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EFFECT OF JAVANESE GINSENG MUCILAGE AND ORANGE OIL CONCENTRATIONS ON PROPERTIES AND OXIDATIVE STABILITY OF BEVERAGE EMULSIONS

Nor Hayati Ibrahim[1] ✉, Tengku Nur Dayana Tengku Zakaria[1], Yusnita Hamzah[1], Wan Rosli Wan Ishak[2], Amiza Mat Amin[1]

[1]Faculty of Fisheries and Food Science, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia

[2]School of Health Sciences, Universiti Sains Malaysia, Health Campus, 16150 Kubang Kerian, Kota Bharu, Kelantan, Malaysia

✉yati@umt.edu.my

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ABSTRACT

The objective of this study was to investigate the effect of Javanese ginseng mucilage and orange oil concentrations on the properties (viscosity and droplet size) and oxidative stability (total oxidation value) of beverage emulsions. The mucilage was firstly extracted from the fronds of Javanese ginseng and determined to exhibit a lower IC_{50} (i.e. the concentration of sample required to scavenge 50% of free radicals) as compared to gum Arabic, reflecting its higher antioxidant capacity. Fourteen emulsions varying in mucilage (5 – 10%) and orange oil (6 – 10%) concentrations were further prepared based on a two-factor central composite design. The emulsions exhibited a thixotropic behaviour with the viscosity range of 3920 – 20050 mPa.s and the droplet size range of 3.14 – 16.49 µm. All emulsions were stable towards lipid oxidation with low total oxidation values (< 10). The data were subsequently fitted to the quadratic model using a response surface modelling to further elucidate the effects of both factors on each response. There was a significant ($p < 0.05$) synergistic interaction between mucilage and orange oil which positively increased the emulsion viscosity. However, significant ($p < 0.05$) quadratic effects of orange oil resulted in too much increase in the viscosity and thus undesirably led to the formation of large droplets and high total oxidation value. The results suggested that the mucilage and oil should be used around 8 – 9.5% and 6 – 8.5%, respectively in order to provide the emulsions with desired properties and oxidative stability.

1. Introduction

Javanese ginseng, botanically known as *Talinum paniculatum* (Jacq.) *Gaertn*. is a succulent subshrub from the family of Talinaceae. This edible ornamental plant is one of the perceptible medicinal plants, which is also commonly known as Fameflower, Som Java and Jewels-of-Opar. A leaf cross-sectional of this plant exhibits a single-layered epidermis covered with a thin cuticle and contains a large number of mucilaginous cells (Tolouei *et al.*, 2019). The abundance of mucilaginous cells in the leaves (and also in young stems) makes it possible of extracting a significant amount of a complex soluble polysaccharide called mucilage. The mucilage could be easily obtained via a hot water extraction, followed by a solvent (e.g. ethanol) precipitation. With extraction conditions that vary in water to fronds (young leaves and stems) ratio (0.5:1 – 12:1),

temperature (25 – 90˚C) and pH (3 – 11), the range of mucilage that could be obtained is 2.32 – 4.90% (Nor Hayati *et al*., 2019). The mucilage exhibits a significant surface-active property, a key functional property for application in emulsion-based food products. The surface-active property of Javanese ginseng mucilage is strongly associated with its significant protein content (up to 30.97%). Interestingly, the mucilage could be proposed as a functional food ingredient with health-promoting properties. Tolouei *et al*. (2019) demonstrated that the leaf extract (or mucilage) obtained by ethanol precipitation has significant diuretic and cardioprotective effects as well as non-toxicity. The extract also contains bioactive compounds such as chlorogenic acids, organic acids, and O-glycosylated flavones, which are thought to be responsible for its antioxidant and related medicinal properties (Lestario *et al*., 2009; Thanamool *et al*., 2013; Reis *et al*., 2015).

For decades, food hydrocolloids have become important ingredients for the food industry. Besides, it was made constant efforts from the scientists to search for new sources and varieties of food hydrocolloids to function as emulsifiers, stabilizers and texturizers mainly in emulsion-based food products. In addition to *T. paniculatum* mucilage, other examples of leaf mucilage that exhibit the said functionalities are the mucilage extracted from leaves (or young fronds) of *Asplenium australasicum* (Lai and Liang, 2012), *Pereskia aculeate* (Martin *et al.*, 2017; Lago *et al.*, 2021), *Perekia bleo* (Nurul Farhanah *et al*., 2019), *Basella alba* (Hung and Lai, 2019), and *Malva parviflora* (Munir *et al.*, 2021). However, very limited studies concern about their functionality performance in model or real food emulsion systems. To the best of our knowledge, there is one related study done by Lago *et al*. (2019) who have examined the performance of *P. aculeate* leaf mucilage in soybean oil-in-water nanoemulsions. The study highlighted that the mucilage concentration should be set at 1.0 to 1.5% with the oil concentration should be less than 5% to obtain the nanoemulsions with the desired properties and stability. In particular, no information is available regarding the performance of Javanese ginseng mucilage in any food system including beverage emulsions. Moreover, the effectiveness of the mucilage in playing a role as a natural antioxidant in such a complex system is yet to be investigated.

