

Formation and stability of an oil in water emulsion containing lecithin, xanthan gum and sunflower oil

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Abstract

The optimisation of the formation and stability of an oil in water emulsion containing lecithin, xanthan gum and sunflower oil was evaluated using Response Surface Methodology (RSM) and nonlinear regression. The main and combined effects of three independent variables; concentration of sunflower oil (10-20% v/v), soy lecithin (1-5% w/v) and xanthan gum (0.01-3% w/v) on the responses were examined. The main objectives of the study were to model and optimise maximum emulsion storage stability and to study interactive effects of emulsion ingredient. Emulsion stability and mean droplet diameter were measured over 14 days of storage using an image processing procedure developed. Xanthan gum and lecithin were found to have significant influences on emulsion stability and mean droplet diameter. Optimum concentrations were found to be sunflower oil 19.02% v/v, soy lecithin 1.2% w/v and xanthan gum 0.28% w/v.

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Introduction

An emulsion is traditionally defined as a dispersion of droplets of one liquid in another, the two being immiscible (McClements, 2005). From a physiochemical point of view, emulsions are thermodynamically unstable systems. Over a period of time, an emulsion can rapidly or slowly separate into two immiscible phases. The most common processes of emulsion destabilisation are droplet-droplet coalescence, flocculation, creaming, and Ostwald ripening (Tcholakova *et al.*, 2006). Aggregation of droplets greatly influences shelf life and texture of emulsions (Dickinson & McClements, 1995). The food industry is one of many industries that heavily rely on the use of emulsions and emulsifiers. Emulsions play an important role in the formulation of foods; some food emulsions (salad dressings, mayonnaise, cream liqueurs, etc.) are end products themselves (Charcosset, 2009). Food emulsions can also be ingredients which participate in the formation of more complex products such as yoghurts, ice creams and whipped products (Leal-Calderon *et al.*, 2007).

Forming a kinetically stable emulsion for a period of time to increase shelf-life is one of the main challenges of food product formulation. This can be achieved through the addition of emulsifiers and stabiliser, emulsifiers are surface-active

molecules which lower surface tension and prevent droplet flocculation by absorption on the droplet surfaces (Krstonosic *et al.*, 2009). Lecithin is a small molecule surfactant which is one of the most commonly used emulsifiers in the food industry (Whitehurst, 2004). Lecithin is accepted as a natural ingredient by consumers, and legislators classify it as generally recognised as safe (GRAS) (Bylaite *et al.*, 2001). Polysaccharides employed as thickeners of emulsions, are commonly added to the aqueous phase of oil in water emulsions to confer long term emulsion stability and prevent creaming through viscosity modification of the aqueous continuous phase (Dickinson, 2003). Xanthan gum, one of the most employed thickeners in food emulsions, has the ability to increase the viscosity of the aqueous continuous phase at relatively low concentrations, therefore it is commonly used to stabilise dispersed oil droplets in pourable salad dressings and sauces (Hemar *et al.*, 2001).

Emulsion stability refers to the ability of an emulsion to resist changes in its properties over time: the more stable the emulsion, the more slowly its properties change (McClements, 2005). The perceived quality of emulsion based food products is strongly influenced by their stability, rheology and appearance (Mirhosseini *et al.*, 2008a). A main indicator of loss of stability is an increase in droplet mean diameter of the emulsion, and the growth rate of the droplets can

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reveal the mechanism responsible (Silva *et al.*, 2010). Enhancing an emulsion based product's resistance to destabilisation can be enhanced by reducing the droplet size (McClements, 2005).

Response Surface Methodology (RSM) and design of experiments are scientific tools that can help to optimise a formulation, and have previously been used to study the dependence of emulsion stability on its ingredients (Gharibzahedi *et al.*, 2012). In the present work, RSM and nonlinear regression were applied as statistical tools to (1) model and optimise the emulsion ingredient levels (soy lecithin, xanthan gum and sunflower oil) leading to maximum emulsion storage stability ratio (SR) and minimum average droplet diameter (DS) and (2) study the linear and quadratic effects of emulsion ingredient concentration on SR and DS. The responses used for optimisation of formulation and stability of the oil in water emulsion were SR and DS. The experimental conditions selected were applied in order to obtain a minimum DS and maximum SR.

Materials and methods

Materials

Soy lecithin (Kelkin, Dublin, Ireland) was purchased in a local supermarket (Dunnes Stores, Dublin, Ireland). Xanthan gum was kindly donated by Chemcolloids Ltd. (Cheshire, UK). Sunflower oil (Basso Fedele and Figli, Avellino, Italy) was purchased from a local food supplier.

