Effect of chitosan-palm olein emulsion incorporation on tapioca starch-based edible film properties

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Abstract

The incorporation of hydrophobic substance to improve edible film properties is normally carried out using emulsifier. The present work was aimed to evaluate the effect of chitosan (CH)/ palm olein (PO) emulsion addition into tapioca starch (TS)-based edible film properties in the absence of emulsifier. Six combinations of CH/PO emulsion were prepared from CH (0.5% and 1% w/v) and PO (0%, 0.4 and 0.8% v/v) according to 2×3 factorial design. The emulsions were added to the tapioca-starch suspension and brought to gelatinisation temperature prior to drying by a casting technique. The result showed that the proposed method has successfully stabilised PO droplets in the film matrix. Elongation at break (EAB) and water vapour permeability (WVP) were significantly influenced by PO concentration in which higher PO concentration resulted in higher EAB and lower WVP. The lowest WVP was 0.240×10^{-5} g·m/Pa·h·m². Higher value of tensile strength was observed from increasing CH concentration. Meanwhile, both CH and PO did not have significant influence towards the thickness and total soluble matter (TSM). Microscopy observation further confirmed the successful incorporation of lipid (PO) into the polysaccharides (TS and CH) matrix.

Introduction

Tapioca starch (TS) and chitosan (CH) are both polysaccharides able to form edible film polymers. The use of both substances in edible films have been the subject of several studies, both as individual substance (Flores *et al*., 2007; Suseno *et al*., 2014; Kim *et al*., 2015; Shi *et al*., 2016) or as a composite (Chillo *et al*., 2008; Zhong and Xia, 2008; Vásconez *et al*., 2009). TS usage grows rapidly, especially because the price is lower as compared to that of starches from other sources (Flores *et al*., 2007). Meanwhile, the interest on using CH as edible film has also increased significantly, especially due to its abundant functional properties such as antioxidant, emulsifier and anti-microbial (Aranaz *et al*., 2009; Elsabee and Abdou, 2013; Ngo *et al*., 2015; Kaya *et al*., 2018).

Forming composite of polymers has been the strategy to improve the properties of film packaging (Robertson, 2006). In general, polysaccharide-based edible films have less protection towards water vapour due to its hydrophilic character (Galus and Kadzińska, 2015). Hydrophobic substance such as lipid is often added to polysaccharide-based edible films to improve its water vapour permeability (Robertson, 2006). On contrary, mixing TS with lipid is not easy and should resort to the use of additional emulsifier. Nindjin *et al*. (2015) has reported that TS-lipid edible film which was made without emulsifier led to phase separation during drying process.

CH has been reported to have good emulsifying capability (Pereda *et al*., 2012). Hence, several lipid-CH emulsion edible films have been studied (Vargas *et al*., 2011; Dimzon *et al*., 2013; Akyuz *et al*., 2018; Kaya *et al*., 2018) which include sunflower oil, olive oil, corn oil, seed oil and animal fats as the hydrophobic substance. To the best of our knowledge, although various studies on TS-CH and CH-lipid edible films have been published, the data on TS-CH-lipid composite film are still lacking especially when using palm olein (PO) as the lipid source. PO is the liquid fraction of palm oil, which is currently one of the most produced oils in the world. Different from its counterpart, palm stearin, which is solid at ambient temperature, PO maintains its liquid form rendering it to be easily handled during edible film production. Therefore, the present work was aimed to develop a method to prepare edible films using three components which were TS, CH and PO, and to evaluate their properties such as film forming solution viscosity, thickness, water vapour

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permeability, tensile strength, elongation at break, total soluble matter, and its optical microstructure.

Materials and methods

Experimental design and preparation of CH/PO emulsions

CH/PO emulsions were prepared based on Pereda *et al.* (2012) with slight modifications. Appropriate amount of commercial high degree (92%) deacylated CH powder (purchased from Che-Mix, Yogyakarta-Indonesia) was dissolved in 0.5% (v/v) acetic acid solution (Merck, Germany) at room temperature to achieve CH concentrations of 0.5% and 1% (w/v). The CH solutions were stirred on a magnetic stirrer for approximately 30 min until fully dissolved. PO (purchased in Semarang, Indonesia) was added to the CH solution with ratios of 0%, 0.4%, and 0.8% (v/v) thus resulting in a factorial design of 2×3 with six combinations: CH-0.5/PO-0, CH-0.5/PO-0.4, CH-0.5/PO-0.8, CH-1/PO-0, CH-1/PO-0.4, and CH-1/PO-0.8. The mixtures were then subjected to homogenisation (IKA T25 Ultra Turrax Homogeniser, Germany) for 10 min at 15,000 rpm before further prepared into edible films.

