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Optimisation of Cinnamaldehyde-in-water Nanoemulsion Formulation using Central Composite Rotatable Design

(Pengoptimuman Formulasi Nanoemulsi Sinamaldehid dalam Air Menggunakan Reka Bentuk Komposit Putaran Tengah)

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ABSTRACT

*Thirteen formulations of cinnamaldehyde/non-ionic surfactant/water system nanoemulsions were prepared using high-pressure homogenisation. The result showed that varying the cinnamaldehyde/surfactant ratio had effect significantly (*p*<0.05) to mean droplet diameter, polidispersity index, ζ-potential, turbidity and whiteness index, while no significant effect (*p*>0.05) to viscosity. The mean droplet diameter ranged from 50.48 to 106.4 nm, polydispersity index from 0.06 to 0.28 and ζ-potential from -4.11 to -6.98 mV. The smallest droplet size was produced using 5% cinnamaldehyde and 5% Tween 80. Response surface for droplet diameter showed that the higher the cinnamaldehyde and surfactant concentrations, the larger the droplet diameter, polydispersity index and whiteness index. However, the ζ-potential increased as the cinnamaldehyde concentration decreased and Tween 80 increased. Increasing the cinnamaldehyde concentration led to an increase in turbidity. Formulation of 5% cinnamaldehyde and 6.23% Tween 80 gave no observable separation of the nanoemulsion with minimum droplet size, polidispersity index, viscosity, turbidity, whiteness index and maximum ζ-potential in modulus. The stability of the optimum formulation was sustained for 10 days upon storage at 4°C. The values of droplet diameter, PDI* and *ζ-potential were 55.50 nm, 0.08 and -5.38 mV, respectively.*

Keywords: Cinnamaldehyde; formation; high-pressure homogenisation; nanoemulsion; optimisation

ABSTRAK

*Tiga belas formulasi nanoemulsi sistem sinamaldehid/surfaktan bukan ion/air telah disediakan menggunakan homogenisasi tekanan tinggi. Keputusan menunjukkan bahawa perlakuan nisbah sinamaldehid/surfaktan memberi kesan yang signifikan (*p*<0.05) terhadap rerata diameter titisan, indeks polidispersiti, ζ-potensial, kekeruhan dan indeks keputihan, namun tidak berpengaruh signifikan (*p*>0.05) terhadap kepekatan. Nilai rerata diameter titisan berkisar antara 50.48 hingga 106.4 nm, indeks polidispersiti daripada 0.06 hingga 0.28,* dan *ζ-potensial dari -4.11 hingga -6.98 mV. Saiz titisan terkecil dihasilkan menggunakan sinamaldehid 5% dan 5% Tween 80. Tindak balas permukaan untuk diameter titisan menunjukkan bahawa lebih tinggi kepekatan sinamaldehid dan surfaktan, semakin besar pula diameter titisan, indeks polidispersiti dan indeks keputihan. Walau bagaimanapun, potensi ζ meningkat apabila kepekatan sinamaldehid menurun dan Tween 80 meningkat. Peningkatan kepekatan sinamaldehid menyebabkan peningkatan kekeruhan. Formulasi sinamaldehid 5% dan 6.23% Tween 80 tidak ditemukan adanya pengasingan nanoemulsi dengan diameter saiz titisan, indeks polidispersiti, kelikatan, kekeruhan dan indeks keputihan yang minimum dan maksimum nilai ζ-potensial dalam modulus. Kestabilan formulasi optimum dikekalkan selama 10 hari tempoh penyimpanan pada suhu 4°C. Nilai diameter titisan, indeks polidispersiti dan ζ-potensial masing-masing adalah 55.50 nm, 0.08 dan -5.38 mV.*

Kata kunci: Homogenisasi tekanan tinggi; nanoemulsi; pembentukan; pengoptimuman; sinamaldehid

INTRODUCTION

Nanoemulsions are fine oil-in-water or water-in-oil dispersions and non-equilibrium systems which have a remarkably small oil droplet dispersed within a continuous watery phase, with each oil droplet being surrounded by a protective coating of surfactant molecule as emulsifiers (McClements 2005). The appropriate size range of a nanoemulsion is defined differently by different authors, such as 1-100 nm (Kourniatis et al. 2010), 20-200 nm (Ee et al. 2008; Gutiérrez et al. 2008; Solans et al. 2005) and 100-500 nm (Shah et al. 2010; Tang et al. 2013). Nanoemulsions cannot form spontaneously. Consequently, energy input, generally from a mechanical device or from the chemical potential of the components, is necessary (Delmas et al. 2011). Under mechanical energy, the interface between the two phases is deformed to such an extent that droplets form, which are subsequently broken up or disrupted into smaller droplets (Floury et al. 2000).

A number of factors will affect the production of stable nanoemulsions, such as composition variables and preparation variables. The ratio of the oil phase and surfactant concentration can influence the formation and

stability of the emulsions (Gutiérrez et al. 2008; Komaiko & McClements 2015a). In an emulsion, the surfactant acts to lower the interfacial tension between the internal and external phases in order to allow the formation of droplets (Silva et al. 2012). The amount of surfactant needed to produce a stable emulsion depends on the total surface area of the droplets (McClements 2005). Nanoemulsion formulation requires a low amount of surfactant compared to micro- or macroemulsions. For example 20-25% surfactant is required for the preparation of a microemulsion, whereas 5-10% surfactant is sufficient to result a fine nanoemulsion (Asmawati et al. 2014; Tadros et al. 2004). The smallest droplet sizes of nanoemulsions using a high-energy method, can be reached at low oil-to-surfactant ratio (Ghosh et al. 2013; Gutiérrez et al. 2008; Yang & McClements 2013).

