

PHYSICAL PROPERTIES OF DRESSING-TYPE OIL IN WATER EMULSIONS AS AFFECTED BY OKRA GUM-XANTHAN GUM-CORN STARCH INTERACTIONS

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ABSTRACT

Okra gum is one of potential hydrocolloids due to its' suspending and emulsifying abilities and blending it with other gums shall widen its application in emulsion-based food products. Therefore, interaction effects of okra gum (OG), xanthan gum (XG) and corn starch (CS) on the physical properties of dressing-type emulsions were investigated in this study using a simplex-centroid mixture design. Application of OG:CS blend resulted in smaller droplet size range (5.40 – 11.22 μm) compared to XG:CS (4.82 – 13.97 μm) with the same gum ratio (i.e. 1:1), due to emulsifying property of OG. Emulsion prepared with this blend also exhibited better viscosity (30.43 \pm 9.81 Pa.s) and turbidity (Absorbance value = 0.95 \pm 0.12) compared to other emulsions with individual OG or CS. Regression modelling further confirmed the occurrence of synergistic interaction in OG:CS blend which positively affected these emulsion properties. Droplet size and turbidity responses were successfully fitted with a special cubic model while viscosity response was successfully fitted with quadratic model (no lack-of-fit, $p > 0.05$). The models are useful for predicting the respective responses to any blend combination of the components. Results of this study signify that OG is a promising hydrocolloid showing synergism with CS and this synergism can be manipulated to overcome lack of OG performance in food emulsion.

Key words: Emulsion, okra gum, polysaccharide blends, synergistic effect, properties

INTRODUCTION

Okra (*Abelmoschus esculentus* L.) or commonly known as lady's fingers belongs to Malvaceae family is a tropical perennial crop growing natively throughout Africa, Middle East, Asia and the southern states of USA. Soluble polysaccharide or gum from seedless okra pods can be extracted using aqueous medium with acetone precipitation (Zaharuddin *et al.*, 2014). When dispersed in water, Okra gum (OG) produces viscous and slimy dispersion due to its polysaccharide nature, which can be applied as natural food-grade thickener and stabilizer (Georgiadis *et al.*, 2010). The gum however have a very weak gelling property which is otherwise commonly exhibited by pectin, another type of soluble polysaccharide (Nazaruddin *et al.*, 2011). As evidenced in its infrared spectrum, OG is

identified as a random coil polysaccharide mainly consists of galactose, rhamnose and galacturonic acid represented by C-O stretching within infrared frequency of 1200 – 1000 cm^{-1} (Zaharuddin *et al.*, 2014). The repeating units comprised of (1-2) rhamnose and (1-4) galacturonic acid residues with disaccharides side chains (Alamri *et al.*, 2012). The side chains composed of galactose attached to O-4 of half of the rhamnose residues (Tomada *et al.*, 1980). The viscosity of OG dispersion arises from physical entanglement of the random coil in its disordered conformation (Saha & Bhattacharya, 2010). In addition to its galactose constituent, OG has been reported to contain a covalently-bound peptides which could also explain its emulsifying capacity (Alamri *et al.*, 2013). Both viscosity producing and emulsifying effects are of important functionalities needed in emulsion-based food products such as mayonnaises, fruit sauces and salad dressings. However, as compared to a commonly

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used gum i.e. xanthan gum (XG), OG exhibits much less thickening ability in food emulsion and thus causes lower viscosity of such products. It is suggested that OG is a more flexible polymer chain which exhibits a smaller chain dimension, giving rise to smaller intrinsic viscosity (Doublier & Cuvelier, 2006) as compared to XG.

Polysaccharide blends are typically employed in order to overcome poor functionality of individual polysaccharide in food emulsion. There is a significant number of study related to blends of XG with various starches including corn starch (CS), aiming to understand their interaction which indirectly could overcome some shortcomings related to starch. According to Sikora *et al* (2007), XG and CS blend is able to provide higher viscosity on strawberry sauces as compared to individual XG or CS. This is due to molecular interactions of XG and CS, causing synergism on their intrinsic viscosity and thus overall viscosity of the sauce. The term “synergism” is used to indicate that a combination of two polysaccharides provides a stronger effect on viscosity than would be anticipated by adding the individual contribution of each polysaccharide. Nevertheless, studies related to blend of OG with other polysaccharides are hardly found. Thus, the present study was undertaken to determine the interaction effects of OG, XG and CS on the physical properties of dressing-type oil-in-water (O/W) emulsions.