Indeed, this research focus is consistent with the recent trend of using food ingredients with high antioxidant activity in the food system (Paraiso *et al*., 2020; Souza *et al*., 2020a). Thus, the objective of the present study was to determine the effects of Javanese ginseng mucilage and orange oil concentrations on the properties and oxidative stability of beverage emulsions. The effects were elucidated in a meaningful way by using response surface modelling, which also allows one to empirically estimate the concentration limit of both ingredients in the formulation.

2. Materials and methods

2.1. Materials

Javanese ginseng fronds were collected from a controlled farm in Kelantan, Malaysia. To obtain the mucilage powder, the extraction was carried out according to our previous report (Nor Hayati *et al*., 2019) using the optimized extraction condition i.e. water to fronds ratio (8.4:1), temperature (90°C) and pH (8). Orange oil was purchased from Mee Soya Sdn. Bhd. Other ingredients for the emulsion formulation were purchased from SIM Company Sdn. Bhd.

2.2. Determination of antioxidant activity of mucilage

To better understand the mucilage's ability to inhibit lipid oxidation in beverage emulsions, the antioxidant activity of the mucilage was first analysed in terms of 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity test according to the modified method of Kong *et al*. (2010). Briefly, 2.0 mL DPPH solution (2 mM ethanol) was added to 2.0 mL of mucilage in water at different concentration of 100 – 800 µg/ml. The mixture was shaken and left to stand for 30 min at room temperature in the dark. The absorbance was measured at 517 nm with a UV–Vis spectrophotometer (UV-160,

Shimadzu, Kyoto, Japan). The DPPH radical scavenging effect was calculated as follows:

$$Scavenging\ activity\ (\%) = \frac{(Ao - (A - Ab))}{Ao} x\ 100 \quad (1)$$

where Ao is the absorbance (A_{517}) of DPPH without sample, A is the A_{517} of sample and DPPH, and Ab is the A_{517} of sample without DPPH. Sample concentration providing 50% scavenging activity (IC_{50}) was determined from the plot of scavenging activity (%) against the sample concentration. Butylated hydroxytoluene (BHT) was used as a positive control and commercial gum Arabic was used for comparison. All assays were carried out for triplicate samples.

2.3. Preparation of beverage emulsions and experimental design

A response surface methodology was used to determine the effect of two most significant emulsion components namely Javanese ginseng mucilage and orange oil on viscosity, droplet size, and total oxidation (TOTOX) of beverage emulsions. Fourteen model beverage emulsions were prepared according to two factor central composite design (face-centred). The two factors involved were mucilage (5-10%) and orange oil (6-10%). Other ingredients namely carboxymethyl cellulose (CMC) (0.5%), sodium benzoate (0.08%), citric acid (2%) and distilled water (mark up to 100%) were constant. Hydrocolloid dispersion was firstly prepared by blending the mucilage with CMC at 80°C for 2 h and subsequently stored at room temperature (25±2 ºC) overnight. The dispersion was then added with distilled water, citric acid and sodium benzoate. The mixture was homogenized for 2 min at 6000 rpm. After that, orange oil was mixed to the mixture and then homogenized for 3 min at 10000 rpm. Homogenization was done using a high-speed homogenizer (Ultra Turrax T-25, IKA Instruments, IKA-Werke GmbH & Co., Germany). The emulsions were observed to be stable after homogenization with desirable pH of 3.0 ± 0.2 (Guzey & McClements, 2006).

2.4. Determination of emulsion viscosity

Determination of viscosity was carried out by using a viscometer (Brookfield DV-III Viscometer, Brookfield, Brookfield Engineering Laboratories, Inc., USA) equipped with a small sample adapter and Spindle No. 1 at 100 rpm were used. The viscosity was measured at room temperature for 12 min.

2.5. Determination of emulsion droplet size and microstructure

The droplet image for the emulsions was captured under a polarized light microscopy at room temperature. Droplet microstructures were visualized by Eclipse 80i Advanced Research microscope (Nikon, Nikon Instruments, Inc., USA) at 100x magnification after being equilibrated for 2 min. The average size of the predominant droplets in each emulsion were recorded. In addition, the smallest, medium and largest size of the droplets were also determined in order to estimate the emulsion polydispersity.

2.6. Determination of emulsion oxidative stability

Peroxide value (PV) and Anisidine value (AV) of the stored emulsions (30 days at 5°C in order to stimulate the normal storage condition) were determined by the standard iodometric AOAC Method 965.33 (AOAC, 1990) and based on Egan *et al*. (1981), respectively. The oxidative stability was expressed in a TOTOX value calculated as 2PV + AV.

2.7. Statistical analysis

A one-way ANOVA with Tukey's post-hoc test was applied to the DPPH radical-scavenging data. For other data, the statistical analysis was done based on a response surface regression analysis involving determination of estimated coefficients of model terms and ANOVA. Independent variables of mucilage (X_1) (5 – 10 %) and orange oil (X_2) (6 – 10 %) and their relationship with dependent variables (responses) (Y_i) was expressed by a multiple linear regression equation as follows:

$$Y_i = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{12} X_1 X_2 \quad (2)$$

where β_0 is a constant, β_1 and β_2 are regression coefficients for the linear effects, β_{11} and β_{22} are coefficients for the quadratic effects and β_{12} is coefficient for the interaction effects. The interaction effect between independent variables on the responses were also shown in the response contour plots. Model lack-of-fit and coefficient of determinations (R^2) were used to judge the model adequacy. All analyses were carried out by using a Minitab statistical software package (Release 14, Minitab Inc, USA). The level of confidence used was at α-0.05.