Nanoemulsions are used as a delivery system for preservatives and bioactive compounds in foods (Neethirajan & Jayas 2011) such as antimicrobial and antioxidant agents (Donsi et al. 2012; Kentish et al. 2008), non-polar functional components (Tang et al. 2013) such as bioactive lipids, drug, natural flavours and natural colourings (Komaiko & McClements 2015b; Mao et al. 2009). The application of nanoemulsions in food products may lead to the modification of many macroscale characteristics, sensory attributes, process ability and shelf life (Huang et al. 2010). Antimicrobial properties of nanoemulsions and microemulsions are believed to result from the small size of oil particles (Vanhecke et al. 2002) that have a high surface tension. Application of D-limonene nanoemulsified as antimicrobials was increased the antimicrobial efficiency than those of the bulk D-limonene added directly (Maté et al. 2016; Zahi et al. 2017).

At present, owing to the increased consumer awareness and concern regarding of the ill-effects of synthetic chemical additives in foods during preservation (Yildirim et al. 2017), the use of natural additives and antimicrobial compounds in foods instead during preservation, has attracted growing interest (Donsi et al. 2012; Sow et al. 2017). Therefore, some studies had focused on application of natural substances such as essential oils in food products. The application essential oil such as cinnamaldehyde, a major non-polar functional compound of cinnamon bark essential oil, as flavouring can improve the texture, aroma and flavour, also in order to extend the shelf-life of food product. Cinnamaldehyde has traditionally been used to preserve foods and to enhance flavour and odour (Otoni et al. 2014). In Europe countries, cinnamaldehyde is used as flavor in meat and fast food products, sauces and pickles, confectionery, cola-type drinks, dental and pharmaceutical. Cinnamaldehyde also has antioxidant, antimicrobial and antifungal to slow meat spoilage (Singh et al. 2007; Wei et al. 2011).

However, the application of cinnamaldehyde in meat product is limited owing to poor solubility (high hydrophobicity) in aqueous media and low bioavailability (Abd-Elsalam & Khokhlov 2015). Therefore, through nanoemulsion using food-grade stabilizer (Tween 80), the solubility and availability of cinnamaldehyde in food products can be enhanced including antioxidant and antimicrobial activity. In addition, during marination of intact muscles, the rate and extend of marination is often limited and localized to the meat surface. These problems can be overcome through encapsulation technique at the nanoscale to provide the function of cinnamaldehyde, promoting solubility and dispersibility in aqueous phase. In addition, the encapsulated essential oil in appropriate delivery systems, will reduce the doses required of essential oil and increase the physical stability of active substances (Weiss et al. 2009), thus minimizing the impact on aroma, flavor and taste (Donsi et al. 2012).

In this model study, cinnamaldehyde was used as the oil phase in the oil/water emulsion system. The aim of this study was to determine the optimum cinnamaldehyde (essential oil) and polyethylene glycol sorbitan monooleate (Tween 80) (non-ionic surfactant) concentration to produce a stable cinnamaldehyde nanoemulsion via response surface methodology (RSM). The physical properties of the nanoemulsion and storage stability of the optimum formulation was also observed in this study.

Materials and Methods

Materials

Cinnamaldehyde (95% pure, C_9H_8O) and food-grade surfactant Tween 80 (polyethylene glycol sorbitan monooleate) were purchased from Sigma Aldrich Chemical Company (USA). Deionised water was freshly obtained from Milli-Q Plus apparatus (Millipore, Billerica, USA) and was used throughout the experiments.

Preparation of the nanoemulsions

A coarse emulsion was prepared at room temperature (25°C) by blending cinnamaldehyde (oil phase) and Tween 80 (emulsifier); then, deionised water (aqueous phase) as generated by RSM was added slowly and homogenised using Ultra Turrax digital T25 Basic, Janke Kumkel, IKA-Germany (12000 rpm for 5 min) at room temperature. The droplet size was then reduced further by passing the coarse emulsion through an APV 2000 high-pressure homogeniser (APV, Germany) for two passes at 900 bar. Each experiment was then analysed for droplet size, polydispersity index (PDI), ζ-potential, viscosity, turbidity and whiteness index (WI).

Characterisation of the nanoemulsions

The mean droplet size and PDI of the emulsions were measured by dynamic light scattering (DLS) with a Zetasizer Nano-ZS laser diffractometer (Malvern Instruments Ltd, Worcestershire, UK) working at 633 nm at 25°C and equipped with a backscatter detector (173°). Nanoemulsions samples were diluted 1:10 with deionised water before the measurement. Each sample

was poured into square disposable polystyrene cuvettes (DTS0012) from Malvern Instruments Ltd, UK. The oil droplet size (nm) was characterised by distribution curves in terms of intensity (%) and average mean droplet size. The polydispersity index (PDI) of nanoemulsions were reported because PDI indicates the heterogeneity of droplet dimension which is the width of the droplet size distribution in range from 0 to 1.

The ζ-potential of the oil droplets was measured by phase-analysis light scattering (PALS) with a Zetasizer Nano-ZS laser diffractometer (Malvern Instruments Ltd, Worcestershire, UK) to determine the surface charge at the surface of the droplets. The samples were diluted with deionised water prior to analysis. The ζ-potential is an important tool for understanding the state of the nanoparticle surface and for predicting the long-term stability of the nanoparticle.

The viscosity of each emulsion was determined using a Brookfield LV III Ultra viscometer (Brookfield Engineering Laboratories, Inc., USA). The operation principle of a viscometer is to drive a spindle (which is immersed in the sample) through a calibrated spring. The viscous drag of the fluid against the spindle is measured by the spring deflection. Spring deflection is measured with a rotary transducer. The measuring range of a DV-III (in centipoises, cP) is determined by the rotational speed of the spindle, the size and shape of the spindle, the container the spindle is rotating in as well as the full-scale torque of the calibrated spring.

