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Emulsifying properties of extracted Okra (*Abelmoschus esculentus* L.) mucilage of different maturity index and its application in coconut milk emulsion

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Abstract

The emulsifying properties of extracted okra (*Abelmoschus esculentus* L.) mucilage at different maturity indices (1, 2 and 3) were studied. The okra mucilage was prepared using water extraction method and was determined their viscosity at different temperature (10, 30, 50 and 70°C), water holding capacity (WHC), oil holding capacity (OHC), as well as their emulsion capacity (EC) and emulsion stability (ES). Results found that okra with maturity index 2 produced the highest percentage yield of mucilage (1.46%) and followed by index 1 (1.10%) and index 3 (0.31%) ($p<0.05$). The viscosity of okra mucilage with maturity index 3 was the lowest, and it decreased as temperature was increased in the reaction. The WHC of okra mucilage with maturity index 3 obtained the highest capacity (4.84%), while the OHC of okra mucilage with maturity index 2 obtained the highest capacity (8.54%). Based on these findings, okra mucilage index 2 was selected to be added into oil-in-water (O/W) emulsion system of coconut milk at different percentage of 0.25%, 0.50% and 1.0%. Results revealed that okra mucilage (maturity index 2) at 1.0% percentage in coconut milk obtained the highest value in emulsion capacity (EC) and emulsion stability (ES). Thus, this study concluded that okra plant have potential to be used as emulsifying agent in food emulsion system.

Introduction

Okra (*Abelmoschus esculentus* L.) is a flowering plant of the *Malvacae* family which is also known as lady's fingers, gumbo, bamya or bamia or bhindi (Georgiadis *et al*., 2011). This plant was formerly name as *Hibiscus esculentus*, and was originated from India but now it is grown widely in other areas including Middle East, Africa and Southern State of USA. Okra has three different sizes; large (L), medium (M) and small (S) with length of more than 15 cm, between 10 to 15 cm and between 7 to 10 cm, respectively. Its quality can be maintained up to 7- 9 days if was kept at temperature ranged from 8 to 12°C with relative humidity of 90 to 95% (FAMA, 2008).

Mucilage is a plant hydrocolloid which is a polymer of a monosaccharide or mixed monosaccharide (Deogade *et al*., 2012). In fact, polysaccharide mucilage is highly hydrophilic substances with high molecular weight molecules. The polysaccharides are soluble and dispersible in water due to their ability to interact with water and swell. The swelling properties are characterised by the entrapment of large amount of water between the polymer chains and branches. Thus, mucilage can be used as one of the food additives, to modify the food quality in terms of food stability, texture and appearance properties by acting as emulsifiers, thickeners, gelling agents or texture modifiers.

The chemical compositions, molecular structures, monosaccharide sequences, glycoside linkages configuration and position in the backbone and side chains are some of the factors that can affect the functional properties of natural plant mucilage (Mirhosseini and Amid, 2012a).

Previously, there were many studies on the physicochemical and functional properties of polysaccharide from other plants include Durio zibethinus seed (Mirhosseini and Amid, 2012b), Ferula galbaniflua (Milani *et al*., 2007), and rhizomes of lotus (Shad *et al*., 2011). However, there were very little information had been reported on the functionality of okra mucilage and its application in foods. Therefore, the objectives of this study were to evaluate the emulsifying properties of okra mucilage and its application as emulsifier in coconut milk.

Materials and Methods

Samples of okra

Okra plant with different maturity index (1, 2

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and 3) was purchased fresh from supplier in Tanjong Karang, Selangor, Malaysia. The maturity indices were evaluated based on color and texture of okra as stated by Federal Agricultural Marketing Authority (FAMA) (2008). The maturity indices of okra are determined as in Table 1.

Table 1. Maturity indices of okra

Maturity indices	Physical properties
1	Light green coloured with soft texture
2	Light green coloured, but its texture is hard
3	Green whitish or green yellowish, the harde texture and its end is not easily broken

Extraction of okra mucilage

Prior to the extraction, the okra was washed to remove any unnecessary dirt from their surfaces. The cleaned pods without seeds were then immediately frozen and kept at -20°C to prevent any color changes resulting from natural oxidation and browning process.

Okra mucilage extraction

The extraction of okra mucilage was carried out according to the method done by Ameena *et al.* (2010) with modifications. Initially, 1 kg of cleaned and sliced okra with different maturity indices was blended using ~5°C distilled water containing 1% (w/v) of sodium metabisulphite (Sigma-Aldrich, UK). Then, the slurry was centrifuged at 3000 rpm for 5 minutes and precipitated from the supernatant using pure acetone (99.5%) (Sigma-Aldrich, UK). The precipitated mucilage was washed several times with acetone and was freeze dried (Christ Alpha 1-4LD Plus, Germany). Then the dried okra mucilage was grinded into powder form using commercial dry blender.