Emulsion preparation

The surfactant solutions were prepared by dispersing the lecithin (1-5% w/v) and xanthan gum (0.01-0.35% w/v) in distilled water and mixing for 1.5 hours at 60°C (Sznitowska *et al.*, 2002) using a magnetic stirring bar and magnetic stirrer hotplate (Stuart CB162, Bibby-scientific, Staffordshire, UK). The lipid phase (10-20% v/v) was then heated to 60°C and slowly added to the aqueous phase while stirring. This pre-emulsion was allowed to mix for a further 5 minutes before being homogenised with a high shear blender for 3 minutes at 8,000 rpm. The resulting emulsion was allowed to cool to room temperature and was placed into the storage vials in a darkened incubator at room temperature (23°C ± 1°C) for 14 days.

Experimental Design

A Box Behnken experimental design was chosen, and 15 random order (2 centre point replicates) experimental settings were generated with 3 factors (x_1 - x_3) using R 2.14.1 software package (R

Development Team, Vienna, Austria). The effect of three independent coded variables on day 14 of storage, x_1 (sunflower oil concentration 10-20% v/v), x_2 (lecithin concentration 1-5% w/v) and x_3 (xanthan gum concentration 0.01-0.3% w/v) on emulsion stability (SR) was studied. For statistical analysis the variables were centred in the interval [-1, 1] by:

$$x_n = (x - x_{\min}) / ((x_{\max} - x_{\min}) / 2) \quad (\text{Eq. 1})$$

Where x and x_n are the original and the coded variable respectively and x_{\max} and x_{\min} are the maximum and minimum of the variable x .

SR measurements were taken on day 14, measurements were repeated three times, and experiments were conducted in triplicate, resulting in 9 measurements in total for each experimental condition for SR. DS was measured at day 0, 1, 7 and 14, and experiments were conducted in triplicate. Table 1 lists the independent variables and experimental design factor setting for the Box Behnken design including the values corresponding to the levels of factors and treatments, assuming three factors, each with low, medium and high settings. The optimal level of 3 independent variables (x_1 , x_2 and x_3) which led to the desirable multi response goals (minimise DS and maximise SR) was determined by graphical and numerical optimisation procedures. Contour plots and canonical analysis were employed in order to deduce workable optimum conditions.

Emulsion stability

30 mL of emulsion were transferred to a graduated 40 mL plastic test tube (84 x 30 mm polypropylene with screw cap, Sarstedt Ltd., Wexford, Ireland), tightly sealed with a plastic cap, and then stored for 14 days at ambient room temperature (23°C ± 1°C). SR was calculated as a percentage of the initial emulsion height in the tube (HE), height of cream layer (HC) and height of sedimentation phase (HS) (Mirhosseini *et al.*, 2008b):

$$\text{SR} = 100 \times (\text{HE} - (\text{HC} + \text{HS}) / 100) \quad (\text{Eq. 2})$$

Experimental determinations were carried out in triplicate and each measurement was recorded three times resulting in 9 measurements in total for each sample.

Mean droplet diameter by image analysis

Image acquisition was conducted using an optical microscope (Olympus BH2) with a 400 magnification coupled with a digital camera (Canon DSLR EOS D30). The images were then transferred to a personal

computer and the image processing procedure followed the method sequence of other studies (Freire *et al.*, 2005; Trindade *et al.*, 2008; Silva *et al.*, 2010) involving binarization and droplet quantification. The image processing procedure was developed using an automated program developed in MATLAB 7.0 (The Math Works, Inc., Natick, MA, USA). The Image J software (Version 1.451, U.S National Institute of Health, Bethesda, Maryland, USA) and a stage micrometer (OB-M stage micrometer, Olympus Imaging and Audio Ltd., Essex, England) of known size were used for calibration of the droplet diameter. Before analysis each sample was diluted with distilled water to 1:1000. On each day of analysis, an average of 23 droplets per image and an average of 22 different images per sample were analysed providing an average of 500 droplets for each sample and an average of 1500 droplets for each condition for each point. At the end of the experiment over 4000 images were analysed in total.

Regression models

The effects of the independent variables on the emulsion stability (SR) of an oil in water emulsion and their interaction were analyzed using polynomial regression analysis. A second order polynomial equation for the three dependent variables (x_1 - x_3) was established to fit the experimental data;

$$SR = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{23} x_2 x_3 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{33} x_3^2 + \varepsilon \quad (\text{Eq. 3})$$

Where SR is the Stability Ratio response, $x_1, \dots, x_1^2, \dots, x_1 x_2, \dots$ are the independent linear, quadratic and interaction terms. In the model, 0 is the intercept term, $\beta_1, \dots, \beta_{11}, \dots, \beta_{12}, \dots$ are the regression coefficients for the linear, quadratic and interaction effect terms and ε is an error term corresponding to an independent normal distribution. Estimation of the regression parameters and their standard errors was done using the least-square technique (Myers *et al.*, 2009).