The turbidity of nanoemulsion was determined without dilution by use of a UV-Visible spectrophotometer for absorbance at 600 nm. Deionised water was used as a reference to the blank cell. The colour of the emulsions was measured using a Minolta Chromameter CR-400 (Osaka, Japan) at room temperature. CIE L^*, a^* and b^* values (representing lightness, redness and yellowness, respectively) were determined and the WI was calculated based on the following equation (Salvia-Trujillo et al. 2013a; Vargas et al. 2008):

$$WI = 100 - [(100-L)^2 + (a^2 + b^2)]^{½}$$

EXPERIMENTAL DESIGN AND OPTIMISATION

Response surface methodology (RSM) was used to observe the effects of the independent variables, cinnamaldehyde (X_1) and surfactant/Tween 80 (X_2) concentrations, on the formation and stability of cinnamaldehyde nanoemulsions. The experiments were designed according to the central composite rotatable design (CCRD) using Design Expert 7.0.3 Wiley software. A total of 13 experiments, including five replicates of the central points, were carried out in a random run order. The responses were droplet size (Y_1), PDI (Y_2), ζ-potential (Y_3), viscosity (Y_4), turbidity (Y_5) and WI (Y_6). The optimisation criteria to obtain a stable cinnamaldehyde nanoemulsion were set for minimum of droplet size, PDI, viscosity, turbidity and WI, the optimum ζ-potential value was set as maximum in modulus.

OPTIMUM FORMULATION STABILITY

The stability of the optimum formulation was monitored by physical observation (change in droplets size, PDI, ζ-potential and distribution of droplets size) during storage for 10 days at temperatures of 4 and 25°C. The significance of storage time and temperature were analysed using the general linear model (GLM) with post-hoc Duncan's test using SAS 9.2 (Statistical Analysis System, SAS Inc., Chicago, USA).

RESULTS AND DISCUSSION

OPTIMISATION FOR CINNAMALDEHYDE NANOEMULSION FORMATION FITTING THE MODELS

The experimental data were used to calculate the coefficients of the polynomial equation and the derived equation was then used to predict the response values. The experimental values agreed well with the predicted values obtained from the RSM design. The statistical model representing the response surface of all responses is shown in Table 1. The models were deemed a good fit if the model was significant and gave p values lower than 0.05. Thus the result obtained from this experiment showed the lack-of-fit test was not significant and gave p values higher than 0.05 and the R^2 and adjusted R^2 values were more than 0.75. The smaller the p value, the higher the significance of the corresponding coefficient (Table 2). The concentration of cinnamaldehyde had a significant effect ($p<0.05$) on the droplet size, whereas the surfactant concentration was insignificant ($p>0.05$), as shown in Table 2. The interaction of independent variables x_{22} and x_{12} shows significance ($p<0.05$) in terms of the droplet size. The positive coefficient value for cinnamaldehyde concentration showed that, as the concentration of surfactant increased, the droplet size also increased. The model coefficients for PDI for both of the independent variables had significant effects ($p<0.05$). All interactions between the variables also had significant effects ($p<0.05$) on the PDI. The linear coefficients for x_1 showed a positive value, whereas the linear coefficient for surfactant concentration (x_2) showed a different result. Model coefficients that were significant ($p<0.05$) for ζ-potential were x_1, x_2, x_{11} and x_{22}. The coefficient of cinnamaldehyde concentration was a negative, whereas the coefficient of surfactant concentration was a positive. Viscosity had a significant effect ($p<0.05$) in the model and lack-of-fit, in which the R^2 values for both models were higher than 0.75. Analysis of the coefficients for turbidity demonstrated that the cinnamaldehyde and surfactant concentrations as a single independent variable had a significant effect ($p<0.05$) on the turbidity, whereas the interaction of both independent variables did not show significant effect for turbidity. The coefficient for cinnamaldehyde concentration was a positive value and negative coefficient was found for surfactant concentration. The WI results showed that all of the independent variables and interaction variables have a significant effect ($p<0.05$) on the WI.

TABLE 1. Model equations fitted by analysis of variance (ANOVA) for the droplet size (DS), polydispersity index (PDI), zeta potential (ζ-P), pH, viscosity, turbidity and whiteness index (WI)

Response	Model equation	Model sig.	Lack of Fit	R^2	Adj R^2
DS, nm	Coded: $Y = 77.44 + 9.11x_1 + 1.76x_2 - 11.27x_1x_2 - 3.72x_1^2 + 13.58x_2^2 + 8.22x_1^2x_2 + 7.15x_1x_2^2$	0.0047	0.7872	0.9535	0.8884
(Y_1)	Actual: $DS = -382.93 + 111.03X_1 + 62.74X_2 - 16.56X_1X_2 - 4.54X_1^2 - 1.26X_2^2 + 0.53X_1^2X_2 + 0.46X_1X_2^2$				
PDI	Coded: $Y = -2.41 + 0.27x_1 - 0.53x_2 - 0.09x_1x_2 + 0.12x_1^2 + 0.18\,x_2^2 + 0.48x_1^2x_2 - 0.17x_1x_2^2$	0.0012	*0.4426*	0.9737	0.9370
(Y_2)	Actual: $PDI = -8.23 + 2.81X_1 - 0.02X_2 - 0.32X_1X_2 - 0.21X_1^2 + 0.12X_2^2 + 0.03X_1^2X_2 - 0.01X_1X_2^2$				
ζ-P, mV	Coded: $Y = -4.49 - 0.42x_1 + 0.98x_2 - 0.68{*}x_1^2 - 0.54x_2^2$	0.0001	*0.3328*	0.9254	0.8881
(Y_3)	Actual: $\zeta\text{-P} = -17.12 + 1.46X_1 + 1.68X_2 - 0.11X_1^2 - 0.09X_2^2$				
Viscosity, cP	Coded: $Y = 2.15 + 0.04x_1 - 0.18x_2 + 0.16\,x_1x_2 + 0.01x_1^2 - 0.08x_2^2 + 0.18x_1^2x_2 + 0.24x_1x_2^2$	0.0460	*0.0004*	0.8769	0.7045
(Y_4)	Actual: $viscosity = -8.06 + 1.98X_1 + 2.33X_2 - 0.38X_1X_2 - 0.09X_1^2 - 0.13X_1X_2^2 + 0.01X_1^2X_2 + 0.02X_1X_2^2$				
Turbidity	Coded: $Y = 3.63 + 0.07x_1 - 0.12x_2 + 0.07x_1x_2 - 0.21x_1^2 - 0.27\,x_2^2 + 0.12x_1^2x_2$	0.0003	*0.6588*	0.9692	0.9385
(Y_5)	Actual: $turbidity = -3.12 + 1.30X_1 + 0.95X_2 - 0.10X_1X_2 - 0.09X_1^2 - 0.04X_2^2 + 7.60\text{E-}03X_1^2X_2$				
WI (Y_6)	Coded: $Y = 68.54 + 2.03x^1 - 5.69x^2 + 4.50x^1x^2 - 0.80x_1^2 - 4.26x_2^2 - 0.06x_1^2x_2 + 3.69x_1x_2^2$	< 0.0001	*0.0964*	0.9985	0.9965
	Actual: $WI = -23.57 + 10.19X_1 + 28.91X_2 - 2.77X_1X_2 - 0.10X_1^2 - 2.45X_2^2 - 3.68\text{E-}03X_1^2X_2 + 0.24X_1X_2^2$				