MATERIALS AND METHODS

Materials

Okra was purchased from Jabatan Pertanian Lundang, Kelantan. Xanthan gum and CS were purchased from Sigma-Aldrich Company. The materials for dressing-type emulsions such as soybean oil, vinegar and eggs were purchased from local market.

Extraction of okra gum

Okra gum was extracted from seedless pods of okra fruits. The pods (100 g) were homogenized with distilled water at 1:4 ratio by using a Waring blender. The viscous homogenate was heated at 70°C for 30 minutes in a shaking water bath (PROTECH 903). The viscous homogenate was cooled and filtered through a white muslin cloth. Acetone was added to the filtrate (3:1) to precipitate the gum. The gum obtained was dried overnight in the oven at 40°C followed by pulverizing and screened with a 0.125 mm stainless steel sieve. Powdered form of OG was stored in a capped amber container for further uses (modified from Ameena *et al.*, 2010).

Preparation of polysaccharide blends

The OG, XG and CS dispersions were prepared by dispersing 1.25 g of polysaccharide powder in 100 g distilled water with vigorous stirring at 70°C for 2 hours. The dispersions were then cooled to room temperature and left overnight prior to blend preparation to ensure a complete hydration. Further, binary blends of OG:XG, OG:CS and XG:CS were prepared at 1/2:1/2 ratio. Ternary blends of OG:XG:CS was prepared at 1/3:1/3:1/3 ratio (modified from Nor Hayati *et al.*, 2009).

Preparation of emulsions

The emulsions (125 g) were prepared in a lab-scale proportion using 40% soybean oil, 10% distilled water, 4% vinegar, 6% egg yolk and 40% polysaccharide dispersion. The final percentage of polysaccharide in total emulsion formulation was 0.5%. A premix was firstly prepared by homogenizing distilled water, vinegar and egg yolk at 6000 rpm. for 1 min. Soybean oil and polysaccharide dispersion were added alternately while homogenizing for 4 min at 10000 rpm. Final homogenization was done 14000 rpm. for 1 min. Homogenization was done by using a Diach 900 high-speed homogenizer (Heidolph Inst. GmbH & Co. Kg, Schwabach, Germany) under room temperature (Nor Hayati *et al.*, 2009).

Droplet size analysis

The droplet microstructure of the emulsions was observed under a polarized light by using a Nikon Advanced 80i Eclipsed microscope with eye piece camera (Dino-eye AM423x, AnMo Electronics Corporation, Taiwan). A small drop of emulsion was placed onto the microscope slide and carefully covered. After being equilibrated for 2 min under 100X magnifications, the smallest and largest size of droplet groups were determined for each emulsion based on the internal microscope scale (in μm) (modified from Nor Hayati *et al.*, 2016).

Viscosity determination

Viscosity measurements were carried out on the emulsion samples at room temperature (25°C) with a Brookfield viscometer (Brookfield DV II Pro+, Programmable Viscometer, Middleboro, MA, USA) at 1.0, 2.5, 5.0, 10.0, 20.0 r.p.m. with RV spindle (RV4 type). The disk spindle was used in accordance with the sample nature to get all readings within the scale. First measurements were taken 2 minutes after the spindle was immersed in each sample to allow thermal equilibrium in the sample and to eliminate the effect of immediate time dependence. All data were then taken after 1 minute interval for different r.p.m. The experimental data (r.p.m.) obtained were converted to shear rate was obtained from

conversion of spindle factors (torque and speed spindle) of the viscometer to viscosity function as described by Heidarinasab *et al* (2010). Graph of viscosity (Pa.s) versus shear rate (1/s) was plotted to determine the rheological behavior.

Turbidity

Turbidity was determined by measuring absorbance of diluted emulsion at 500 nm by means of a UV-visible spectrophotometer (UV-1700, Shimadzu Corp., Kyoto, Japan). The emulsions were diluted to 0.25% (w/w) in a 10% sugar solution and were stored at 1 L blue cap bottles at room temperature before the absorbance reading (Nor Hayati & Shamini, 2011).