Measurement of viscosity

The measurement of viscosity of extracted okra mucilage was carried out using method done by Amin *et al.* (2007). The analysis was carried out using Physica MCR 300 rheometer (Physica Messtechnic GmbH, Stuttgart, Germany) after 24 hours of hydration of mucilage. First, 1% of mucilage solution was prepared by dissolving 2 g of okra powder with 200 g of distilled water. The mixture was agitated vigorously for 15 minutes or until the solutions become homogeneous and viscous. The viscosity of the mucilage was measured at constant shear rates (10 s^{-1}) at different temperatures (10, 30, 50, and 70°C).

Measurement of water holding capacity (WHC) and oil holding capacity (OHC)

The WHC and OHC of okra mucilage were determined according to the method of Galla and Dubasi (2010). One gram of okra powder was added into 10 ml of distilled water (for WHC) or corn oil (for OHC). The mixture was then vortexed for 2 minutes. Next, the mixture was centrifuged at 3000 rpm for 30 minutes before any excessive water or oil was decanted. The WHC and OHC were then calculated by dividing the weight (g) of water or oil absorbed by 100 g of okra mucilage.

Measurement of emulsifying properties

The emulsifying properties of okra mucilage were determined by evaluating its emulsifying capacity (EC) and emulsifying stability (ES). Both were done according to the method of Naji *et al.* (2013). For EC, an oil-in-water emulsion system was prepared at room temperature (~26°C) by adding 6 mL of corn oil into 60 mL of 1% (w/w) of mucilage solution. Next, the emulsion was mixed at 2000 rpm for 10 minutes. After that, the emulsion was homogenised at 9600 rpm for 1 minute. Finally the suspension was centrifuged at 4000 rpm for 10 minutes. However, for ES, the emulsion was heated first in water bath (80°C) for 30 minutes and subsequently cooled under running tap water before the mixture was centrifuged at 4000 rpm for 10 minutes. The EC and ES of okra mucilage were calculated using following formula:

$$\text{EC or ES (\%)} = \frac{e_v}{t_v} \times 100$$

where, e_v is the volume of emulsion after being centrifuged and is total volume of mixture.

Emulsifying properties of okra mucilage in coconut milk

Coconut milk system was prepared according to Chappellaz *et al*. (2010) with modifications. First, fresh shredded coconut (purchased from supplier in Shah Alam, Selangor, Malaysia) was mixed using magnetic stirrer with warmed water (60°C) at a ratio of 1:1 (w/v) for 5 minutes before the coconut milk was extracted. Then, the coconut milk was added with 0.25%, 0.50% and 1.00% okra mucilage solutions. The mixture was first mixed using magnetic stirrer at 50°C for 5 minutes, and were continuously mixed for another half an hour at room temperature (~26°C) and homogenised at 9600 rpm for 1 minute. This mixture were then analysed for EC and ES according to the method by Naji *et al*. (2013) as described above.

Statistical analysis

All analyses were performed in triplicate for each treatment. Data was subjected to analysis of variance (ANOVA) to determine the significant difference among treatments at 5% level using SPSS Version 15.0.

Results and Discussion

Yield extraction of okra mucilage

This study found that among three different maturity indices of okra, the okra with maturity index 2 produced the highest mucilage yield (1.46%) followed by okra with maturity index 1 (1.10%) and maturity index 3 (0.31%). This result was in line with the study conducted by Sreeshma and Nair (2013) that reported the okra mucilage content increased from index 1 to index 2 and then gradually decreased from the fruit tissues at maturity index 3.

The increasing in mucilage content from okra with maturity index 1 to index 2 was due to growth and development of the okra itself. This was supported by Sreeshma and Nair (2013) who explained that mucilage contribute to moisture balance of the fruit and prevent it from drying out. In contrast, the declining in mucilage content as okra matures (index 2 to index 3) is due to degradation process. From the results in this study, it shows that mucilage content of okra at maturity index 3 decreased and it could be due to drying out as the fruit matures. According to Western *et al.* (2000), mucilage is the pectinous matrix of cell layers which undergoes degradation process as it enters senescence period.

Viscosity of okra mucilage

The viscosities of okra mucilage at different maturity index decreased as the temperature increased from 10 to 70°C (Figure 1).