X1/x1: Cinnamaldehyde concentration (%).X2/x2: Surfactant (Tween 80) concentration (%).

TABLE 2. Model coefficients for droplet size (DS), polydispersity index (PDI), ζ-potential (ζ-P), viscosity, turbidity and whiteness index (WI) of cinnamaldehyde-in-water nanoemulsion (x1: cinnamaldehyde concentration, %; x2: Surfactant (Tween 80) concentration, %)

Responses	Model coefficient					
	x_0	x_1	x_2	x_{11}	x_{22}	x_{12}
DS (d, nm)						
Coeff.	77.44	9.11	1.67	-3.72	13.58	11.27
F	14.64	8.94	0.33	2.59	34.55	13.68
Prob<*F*	0.0047	0.0305	0.5880	0.1685	0.0020	0.0140
PDI	-2.41	0.27	-0.53	0.12	0.18	-0.09
Coeff.	26.48	29.93	116.27	9.75	24.79	3.14
F	0.0012	0.0028	0.0001	0.0262*	0.0042*	0.0364
Prob<*F*						
ζ-P (mV)	-4.49	-0.42	0.98	-0.68	0.54	-
Coeff.	24.80	10.16	55.39	23.23	14.68	-
F	0.0001	0.0129	<0.0001	0.0013*	0.0050*	-
Prob<*F*						
Turbidity	3.63	0.07	-0.12	-0.21	-0.27	0.07
Coeff.	31.50	8.39	13.73	66.78	115.77	4.49
F	0.0003	0.0275	0.0100	0.0002*	<0.0001	0.0785
Prob<*F*						
WI	68.54	2.03	-5.69	-0.80	-4.26	4.50
Coeff.	490.51	91.44	720.74	24.71	704.72	451.10
F	<0.0001	0.0002	<0.0001	0.0042	<0.0001	<0.0001
Prob<*F*	1					

ANALYSIS OF RESPONSE SURFACE

In order to study the relationship between the independent and dependent variables, the response of surface and contour plots of the quadratic polynomial model were elucidated by two of the independent variables being varied within the experimental range while the other parameters were held constant at the central point. The response surface plots for the droplet size are presented in Figure 1(a) as a function of cinnamaldehyde and surfactant concentration. The cinnamaldehyde nanoemulsion had the smallest droplet size at the lowest cinnamaldehyde and surfactant concentrations and the larger droplet size at the higher cinnamaldehyde and surfactant concentration. Similar results also showed that the higher ratio oil surfactant concentration, the larger the size of the obtained droplet (Guttoff et al. 2015; Komaiko & McClements 2014). However Komaiko and McClements (2015b), emulsions prepared by spontaneous emulsification showed smaller droplets being formed at higher surfactant-to-oil ratios. In this study, the nanoemulsion was not spontaneously formed but rather mainly owing to the energy input in the high pressure homogeniser and supplemented by the presence of Tween 80 as surfactant. The size of the droplets was affected/closely related to the concentration of surfactant. As the surfactant concentration increased, while maintaining the cinnamaldehyde concentration constant, an increasing trend in droplet size of the nanoemulsion was observed. Moreover, when the surfactant concentration was doubled over the cinnamaldehyde, the droplet size increased due to the excess of the surfactant in the emulsion system. This finding was in conforment with result reported by Guttoff et al. (2015) and Li and Chiang (2012).

Tween 80 was used in this study, which was more effective in minimising the mean droplet size compared to polymers, owing to its rapid adsorption onto the droplet surface (Qian & McClements 2011). The response surface plots for PDI are presented in Figure 1(b) as a function of oil and surfactant concentration. The PDI increased with increasing cinnamaldehyde concentration and decreasing surfactant concentration. In this study, low PDI values were observed for all formulations, indicating uniformity in droplet size within each formulation. The PDI of cinnamaldehyde nanoemulsion was less 0.3, which indicates good monodispersity of the nanoemulsions. A PDI <0.08 indicates an almost monodisperse sample, whereas 0.08-0.70 represent mid-range PDI values, but it is the range over which the distribution algorithms best operate. A PDI close to 1 (>0.7) indicates a very broad distribution of droplet size (Kwon et al. 2015).

