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Tamarind kernel powder, gum arabic and maltodextrin as a novel combination for encapsulating agents of phenolic antioxidants

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Abstract

The effect of tamarind kernel powder (TKP, 0-2.7% w/w), gum arabic (GA, 0-18% w/w) and maltodextrin (MD, 0-27% w/w) on droplet size, rheological properties, ζ-Potential, encapsulation efficiency and the stability of W/O/W emulsions from methyl gallate (MG) and mango seed kernel (MSK) extract was investigated. The result shows that increasing GA and MD reflected to small droplet size. The viscosity was directly related to GA, MD and TKP. The higher concentration of MD and GA reflected to lower creaming layer. The higher encapsulation efficiency was found with increasing the concentration of GA and MD. The stability and encapsulation efficiency results of MSK extract in W/O/W double emulsion was higher than that of MG emulsion. The results suggested that the novel combination of TKP, GA and MD has a potential usage as wall material in phenolic encapsulation.

Introduction

Several factors have been reported as affecting the stability of W/O/W emulsions. These include the method of preparation, the composition of the emulsion, i.e. the nature of oil phase, type of emulsifiers and the nature of entrapped materials (Florence and Whitehill, 1985; Omotosho *et al*., 1986). There were many reports showed that the double emulsions properties were enhanced when the adsorbed biopolymer complex was thicker (Benichou *et al*., 2007) Nevertheless, the characteristics of adsorbed complexes, and the structures of mixed biopolymer interfaces, are still poorly understood (Dickinson, 2008). Generally, polysaccharides contribute to the stability of emulsions through their thickening and steric stabilizing characteristics (Dickinson, 2011).

Natural polysaccharides due to their stability at the interface or their functions as stabilizer can be used in W/O/W emulsion alone or in combination with other polysaccharides. These polysaccharides may be included in the external aqueous phase of W/O/W emulsion to improve the encapsulation efficiency (EE) and stability.

Tamarind (*Tamarindus Indica*) seed gum has a β(1→4) linked glucopyranosyl backbone which is frequently branched at O-6 position with short chains of one or two D-xylopyranosyl capped with D-xylopyranosyl, D-galactopyranosyl or L-arabinofuranosyl units. Tamarind kernel powder (TKP) disperses and hydrates quickly in cold water (Rao and Srivastava, 1973). The viscosity is higher if it heated at 100°C for 20-30 min (Rao and Srivastava, 1973). It is a good creaming agent, a good source of gelling polysaccharides called 'polyose' and having good film forming properties (Glicksman, 1986). Tamarind seed polysaccharide has excellent stability over the acid pH range (Rao and Srivastava, 1973). TKP is used as a thickening, stabilizing and gelling agent in the food industry, particularly in Japan where it is a permitted food additive (Glicksman, 1986; Gidley *et al*., 1991).

Gum Arabic (GA) is a complex hetero-polysaccharide containing about 2% proteinaceous matter of which a substantial part of the protein consists of the amino acids hydroxyproline, serine and proline (Osman *et al*., 1993). The chemical structure of GA commonly referred to as the arabinogalactan–protein (AGP, about 10 wt% of total), arabinogalactan (AG, about 90–99 wt% of total) and glycoprotein (GP, about 1 wt% of total) (Phillips and Williams, 2000). It has been shown that the protein-containing fraction of this gum of AGP adsorbs on the oil-water interface. Therefore, GA can be used not only as a stabilizer for increasing emulsion viscosity, but it also contributes as emulsifier to create surface active layer at water-

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oil interface. Problems associated with the use of GA in emulsions are high cost and limited supply.

Maltodextrins (MD) are defined as starch hydrolysis products with dextrose equivalent (DE) less than 20. Starch degradation products in MD extend from oligomeres to macromolecules and different DE value. MD contain linear amylose and branched amylopectin degradation products. MD has different physicochemical properties like solubility, freezing temperature, viscosity etc (Dokić *et al*., 1998; Wang and Wang, 2000). MD can form weak gels that are results of interactions between amylose fractions characterised by helical regions and branched and linear chains of amylopectin molecules (Chronakis, 1998). MD is not particularly surface-active, and so their main stabilizing action in emulsions is believed to be through viscosity modification or gelation of the aqueous continuous phase (Dickinson, 2003).

The extract of mango seed kernel (MSK) was shown to be good source of phenolic antioxidants with metal chelating and tyrosinase inhibitory activity (Maisuthisakul and Gordon, 2009.). The components present in MSK extract included gallotannins and condensed tannin-related polyphenols (Arogba, 1997). The extract from the MSK cultivar Chok-Anan contained total phenolics at a concentration of 194.1 mg GAE/g dry weight of MSK, of which 85.7% was identified as methyl gallate (Maisuthisakul and Gordon, 2011a.). In addition, 1,2,3,4,6-penta-O-galloyl-beta-D-glucopyranose, methyl gallate and gallic acid have been identified as components of an ethanolic extract of the Thai mango seed kernel cultivar Fahlun (Nithitanakool *et al*., 2009).