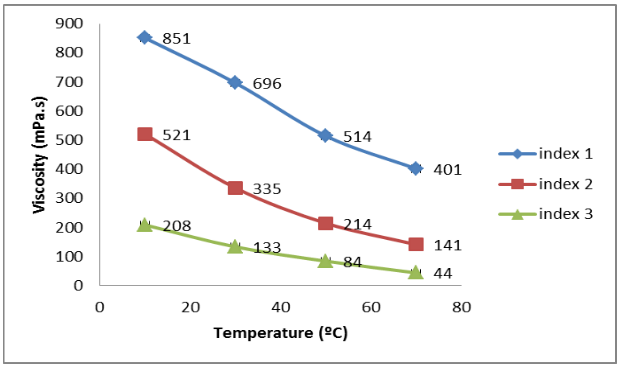

Figure 1. Effect of different temperature on the viscosity of okra mucilage with maturity index 1, 2 and 3

Similar findings was also reported by Amin *et al.* (2007) that viscosities of gums such as guar gum, durian seed gum and locust bean gum decreased as the temperature increases. In general, the decreasing in viscosity of mucilage upon heating was due to changes in polymer conformation into random coil structure, leading to less molecule combination followed with decreasing solution viscosity (Thanatcha and Pranee, 2011). The maturity index 1 of okra mucilage showed the highest viscosity at all temperature compared to other mucilage indices. This might be attributed to utilisation of carbohydrate in metabolic process as the okra growth, lead to reduction in mucilage viscosity of maturity index 2 and 3 (Adetuyi *et al.*, 2008). Ibe and Madukwe (2010) also reported that the higher the amount of mucilage, the more viscous the solution will be produced.

Water holding capacity (WHC) and oil holding capacity (OHC) of okra mucilage

Water holding capacity (WHC) was defined as the ability of a substance to associate with water under limited water conditions (Singh, 2001). The ability of gums to hold water producing gels or highly viscous solution is desirable in industrial application (Tosin *et al.*, 2010). This characteristic was also important to reduce vaporisation rate and alter freezing rate (Amid and Mirhosseini, 2012). As shown in Figure 2, the WHC of okra mucilage with maturity index 3 was significantly highest ($p<0.05$) as compared to maturity index 2 and index 1.

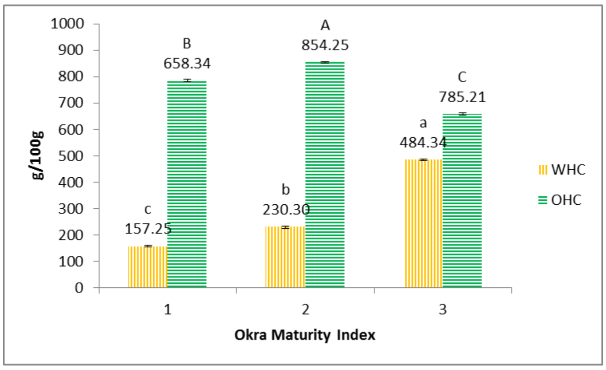

Figure 2. Water holding capacity (WHC) and oil holding capacity (OHC) of okra mucilage with maturity index 1, 2 and 3

Indeed, the high value of WHC of okra mucilage can be related with the presence of polysaccharide in mucilage (Al-Sayed *et al.*, 2012) including rhamnose, galacturonic acid, galactose, glucose and glucuronic acid (Lengsfeld *et al.*, 2004; Deters *et al.*, 2005). Miguel and Belloso (1999) stated that WHC represents the percentage of hydrophilic fraction, which has a greater affinity to absorb water. Thus, it might be concluded that okra mucilage maturity index 3 has highest polysaccharide fraction as compared to mucilage with maturity index 1 and 2. However, further study is needed in order to confirm the interaction in this food system.

Oil holding capacity (OHC) of a substance is the capacity of the substance for oil absorption (Phimolsiripol *et al*., 2011). In this study, different maturity indices of okra led to a significant different (p<0.05) in OHC). The okra mucilage with maturity index 2 has the highest OHC as compared to other indices. However, okra mucilage with index 1 has the lowest of both WHC and OHC. Its stabilising effect on an emulsion could arise mainly from its high thickening property where it has the highest viscosity compared to other okra indices. According to Thanatcha and Pranee (2011), high OHC value of okra mucilage was due to the presence of non-polar side chains and hydrophobic fraction such as protein and fat which may bind the hydrocarbon units of oil, thus inducing a higher capacity of oil absorption.

Emulsifying capacity (EC) and emulsifying stability (ES) of okra mucilage

Emulsifying capacity (EC) measures the ability of emulsion to retain its system after subjected to centrifugal force. As shown in Figure 3, the EC of okra mucilage with index 3 was significantly highest (p<0.05) as compared to other maturity indices.