The size distribution is a plot of the relative intensity of light scattered by particles in various size classes. The effect of oil and surfactant concentrations on the size distribution in the cinnamaldehyde nanoemulsion is shown in Figure 2. A wider droplet size distribution indicates that the nanoemulsion did not provide a good dispersion in terms of droplet size. In this study, the narrowest of the size distributions was observed in

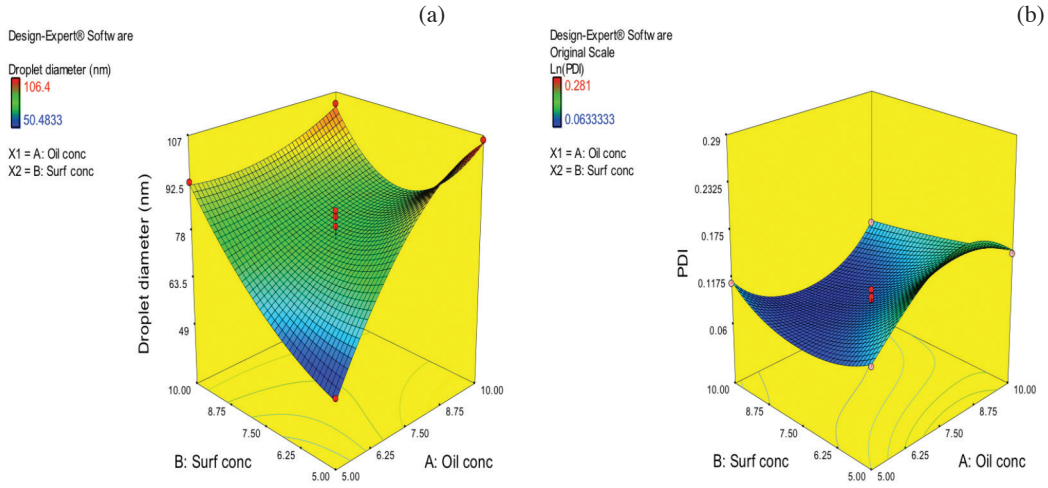

FIGURE 1. Response surface plots of (a) droplet size (d, nm) and (b) the PDI of cinnamaldehyde nanoemulsion as a function of oil and surfactant concentration

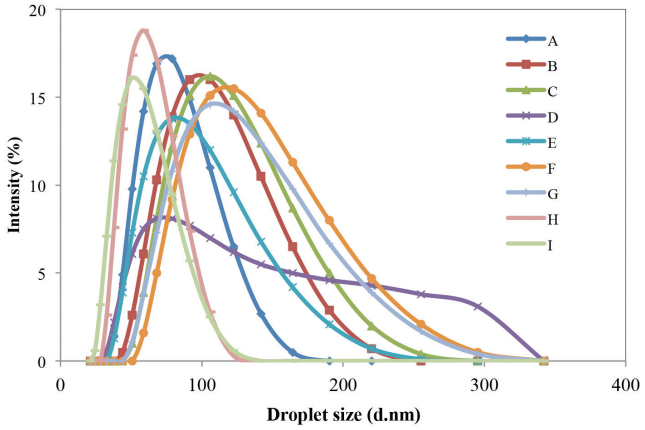

FIGURE 2. Droplet size distribution of the cinnamaldehyde nanoemulsion for cinnamaldehyde-to-Tween 80 ratios of A) 7.5:7.5, B) 5:10, C) 10:10, D) 7.5:3.96, E) 11.04:7.5, F) 7.5:11.04, G) 10:5, H) 3.96:7.5 and I) 5:5

the cinnamaldehyde nanoemulsion produced with 5% cinnamaldehyde and 5% surfactant.

These results showed that the ζ-potential became more positive by increasing the surfactant concentration. Dispersions with a low ζ-potential value will eventually aggregate, owing to van der Waals inter-particle attractions. The ζ-potential value range of -30 to +30 mV shows unstable emulsions, whereas the stable emulsions occur when the value is more negative than -30 mV or more positive than +30 mV (Hertault et al. 2003). At high stabiliser (surfactant) concentrations, well above of the plateau of the adsorption isotherm, electrolyte stabilisers can cause a decrease in the diffuse layer, leading to a decreased ζ-potential and decreased physical stability. In this study, the cinnamaldehyde nanoemulsion showed negative ζ-potential value in range of -3 to -12 mV, thus categorised as unstable emulsions owing to the fact that the surfactant used was Tween 80, which is a non-ionic surfactant (Sari et al. 2015). Mei et al. (2011) stated that the negative surface charge of a nanoemulsion was obtained from the specific adsorption of hydroxyl ions. This phenomenon is caused by the formation of hydrogen bonds between the hydroxyl ions and water molecules in the boundary layer.

The viscosity had a significant effect (p<0.05) in the model and lack-of-fit, for which the R^2 values for both models were higher than 0.75. Whereas, the viscosity response showed that it did not satisfactorily fit the model, because the lack-of-fit test was significant, so cannot be optimised. In general, there are several factors that affect the viscosity of emulsions, such as the ratio of oil and the surfactant concentration in the system (Polychniatou & Tzia 2014), the structure of the emulsions (Garti et al. 2005) and the energy input from a mechanical device (Salvia-Trujillo et al. 2013a). The viscosity of nanoemulsions decreased with increasing droplet size (Salvia-Trujillo et al. 2013b).

The turbidity of the cinnamaldehyde-in-water nanoemulsions showed the higher the concentration of

cinnamaldehyde, the higher the turbidity. In contrast, there was a sharp decrease in turbidity, in fact becoming more transparent with an increased surfactant concentration. According to Komaiko and McClements (2015b), decreasing turbidity corresponds to a decreasing droplet size.

The average WI of the cinnamaldehyde nanoemulsions increased with increasing concentration of cinnamaldehyde (Figure 3). The appearances of cinnamaldehyde nanoemulsions in this work were milky white. McClement (2002) confirmed that the colour of emulsions is influenced by the refractive index of the dispersed and continuous phase as well as the size of the droplet. According to the results of Salvia-Trujillo et al. (2013a, b), the WI descended with increasing droplet size.

OPTIMUM CONDITIONS FOR PREPARING CINNAMALDEHYDE NANOEMULSIONS

The optimum conditions to obtain a cinnamaldehyde nanoemulsion using a high-pressure homogeniser were determined based on the statistical highest desirability to the responses. The criteria applied for graphical optimisation were minimum droplet size, PDI, viscosity, turbidity and WI as well as maximum ζ-potential in modulus. The 3D surface plot of desirability produced an optimum region for the criteria set. The optimum formulation of cinnamaldehyde and Tween 80 was 5 and 6.23%, respectively. At this optimum point, the values of droplet size, PDI and ζ-potential were 55.50 nm, 0.08 and -5.38 mV, respectively.