Preparation of edible films

TS powder (purchased in Semarang, Indonesia) was suspended in distilled water at concentration of 4% (w/v). The suspension was then added with each CH/PO emulsion (ratio 1:1) while continuously stirred with a magnetic stirrer. An appropriate amount of glycerol (MKR Chemicals Ltd, Semarang-Indonesia) was subsequently added to achieve 0.3 mL glycerol/gram TS as a plasticiser. The mixture was then homogenised for 3 min at 5,000 rpm at 50°C. Subsequently, the temperature was increased to 75°C while kept stirred for 5 min to ensure the gelatinisation of TS. To form a film, 55 mL of the obtained emulsion was then poured onto a Teflon pan (diameter 18 cm) and dried in a cabinet dryer for at least 18 h at 35±2°C. The films formed from exactly the same procedure were then peeled off from the pan and equilibrated at ambient temperature (25±3°C) and RH (65±3%) for at least 24 h.

Viscosity of film forming solution

The viscosity of film forming solution was measured using a uni-cylinder rotational viscotester Rion VT-06 (Tokyo, Japan). A suitable rotor type was submerged in film forming solution at 50°C. The measurements were done in triplicate for each film forming solution.

Thickness of film

The thickness of films was measured using hand micrometre with accuracy of 0.001 mm. The measurements were done in five random locations for each film.

Tensile strength and elongation at break of film

The tensile strength (TS) and elongation at break (EAB) analyses were conducted using TA Plus texture analyser (Lloyd Instrument, United Kingdom). Films were cut into 1 cm width strand prior to analysis. The films were fixed on the texture analyser crossheads with 5 cm gap. Crossheads speed was 8.33 mm/s. The TS was expressed as N/mm^2, while EAB was presented as % of additional length over the initial film length upon break. Measurements were done in triplicate for each film sample.

Water vapour permeability of film

The water vapour permeability (WVP) analysis was conducted based on ASTM Method E96-95 (ASTM, 1995) with slight modification. The film specimens were equilibrated in a chamber (Krisbow drying cabinet AD-160h, China) at 44% RH and 20°C for 24 h. Subsequently, the films were sealed on 5 cm inner diameter glass permeation cups filled with distilled water (maximum-height 2.54 cm below the film) in order to create 100% RH condition and equilibrated at the same condition. The weights of the cups were measured at 30 min intervals for 5 h. A linear regression was used to calculate the slope of cups' weight data plotted in g/h. The WVP of the films, expressed as $g \cdot m/Pa \cdot h \cdot m^2$, were calculated using the following equation.

$$WVP = WVTR \times \frac{thickness}{p2 - p1}$$

Water vapour transmission rate (WVTR) = ΔW $[\Delta t \, A]^{-1}$ where ΔW was the weight difference of the cup while Δt was the time interval of weighing. Therefore, $\Delta W / \Delta t$ was the slope of the fitted data of cup's weight over time. A was the cup mouth area (m^2), while p^2 and p^1 were vapour pressure (Pa) calculated from the relative humidity of inside and outside permeation cup (the chamber), multiplied by saturated vapour pressure at corresponding test temperature. The measurements were done in triplicate for each film.

Total soluble matter of film

The total soluble matter (TSM) was expressed as the percentage of dry matter of film soluble in distilled water after 24 h immersion. This wet method was proposed by Rhim *et al.* (1998) and performed

with slight modifications to determine the TSM of the edible films. Film specimens were cut into 3 cm × 1cm piece, and the weight were measured (±0.0001 g) prior to immersion in 50 mL distilled water containing 0.02% potassium sorbate to prevent microbial growth. After 24 h, the films were taken and dried in drying oven at 105°C until a constant weight was achieved to obtain the dry weight of insoluble film (m_u). In order to get the initial dry weight, other pieces of the same specimen film were not immersed in distilled water and dried within the same method. The difference in the initial dry weight of film (m_i) and dry weight of insoluble film (m_u) was considered as the soluble matter of the film and expressed in per cent using the following equation.

$$TSM = \frac{m_i - m_u}{m_i} \, x \, 100\%$$

Optical microscopy of film

The surface morphology of the films was observed under an Olympus CX22 microscope (Tokyo, Japan), coupled with Mshot-MD50 (China) camera. A piece of film was mounted on the glass slide and observed under controlled light to obtain a clear image. The images were captured under 400× magnification.