In this work, our objective was to systematically study the effects of polysaccharide combination of TKP, GA and MD on droplet size characteristics, rheological properties, ζ-Potential, encapsulation efficiency and the stability of W/O/W emulsion from MSK extract and methyl gallate.

Materials and Methods

Materials

Sun dried seeds from ripened mango (*Mangifera indica* cultivar Chok-Anan) were donated from a mango processing manufacturer in Thailand from March to June in 2008 as by-products. Moisture content on a dry weight basis according to AOAC (AOAC, 1990) of dried MSK equalled to 9.81±0.34%. The dried material was kept in freezer at -20°C no longer than two months. Soybean oil was purchased from local supermarket. Polyglycerol polyricinoleate (PGPR 4125, HLB~1.5, Palsgaard, Denmark) was obtained from Palsgaard Industri de Mexico (St. Louis, MO). As stated by the manufacturer, the polyglycerol moiety of the PGPR was predominantly di-, tri-, and tetraglycerols (minimum of 75%) and contained not more than 10% of polyglycerols equal to or higher than heptaglycerol. MD (DE 20) was donated by CornProduct (Thailand) Co., Ltd. (Bangkok, Thailand). They are enzyme hydrolysis products from corn starch with dextrose equivalent 16. Gelatin (Type A: Bloom 100, pI 5.2) were obtained as gift sample from Cartino gelatin Co., Ltd. (Samutprakarn, Thailand). TKP (75 µm mesh sieve) contains 69.84% polysaccharide (Mw 2.413 × 10^6 g/mole), 18.82% protein (aspartic acid 11.59-11.82 g/16gN, glutamic acid 16.91-18.53 g/16gN, serine 4.78-7.741 g/16gN and glycine 4.62-9.12 g/16gN (Marangoni *et al*., 1988)), and 8% fat (Poommarinvarakul *et al*., 2010) were obtained from G.M Ichihara (Bangkok, Thailand). GA was purchased from Bronson and Jacobs International Co. (Bangkok, Thailand). Polyoxyethylene sorbitan monoleate (Tween 80, HLB~15) purchased from Rankem (New Delhi, India), analytical grade sodium chloride (NaCl) was purchased from Merck (Darmstadt, Germany) methyl gallate was purchased from fluka (Buchs, Switzerland) and sodium azide (NaN_3) was purchased from Lab-chem (New South Wales, Australia). Folin–Ciocalteau reagent was obtained from Sigma Chemical Co., Ltd (St. Louise, USA).

Extraction of mango seed kernel

The freezing kernel (80 g) was blended for 1 min with 95% ethanol and refluxed with 1.2 M hydrochloric acid in ethanol for 3 h. The supernatant, after filtration through cheesecloth and Whatman No 4 filter paper, was evaporated under vacuum. Sample was dried in a freeze dryer and stored in aluminum foil after flushing with nitrogen at -20°C until usage.

Preparation of W1/O/W2 emulsions

$W_1/O/W_2$ emulsions were prepared using a modified two-step emulsification process. The inner aqueous phase (W_1) was prepared by hydrating gelatin (0.02% w/w), NaCl (0.012% w/w) and methyl gallate (0.02% w/w) in distilled water at 40°C for 2 min using moderate magnetic stirring. The oil phase was prepared by dispersing 0.64 wt % PGPR into soy bean oil (8%, w/w) and heating to 50°C for 2 min under agitation with a magnetic stirrer and then blended together using a hand homogenizer at 12,000 rpm for 2 min (IKA-Ultra-Turrax T25, Germany). The coarse emulsions were passed through high-pressure homogenizer (Armfield model FT9, UK) three times at 3000 psi.

The outer aqueous phase (W_2) was prepared by hydrating Tween 80 (0.45% w/w), TKP (0-2.7% w/w) GA (0-18% w/w) and MD (0-27% w/w) in distilled water under moderate magnetic stirring conditions. After adding the water-in-oil (W_1/O, 10 vol%) to the outer phase (W_2, 90vol%), the emulsion was homogenised for 2 min using a hand homogenizer at 12,000 rpm. The emulsions were heated at 50°C for 30 min and then add sodium azide (0.02% w/w) as an antibacterial agent.

Emulsion droplet size

Average droplet sizes of W/O/W emulsions were measured using a dynamic light scattering instrument (Zeta sizer nano, Malvern Instruments, Worcestershire, U.K.). To avoid multiple scattering effects, W/O/W emulsions were diluted to a droplet concentration of approximately ~0.005 wt % using distilled water.

ζ-Potential

ζ-Potential of W/O/W emulsions were measured using dynamic light scattering instrument (Zeta sizer nano, Malvern Instruments, Worcestershire, U.K.)

Rheological measurement

The viscosity of each W/O/W double emulsion was determined using a rotational rheometer (MCR 300, Physica, Stuttgart, Germany). W/O/W double emulsion was transferred to the double gap cylinder geometry DG 26.7 and equilibrates for 3 min at 25°C. The samples were subjected to the specified shear at 30 s^{-1}.