VERIFICATION OF THE MODELS

In order to ascertain the adequacy of the response surface equations, the predicted values were compared with experimental values. The optimised formulation of the cinnamaldehyde nanoemulsion had a particle size (nm) of 54.40 ± 0.43, PDI of 0.105 and ζ-potential (mV) of -5.44 ± 0.03. According to the results, no significant difference (p>0.05) was noted between the experimental and theoretically predicted values. The accuracy of the RSM model was verified based on the experimental data obtained.

STABILITY OF THE OPTIMUM NANOEMULSION FORMULATION

The optimum formulation for preparing a cinnamaldehyde nanoemulsion from the central composite rotatable design (CCRD) was characterised further in terms of stability (Figure 4). The changes in means droplet size and size distribution for cinnamaldehyde nanoemulsions during storage are presented in Figure 4(a) and 4(b), respectively. The result showed the range of droplet sizes over 10 days storage at 4 and 25°C were 54.48-123.95 nm and 54.48-239.57 nm, respectively. The mean droplet size increased slowly with increasing storage time, but the size of the droplets increased drastically on the fifth day of storage at room temperature. An increase in droplet size was caused by the coalescence of cinnamaldehyde droplets (Rebolleda et al. 2015). The ζ-potential of cinnamaldehyde nanoemulsions (Figure 4(c)) at 25°C were found to be more negatively charged, with the increasing storage time indicating the unstability of the emulsion at 25°C. At room temperature, the formation of a sedimentation front at the bottom of the sample was observed after fifth day for oil of surfactant ratio of 3.96 : 7.5 and 7.5 : 11.04, owing to the fact that they have a higher density than the surrounding liquid. The process of sedimentation was caused by gravitational separation (McClements 2005).

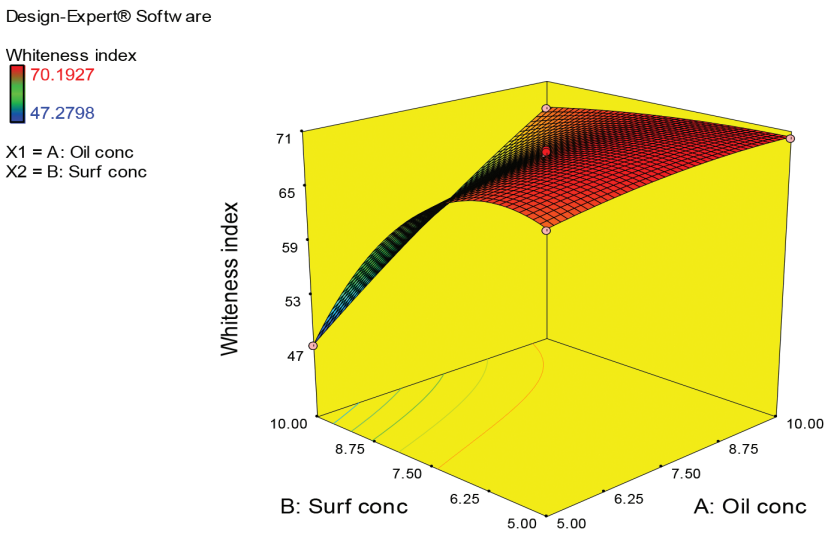

FIGURE 3. Response surface plots for the WI of cinnamaldehyde nanoemulsions as a function of oil and surfactant concentration

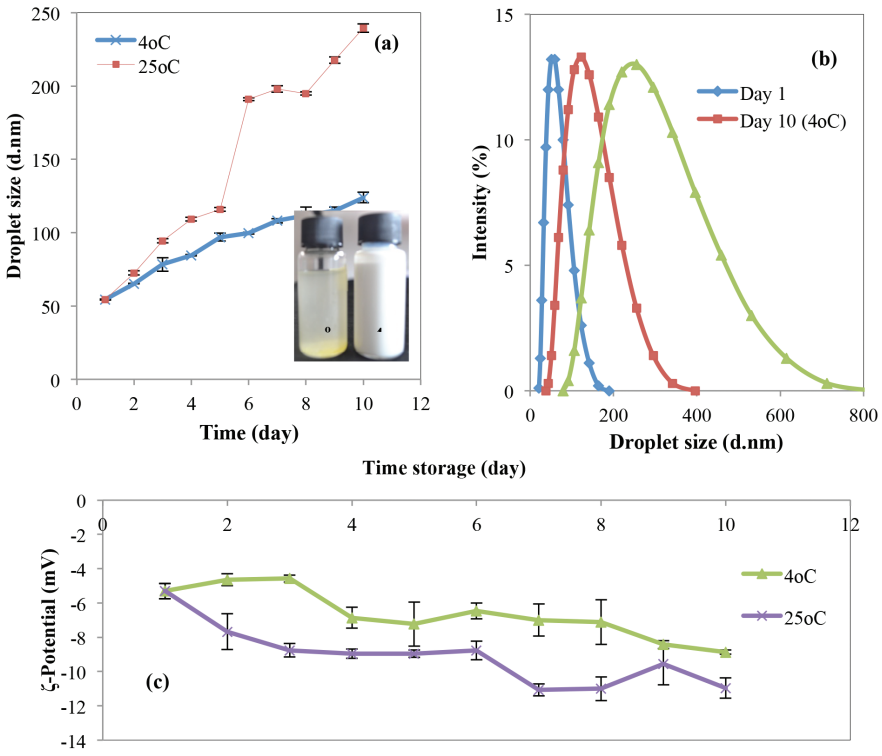

FIGURE 4. Effect of storage condition of the optimum cinnamaldehyde nanoemulsion: (a) droplet size (nm) during storage, (b) droplet size distribution, and (c) ζ-potential as a function of time and temperature