The effects of the independent variables on the mean droplet diameter (DS) of an oil in water emulsion and their interaction were analyzed using a primary model to describe the dependence of the DS on storage time and a secondary model to describe the dependence of the primary model parameters on the design variables (Aguirre *et al.*, 2009). The primary model chosen to best describe the kinetics of DS during storage after graphical analysis was an apparent 1st order kinetic model described by;

$$DS = Asym + (R_0 + (R_0 - Asym)e^{-lrc \cdot \text{time}}) + \varepsilon \quad (\text{Eq. 4})$$

Where *Asym*, R_0 and *lrc* are the asymptotic DS at long storage time, R_0 is the initial droplet diameter, *Asym* is the final droplet diameter and *lrc* is the natural logarithm transformation of the apparent first order rate constant (Day^{-1}). The secondary modelling of the three parameters *Asym*, R_0 and *lrc* was done using a second order polynomial dependence on the coded variables x_1 , x_2 and x_3 . The building procedure to obtain a model with a minimum set of dependence of the three parameters with the experimental variables followed these steps:

1. An initial model was built using a second order polynomial dependence of the parameters *Asym*, R_0 and *lrc* with the normalised variables x_1 , x_2 and x_3 .
2. A summary of the model was produced with *t*-statistics for each individual model coefficient.
3. Based on the *t*-test statistics of significance, non-significant terms of the model were eliminated (Pinheiro & Bates, 2000) and a new model was built.
4. A summary of the new model was produced with new *t*-statistics for each model term.
5. A logarithm likelihood ratio test (Bates & Watts, 1988) was employed to compare the new model with the previous one and decide if the simplification obtained was statistically significant or not in terms of capacity to describe the data (i.e. the log-likelihood).
6. Steps 1–5 were repeated with new simplifications proposed until a satisfactory model reduction was achieved.

The adequacy of the model was determined using graphical analysis and examining the coefficient of determination (R^2) (Lee *et al.*, 2000). Analysis was performed in triplicate and data was reported as the estimated parameter \pm standard error (SE). To determine the significance of a model parameter, the *t*-student test was used. Differences were considered to be statistically significant at $p \leq 0.05$. The RSM libraries (Lenth, 2009) and nls (nonlinear least squares) from the R software (Version 2.14.1, R Development Team, Vienna, Austria) were used.

Multicriteria optimisation

The optimal conditions for the targeted responses were generated by R 2.14.1 software package. The NSGA II MOEA numeric optimisation algorithm from the R library MCO (Multi Criterion Optimisation) (Trautmann *et al.*, 2010) was employed to study the simultaneous optimisation of SR and DS. A Pareto front from the multiple response optimisations was defined to maximise the SR and minimise the DS

within the experimental range studied.

Validation of optimal conditions

The adequacy of response surface models for predicting optimum response values was verified by conducting experiments under one of the sets of optimum conditions devised in the Pareto front. The experimental predicted values of the responses were compared in order to check the validity of the models.

Results and Discussion

Fitting the response surface models

Regression analysis was carried out to fit mathematical models to the experimental data. Table 1 illustrates experimental design settings, the mean values and the corresponding standard deviations of the two responses: the emulsion stability (SR) and mean droplet diameter (DS). The regression coefficients and analysis of variance of the coded independent variables for SR are presented in Table 2. Besides showing the optimal conditions for maximum SR and minimum DS, the mathematical model was able to identify and describe significant effects of the independent variables, their relative sensitivity and some interesting interactions between the variables on both responses. The R^2 statistic indicated that the response surface model accounted for 89% of the variation for SR and 86% for DS. When the residual plots were investigated, they appeared to follow a normal distribution and to be independent from any of the variables. The repeatability of the experimental procedure was evaluated by calculating the relative standard deviation percentage (RSD %) of three replicates (data not shown) of each experimental condition. The RSD % for SR ranged from 0 to 8.78, with a RSD % average of 2.62, while the RSD % for average DS ranged from 0.24 up to 5.71, with a RSD % average of 2.56, indicating that the replication within each experimental condition was good (lower than 5%).

Emulsion stability

Polynomial parameter estimates for xanthan gum indicate that linear (x_3), quadratic (x_3^2) and interactive ($x_1 \cdot x_3$ and $x_2 \cdot x_3$) effects were significantly influential ($p \leq 0.05$) on SR (Table 2). Furthermore, all these effects proved to positively influence SR. Additionally, the magnitude of xanthan gum's influence on SR can be seen in the Pareto effects chart (Figure 1). The linear (x_3) and quadratic terms (x_3^2) were located in the upper end of the Pareto effects chart implying they were highly influential, while the interactive terms were in the lower end.