Experimental design and statistical analysis

Binary and ternary blends of OG, XG and CS were prepared based on the simplex-centroid mixture design with 7 points. The experimental domain of this research consisted of different proportions of component of X_1 (OG), X_2 (XG) and X_3 (CS) between zero and one ($0 \leq X_i \leq 1$; $\sum X_i = 1$). The experimental domain was within an equilateral triangle (regular simplex). The three vertexes of the simplex represented the pure components, the three points at the edges of the triangle represented the two component blends and the centroid point within the triangle represented the three component blends. The component proportion of blends is illustrated in Table 1. All blends were prepared in three independent replications for all physical properties analysis and stability analysis in order to allow error estimation. Hence, a total of 21 blends were needed. A detail statistical analysis description can be found elsewhere (Nor Hayati *et al.*, 2016). Briefly, a one-way ANOVA with Tukey's Multiple Comparisons was carried out to determine significant differences between means at 95% level of significance. Mixture regression analysis was performed to determine estimated coefficients, significance of model terms, *F*-test and coefficient of determinations

(R^2). The experimental design, data analysis, contour and surface plots were developed by using a Minitab (Release 14) statistical software package.

RESULTS AND DISCUSSION

Dressing-type O/W emulsions prepared in the present study contained individual OG, XG, CS and blends thereof as thickeners. Both XG and CS were of commercial samples while OG was prepared from a laboratory aqueous extraction of seedless okra pods. Table 1 summarizes effects of OG, XG, CS and the blends on emulsion droplet size range, viscosity and turbidity. Emulsions containing OG and OG:CS pronouncedly recorded the smallest droplet size range. This reflects their low polydispersity characteristic which might positively affect their rheology and stability.

Hydrophobicity and substantial surface activity of OG, mainly correspond to galactose content (Alamri *et al.*, 2012), are believed to reduce the droplet surface tension and thus favored formation of small droplets during homogenization. This was in line with previous findings which proposed that okra pectin (gum constituent) can be utilized as emulsifying agent (Sengkhamparn *et al.*, 2009). Moreover, Alba *et al* (2013) have demonstrated that average droplet diameters remained stable after 30 days of storage upon addition of okra pectin in acidic emulsions. The droplet size response was fitted with a special cubic model (no lack-of-fit, $p = 0.99$). The lowest positive coefficient in the model ($10.864X_1 + 12.986X_2 + 11.454X_3 + 7.013X_1X_3 + 32.550 X_1X_2X_3$) confirmed that the highest antagonism (desired) on producing large droplet size could be obtained by using binary blend of OG:CS. This effect is well demonstrated in Figure 1, showing that smaller droplets can be obtained in the area of OG:CS blend approaching higher level of OG.

Table 1. Droplet size range, viscosity and turbidity of emulsions

Emulsion code	Polysaccharide proportion			Droplet size range (μm)	Viscosity at 1.0 rpm (Pa.s)	Turbidity (Absorbance)
	X_1	X_2	X_3			
OG	1	0	0	4.67–10.84	26.33 ^{ab} \pm 5.07	0.91 ^a \pm 0.07
XG	0	1	0	4.35–12.99	55.67 ^a \pm 24.24	0.55 ^b \pm 0.03
CS	0	0	1	5.14–11.43	0.40 ^b \pm 0.01	0.79 ^{ab} \pm 0.18
OG:XG	1/2	1/2	0	4.46–11.92	36.13 ^a \pm 2.64	0.62 ^{ab} \pm 0.14
XG:CS	1/2	0	1/2	4.82–13.97	30.87 ^{ab} \pm 11.46	0.63 ^{ab} \pm 0.16
OG:CS	0	1/2	1/2	5.40–11.22	30.43 ^{ab} \pm 9.81	0.95 ^a \pm 0.12
OG:XG:CS	1/3	1/3	1/3	4.07–13.75	32.33 ^{ab} \pm 13.20	0.50 ^b \pm 0.08

Data are presented in mean \pm standard deviation of triplicate results. ^{a,b}Means with different superscripts are significantly different ($p < 0.05$). OG (X_1), Okra gum; XG (X_2), xanthan gum; CS (X_3), corn starch.