Statistical analysis

The collected data of all parameters were analysed for their variances and followed up by Duncan post hoc analyses on the significantly different ($p <$ 0.05). Significant interactions between CH and PO concentration were included in the mode. The results were presented as least squares means (LSMean) and standard error (SE). The analyses were carried out by using SAS (Statistical Analysis System, Version 9.13).

Results and discussions

The homogenisation was applied twice in the edible film preparation; during preparation of CH/PO emulsion and after the complete mixture of TS/CH/PO. The first homogenisation was to help PO dispersion in the CH solution. Similar to the result of Pereda *et al.* (2012), CH was able to stabilise the PO droplets in any tested combinations. This was due to the amphiphilic property of CH. The inclusion of PO made the CH solution became visually milky from initially transparent, most probably due to oil droplet's ability to scatter light. The second homogenisation was performed after the complete mixture reached 50°C with the objective to reach homogenous mix between two polysaccharides (TS and CH) before the TS gelatinised. The subsequent increase in heating temperature to 75°C should suffice to gelatinise the TS since it could gelatinise at a temperature range approximately 65 – 75°C as determined by its thermal properties (Han *et al.*, 2012), and TS starts to change its viscosity at 72°C as determined by its pasting properties (Huang *et al.*, 2017). The gelatinised TS was therefore expected to form a CH/PO containing matrix and kept them stable.

Viscosity of film forming solution

Table 1 shows the viscosity of film forming solution, tensile strength and elongation at break. Viscosity of the film forming solution influenced the spread-ability of the solution when it was poured onto the casting pan. The thicker the solution, the harder it was to spread evenly. Another factor affecting spread-ability of edible emulsion during casting technique is cohesion which determines the flow behaviour of the solution. Flow behaviour is determined by concentration, molecular size and molecular weight of discontinuous phase (Rao *et al.*, 2014). Since film forming solution was prepared from TS, CH, and PO, they influenced the viscosity. Concentration of CH and PO changed as the treatment and they influenced the viscosity as presented in Table 1. Tapioca starch on the other hand was kept constant. However, at 50°C, tapioca starch could generate viscosity as affected by gelatinisation. As can be seen in Table 1, viscosity increased very significantly as both CH and PO concentration increased ($p < 0.01$). However, the *p* value of CH*PO was 0.431 (> 0.05) which indicated that the effect of interaction between both compounds towards viscosity was not statistically significant. According to Mcclements (2005), as biopolymer molecules, CH suspension can be categorised as a dilute suspension of rigid non-spherical particles. Furthermore, CH suspension disrupts the fluid flow of continuous phase and causes greater energy dissipation due to the friction thus increases the viscosity.

Table 1. Film forming solution viscosity, elongation at break and tensile strength of edible film

CH	0.5%			1%			SE	p value [z]		
PO	0%	0.4%	0.8%	0%	0.4%	0.8%		CH	PO	CH*PO
Viscosity (dPas)	0.667	0.967	1.233	0.767	1.300	1.467	0.087	0.007	<0.001	0.431
EAB (%)	17.32	34.37	67.47	43.61	55.76	59.64	7.044	0.067	0.004	0.066
Tensile strength (N mm^{-2})	4.808	5.279	5.633	7.109	7.688	9.534	1.074	0.004	0.300	0.714

[z] CH*PO indicates the significance of the interaction between CH and PO

Oil such as PO emulsion should be classified as a dilute suspension of fluid spherical particles which on contrary lessens the energy loss due to friction and therefore lowers the viscosity. However, the result obtained in the present work is not in agreement with former explanation. A possible explanation was that PO droplets were stabilised by the amphiphilic action of CH thus it could be safely assumed that the droplets were covered by rigid CH layer. Therefore, the PO droplets could no longer be considered as fluid spherical particles, but more as rigid particles. Consequently, it increased the viscosity following aforementioned mechanism.