3. Results and discussions

3.1. Antioxidant activity of Javanese ginseng mucilage

In the DPPH assay, BHT served as a positive control since it is known as a synthetic antioxidant which typically exhibits higher antioxidant activity in most antioxidant analyses as compared to plant extract. The result for BHT will demonstrate that the procedure used in the present study was appropriate. In addition, commercial gum Arabic served as a sample for comparison. This is due to the fact that it is a plant extract that has been shown to have antioxidant activity, as well as some functional similarities (e.g. stabilizing/emulsifying effects on emulsion) with Javanese ginseng mucilage. As shown in Table 1, the DPPH radical-scavenging activities gradually increased as the sample concentration increased from 100 to 800 µg/ml. For each concentration tested, there were significant differences ($p < 0.05$) among the samples.

One promising finding was that Javanese ginseng mucilage had nearly twice the free radical scavenging activity as gum Arabic at all concentrations tested. The average 50% inhibitory concentration (IC_{50}) values of Javanese ginseng mucilage and gum Arabic were 536 and 1327 µg/ml, respectively, indicating that the mucilage has a higher antioxidant activity.

The IC_{50} of Javanese ginseng mucilage was lower than that of Hsian-tsao leaf mucilage (Lai *et al*. (2001) and partially purified mucilage from Japanese yam (860 µg/ml) (Hou *et al*., 2002). Moreover, the value was within the IC_{50} range (380 – 650 µg/ml) of Balangu (*Lallemantia royleana*) seed gums as reported by Sardarodiyan *et al*. (2019). Meanwhile, Lin *et al*. (2001) reported a comparable value of the IC_{50} (547 µg/ml) for *Dioscorea alata* L. cv. Tainong 2 (Taiwanese yam) mucilage.

Table 1. Scavenging activity of Javanese ginseng mucilage as compared to butylated hydroxytoluene (BHT) and gum Arabic

	Scavenging activity (%)[1]		
Concentration (µg/ml)	**BHT**	**Gum Arabic**	**Javanese ginseng mucilage**
100	42.24 ± 0.07[a]	9.35 ± 3.52[c]	18.06 ± 5.26[b]
200	61.87 ± 0.18[a]	12.98 ± 1.42[c]	25.88 ± 2.51[b]
400	84.16 ± 0.01[a]	20.24 ± 4.05[c]	41.60 ± 0.74[b]
800	98.12 ± 0.01[a]	32.32 ± 2.98[c]	68.96 ± 0.34[b]
Regression model ($R^2 > 0.98$)	Y = 0.1287x + 19.903	Y = 0.0769x + 8.8143	Y = 0.0329x + 6.346
IC_{50} (µg/ml)[2]	146	1327	536

[1] Means with standard deviation from three independent replications. Means with different superscripts within the same row are significantly different ($p < 0.05$)

[2]The concentration of sample required to scavenge 50% of free radicals (determined by the respective regression models).

In contrast, The IC_{50} of Javanese ginseng mucilage was substantially lower than that of the mucilage from *M. parviflora* leaves, with an IC_{50} of 154.27 µg/ml (Munir *et al*., 2021). According to some previous studies (Ragavee *et al*., 2018; Gemede *et al*., 2018; Souza *et al*., 2020b), the

antioxidant activity of the mucilage or gum could be well related with their phenolic acid and flavonoid compounds. As for Javanese ginseng mucilage, chlorogenic acids and O-glycosylated flavones (Tolouei *et al*., 2019) are examples of the said compounds possibly responsible for its antioxidant activity.

3.2. Fitting the experimental data of emulsions to the quadratic model

The experimental data obtained for the prepared emulsions in terms of viscosity, droplet size and TOTOX values (responses) are presented in Table 2 and the data were fitted to a quadratic model using response surface modelling. Table 3 depicts the regression model for each response addressing the significance of the coefficient of estimation for each linear, quadratic and interaction terms. All the fitted models were found to be significant ($p < 0.05$) and thus pertinent to indicate the relationship between the factors and the responses. The high values (> 0.8) of R^2 indicate that both mucilage and orange oil as factors in the model could explain well the variation observed in the responses. There were insignificant lack-of-fits ($p > 0.05$), suggesting that the models fitted well to the data and are able to accurately predict future responses. This study also found that removal of non-significant terms from the initial models fitted to the viscosity and TOTOX value did not significantly increase the R^2 and thus the initial models were unreduced.

In contrast, the removal of non-significant interaction term from the initial model fitted to the droplet size data seemed to be essential to improve the goodness of fit.