Creaming stability measurement

Ten grams of emulsion were transferred into a test tube (internal diameter 15 mm, height 125 mm) and then stored for 4 hours at room temperature. After storage, some emulsions separated into an optically opaque "cream" layer at the top and a transparent (or turbid) "serum" layer at the bottom. We defined the serum layer as the sum of any turbid and transparent layers. The total height of the emulsions (HE) and the height of the serum layer (HS) were measured. The extent of creaming was characterized as % serum = 100 (HS/HE) (Surh *et al*., 2007).

Determination of encapsulation efficiency

The multiple emulsions were characterized in term of encapsulation efficiency (EE) by measuring the concentration of phenolics leaked immediately after preparation of the double emulsion and after a storage period of 24h. Briefly, samples of emulsions (50 g) were centrifuged at 15,000 g at 4°C for 30 min, after which the lower layer was carefully removed and filtered using a 0.22 µm Millipore syringe filter (Milex-GV, Millipore, MA, USA). The phenolic content was determined with Folin Ciocalteu's reagent (modified from Kähkonen *et al*., 1999). The concentration of total phenolic compounds in all samples was expressed as mg of methyl gallate equivalent per g dry weight of MSK extract using a linear equation. The EE (%) was then calculated according to Maisuthisakul and Gordon (2011b).

Table 1. Central composite design: independent (X_i) and response variables ($Y_{j)}$ (mean±SD)$^{\phi}$

Run No.	Independent variable (%wt)			Response variable		
	GA (X_1)	MD (X_2)	TKP (X_3)	Droplet size (Y_1,µm)	Viscosity (Y_2,mPa.s)	Creaming index (Y_3, %)
1	9.00	0.00	1.35	0.70±0.30	20.95±0.07	56.87±0.32
2	14.40	21.60	2.15	0.33±0.10	267.00±1.41	7.14±0.86
3	14.40	5.40	0.55	0.98±0.60	42.80±0.00	50.73±0.74
4	3.60	21.60	0.55	0.66±0.70	24.75±0.21	66.99±0.76
5	14.40	21.60	0.55	0.43±0.20	211.00±2.83	4.10±0.85
6	3.60	5.40	0.55	1.15±0.20	10.65±0.21	77.35±0.37
7	3.60	21.60	2.15	0.57±0.80	54.70±0.00	47.96±0.41
8*	9.00	13.50	1.35	1.40±0.70	42.15±0.07	52.50±0.46
9	9.00	13.50	2.70	0.75±0.30	68.45±0.07	33.99±0.29
10*	9.00	13.50	1.35	1.22±0.40	41.50±0.14	53.93±0.53
11*	9.00	13.50	1.35	0.96±0.50	42.70±0.28	53.92±0.85
12	14.40	5.40	2.15	1.59±0.70	68.70±0.14	20.59±0.30
13*	9.00	13.50	1.35	0.72±0.30	42.70±0.71	49.75±0.43
14	3.60	5.40	2.15	1.80±0.50	74.80±0.28	48.13±0.38
15	9.00	13.50	0.00	0.69±0.40	25.60±0.42	52.67±0.48
16*	9.00	13.50	1.35	0.83±0.10	41.90±0.00	59.89±0.69
17	0.00	13.50	1.35	1.32±0.20	22.15±0.07	70.48±0.82
18*	9.00	13.50	1.35	0.80±0.00	42.30±0.57	56.71±0.65
19	18.00	13.50	1.35	0.63±0.4	208.00±1.41	7.54±0.07
20	9.00	27.00	1.35	0.40±0.1	167.00±0.00	3.11±0.07

$^{\phi}$GA: Gum arabic, MD: Maltodextrin ,TKP: Tamarind kernel powder.
*Center point.

Experimental design and statistical analysis

A Central Composite Design was used to study the response pattern (encapsulation efficiency, ζ-Potential, droplet size, creaming index and viscosity) and to determine the optimum combination of GA, MD and TKP concentrations. Twenty emulsion samples were established based on the CCD with three independent variables at five levels on each variable (Table 1). Multiple regression analysis was applied to predict the linear, quadratic and interaction terms of the independent variables in the response surface models (RSM). The RSM was applied to the experimental data using Design-Expert version 6.0.2 (Trial version) (Statease Inc., Minneapolis, USA). The generalized response surface model for describing the variation in response variables is given below:

$$Y = b0 + b1x1 + b2x2 + b3x3 + b11x12 + b22x22 + b33x32 + b12x1x2 + b13x1x3 + b23x2x3 + \varepsilon \quad (1)$$

Data were modeled by multiple regression

analysis adopting stepwise analysis. The variables significant at $p<0.05$ levels were only selected for the model construction. The significant terms in the model were found by analysis of variance (ANOVA) for each response. The adequacy of model was checked accounting for lack of fit, adequate precision and pure error.