Generally, all emulsions portrayed shear-thinning (or pseudoplastic) behaviour as characterized by progressive decreases in their viscosity with increasing shear rate (Figure 2). In an O/W emulsion system, shear-thinning effect is resulted from disruption droplet flocs (of oil dispersed phase) as the shear rate increased, leading to subsequent decrease in the viscosity (Peamprasart & Chiewchan, 2006). This behaviour is favourable in dressings, as the viscosity decreases with the increasing shear rate, the product becomes easy to pour upon stress application. However, this behaviour was not clearly observed for emulsion with pure CS. This emulsion also had the lowest

apparent viscosity which was significantly different ($p < 0.05$) from the emulsion containing XG (Table 1). Furthermore, the emulsion viscosity is also governed by the viscosity of the continuous phase containing polysaccharide (Nor Hayati *et al.*, 2009). As for XG emulsion, the unique rigid, rod-like conformation of XG caused it more responsive to shear than a random-coil conformation of CS (Urlacher & Noble, 1997). This conformation is due to close alignment of the trisaccharide side chains (mannoses and glucuronic acids) with (1,4)-linked β -D-glucan (cellulose) backbone (Nussinovitch, 1997). In relation to this, application of XG:CS resulted in much higher viscosity profile of the

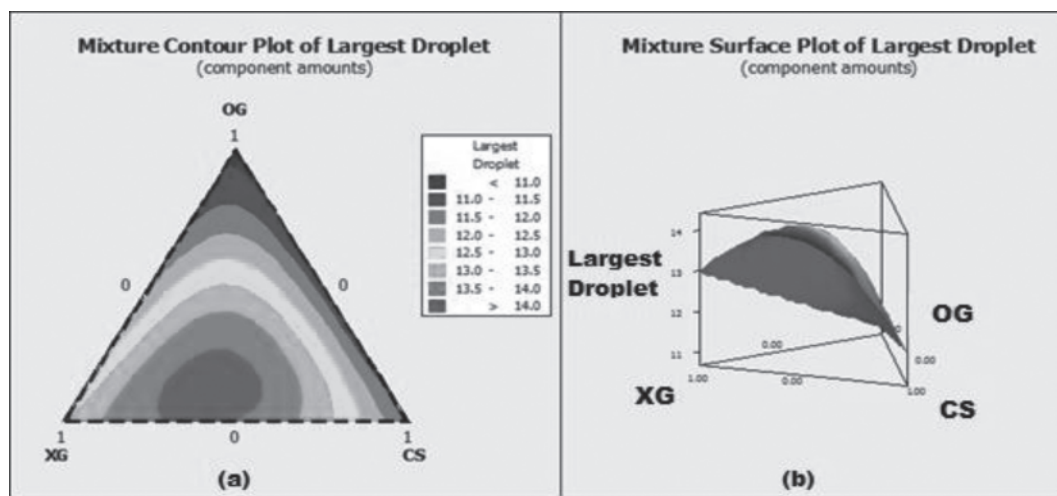


Fig. 1. Contour (a) and surface (b) plots for emulsion droplet size (μm) fitted by a special cubic model. OG, okra gum; XG, xanthan gum; CS, corn starch.