Table 2. A Central Composite Design with the experimental data of beverage emulsions

Run	Sample code	Factors (Actual value)		Responses		
		Mucilage (%)	Orange oil (%)	Viscosity (mPa.s)	Droplet size (µm)	Total oxidation (TOTOX)
1	7.5%M-8%O	7.5	8	11040	8.77	5.67
2	7.5%M-10%O	7.5	10	12560	9.68	9.37
3	7.5%M-8%O	7.5	8	9760	7.39	7.12
4	10%M-8%O	10	8	17440	7.22	3.86
5	5%M-8%O	5	8	4000	3.14	5.84
6	7.5%M-8%O	7.5	8	6960	12.39	4.50
7	7.5%M-6%O	7.5	6	8960	7.60	4.46
8	5%M-6%O	5	6	3920	6.20	6.73
9	7.5%M-8%O	7.5	8	7600	12.72	6.70
10	7.5%M-8%O	7.5	8	9520	8.80	4.81
11	10%M-10%O	10	10	20050	16.49	7.07
12	10%M-6%O	10	6	15120	10.36	2.94
13	7.5%M-8%O	7.5	8	9920	10.13	4.22
14	5%M-10%O	5	10	6800	8.13	8.39

Note: All beverage emulsions had the same composition of carboxymethyl cellulose (CMC) (0.5%), sodium benzoate (0.08%), and citric acid (2%) except that distilled water concentration was varied between 79.42 – 86.42%, following the composition of mucilage (5 – 10%) and orange oil (6 – 10%).

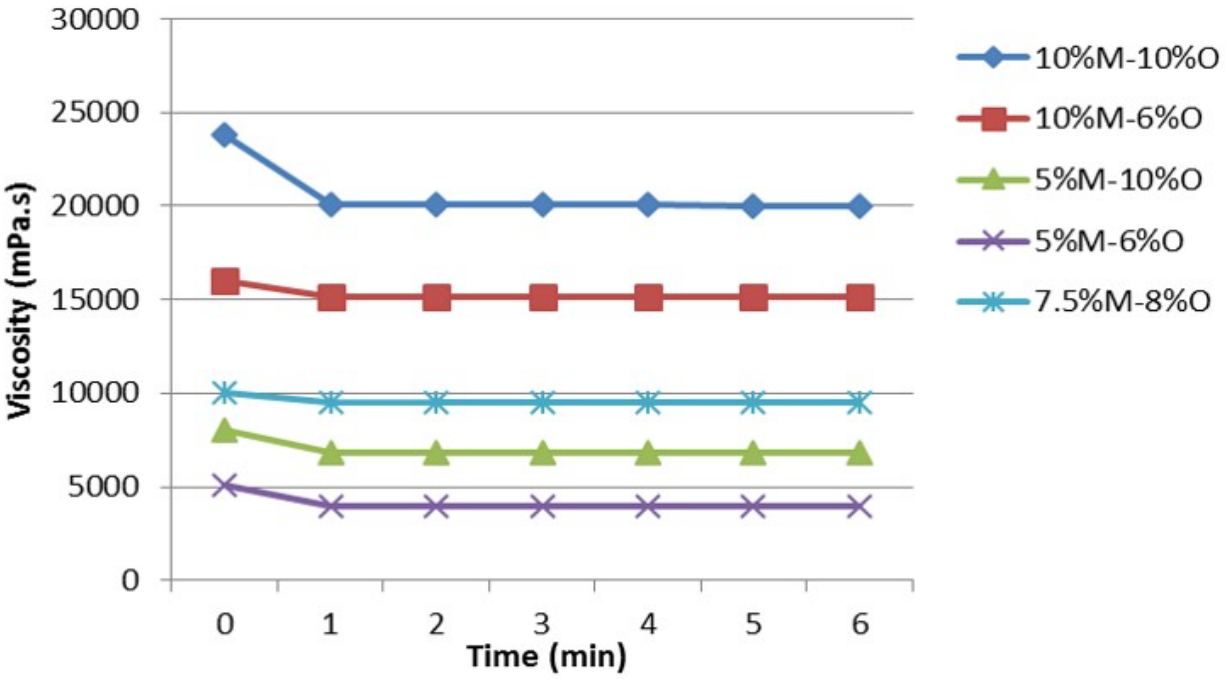


Figure 1. Viscosity profile of representative beverage emulsions varying in Javanese ginseng mucilage (M) and orange oil (O) concentrations. See Table 2 for the description of each sample code.

3.3. Effect of mucilage and orange oil concentrations on emulsion viscosity

The viscosity values of the emulsions were in the range of 3920–20050 mPa.s which were greatly influenced by the mucilage and oil concentrations (Table 2). The viscosity of all emulsions notably decreased within the first 2 min of measurement as shown by the representative emulsions in Figure 1, revealing a thixotropic behavior. This behavior is due to the fact that aggregated droplets (held by weak forces) were gradually disrupted and collapsed because of the shearing force, causing reduction of the viscosity over time (McClements, 2005).

According to the results, the combination of mucilage and orange oil at their highest concentrations (10%) could produce an emulsion with higher viscosity than other combinations. This is because a higher mucilage concentration contributes to a higher emulsion continuous phase viscosity. Similarly, increasing the oil concentration in the dispersed phase will result in more droplet-droplet interactions, making the emulsion more resistant to flow, as indicated by an increase in viscosity. According to Zeng and Lai (2014), increasing the volume ratio of oil has increased the emulsion viscosity and slowed the potential of phase separation in
the emulsions containing *A. australasicum* mucilage. However, in the present case, it was discovered that the emulsions with the lowest amounts of mucilage (5%) and oil (6%) displayed the lowest viscosities, which may encourage the phase separation during storage.