Results and Discussion

To understand the effect of GA, MD and TKP as wall materials in W/O/W emulsions for encapsulation MSK extract and methyl gallate, which is main active ingredient, are used to study. The experiment was optimized with respect to the stability of the emulsion and the encapsulation efficiency by using response surface methodology (RSM). The double emulsions containing MSK extract were used to compare with the optimized formulation emulsion of methyl gallate to elucidate other minor ingredients (such as other phenolic substances, protein, sugar, etc) of MSK extract affected encapsulation emulsion stability.

ζ-Potential of W/O/W emulsions was not modified by GA, MD and TKP content. Average ζ-Potential was not significantly different for each treatment. It was found that the ζ-Potential value for the emulsions ranged from (-44.75) – (-54.5) mV (data not show).

Droplet size

The droplet size distribution of the double emulsion droplets was determined using dynamic light scattering. The effect of polysaccharides on the size of emulsion droplets is essential and highly dependent on its viscosity and concentration. Figure 1 show the perturbation plots which were drawn to illustrate the main effect of the independent variables on dependent ones. The droplet size decreased by increasing the concentration of MD and GA, while increasing the content of TKP had no effect on the droplet size. The result was consistent to fitting model (Table 2). The coefficient value of MD and GA were -0.30 and -0.15, respectively, that mean the MD had affected on the droplet size higher than GA. The minus value means the higher concentration; the lower size will be obtained. Fitting of the data to various models (linear, two factorial interactions (2FI), quadratic and cubic) showed that the reactions of droplet size was most suitably described by a linear model (adjust R^2 = 0.42) (Table 2).

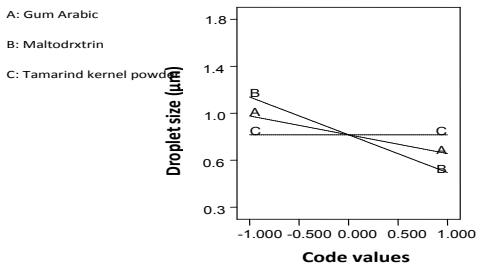

Figure 1. The perturbation plots for the effect of gum Arabic (GA), altodextrin (MD) and tamarind kernel powder (TKP) concentrations on the mean diameter of double emulsion droplets.

The fitting of the model was checked by the determination of lack of fit, adequate precision and pure error. The lack of fit test measured a variation of the data around the fitted model. If the model did not fit the data well, the lack of fit test would be significant (Montgomery, 2001). The analysis of variance to the models source revealed that the lack of fit test for the linear model was non-significant

Table 2. Table of ANOVA for the experimental variables as a linear, quadratic and interaction term of each response variable and corresponding coefficients for the predictive models

Source	DF	Droplet size (µm)			DF	Viscosity (mPa.s)			DF	Creaming index (%)			DF	Encapsulation efficiency (%)		
		Coefficient	Sum of squares	p-value		Coefficient	Sum of squares	p-value		Coefficient	Sum of squares	p-value		Coefficient	Sum of squares	p-value
Model	2	0.90	1.49	0.0036	7	39.39	1.028E+005	< 0.0001	7	51.79	9582.54	< 0.0001	4	66.93	1637.26	0.0002
Linear																
b_1	1	-0.15	0.30	0.0930	1	53.98	39790.03	< 0.0001	1	-19.31	5092.76	< 0.0001	1	-0.32	*1.39*	*0.8454*
b_2	1	-0.30	1.19	0.0023	1	44.38	26901.40	< 0.0001	1	-11.79	1898.57	< 0.0001	1	-3.83	*200.17*	*0.0310*
b_3	-	-	-	-	1	16.16	3565.03	0.0002	1	-8.71	1035.32	0.0001	1	5.81	*460.42*	*0.0026*
Quadratic																
b_{11}	-	-	-	-	1	27.41	10830.85	< 0.0001	1	-4.32	271.09	0.0139	-	-	-	-
b_{22}	-	-	-	-	1	19.95	5738.31	< 0.0001	1	-7.50	819.67	0.0003	-	-	-	-
b_{33}	-	-	-	-	1	6.24	560.53	0.0550		-	-	-	-	-	-	-
Interaction																
b_{12}	-	-	-	-	1	46.56	17344.53	< 0.0001	1	-6.19	306.90	0.0099	1	11.04	*975.27*	*< 0.0001*
b_{13}	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-
b_{23}	-	-	-	-	-	-	-	-	1	5.42	235.12	0.0201	-	-	-	-
Residual	17		1.59		12		1488.46		12		393.38		15		530.05	
Lack-of-fit	12		1.17	0.4623	7		1255.91	0.0782	7		367.35	0.0108	*10*		*460.24*	*0.1000*
Pure error	5		0.42		5		232.55		5		26.03		5		*69.81*	
Total	19		3.08		19		1.043E+005		19		9975.92		19		2167.31	
R^2		0.4837				0.9857				0.9606				0.7554		
Adj-R^2		0.4229				0.9774				0.9376				0.6902		
CV		34.10				14.66				13.10				8.88		
Adeq precision		8.394				33.135				21.987				13.913		