Table 1. Experimental design with level of factors, mean values and standard deviation for responses according to the Box Behnken design

Sunflower oil (% v/v)	Lecithin (% w/v)	Xanthan gum (% w/v)	SR (%)	SD	DS (μ m)	SD
15	5	0.01	67.78 \pm 5.95	5.74 \pm 0.24		
10	3	0.3	100 \pm 0	4.94 \pm 0.11		
10	3	0.01	88.91 \pm 1.03	5.4 \pm 0.05		
15	3	0.155	50.95 \pm 2.84	5.31 \pm 0.05		
10	1	0.155	92.13 \pm 1.55	5.24 \pm 0.04		
15	1	0.01	81.30 \pm 1.79	5.13 \pm 0.08		
10	5	0.155	54.30 \pm 2.80	5.08 \pm 0.18		
20	3	0.01	70.98 \pm 1.06	6.05 \pm 0.35		
20	5	0.155	62.94 \pm 2.00	5.26 \pm 0.29		
15	5	0.3	100 \pm 0	4.69 \pm 0.21		
15	3	0.155	48.52 \pm 2.93	5.48 \pm 0.15		
15	3	0.155	50.66 \pm 2.03	5.41 \pm 0.09		
20	1	0.155	100 \pm 0	5.28 \pm 0.12		
20	3	0.3	100 \pm 0	4.91 \pm 0.12		
15	1	0.3	100 \pm 0	4.96 \pm 0.12		

^ccenter point.
SD, Standard Deviation.

Table 2. Regression coefficients and analysis of variance of coded independent variables for stability ratio (SR)

Regression coefficients	SR	SE
0	50.05 ***	\pm 37.89
1	-0.85	\pm -1.02
2	-10.40 ***	\pm -12.51
3	11.38 ***	\pm 14.07
12	-1.13	\pm -0.93
13	4.48 ***	\pm 3.92
23	3.38 **	\pm 2.96
1 ²	15.70 ***	\pm 13.00
2 ²	12.97 ***	\pm 10.76
3 ²	24.26 ***	\pm 20.12
R²	0.8921	
Regression (P-value)	< 2.2e-16^c	
Lack of fit (P-value)	< 2.2e-16^c	

β_0 : estimated regression for the main linear effects.

β_1, β_2 : the estimated regression coefficients for the quadratic effects.

$\beta_{12}, \beta_{13}, \beta_{23}$: the estimated regression coefficients for the interaction effects.

^c Significant ($p \leq 0.05$), ** Significant ($p \leq 0.01$), *** Significant ($p \leq 0.001$)

SE, Standard Error

These results suggest that varying the concentration of xanthan gum was the most influential variable on SR, and additions of xanthan gum resulted in an increase in SR, consequently enhancing its resistance to gravitational separation. Nevertheless, Figures 2 (a) and (b) seem to indicate that when xanthan gum concentrations were initially low (0.01 % w/v), SR range was 70-80%, however, at intermediate xanthan gum concentrations (0.15% w/v), SR further decreased to 50-60%. On the other hand, once levels of xanthan gum passed this intermediate point (< 0.15% w/v), SR subsequently increased to 100%.

Dickinson (2009) found that at low concentrations, added hydrocolloids (such as xanthan gum) can

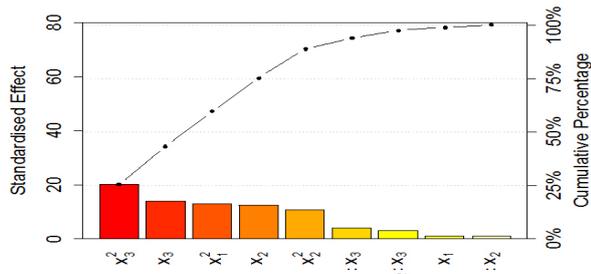


Figure 1. Pareto effects chart for stability ratio (SR)

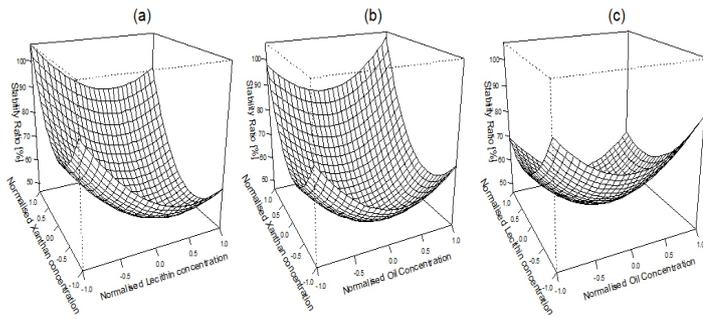


Figure 2. Response surface plots for emulsion stability as a function of (a) xanthan gum and lecithin concentration when sunflower oil is fixed at 0.08, (b) xanthan gum and sunflower oil concentration when lecithin is fixed at 0.44 and (c) lecithin and sunflower oil concentration when xanthan is fixed at -0.27