Changes in mucilage and oil concentrations caused abrupt changes in emulsion viscosity, implying that their respective effects may not be simply linear. According to the fitted regression model (Table 3), the quadratic (square) and interaction terms of mucilage and orange oil had significant ($p < 0.05$) increasing effects on viscosity (i.e. positive sign of the estimated regression coefficients). This is consistent with previous research, which found that increasing the concentration of beet pectin increased the viscosity of secondary lactoferrin-coated orange oil emulsions (Zhao *et al*., 2015). The quadratic effect of oil (X_2), on the other hand, appeared to be stronger than that of mucilage (X_1) as indicated by its higher coefficient (221). Moreover, it is worth noticing an interesting

finding on a synergistic interaction effect of mucilage and oil on the emulsion viscosity.

With a positive coefficient of estimation (104 of X_1X_2), the result revealed that when the mucilage and oil concentrations increased, there would be a sudden increase in the viscosity in a non-linear manner. The contour plot (Figure 2a) gives a better picture of the interaction effect on the emulsion viscosity when both mucilage and orange oil concentration increased. The interaction could be attributed to movement restriction of oil dispersed droplets due to increased viscosity caused by the increase in the mucilage concentration. At the same time, an increase in oil concentration would increase the packing fraction of the droplets which in turn would also restrict the droplet movement.

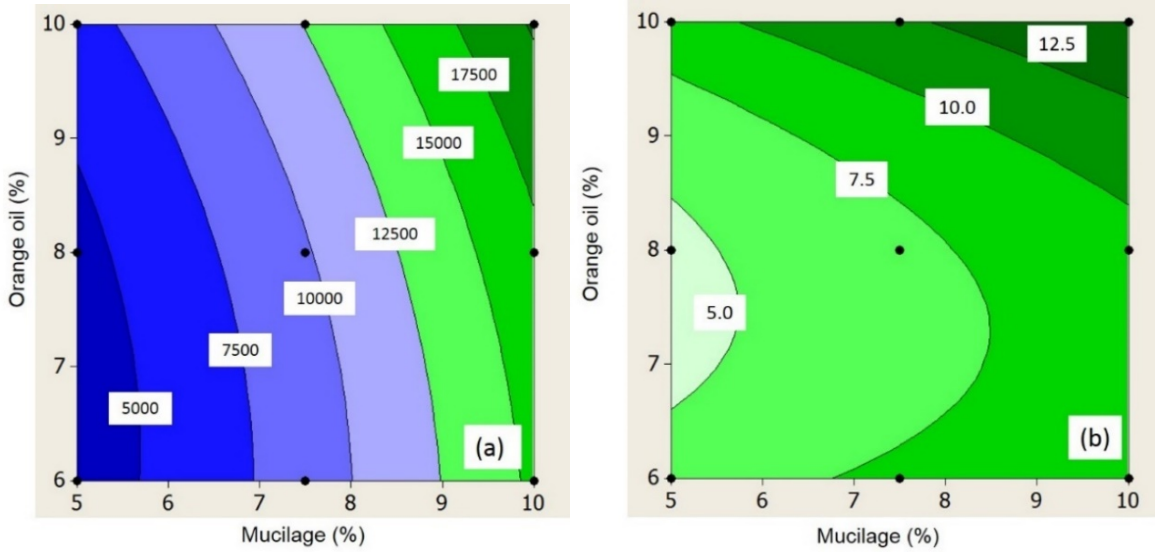
3.3. Effect of mucilage and orange oil concentrations on emulsion droplet size

Droplet size was measured using the average size of predominant droplets ranging from 3.14 to 16.49 µm (Table 2) among the emulsions due to the large difference in mucilage-oil concentration. Furthermore, the presence of droplets of various sizes in the representative micrographs (Figure 3) demonstrated the polydispersity characteristic of each emulsion.The emulsions demonstrated a typical droplet packing of emulsions with a low amount of oil (6 – 10%), with the flocculated droplets being scarcely visible, especially for the emulsion with the lowest amounts of mucilage-oil (Figure 3a), which had a narrower size range of 0.91 – 13.77 µm in comparison to others.

Table 3. Summary of ANOVA results for the fitted regression models

Responses	Fitted regression models	R^2	Lack-of-fit
Viscosity (mPa.s)	$11240 - 335X_1 - 3371X_2 + \mathbf{135X_1X_1^*} + \mathbf{221X_2X_2^*} + \mathbf{104X_1X_2^*}$	0.998	0.184
Droplet size (µm)	$48.5 + 0.1X_1 - \mathbf{12.4X_2^*} + \mathbf{0.8X_2X_2^*} - 0.1X_1X_2$	0.810	0.090
TOTOX value	$34.5 - 0.8X_1 - \mathbf{7.1X_2^*} - 0.1X_1X_1 + \mathbf{0.4X_2X_2^*} + 0.1X_1X_2$	0.928	0.400

X_1, Javanese ginseng mucilage; X_2, orange oil; TOTOX, total oxidation. *Significant at $p > 0.05$.