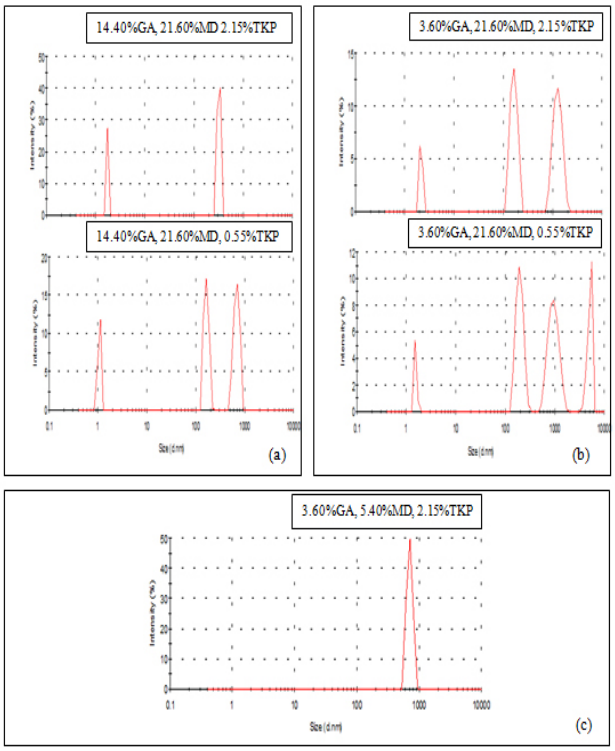

Figure 2. Droplet size distributions of (a), (b) some oil globules in W/O/W emulsion with higher and lower concentration of tamarind kernel powder (TKP) at similar concentration of gum Arabic (GA) and maltodextrin (MD); (c) the oil globules in W/O/W emulsion at the concentration 3.60% gum Arabic (GA), 5.40% maltodextrin (MD) and 2.15% tamarind kernel powder (TKP).

at 0.46. The adequate precision measures signal to noise ratio was computed by dividing the difference between the maximum predicted response and the minimum predicted response by the average standard deviation of all predicted responses. Ratios greater than 4 are desirable (Montgomery, 2001). In this result the value was 8.39 which were well above 4, indicated adequate signals to use this model to navigate the design space. And the pure error was very low (0.42), indicating a good reproducibility of the experimental data.

The droplet size distribution was found to be comparatively different among the emulsions in terms of their peak and maximum. Although TKP did not affect average droplet size diameter, yet at the higher concentration of TKP, the number of peak distribution was less than that at lower concentration (Figure 2a and b). The mono-dispersed emulsion was found in the emulsions prepared with 3.60% GA, 5.40%MD and 2.15%TKP (Figure 2c). The results showed that TKP lead to a creation of uniformity of droplet. It might be explained by a higher viscosity at the higher concentration (>2.00%) of TKP.

The viscosity change for TKP dispersion was substantial (15.64×10^{-2} Pa s at 1.15% gum to 29.50 Pa s at 2.75% gum) (Khounvilay and Sittikijyothin, 2012) compared to the GA and MD dispersion which was only 1.32×10^{-2} and 0.74×10^{-2} Pa s at 18.00% gum (Imagi *et al*., 1990).

Viscosity

The viscosity of an emulsion is an important characteristic since it influences the rate of creaming, the physical shelf-life of the product. The viscosity was directly related to GA, MD and TKP and surprisingly interaction effect of GA and MD (Table 2). The results also revealed that, at low MD concentration, GA did not have much effect on the viscosity of the emulsion, while at higher concentration of MD, increasing the GA content increased the emulsion viscosity. It might be explained that the larger thickness of the membranes surrounding the particles affected to the apparent viscosity of the emulsions (Pal, 2011) because the droplets come into close proximity.

The linear terms of GA, MD and TKP along with their quadratic and interactions had positive effects on the viscosity of the emulsions (Table 2). The coefficient values of GA, MD and TKP were 53.98, 44.38 and 16.16, respectively, mean that the GA had affected on the viscosity higher than MD and TKP. The model also showed non-significant lack of fit test for the quadratic model. The high value of adequate precision (33.135) indicated an adequate signal. The coefficient of determination was adjusted R^2 = 0.9774. This indicates that the accuracy and general availability of the polynomial model were good. Moreover, the predicted values (data not shown) were obtained by a model fitting technique using the software design expert version 6.0.2 (Trial version) and were seen to be sufficiently correlated to the observed values.