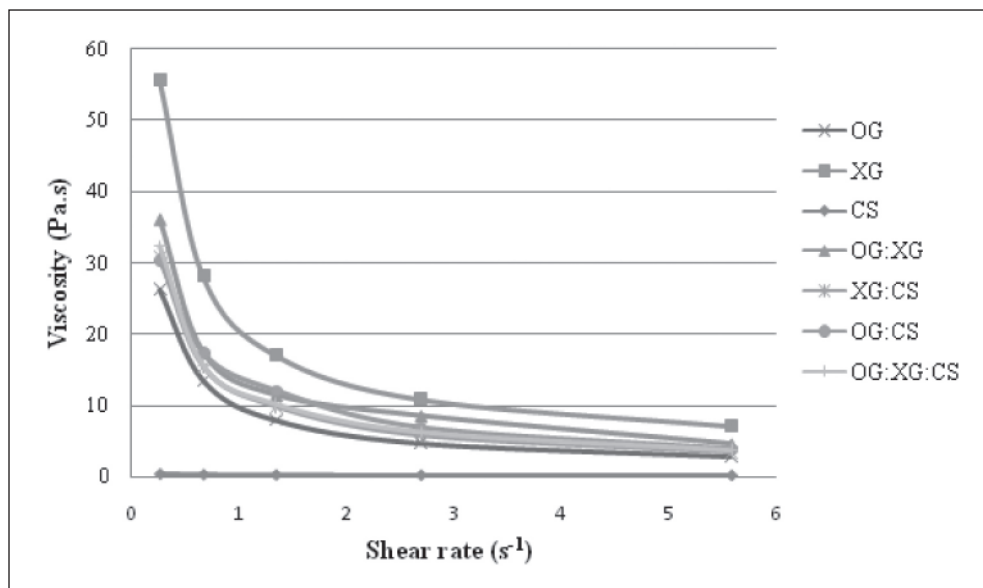


Fig. 2. Viscosity of emulsions presented as mean from three replications ($n=3$). OG, okra gum; XG, xanthan gum; CS, corn starch.

emulsion as opposed to CS alone (Figure 2). It has been reported that XG is capable to enwrap the starch granules (Cai *et al.*, 2011) and thus increases the blend viscosity. In addition, xanthan molecules are proposed to be interact with dissolved amylose molecules but not with amylopectin molecules.

Interestingly, binary OG:CS emulsion exhibited higher viscosity (30.43 Pa.s) compared to emulsions either with pure OG (26.33 Pa.s) or CS (0.40 Pa.s) (Table 1) indicating their synergistic interaction. The interaction between OG and CS might occur *via* OG's disaccharide side chain (Tomada *et al.*, 1980) with amylose molecules of CS due to the formation of junction zones. This could be attributed to clustered galactose branch points that favour the network dispersability via hydrogen bonding with the water molecule (Huang *et al.*, 2007). However, further study on molecular interaction should be undertaken to confirm this mechanism. Viscosity response was successfully fitted with quadratic model (no lack of fit, $p = 0.89$). Regression equation ($24.381X_1 + 54.826X_2 + 1.527X_3 + 66.434X_1X_3$) with the highest positive coefficient as well as contour/surface plots (Figure 2) verified that the strongest synergism on viscosity could be obtained with utilization of OG:CS binary blend approaching higher level of OG which far away from XG.

In addition, OG:CS emulsion demonstrated the highest turbidity which was significantly different ($p < 0.05$) from XG and OG:XG:CS emulsions (Table 1). It should be mentioned that, lipoprotein (from egg yolk) was main the emulsifier involved

in emulsifying the oil into dispersed droplets. According to Clitor *et al* (2013), the presence of anionic polysaccharide in such system allows for formation of complexed protein-polysaccharide films covering the droplet surface with high negative charge density. In the present case, anionic nature of OG:CS is also believed to complex with yolk lipoprotein and therefore induced more negative droplets which increased the electrostatic repulsion among droplets, maintaining the emulsion turbidity. Rasnani *et al* (2011) also reported that negative-charged pectin adsorbed to the positive-charged sodium caseinate (emulsifier) increasing the overall negative charge on the droplets, minimizing droplet aggregation and maintaining emulsion turbidity. Small droplets found in this emulsion also contributed to its turbidity. Generally, small droplet size will result in higher turbidity value and *vice versa* (Nor Hayati & Shamini, 2011). Otherwise, OG:XG:CS emulsion recorded the lowest turbidity value attributable to the decreased number of droplets in an emulsion due to the coalescence (Rios *et al.*, 1998). The coalescence was believed to be induced by XG or the small concentration of OG rendering insufficient surface activity. Turbidity response was fitted with special cubic model ($0.870X_1 + 0.508X_2 + 0.784X_3 + 0.482X_1X_3 - 7.404X_1X_2X_3$) confirming that the strongest synergism on turbidity value could be obtained by using OG:CS blend (no lack-of-fit, $p = 0.40$) which can be further visualized in Figure 4.