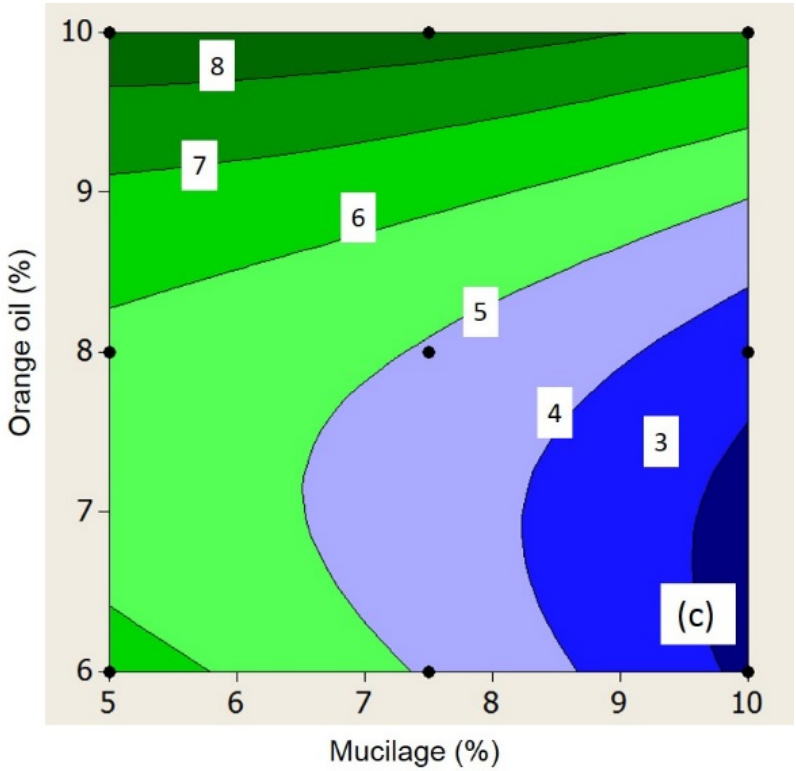
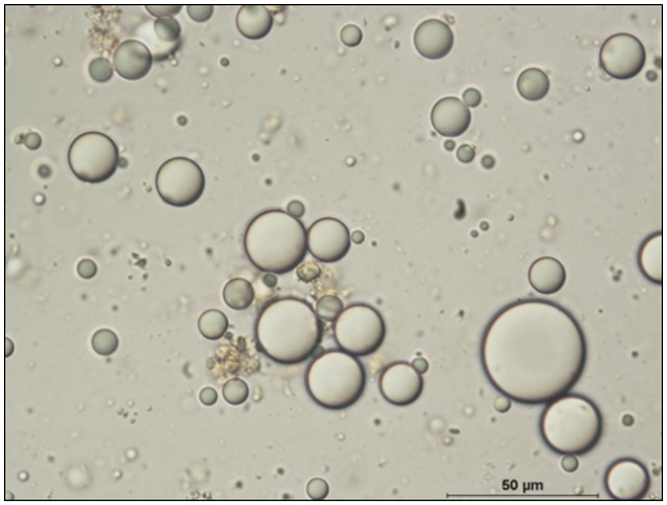
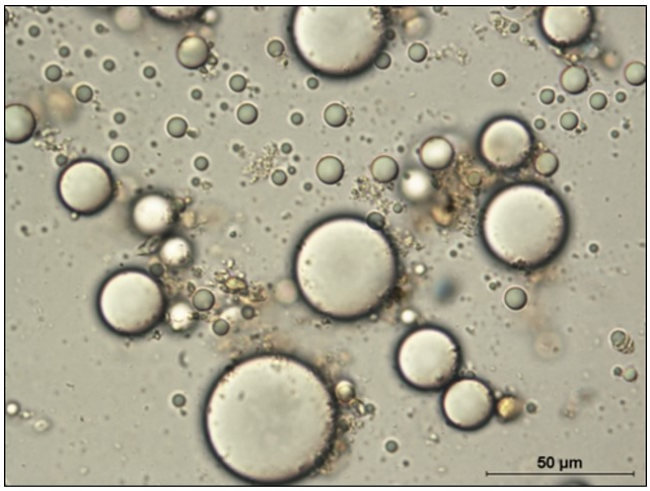


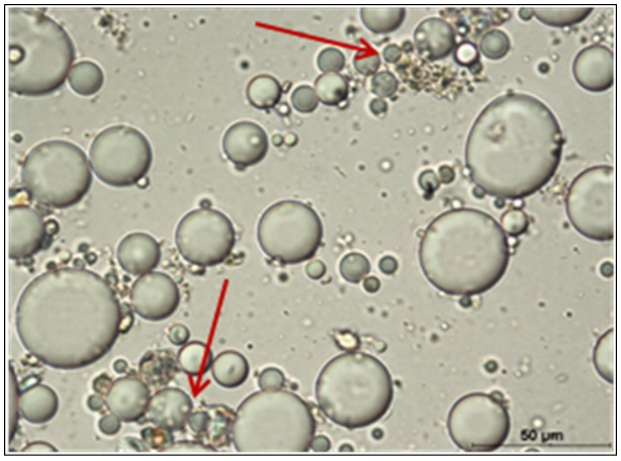
Figure 2. Contour plots showing the interaction effect of mucilage and oil on (a) Viscosity, mPa.s (measured at 0 min); (b) Droplet size, μm and (c) Total oxidation value of beverage emulsions.