Creaming Index

Creaming is one of the emulsions instabilities which are caused by gravity, and we measured changes in creaming index by direct observation of emulsion separation. Over time the droplets moved upwards due to gravity (Chanamai and McClements, 2001; Tudors, 2004). Changes in creaming index during storage of emulsions formed with different polysaccharide concentrations are shown in Table 1. For emulsions containing smaller droplet size, it showed high emulsions viscosity and forming a lower creaming rate. It is well known that an increase in droplet mean diameter is an indictment of droplet coalescence during storage; hence the larger droplet of emulsion is easy to combined together or rapid creaming. Moreover, the results showed that the increasing MD and GA concentrations gave smaller droplets. A further increase in biopolymer concentration caused an appreciable decrease in

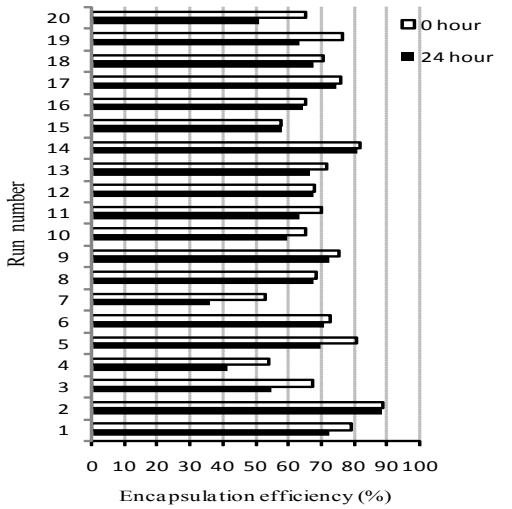

Figure 3. Effect of tamarind kernel powder (TKP), gum Arabic (GA) and maltodextrin (MD) concentrations on the encapsulation efficiencies of W/O/W emulsions at (a) 0 hour and (b) 24 hour. The polysaccharide compositions of each run number of ouble emulsion were shown in Table 1

creaming rate because the increase in aqueous phase viscosity caused the upward movement of the flocs to be retarded (Chanamai and McClements, 2001).

The data given in Table 2 indicate that the creaming index was directly related to the linear and quadratic effect of GA, MD and TKP concentration, and interaction effect of GA with MD and GA with TKP content ($p < 0.05$). Based on the sum of squares, the variables with the largest effects were the linear term of GA, MD, TKP content followed by the quadratic term of MD, interaction effect of GA with MD concentrations including the quadratic term of GA, interaction effect of MD with TKP concentrations (Table 2). All terms of GA, MD and TKP along with their interactions had negative effects on the creaming stability of the emulsions; in contrary, the response value was positively influenced by the interaction effect of MD with TKP. The results also performed that, at low TKP concentration, MD had much effect on the creaming index of the emulsion, while at higher concentration of TKP, increasing the MD content slightly decreased the emulsion creaming (data not shown). The higher concentration of MD and GA reflected to lower creaming layer. As mention before, GA and MD reflected to small droplet size but TKP did not affect size of the double emulsions. It seems that under these conditions the influence of increased viscosity is more largely compensated by the droplet size. Similar trend has been reported by Dickinson and Galazka, (Dickinson and Galazka, 1992).

Encapsulation efficiency

Encapsulation efficiency (EE) increases with increasing polymer concentration (Mehta *et al*., 1996; Rafati *et al*., 1997; Li *et al*., 1999). High viscosity and solidification of the dispersed phase contributed to reducing porosity of the microparticles as well (Schlicher *et al*., 1997). In this experiment, run number 2 which was composed of 14.40%GA, 21.60%MD and 2.15%TKP (total gum = 38.15%), found to have the highest EE both at 0 and 24 hour. Although run 20 be composed similar of total gum (37.35%), which was 9.00%GA, 27.00%MD and 1.35%TKP, found that EE was not stable from 0 to 24 hour (Figure 3). Hence, the type of polysaccharide affected the efficiency. This can be explained by viscosity of the system (Table 1). The high polymer concentration on the surface of the dispersed phase can prevents active ingredient diffusion across the phase boundary (Mehta *et al*., 1996) make the EE higher than the lower one. Another reason to obtain high EE is the viscosity of the solution. It can be delay the ingredient diffusion within the polymer droplets (Bodmeier and McGinity, 1988). This indicates that the polysaccharides composition and concentration contributed to stabilization of the double emulsion and efficient internalization of methyl gallate.

The EE decreased with decreasing TKP loadings (Table 2) or increasing GA and MD. The interaction term of GA and MD was positive effect to EE. Most emulsions showed less EE at 24 hours comparing to that at 0 hour (Figure 3). One of the major reasons for the decrease in the EE after storage of the double emulsions may be Ostwald ripening. Ng *et al*. (1996) suggested that the alleviators of Ostwald ripening are (a) an oil-insoluble solute in the inner-water phase and (b) an osmotic pressure excess of the outer-water phase compared to the inner-water phase. The osmotic balance is also known to be one of the major reasons for the instability of the W/O/W emulsions, but the osmotic balance of the two water phases in this study would influence as an inhibitor of bursting, because of the higher osmotic pressure in the outer-phase solution due to high concentration of polysaccharides compared to the inner one. On the other hand, the gradation of the osmotic balance would drive the leakage of water from inner-water phase to the outer one. The leaked water from the inner-water phase to the outer-one driven by the osmotic balance might dilute the outer-phase concentration of polysaccharides and make them instability and have lower EE after storage.