CONCLUSION

It is proved that CCRD and RSM has given the optimum formulation cinnamaldehyde emulsified by Tween 80 in nano scale. This work has a potential in flavour enhancement and it's being carried out in the marination of meat product. The cinnamaldehyde and surfactant concentrations had significant effects on droplet formation, polydispersity index (PDI) and ζ-potential of the nanoemulsions. The smallest diameter and the narrowest size distribution of the particles were observed in the cinnamaldehyde nanoemulsion produced with 5% cinnamaldehyde and 5% surfactant. The higher the cinnamaldehyde and surfactant concentrations, the larger the droplet size. An increase in PDI and turbidity was observed with increasing cinnamaldehyde concentration and decreasing surfactant concentration. The ζ-potential became more positive with increasing surfactant concentration. The average WI of the cinnamaldehyde nanoemulsion increased at increasing concentrations of cinnamaldehyde. The appearance of cinnamaldehyde nanoemulsions were milky white. By selectively changing the composition variables, that is, the cinnamaldehyde and surfactant concentrations, the optimum conditions for nanoemulsion preparation were found to be a combination of 5% cinnamaldehyde and 6.23% surfactant. Nanoemulsions with the optimum formulation showed good stability when stored at 4°C without sedimentation occurring during storage.

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REFERENCES

Abd-Elsalam, K.A. & Khokhlov, A.R. 2015. Eugenol oil nanoemulsion: Antifungal activity against *Fusarium oxysporum* f. sp. *vasinfectum* and phytotoxicity on cottonseeds. *Application Nanoscience* 5: 255-265.

Asmawati, M.W.A.W., Yusop, S.M., Maskat, M.Y. & Shamsuddin, A.F. 2014. Characteristics of cinnamaldehyde nanoemulsion prepared using APV-high pressure homogenizer and ultra turrax. *AIP Conference Proceeding* 1614: 244-250.

Delmas, T., Piraux, H., Couffin, A., Texier, I., Vinet, F., Poulin, P., Cates, M.E. & Bibette, J. 2011. How to prepare and stabilize very small nanoemulsions. *Langmuir* 27(5): 1683-1692.

Donsi, F., Annunziata, M., Vincensi, M. & Ferrari, G. 2012. Design of nanoemulsion-based delivery systems of natural antimicrobials: Effect of the emulsifier. *Journal of Biotechnology* 159: 342-350.

Ee, S.L., Duan, X., Liew, J. & Nguyen, Q.D. 2008. Droplet size and stability of nano-emulsions produced by the temperature phase inversion method. *Chemical Engineering Journal* 140: 626-631.

Floury, J., Desrumaux, A. & Lardières, J. 2000. Effect of high-pressure homogenization on droplet size distribution and rheological properties of model oil-in-water emulsions. *Innovative Food Science & Emerging Technologies* 1: 127-134.

Garti, N., Spernath, A., Aserin, A. & Lutz, R. 2005. Nano-sized self assemblies of nonionic surfactants as solubilization reservoirs and microreactors for food systems. *Soft Matter.* 1: 206-218.

Ghosh, V., Mukherjee, A. & Chandrasekaran, N. 2013. Ultrasonic emulsification of food-grade nanoemulsion formulation and evaluation of its bactericidal activity. *Ultrasonic Sonochemistry* 20: 338-344.

Gutiérrez, J.M., González, C., Maestro, A., Solè, I., Pey, C.M. & Nolla, J. 2008. Nano-emulsions: New applications and optimization of their preparation. *Current Opinion Colloid Interface Science* 13: 245-251.

Guttoff, M., Saberi, A.H. & McClements, D.J. 2015. Formation of vitamin D nanoemulsion-based delivery systems by spontaneous emulsification: Factors affecting particle size and stability. *Food Chemistry* 171: 117-122.

Heurtault, B., Saulnier, P., Pech, B., Proust, J.E. & Benoit, J.P. 2003. Physicochemical stability of colloidal lipid particles. *Biomaterials* 24: 4283-4300.

Huang, Q., Yu, H. & Ru, Q. 2010. Bioavailability and delivery of nutraceuticals using nanotechnology. *Journal Food Science* 75: R50-R57.

Kentish, S., Wooster, T.J., Ashokkumar, M., Balachandran, S., Mawson, R. & Simons, L. 2008. The use of ultrasonics for nanoemulsion preparation. *Innovative Food Science & Emerging Technologies* 9(2): 170-175.

Komaiko, J. & McClements, D.J. 2015a. Food grade nanoemulsion filled hydrogels formed by spontaneous emulsification and gelation: Optical properties, rheology, and stability. *Food Hydrocolloids* 46: 67-75.

Komaiko, J. & McClements, D.J. 2015b. Low-energy formation of edible nanoemulsions by spontaneous emulsification: Factors influencing particle size. *Journal of Food Engineering* 146: 122-128.

Komaiko, J. & McClements, D.J. 2014. Optimization of isothermal low-energy nanoemulsion formation: Hydrocarbon oil, non-ionic surfactant, and water system. *Journal of Colloid and Interface Science* 425: 59-66.

Kourniatis, L.R., Spinelli, L.S., Piombini, C.R. & Mansur, C.R. 2010. Formation of orange oil-in-water nanoemulsions using nonionic surfactant mixtures by high pressure homogenizer. *Colloid Journal* 72(3): 396-402.

Kwon, S.S., Kong, B.J., Cho, W.G. & Park, S.N. 2015. Formation of stable hydrocarbon oil-in-water nanoemulsions by phase inversion composition method at elevated temperature. *Korean Journal of Chemical Engineering* 32(3): 540-546.

Li, P. & Chiang, B. 2012. Process optimization and stability of D-limonene-in-water nanoemulsions prepared by ultrasonic emulsification using response surface methodology. *Ultrasonic Sonochemistry* 19: 192-197.

Mao, L., Yang, J., Xu, D., Yuan, F. & Gao, Y. 2009. Effect of homogenization models and emulsifiers on the physicochemical properties of β-carotene nanoemulsions prepared by high pressure homogenization. *Food Technology and Biotechnology* 47: 336-342.

Maté, J., Periago, P.M. & Palop, A. 2016. When nanoemulsified, D-limonene reduces Listeria monocytogenes heat resistance about one hundred times. *Food Control* 59: 824-828.