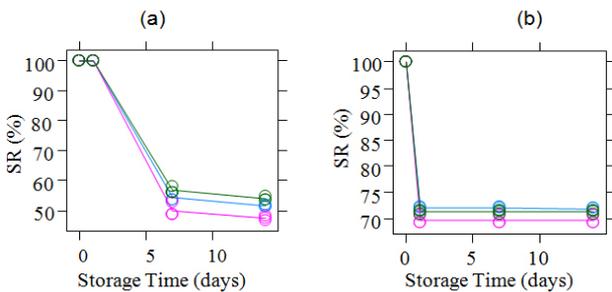


Figure 3. Stability ratio kinetics plot (a) experimental condition 4 and (b) 8

*Different colour lines indicate different replicated conditions.

actually have a destabilising effect on emulsions due to a mechanism known as depletion flocculation. This mechanism is induced by the excess non-absorbing hydrocolloid and/or surfactant forming micelles. In fact, xanthan gum is known as a common depletion flocculant, Hemar *et al.* (2001) observed that although increases in xanthan gum content caused more extensive flocculation of droplets, overall emulsion stability was subsequently improved. Furthermore, xanthan gum’s stabilising effect has been previously attributed to its ability to increase the viscosity of the continuous phase, thereby minimising droplet mobility and decreasing droplet collision numbers (Ye *et al.*, 2004). Hence, in this study, it may be suggested that a critical concentration of xanthan gum (0.15% w/v) was present, at which a destabilising effect from xanthan gum was prevalent, once surpassed, the apparent viscosity of the continuous

phase was increased, thereby reducing the mobility of the emulsion droplets inhibiting aggregation or coalescence (Sworn, 2000).

Specific concentrations for xanthan gum in emulsions with respect to such mechanisms of emulsion stability have also been reported. In these studies initial increases in xanthan gum concentration in emulsions had a destabilising effect and accelerated the creaming process due to the promotion of droplet flocculation through the depletion flocculation mechanism. It was established that a critical concentration (0.12% w/v) existed, above which, re-stabilisation of emulsion droplets began to occur (Ye *et al.*, 2004). Emulsions with 0.3% (w/v) xanthan gum were found to show no sign of phase separation after 150 days of storage (Kiosseoglou *et al.*, 2003).

Although lecithin wasn’t found to be as influential as xanthan gum on SR, lecithin was found to have significant ($p \leq 0.05$) linear (x_2), quadratic (x_2^2) and interactive ($x_2 \cdot x_3$) effects on SR (Table 2). The magnitude and importance of its influence on SR is clear from the (Figure 1), the lecithin’s linear (x_2) and quadratic effects (x_2^2) are positioned high on the Pareto effects chart with a relatively high magnitude of standardised effect. Hence, varying lecithin concentrations had an impact on SR. On the other hand, Figures 2 (a) and (c) suggest that this influence was a negative, higher levels of lecithin ($> 2.5\%$ w/v) appeared to have a destabilising effect. A similar depletion mechanism as that of xanthan gum, where excess non-absorbed surfactant promoted phase separation may be reason for this phenomena.

With regard to sunflower oil, the quadratic effect (x_1^2) and the interactive effect with xanthan gum ($x_1 \cdot x_3$) were the only significant effects ($p \leq 0.05$) found (Table 2). Changes in sunflower oil concentration (x_1) had less of an influence in comparison with the other design variables on SR (Figures 2 (a-c)). It is worth noting that intermediate levels of all independent variables produced the least desirable SR in the present study. Overall, the graphical results seem to indicate that the optimal SR conditions are when high xanthan gum, low lecithin and high sunflower oil are used.

Figures 3 (a) and 3 (b) depicts SR kinetic plots (triplicate samples) through storage time for experimental conditions 4 and 8 respectively. In the case of experimental condition 4 (Figure 3 (a)), initially, SR remained at 100% for day 0 and day 1, however, a sharp decline can be seen from day 1 to day 7 (59.36 %) with a levelling out or “plateau” period to day 14 with little or no change. A similar behaviour can be seen for experimental condition 8 (Figure 3 (b)) with SR declining sharply from

Table 3. Regression coefficients and analysis of variance of coded independent variables for mean droplet diameter (DS)