Comparison of MSK extract and methyl gallate double emulsion

The double emulsion using 0.029% (w/w) of MSK extract was prepared according to run number

14 (3.60%GA, 5.40%MD and 2.15%TKP). The preparation procedure was according to 2.2. The results of droplet size and viscosity of MSK extract emulsions were similar to those of methyl gallate emulsion (data not shown). However, the EE obtained from MSK extract emulsion (96.02%) was higher than that of methyl gallate (81.95%). Interestingly, creaming index of MSK extract emulsion equaled to zero, whereas the creaming index of methyl gallate emulsion was 48.13%. The results suggested that minor composition of MSK extract had greatly affected to emulsion stability.

The response optimization was achieved with respect to the desired criteria, and was based on the optimization of the emulsion stability and the encapsulation efficiency, which are thought to be the most important parameters in product development studies. Apart from this, the optimized viscosity was also taken into account. The solutions were obtained from the software, which sought to maximize the desirability function by being at random starting points and proceeding on the path of the steepest slope to a maximum. The optimum emulsion composition containing the formulation which was practically prepared and corresponding criteria for maximum of EE value, droplet size is in range 0.33-1.8 μm, minimum of creaming index and viscosity is in range 10.65-75 mPa.s. The overall optimum region resulting in a desirable double emulsion was predicted to be obtained with 9.00% (w/w) GA, 13.50% (w/w) MD and 2.48% (w/w) TKP. At this condition, the emulsion properties showed EE equaled 75.17, 0.89 (droplet size), 39.40 (creaming index) and 74.98 (viscosity), respectively. This formulation is further used to produce dry encapsulated powder via spray dry and freeze dry.

For validation the model, the adequacy of the response surface equations was checked by the comparison of experimental and fitted values predicted by the response regression models. No significant difference ($p > 0.05$) was found between the experimental and predicted values (data not shown).

Conclusions

In conclusion, this study has shown that the combination of TKP, GA and MD did not significantly change the ζ-Potential, but affected the droplet size, viscosity, creaming index and encapsulation efficiency. The droplet size and viscosity of MSK extract emulsion were similar to those of methyl gallate emulsion. Whereas MSK extract emulsion gave lower creaming index and higher encapsulation efficiency than methyl gallate double emulsions. Optimum formulation for a stable emulsion was found to be 9.00% (w/w) GA, 13.50% (w/w) MD and 2.48% (w/w) TKP.

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References

AOAC. 1990. Official Methods of Analysis. 15th Ed., Association of Official Analytical Chemists, Arlington, VA. USA .

Arogba, S. S. 1997. Physical, Chemical and Functional Properties of Nigerian mango (*Mangifera indica*) kernel and its processed flour. Journal of the Science of Food and Agriculture 73: 321–328.

Benichou, A., Aserin, A. and Garti, N. 2007. W/O/W double emulsions stabilized with WPI–polysaccharide complexes. Colloids and Surfaces A: Physicochemical and Engineering Aspects 294: 20–32.

Bodmeier, R. and McGinity, J. W. 1988. Solvent selection in the preparation of PLA microspheres prepared by the solvent evaporation method. International Journal of Pharmaceutics 43: 179-186.

Chanamai, R. and McClements, D. J. 2001. Depletion flocculation of beverage emulsions by gum Arabic and modified starch. Journal of Food Science 66: 453-463.

Chronakis, I. S. 1998. On the molecular characteristics, compositional property and structural functional mechanisms of maltodextrins: A review. Critical Reviews in Food Science and Nutrition 38: 599–673.

Dickinson, E. 2003. Hydrocolloids at interfaces and the influence on the properties of dispersed systems. Food Hydrocolloids 17: 25–39.

Dickinson, E. 2008. Interfacial structure and stability of food emulsions as affected by protein–polysaccharide interactions. Soft Matter 4, 932–942.

Dickinson, E. 2011. Double Emulsions Stabilized by Food Biopolymers. Food Biophysics 6: 1-11.

Dickinson, E. and Galazka, V. B. 1992. Emulsion stabilization by protein/polysaccharide complex. In Philips, G. O., Wedlock, D. J. and Williams, P. A. (Eds). Gum and stabilisers for the food industry, Vol. 6, p 351-362. Oxford: IRL Press.

Dokić, P., Jakovljević, J. and Dokić-Baucal, L. 1998. Molecular characteristics of maltodextrins and rheological behaviour of diluted and concentrated solutions. Colloids and Surfaces A: Physicochemical and Engineering Aspects 141: 435–440.

Florence, A. T. and Whitehill, D. 1985. Stability and Stabilization of water-in-oil-in-water multiple emulsions. In Shah, D. O. (Ed). Macro and

Microemulsions: Theory and Application, p. 359–380. Washington, DC: American Chemical Society.

Gidley, M. J., Lillford, P. J., Rowlands, D. W., Lang, P., Dentini, M. and Crescenzi, V. 1991. Structure and solution properties of tamarind-seed polysaccharide. Carbohydrate Research 214: 299-314.

Glicksman, M. 1986. Tamarind seed gum. In M. Glicksman (Ed). Food Hydrocolloids VIII, p. 191-202. Florida: CRC Press Inc.