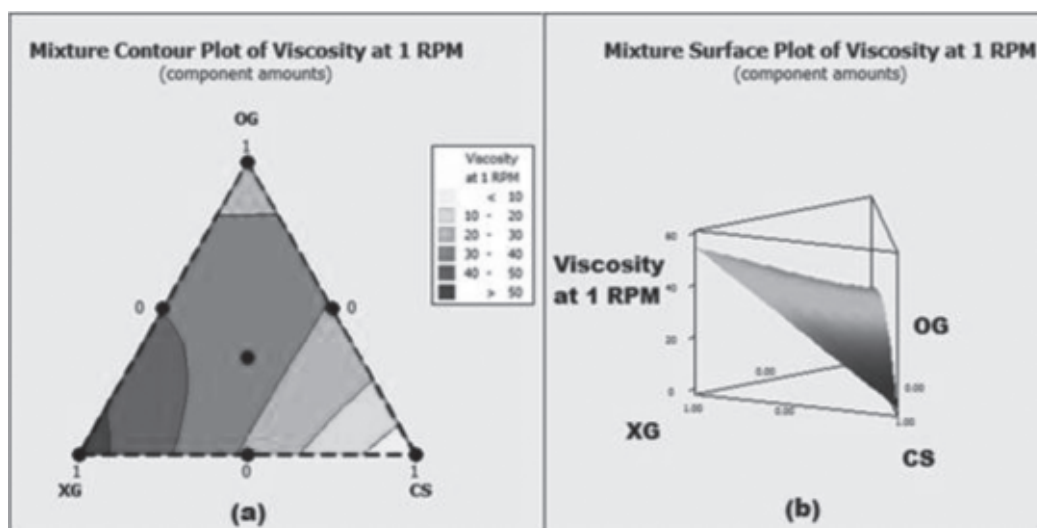


Fig. 3. Contour (a) and surface (b) plots for emulsion viscosity (Pa.s) fitted by a quadratic model. OG, okra gum; XG, xanthan gum; CS, corn starch.

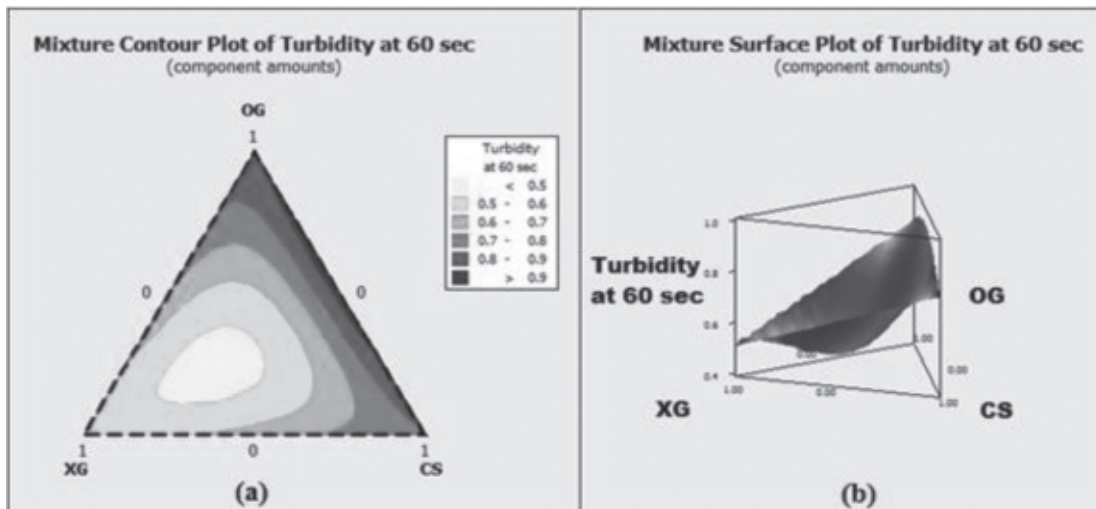


Fig. 4. Contour (a) and surface (b) plots for emulsion turbidity (Absorbance) fitted by a special cubic model. OG, okra gum; XG, xanthan gum; CS, corn starch.

CONCLUSION

In short, this is the first study reported a synergistic interaction between OG and CS which positively affected the emulsion properties and consequently provided more stable emulsions (i.e. able to maintain their original properties). The findings suggested that some drawbacks of OG performance in such food system could be overcome which might open to a new perspective on industrial application of OG. It is also worth to highlight that, the use of mixture design with regression modeling is a valuable tool in better elucidating interaction effects among OG, XG and CS on the emulsion properties.

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