were determined at 1 (wt%) TG, 4 (wt%) WPI and 10 (%, v/v) oleic acid volume fraction. The predicted response values under the optimum conditions for retention and partition coefficient of ethyl acetate were 97.20% and 4.51, respectively. The results showed that there was no significant difference ($p > 0.05$) between experimental and predicted values, suggesting that the response surface equations had high capability to determine the formulation parameters for the highest retention and the lowest partition coefficient of ethyl acetate in emulsion model systems.

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