(a) 5%M-6%O (0.91-13.77 μm)



(b) 10%M-10%O (0.87 – 28.87µm)



(c) 5%M-10%O (0.59-16.41 µm)

Figure 3. Droplet microstructure (100x magnification) of representative beverage emulsions varying in Javanese ginseng mucilage (M) and orange oil (O) concentrations. See Table 2 for the description of each sample code.

Nevertheless, a previous study found that the emulsion droplets became bigger, non-spherical and non-uniform as relative percentage of *A. australasicum* mucilage in the emulsion decreased (Zeng and Lai, 2014). In the present case, the use of maximum levels of mucilage-oil seemed to produce an emulsion with a wider size range (0.87 – 28.87 µm) or higher degree of polydispersity (Figure 3b). It is meaningful to observe that the minimum level of mucilage (5%) appeared to be fairly enough to cover the droplet surroundings at the maximum level of oil (10%) as the emulsion exhibited an intermediate droplet size range of 0.59 – 16.41 µm (Figure 3c). However, as seen in the micrograph, the emulsion flocculated due to an increase in the number of small droplets (as shown by arrows). As referred to the fitted model in Table 3, the linear increment of orange oil was predicted to significantly ($p < 0.05$) reduce the droplet size as indicated by the negative coefficient of estimation (–12.4 for X_2) whilst its quadratic increment would give the opposite effect (0.8 for X_2^2). In this study, the emulsification of oil into fine droplets was primarily caused by the presence of Javanese ginseng mucilage, which has a significant surface property (Nor Hayati *et al*., 2019), as no emulsifier was used in the formulation. The finding implies that the emulsification was only efficient when the

increase in orange oil was linear. However, if the increase was quadratic, it was no longer efficient because the droplets formed were quite large. For this reason, it is important to sustain the interaction term (X_1X_2) in the model despite its insignificant effect, to give insight into the mucilage-oil interaction. As illustrated by the contour plot in Figure 2b, increases in the mucilage concentration approximately from 7 to 10% did not seem to be efficient to produce predominant droplets with small sizes (< 7.5 μm) when the oil concentration increased > 8.5%. Remarkably, when the oil concentration was < 8.5%, the predominant droplets formed were not more than 10 μm in size. One possible explanation for this observation is the increased viscosity of the continuous phase resulting from the increase in the mucilage concentration could have restricted the energy input during homogenization and thus disturbed the mucilage's emulsifying activity. With > 8.5% oil concentration, this drawback seemed to be more prominent as the mucilage-oil interaction had substantially increased the emulsion viscosity while also requiring more oil to be emulsified.

3.5. Effect of mucilage and orange oil concentrations on emulsion oxidative stability

The oxidative stability of the emulsions was evaluated based on the TOTOX value, a comprehensive oxidation index considering hydroperoxides and aldehydes that formed during the primary and secondary stages of lipid oxidation, respectively. Based on Table 1, the TOTOX values were in the range of 2.94 – 9.37 after the emulsions have been stored at 5°C for 30 days. The values < 10 reveal that the prepared emulsions were considerably stable towards lipid oxidation (Rossell & Hamilton, 1986). The result reasonably showed that higher mucilage concentrations at 7.5 and 10% were more efficient to inhibit lipid oxidation in the emulsion system (lower TOTOX values) as opposed to 5%. This is due to the antioxidant activity of the Javanese ginseng mucilage, which is as supported by the DPPH result discussed earlier. Phenolic compounds such as chlorogenic acids may function as hydrogen donators, reducing the consumption of oxygen in emulsion system. Consequently, the amount of hydroperoxides that decomposed into the secondary oxidation products, could be successfully reduced. The lowest TOTOX value (2.94) can be achieved at the minimum orange oil concentration (6%) and maximum Javanese ginseng mucilage concentration (10%). The result is in agreement with a walnut oil-beverage emulsion (28 days of storage) containing 3% walnut oil, 10% gum Arabic and 0.12% xanthan gum giving the lowest TOTOX value (2.347) (Gharibzahedi *et al*., 2012).

The TOTOX value for the emulsions was also successfully fitted with a quadratic model showing that both linear and quadratic terms of orange oil significantly ($p < 0.05$) affected the TOTOX value (Table 3) but in a dissimilar way. The negative coefficient (–7.1) of X_2 suggests that there will be a decrease in the TOTOX value with a linear increase in the orange oil concentration. This is thought to be due to sufficient mucilage coverage surrounding the droplet surfaces which could prevent diffusion of pro-oxidants from the oil-water interface into the oil droplets. Conversely, the value is predicted to be increased when the oil concentration increased in a quadratic manner (0.4 of X_2^2) possibly due to self-interaction of the oil droplets which could favor the oxidation process. Furthermore, it is worth mentioning that increasing the mucilage concentration could increase the oxidative stability of the emulsion even though its' linear and quadratic effects were not significant. This is expressed by the negative sign of the related coefficients (–0.8 of X_1 and –0.1 of X_1^2). This clearly reflects the ability of Javanese ginseng mucilage to inhibit the lipid oxidation in the emulsion system via its high antioxidant activity, as well as formation of the thick layer around the droplet surface. However, the mucilage-oil interaction somehow leveraged this effect as indicated by the positive coefficient (0.1) of X_1X_2. As referred to the contour plot of the TOTOX value (Figure 2c), the decreasing effect is seen towards a higher