McClements, D.J. 2005. *Food Emulsions: Principles, Practice, and Techniques*. 2nd ed. Boca Raton: CRC Press.

Mei, Z., Liu, S., Wang, L., Jiang, J., Xu, J. & Sun, D. 2011. Preparation of positively charged oil/water nano-emulsions with a sub-PIT method. *Journal of Colloid and Interface Science* 361: 565-572.

Neethirajan, S. & Jayas, D.S. 2011. Nanotechnology for the food and bioprocessing industries. *Food and Bioprocess Technology* 4(1): 39-47.

Otoni, C.G., de Moura, M.R., Camilloto, G.P., Cruz, R.S., Lorevice, M.V., Soares, N.F.F. & Mattoso, L.H.C. 2014. Antimicrobial and physical-mechanical properties of pectin/papaya puree/cinnamaldehyde nanoemulsion edible composite films. *Food Hydrocolloids* 41: 188-194.

Polychniatou, V. & Tzia, C. 2014. Study of formulation and stability of co-surfactant free water-in-olive oil nano- and submicron emulsions with food grade non-ionic surfactants. *Journal of the American Oil Chemists Society* 91: 79-88.

Qian, C. & McClement, D.J. 2011. Formation of nanoemulsions stabilized by model food-grade emulsifiers using high-pressure homogenization: Factors affecting particle size. *Food Hydrocolloids* 25: 1000-1008.

Rebolleda, S., Sanz, M.T., Benito, J.M., Beltrán, S., Escudero, I. & San-José, M.L.G. 2015. Formulation and characterisation of wheat bran oil-in-water nanoemulsions. *Food Chemistry* 167: 16-23.

Salvia-Trujillo, L., Rojas-Graü, A., Soliva-Fortuny, R. & Martín-Belloso, O. 2013a. Effect of processing parameters on physicochemical characteristics of microfluidized lemongrass essential oil-alginate nanoemulsions. *Food Hydrocolloids* 30: 401-407.

Salvia-Trujillo, L., Rojas-Graü, A., Soliva-Fortuny, R. & Martín-Belloso, O. 2013b. Physicochemical characterization of lemongrass essential oil-alginate nanoemulsions: Effect of ultrasound processing parameters. *Food and Bioprocess Technology* 6(9): 2439-2446.

Sari, T.P., Mann, B., Kumar, R., Sing, R.R.B., Sharma, R., Bhardwaj, M. & Athira, S. 2015. Preparation and characterization of nanoemulsion encapsulating curcumin. *Food Hydrocolloids* 43: 540-546.

Shah, P., Bhalodia, D. & Shelat, P. 2010. Nanoemulsion: A pharmaceutical review. *Set. Rev. Pharm.* 1: 24-32.

Silva, H.D., Cerqueira, M.Â. & Vicente, A.A. 2012. Nanoemulsions for food applications: Development and characterization. *Food and Bioprocess Technology* 5: 854-867.

Singh, G., Maurya, S., deLampasona, M.P. & Catalan, C.A.N. 2007. A comparison of chemical, antioxidant and antimicrobial studies of cinnamon leaf and bark volatile oils, oleoresins and their constituents. *Food and Chemical Toxicology* 45: 1650-1661.

Solans, C., Izquierdo, P., Nolla, J., Azemar, N. & Garcia-Celma, M.J. 2005. Nano-emulsions. *Current Opinion in Colloid & Interface Science* 10: 102-110.

Sow, L.C., Tirtawinata, F., Yang, H., Shao, Q. & Wang, S. 2017. Carvacrol nanoemulsion combined with acid electrolyzed water to inactivate bacteria, yeast *in vitro* and native microflora of shredded cabbages. *Food Control* 76: 88-95.

Tadros, T., Izquierdo, P., Esquena, J. & Solans, C. 2004. Formation and stability of nano-emulsions. *Advances in Colloid and Interface Science* 108-109: 303-318.

Tang, S.Y., Shridharan, P. & Sivakumar, M. 2013. Impact of process parameters in the generation of novel aspirin nanoemulsions - Comparative studies between ultrasound cavitation and microfluidizer. *Ultrasonic Sonochemistry* 20: 485-497.

Vanhecke, T., Landers, J.J., Hamouda, T. & Baker, J.R. 2002. The fungicidal activity of novel nanoemulsion (X8W60PC)

against clinically important yeast and filamentous fungi. *Mycopathologia* 155: 195-201.

Vargas, M., Cháfer, M., Albors, A., Chiralt, A. & González-Martínez, C. 2008. Physicochemical and sensory characteristics of yoghurt produced from mixtures of cows' and goats' milk. *International Dairy Journal* 18(12): 1146-1152.

Wei, Q.Y., Xiong, J.J., Jiang, H., Zhang, C. & Ye, W. 2011. The antimicrobial activities of the cinnamaldehyde adducts with amino acids. *International Journal of Food Microbiology* 150: 164-170.

Weiss, J., Gaysinsky, S., Davidson, M. & McClements, J. 2009. Chapter 24 - Nanostructured encapsulation systems: Food antimicrobials. In *Global Issues in Food Science and Technology,* edited by Barbosa-Cánovas, G., Mortimer, A., Lineback, D., Spiess, W., Buckle, K. & Colonna, P. New York: Academic Press. pp. 425-479.

Yang, Y. & McClements, D.J. 2013. Encapsulation of vitamin E in edible emulsion fabricated using a natural surfactant. *Food Hydrocolloids* 30: 712-720.

Yildirim, S.T., Oztop, M.H. & Soyer, Y. 2017. Cinnamon oil nanoemulsions by spontaneous emulsification: Formulation, characterization and antimicrobial activity. *LWT - Food Science and Technology* 84: 122-128.

Zahi, M.R., El Hattab, M., Liang, H. & Yuan, Q. 2017. Enhancing the antimicrobial activity of D-limonene nanoemulsion with the inclusion of e-polylysine. *Food Chemistry* 221: 18-23.

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