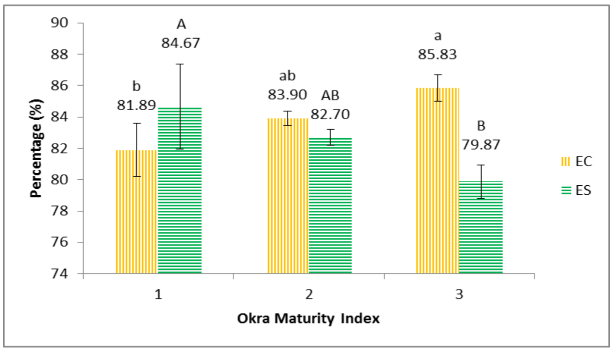

Figure 3. Emulsifying capacity (EC) and emulsifying stability (ES) of okra mucilage with maturity index 1, 2 and 3

Emulsifying stability (ES) is similar with EC except this analysis is to measure the stability of the emulsion that was subjected to high temperature before subjected to centrifugal force. Okra mucilage with maturity index 1 had significantly highest (p<0.05) ES compared to okra mucilage with maturity index 2 and 3. This might be due to its highest viscosity at different temperature. The highest ES exhibited by maturity index 1 of okra mucilage is expected since its emulsion stabilisation mainly due to its thickening property which has slow kinetics of destabilisation (Ndjouenkeu *et al*., 1997).

Contrary to this, EC of maturity index 2 and 3 of okra mucilage is lost by heat treatment at 80°C for 30 minutes (lower ES than maturity index 1). This phenomenon was similar to study conducted by Garti *et al.* (1999) on gum arabic solution. The loss of arabic gum solution EC by thermal treatment was related to denaturation of proteinous matter by heating. Nevertheless, high EC and ES of okra mucilage indicates that mucilage were able to act as stabiliser in an oil-in-water (O/W) emulsion similar to cress seed gum (92%) and xanthan gum (100%) (Naji *et al*., 2012).

Application of okra mucilage in coconut milk

According to the result obtained in this study, okra mucilage with maturity index 2 was chosen to be added into coconut emulsion since it produced the highest yield (1.46%) and OHC (854.25 g/100 g). In addition, the EC and ES properties of okra mucilage with maturity index 2 was also not significantly different (p<0.05) with mucilage of maturity index 1 and 3. This also seems economical extract to be used as stabiliser or emulsifier in coconut milk emulsion as this extracted okra mucilage produced highest yield after extraction.

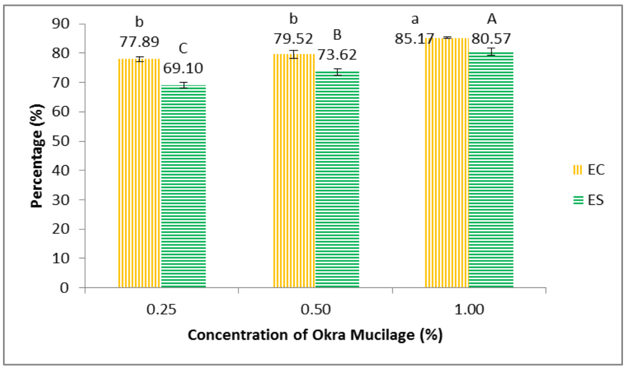

Figure 4. Emulsifying capacity (EC) and emulsifying stability (ES) of coconut milk containing different percentage of okra mucilage with maturity index 2

From Figure 4, okra mucilage with maturity index 2 has the ability to stabilise coconut milk by thickening its aqueous dispersion, where the EC and ES of coconut milk at different percentage, range from 77.9 – 85.2% and 69.1 – 80.6%, respectively. However, the results indicated that the highest EC and ES was at 1.00% mucilage and was significantly different compared to other mucilage percentage (p<0.05).

The ES of the emulsion system for all percentage are lower than EC. According to Onsard *et al.* (2006), the emulsifying properties of coconut proteins are affected by temperature. Coconut proteins have been shown to denature and coagulate when being heated at 80°C (Tangsuphoom and Coupland, 2008), thus effecting the ES. Therefore, high value of EC and ES of coconut milk added with okra mucilage reflect the ability of okra mucilage to act as a stabiliser in the emulsion.

Conclusion

From this study, it was found that okra mucilage have a potential to be used as a natural emulsifier and stabiliser in food especially in food emulsion system. The mucilage with maturity index 2 has highest yield percentage and OHC. It has medium WHC and exhibit medium viscosity at different temperature. Due to these, index 2 mucilage can be categorised as better stabiliser for emulsion and moderate thickener as compared with okra mucilage index 1 and 3 and was chosen to be added in an oil-in-water emulsion (coconut milk) as a stabilizer. However, the application of okra mucilage in food emulsion system needed to be exploited further to clarify the components that provide the improvement of food stability system.

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