Imagi, J., Kako, N., Nakanishi, K. and Matsuno, R. 1990. Entrapment of Liquid Lipids in Matrixes of Saccharides. Journal of Food Engineering 12: 207-222.

Kähkonen, M. P., Hopia, A. I., Vuorela, H. J., Rauha, J.P., Pihlaja, K. and Kujala, T. S. 1999. Antioxidant activity of plant extract containing phenolic compounds. Journal of Agricultural and Food Chemistry 47: 3954–3962.

Khounvilay, K. and Sittikijyothin, W. 2012. Rheological behaviour of tamarind seed gum in aqueous solutions. Food Hydrocolloids 26: 334-338.

Li, X., Deng, X., Yuan, M., Xiong, C., Huang, Z., Zhang, Y. and Jia, W. 1999. Investigation on process parameters involved in preparation of polylactide-poly(ethylene glycol) microspheres containing Leptospira Interrogans antigens. International Journal of Pharmaceutics 178: 245-255.

Maisuthisakul, P. and Gordon, M. H. 2009. Antioxidant and tyrosinase inhibitory activity of mango seed kernel by product. Food Chemistry 117: 332-341.

Maisuthisakul, P. and Gordon. M. H. 2011a. Characterization and storage stability of the extract of Thai mango (*Mangifera indica* Linn. Cultivar Chok-Anan) seed kernels. Journal of Food Science and Technology, Revised.

Maisuthisakul, P. and Gordon. M. H. 2011b. Effect of oil, surfactant and phenolic extract from mango seed kernel on the physicochemical properties of W/O/W emulsions. African Journal of Biotechnology 10: 14937-14947.

Marangoni, A., Alli, I. and Kermasha, S. 1988. Composition and properties activities activities of seeds of the true legume tamarindus indica. Journal of Food Science 53: 1452-1455

Mehta, R. C., Thanoo, B. C. and DeLuca, P. P. 1996. Peptide containing microspheres from low molecular weight and hydrophilic poly (D,L-lactide-co-glycolide). Journal of Controlled Release 41: 249- 257.

Montgomery, D. C. 2001. Design and analysis of experiments (5th ed.), p. 455-492. New York: John Wiley & Sons Inc.

Ng, J. D., Lorber, B., Witz, J., Théobald-Dietrich, A., Kern, D. and Giegé, R. 1996. The crystallization of biological macromolecules from precipitates: evidence for Ostwald ripening. Journal of Crystal Growth 168: 50-62.

Nithitanakool, S. Pithayanukul, P. and Bavovada, R. 2009. Antioxidant and Hepatoprotective Activities of Thai mango seed kernel Extract. Planta Medica 75: 1118-1123.

Omotosho, J., Whateley, T., Law, T. and Florence A. 1986. The nature of the oil phase and the release of solutes from multiple (W/O/W) emulsions. Journal of Pharmacy and Pharmacology 38: 865–870.

Osman, M. E. Williams, P. A. Menzies, A. R. and Phillips, G. O. 1993. Characterization of commercial samples of gum Arabic. Journal of Agricultural and Food Chemistry 41: 71–77.

Pal, R. 2011. Rheology of simple and multiple emulsions. Current Opinion in Colloid & Interface Science 16: 41–60.

Phillips, G. O. and Williams, P. A. 2000. Gum Arabic. In Phillips,

G. O. and Williams, P. A. (Eds). Handbook of Hydrocolloids p. 155-168. Florida: CRC Press, Inc.

Poommarinvarakul, S., Tattiyakul, J. and Muangnapoh, C. 2010. Isolation and Rheological Properties of Tamarind Seed Polysaccharide from Tamarind Kernel Powder Using Protease Enzyme and High-Intensity Ultrasound. Journal of Food Science 75: 253-260.

Rafati, H., Coombes, A. G. A., Adler, J., Holland, J. and Davis, S. S. 1997. Protein-loaded PLGA microparticles for oral administration: formulation, structural and release characteristics. Journal of Controlled Release 43: 89-102.

Rao, P. S. and Srivastava, H. C. 1973. Tamarind. In Whistler R. L. (Ed). Industrial gums (2nd ed) p.369-411. New York: Academic Press.

Schlicher, E. J. A. M., Postma, N. S., Zuidema, J., Talsma, H. and Hennink, W. E. 1997. Preparation and characterization of poly(D,L-lactic-co-glycolic acid) microspheres containing desferrioxamine. International Journal of Pharmaceutics 153: 235-245.

Surh, J., Vladisavljevica, G. T., Mun, S. and McClements, D. J. 2007. Preparation and Characterization of Water/Oil and Water/Oil/Water Emulsions Containing Biopolymer-Gelled Water Droplets. Journal of Agricultural and Food Chemistry 55: 175-184.

Tudors, T. 2004. Application of rheology for assessment and prediction of the long-term physical stability of emulsions. Advances in Colloid and Interface Science 108–109: 227–258.

Wang, Y.J. and Wang, L. 2000. Structures and properties of commercial maltodextrins from corn, potato and rice starches. Starch/Starke 52: 296–304.