Regression Coefficients	DS	SE
$Asym_0$	5.41 ± 0.04	
$Asym_{x_3}$	-0.64 ± 0.04	
$Asym_{x_3 \cdot x_2}$	-0.11 ± 0.05	
R_{00}	4.59 ± 0.04	
R_{0x_1}	0.12 ± 0.03	
R_{0x_2}	-0.21 ± 0.03	
R_{0x_3}	-0.30 ± 0.03	
$R_{0x_1^2}$	0.30 ± 0.04	
$R_{0x_2^2}$	-0.11 ± 0.04	
$R_{0x_1 \cdot x_2}$	0.07 ± 0.03	
$R_{0x_2 \cdot x_3}$	-0.27 ± 0.04	
lrc_0	-1.4 ± 0.2	
lrc_{x_3}	1.51 ± 0.2	
$lrc_{x_2^2}$	-1.2 ± 0.2	
$lrc_{x_3^2}$	1.2 ± 0.2	
$lrc_{x_3 \cdot x_2}$	-0.38 ± 0.16	
$lrc_{x_3 \cdot x_1}$	-1.8 ± 0.3	
R^2	0.86	

* All parameter estimates significant ($p \leq 0.05$)

SE, Standard Error

Subscripts in parameter estimate name indicate the polynomial dependence term in the final secondary model building

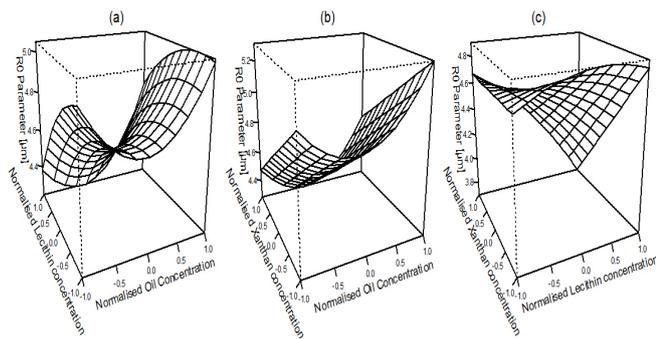


Figure 4. Response surface plots for DS estimated parameter R_0 (Initial DS) as a function of (a) lecithin and sunflower oil concentration (b) xanthan gum and sunflower oil concentration, (c) xanthan gum and lecithin concentration.

100% on day 0 to 60.90 on day 1 and levelling out throughout storage time.

Mean droplet diameter

Statistical significant parameters ($p \leq 0.05$) for the secondary model building of the DS response can be seen in Table 3. The results for initial DS (R_0) showed that all linear, quadratic and interactive variables for sunflower oil (x_1), lecithin (x_2) and xanthan gum (x_3) significantly influenced ($p \leq 0.05$) R_0 (except for the quadratic effect of xanthan gum (x_3^2)). Furthermore, xanthan gum was found to have negative linear (R_{0x_3}) and interactive effects ($R_{0x_2 \cdot x_3}$), which significantly influenced R_0 . While, all effects of lecithin were found to negatively influence R_0 . High concentrations of lecithin and xanthan gum produced the most desirable

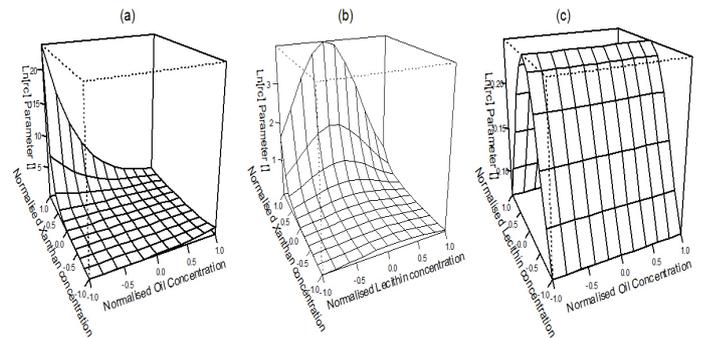


Figure 5. Response surface plots for DS estimated parameter lrc (log of the first order apparent rate constant as a function of (a) xanthan gum and sunflower oil concentration, (b) xanthan gum and lecithin concentration and (c) lecithin and sunflower oil concentration

R_0 . This is depicted in Figures 4 (a-c), where it can be seen that as lecithin and xanthan gum concentrations increased, R_0 subsequently decreased. The influential properties of lecithin in the reduction of initial DS (R_0) in this study would be expected. A primary role of an emulsifier is to migrate to the interface of the newly formed droplets, form a protective layer which prevents aggregation, and reduce the interfacial tension, therefore stabilising against coalescence (McClements, 2005). However, lecithin concentration was found to not be an important influential factor on the emulsion stability and mean droplet diameter in another study (Tirok et al., 2001). Sunflower oil was found to have a positive effect on R_0 , thus, addition of sunflower oil caused an increase in R_0 . Figures 4 (a) and (b) show that intermediate levels of sunflower oil (15% v/v) resulted in a more favourable initial DS (R_0).