concentration of the mucilage (7 – 10%) when the oil concentration was limited to < 8.5%, revealing the effectiveness of the mucilage antioxidant activity. Undesirably, the TOTOX value was substantially increased when the oil concentration increased over the limit, even with > 7% of mucilage present in the system. It is strongly believed that, the mucilage-oil interaction at this stage had led to undesirable increase in the emulsion viscosity (Figure 2a), giving rise to inefficiency of free radical scavenging activity due to restricted mobility of the antioxidant compounds (Nor Hayati *et al*., 2020). In addition, the increase in the droplet size (Figure 2b) as discussed before, is a sign of insufficient mucilage coverage to effectively protect the droplets from the free radical chain reaction.

3.6. Concentration limits of Javanese ginseng mucilage and orange oil in beverage emulsion

Based on the elucidated effects (linear, quadratic and interaction) of Javanese ginseng mucilage and orange oil on the viscosity, droplet size and TOTOX values of the emulsions, it is possible to estimate the concentration limit of both ingredients in the formulation. With the guide of the pattern of their respective contour plots, the desirable range for viscosity, droplet size and TOTOX values were set as 10000 – 20000 mPa.s, 5 – 10 µm and 3 – 5, respectively. The contour plots were then superimposed to give the feasible region of desirable viscosity, droplet size and TOTOX values as depicted in Figure 4. In a formulation with 6 – 8.5% orange oil, it is recommended to employ a concentration of Javanese ginseng mucilage of 8 – 9.5%. Out of these limits, the emulsions were expected to have undesirable high viscosity and large droplet size which will further reduce their oxidative stability.

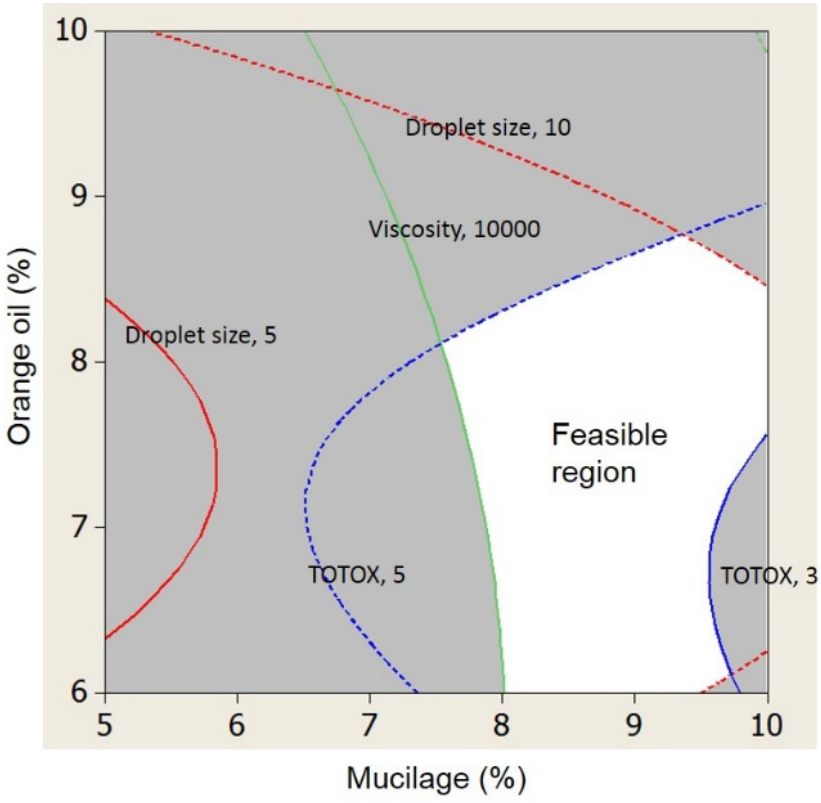


Figure 4. Superimposed contour plots of viscosity, droplet size and total oxidation value (responses) showing the feasible region adhered to concentration limit of the Javanese ginseng mucilage and orange oil.

4. Conclusions

The present findings led to a conclusion that, with certain considerations, it is highly possible to prepare a stable beverage emulsion by using Javanese ginseng mucilage as thickener and emulsifier as well as a natural antioxidant source. It was elaborated that the effects of Javanese ginseng mucilage and orange oil on the viscosity, droplet size and oxidative stability of the prepared emulsions were not simply linear. Increasing the oil concentration in a quadratic manner was predicted to result in too much increase in the emulsion viscosity. This undesirably would favor the formation of large droplet size and also intrude the antioxidant activity of the mucilage to inhibit lipid oxidation in the system. This study also revealed that understanding of interaction between the mucilage and oil is vital in order to formulate the emulsion with desirable properties and stability. Further work is certainly required to better understand a more complex interaction among the mucilage with other ingredients (e.g. protein, salt, sucrose, etc.) with the aim to extend utilization of the mucilage in other various emulsion-based food systems.

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