Based on this finding, the increased viscosity was very likely to happen in oil in water emulsion which was stabilised by biopolymer such as protein and polysaccharide. The information is useful for edible film preparation which uses composition with properties similar with that in the present work, considering viscosity of film forming solution as one of the limiting factors.

Elongation at break and tensile strength of film

As part of film mechanical properties, TS and EAB are undoubtedly among the most important parameters. They define the usability of the film as packaging. A higher TS and EAB of a film are generally preferred due to its durability. As shown in Table 1, the EAB was found to be significantly influenced by PO ($p < 0.05$), but was not influenced by CH ($p > 0.05$). The EAB increased with increasing PO concentration in any CH concentration used. However, the increasing effect of PO on the EAB did not interact with the CH concentration used (p CH*PO > 0.05). Furthermore, PO-0.8 effect at CH-1 (59.64%) was lower than that at CH-0.5 (67.47%). The EAB enhancement as the effect of PO in the present work is in agreement with several previous studies by Pereda *et al.* (2012), Tongnuanchan *et al.* (2015) and Shi *et al.* (2016) in which the incorporation of oil enhanced the film elongation properties. The lipid inclusion has been known to provide plasticising effect which is associated with deformability of lipid droplets during tensile process (Shi *et al.*, 2016). In the case of a relatively low EAB at CH-1/ PO-0.8, the following explanation is proposed.

At a relatively high concentration, the combination of CH and TS made polysaccharide matrix firmer. CH, in a concentration of a slightly higher than PO, trapped its droplets. Since the plasticising effect of PO was hindered by the rigid molecules of CH, the deformability of the matrix was lowered. On the other hand, the hydrophobicity of PO droplets hindered the amphiphilic property of CH to bind water, which consequently limited the plasticising effect of CH. The plasticising effect of CH was studied by Bonilla *et al.* (2013) on wheat starch based film. The effect was attributed to the fact that CH is a charged chain and able to involve greater water molecules in the polymer chain. It has been widely known that water molecules have plasticising effect on edible film (Irissin-mangata *et al.*, 2001).

In regards to TS of film, the present work showed that CH was found to have very significant effect ($p < 0.01$). Higher CH concentration increased the TS of edible film. The result Is in agreement with several other studies (Bangyekan *et al.*, 2006; Bonilla *et al.*, 2013) in which polysaccharide's concentration directly affected the TS. However, the TS was not influenced by PO. Superimposed polysaccharide chains make the back bone of the edible film matrix, thus higher concentration leads to more condensed and stronger matrix. Albeit surrounded by rigid CH molecules, the trapped PO did not give much effect to strengthen the matrix. Consequently, it was shown that CH and PO had no significant interaction (p CH*PO > 0.05) in the change of TS of edible film.

Thickness and total soluble matter

The thickness and TSM observations are presented in Table 2. Thickness is an important property in film application for any purposes while TSM indicates the solubility and durability of the film in the presence of water. In the present work, the thicknesses of films prepared at any combination were even ($p > 0.05$). The thickness is influenced by solid matter incorporated in film matrix. To some extent which depends on density, the higher the concentration of solid matter incorporated, the thicker the film. In this case, the concentration of solid matter is relevant to non-soluble matter. Since the values of TSM were also even among treatments ($p > 0.05$), this indicates

Table 2. Thickness, WVTR, WVP and TSM of edible film

CH	0.5%			1%			SE	p value[z]		
PO	0%	0.4%	0.8%	0%	0.4%	0.8%		CH	PO	CH*PO
Thickness (mm)	0.079	0.076	0.077	0.079	0.075	0.078	0.009	0.995	0.934	0.995
TSM (%)	0.230	0.258	0.240	0.255	0.232	0.230	0.029	0.810	0.930	0.649
WVTR (g h^{-1})	0.105	0.097	0.079	0.090	0.091	0.084	0.011	0.179	0.855	0.515
WVP ($\times 10^{-5}$ g·m/Pa·h·m^2)	0.274	0.272	0.241	0.324	0.279	0.240	0.019	0.250	0.028	0.385