Regarding, the speed of DS growth (lrc), it was found that xanthan gum proved to be the critical influential variable. The linear (lrc_{x_3}), quadratic ($lrc_{x_3^2}$) and interactive ($lrc_{x_3 \cdot x_1}$) and ($lrc_{x_3 \cdot x_2}$) effects of xanthan gum were significantly influential ($p \leq 0.05$). It is clear from Figure 5 (a) that higher levels of xanthan gum (0.15-0.3% w/v) in combination with low levels of sunflower oil (10-15% v/v) appear to have had the effect of increasing the rate of DS growth. The quadratic effect of lecithin ($lrc_{x_2^2}$) also had a significant influence on the lrc . Xanthan gum in combination with lecithin ($lrc_{x_3 \cdot x_2}$) was found to negatively influence lrc , thus, producing a desirable reduction in DS growth rate. Figure 5 (b) demonstrates that when both xanthan gum and lecithin were at lower levels ($\leq 0.15\%$ w/v and $\leq 1.25\%$ w/v respectively), a desirable lrc resulted. However, intermediate levels of lecithin (2.5% w/v) in combination with high levels of xanthan gum (0.24-0.35 w/v) proved to produce an undesirable increase in lrc . Figure 5 (c) appears to indicate that intermediate levels

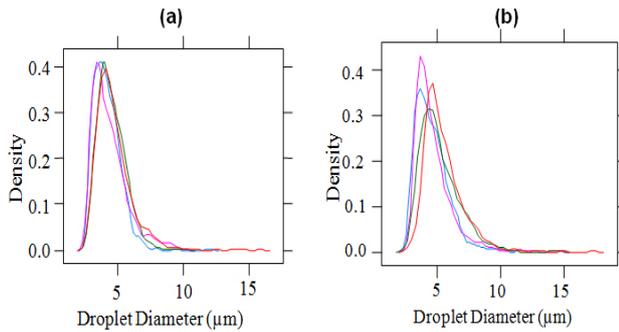


Figure 6. Droplet diameter distribution density plot for emulsion condition 10 (a) condition 11(b).

day 0 — day 1 — day 7 — day 14 —

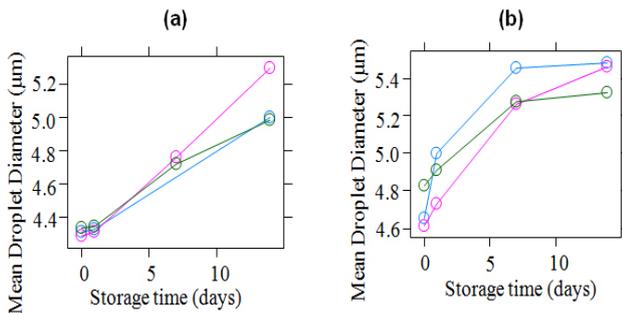


Figure 7. Mean droplet diameter kinetics plot experimental condition 7 (a) and experimental condition 12 (b).

*Different colour lines indicate different replicated experimental points

of lecithin (2.5% w/v) regardless of sunflower oil concentrations resulted in an increase in the rate of DS growth. Hence, the highest rate DS growth in this study was at an intermediate lecithin concentration (2.5% w/v). In terms of final DS ($Asym$), xanthan gum ($Asymx_3$) proved to have a significant influence ($p \leq 0.05$) on $Asym$ (Table 3), increasing xanthan gum concentration reduced the final DS in the emulsions. Additionally, the combination of xanthan gum and lecithin ($Asymx_3 \cdot x_2$) also had a significant influence ($p \leq 0.05$) in producing reducing the final DS (Table 3). A lack of quadratic variables indicated that the response surface was linear, with no maxima or minima found in this study.

Regarding DS as a whole, the results suggest that xanthan gum was the key influential variable in reducing initial DS (R_0), minimising droplet growth rate (lrc) and reducing the final DS ($Asym$). Xanthan gum's ability for minimising droplet mobility can provide the primary benefit of delaying droplet-droplet contact (Hemar *et al.*, 2001; Ye *et al.*, 2004). A secondary benefit of xanthan gum's ability to minimising droplet mobility is that it can provide sufficient time for the emulsifier (lecithin) to migrate to the droplet interface (Cao *et al.*, 1990). Krstonosic *et al.* (2009) observed that an increase in xanthan gum concentration led to a decrease in mean droplet diameter, however, other studies observed that the effective diameter of the droplets in the emulsion

increased because of flocculation caused by xanthan gum (Hemar *et al.*, 2001; Ye *et al.*, 2004).