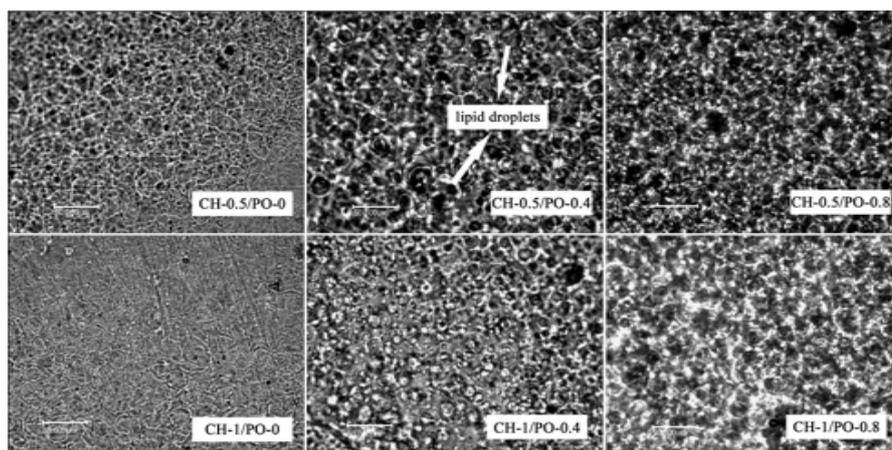[z]CH*PO indicates the significance of the interaction between CH and PO

Figure 1. Light microscopy images of the films (400× magnifications). CH: chitosan; PO: palm olein.

that within the concentrations used, TS, CH, and PO resulted in equal interaction among them to form the film matrix with similar thickness. Insoluble property of CH and PO could be responsible for this film formation (Pereda *et al*., 2012) as indicated by the increased viscosity with increasing both CH and PO in film forming solution. As generally accepted, viscosity increased with increasing concentration, molecular size, and molecular weight (Rao *et al*., 2014).

Water vapour permeability

The value of WVP following equilibration at 44% RH at 20°C for 24 h is presented in Table 2. It is apparent that WVP decreased with increasing PO ($p < 0.05$). Furthermore, no effect on WVP was found as contributed by CH. This phenomenon is in agreement with several previous studies (Pereda *et al*., 2012; Dimzon *et al*., 2013; Shi *et al*., 2016). The use of PO in the present work was intended to improve the barrier property of film. As shown, the hydrophobic properties of PO decreased the edible film permeability against moisture and thereby decreasing the WVP. The result also proves the successful incorporation of PO into CH-TS polysaccharide matrix using the approach demonstrated in the present work. Polysaccharide-based edible films have been known to show excellent barrier properties towards gas and lipid while a bit lacking towards moisture. Therefore, many attempts have been done to improve it by lipid inclusion (Vieira *et al*., 2011).

Optical microscopy

Figure 1 shows the film surface under microscopic observation. Lipid droplets were observed as circle with shiny edge as a result of light diffraction and were scattered in the matrix. This finding shows that PO was dispersed and stabilised within the polysaccharides (TS and CH) matrix. The present work showed that

CH 1% incorporated PO in a smaller droplet size (Fig 1. CH-1/PO-0.4) as compare to that of 0.5% (Fig 1. CH-0.5/PO-0.4) thus suggests a better PO dispersion with higher CH concentration. This phenomenon could be attributed to the emulsifying ability of CH due to its amphiphilic property (Pereda *et al*., 2012) thus higher CH gives higher emulsifying effect.

Conclusions

The present work has shown a successful incorporation of lipid (PO)/CH emulsion in the TS matrix. The newly proposed method offers the benefits of hydrophobic substance addition in a low priced-TS based edible film. CH, in the present work, was not only successfully used to replace emulsifier but also contributed to the improved characteristics of edible film. The approach could be used in other lipid-polysaccharide combination and to reduce the usage of emulsifier. The present work's objective which was to improve the barrier properties of film prepared from TS/CH/PO has been achieved. The WVP increased with increasing PO as a result of successful incorporation. Meanwhile, the application properties such as EAB and TS increased with increasing PO and CH, respectively. There was no significant interaction found between CH and PO on all observed parameters. Based on the results, it is recommended to use 0.5% CH and 0.8% PO since the treatment resulted in highest EAB and a close value of WVP thus more valuable in application due to a wider coverage as a packaging material.

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