Figures 6 (a) and 8 (b) show the droplet diameter distribution over storage time of the two different experimental conditions (condition 10 and 11, respectively). Two contrasting behaviours can be seen in these two plots. Figure 6 (a) displays a relatively narrow distribution curve with a small droplet diameter range and little variation over storage time is displayed in Figure 6 (a). On the other hand, it is can be seen in Figure 6 (b), that the distribution curve on day 0 is relatively narrow at first, however as time increases, the distributions curve becomes wider with a slight shift towards the right indicating that the droplet diameter increased and also that diameter size became less homogenous. Figure 7 (a) shows a kinetic plot of DS (triplicate samples) through storage time for condition 7, it would appear that there was a somewhat linear relationship between DS and storage time for this experimental condition, with DS proportionally increasing through storage time. However, Figure 7 (b) depicts a contrasting behaviour, a more complicated nonlinear relationship between DS and storage time for experimental condition 12 appears to be present. It would appear that after 7 days of storage a "plateau" was reached, in which DS "levels out" with little or no change. This behaviour appears to be present for the majority of the experimental conditions.

Multi Criteria Optimisation and validation of optimal conditions

Multi criteria optimisation was employed to find a compromise between the two response variables in the study using the library MCO for multicriteria optimisation (R 2.14.1 software package). A Pareto front plot for the two optimised responses was produced after generating a population of 1000 individual optimisations (Figure 8). The lack of any curvature in the Pareto front plot, suggests that the optimisation process resulted in no compromise being achieved between the two responses. Thus, any optimisation that attempts to improve the emulsion properties from a particular start up point will result in a benefit in one of the responses that will result in a directly proportional loss in the other response. Therefore, any decrease in DS resulted in a corresponding linearly correlated decrease in SR and vice versa. Hence, a 50/50 weighting was found to be the best condition and was tested to show the ability of the model to optimise the product. These experimental measures were used to follow the validation study.

Stability and average droplet diameter analysis

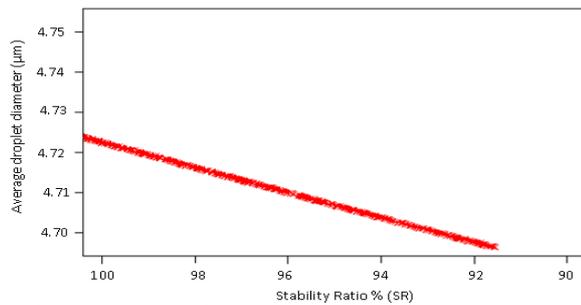


Figure 8. Pareto front plot showing the simultaneous optimisation of the droplet diameter and the stability ratio

were carried out at the optimal conditions to verify the model. A short validation study was conducted employing one set of the optimal conditions from the Pareto front (sunflower oil (x_1) 19.02%, lecithin (x_2) 1.2%, xanthan gum (x_3) 0.28%). The predicted values were $SR \leq 100\%$ and $DS 4.49 \mu\text{m}$, while the experimental values were $SR 100\%$ and $DS 4.35 \mu\text{m}$. It should be noted that both the asymptotic nonlinear regression and polynomial regressions equation were only a statistical empirical model in the selected ranges. An alternative modelling emulsion droplet diameter distribution using the Sauter diameter (D^{32}) was explored (data not shown), however due to variability amongst samples a low R^2 value was achieved and this could not be further examined.

Droplet diameter is a key characteristic of emulsion stability, contributing greatly to the physical stability and organoleptic properties of emulsions, where large globules tend to coalesce faster than the small ones as in Ostwald ripening (McClements, 2005). Stoke's law states that the velocity at which a droplet moves is proportional to the square of the droplet size radius (Dłużewska *et al.*, 2006). Thus, in theory, reducing the size of the droplets should enhance the stability of an emulsion to gravitational separation (Huang *et al.*, 2001). To achieve a decrease in the coalescence rate by a factor of 10-100, a decrease in mean globule diameter by a factor of two is required (Bergenstahl and Claesson, 1997). It was found that a decrease in droplet diameter by $0.02 \mu\text{m}$ resulted in a decrease in emulsion stability by 8%, which translates to a coalescence rate with a factor of 400 (Figure 8).

Conclusions

Results show that both xanthan gum and lecithin were crucial components for extending the emulsions shelf life to 14 days at ambient room temperature. A critical concentration for xanthan gum (0.15% w/v) was established; at which emulsion stability was at its lowest, once surpassed emulsion stability was enhanced. Both initial and final droplet diameter were

minimised with increasing concentrations of xanthan gum. Increasing the lecithin concentration induced emulsion instability, thus, lower levels ($< 2.5\%$ w/v) were found to be optimal. However, increasing lecithin concentration minimised initial droplet diameter. Optimum conditions were found to be sunflower oil 19.02% v/v, soy lecithin 1.2% w/v and xanthan gum 0.28% w/v. The optimisation process resulted in no compromise being achieved between the two responses, a decrease in droplet diameter by $0.02 \mu\text{m}$ resulted in a decrease in emulsion stability by 8%. The knowledge of the stability properties developed in this work is essential for the formulation of emulsion based food products such as sauces, ice cream and salad dressings, using the ingredients selected in this study.

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