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Formation of Coconut Oil By-Product Protein Concentrate-Pectin Through Electrostatic Interaction to Improve Emulsifying Properties

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Abstract

Coconut oil has been rising in popularity because of its health benefits. Coconut oil by-product or *blondo* is obtained during wet processing for virgin coconut oil production. It has a high protein content and can be utilized as an emulsifier in food products. This research aimed to examine the effect of pH, protein and pectin concentration on the stability and emulsification properties of heat-treated *blondo* protein concentrate-pectin complexes. The best conditions of pH, pectin and protein concentration for forming *blondo* protein concentrate-pectin complexes through electrostatic interaction were obtained by mixing *blondo* protein concentrate solution (0.5 to 1.5 wt%) with pectin (0.1 to 0.35 wt%) at different pH conditions (3 to 5). These particles were tested for emulsifying activity, stability and heat stability (85±2 °C; 15 minutes). Complexes formed using 0.5 wt% protein and 0.35 wt% pectin at pH 4 obtained from these experiments had the best zeta potential value and particle size, respectively -25.88 mV and 192.92 nm. Complexation between protein and pectin enhanced the emulsion activity index (EAI), emulsion stability index (ESI) and protection of self-aggregation protein during heating. Complexes that were formed remain stable across a range of pH values (pH 4 to 7). Thus, *blondo* protein concentrate-pectin complexes formed in this research through electrostatic interaction have better functional properties than the *blondo* protein concentrate before complexation. Emulsions created using *blondo* protein concentrate-pectin complexes through electrostatic interaction also had a higher value of emulsifying activity, stability and heat stability than emulsions with *blondo* protein concentrate alone.

Keywords: *blondo* protein concentrate; electrostatic interaction; emulsion; soluble complex; stability

INTRODUCTION

Coconut oil has been rising in popularity because it is a source of lauric acid, which know for its health benefits, such as hypocholesterolemic, anticancer, anti-hepatosteatosis, antidiabetic, antioxidant, anti-inflammatory, antimicrobial and skin moisturizer properties (Lima and Block, 2019; Deen et al., 2021). Coconut oil is produced using two methods, namely the dry method and the wet method. Coconut oil by-product or *blondo* is obtained during wet processing for virgin coconut oil production. In the past, the protein fraction of *blondo* was either discarded or used as animal feed, but it may present a high potential for use as a new protein source or as a valuable compound in the food industry.

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As stated by Permatasari (2015) and Themtawee et al. (2020), the protein content in *blondo* produced through wet processing with physical separation is 24.22%, while Thaiphanit (2016) and Permatasari (2015) stated that the protein content in *blondo* can increase up to 80.30% if it is processed into concentrate. It has a high protein content and can be utilized as an emulsifier in food products. Globulins and albumins are the primary protein fraction in *blondo* (Patil and Benjakul, 2017). They contribute to the surface properties such as hydrophobicity, emulsification and oil binding capacity (Lan et al., 2018). However, the use of *blondo* as an emulsifier in food or beverage formulas still need to be improved due to its poor functional properties.

Emulsions formed from *blondo* protein concentrate were highly unstable to creaming at pH 3 to 5 (Onsaard et al., 2006). It is probably because the emulsification ability of proteins is strongly influenced by pH. Several methods have been used to improve a protein's effectiveness as an emulsifier (Thaiphanit and Anprung, 2016; Fadhila, 2019). Phosphorylation, succinylation, conjugation with maltodextrin, and heat treatment, as previously described by other researchers, could improve the protein's functionality as an emulsifier. These modifications could increase emulsifying activity, but they still had poor thermal stability and were highly unstable to droplet aggregation when pH or ionic strength was modified.

One promising way to improve the ability of proteins as emulsifiers is by forming proteins and soluble polysaccharide complexes (Jones et al., 2009; Salminen and Weiss, 2014; Lan et al., 2018). Forming soluble biopolymer complexes through electrostatic interactions can improve functional properties (Jones et al., 2009; Klassen et al., 2011; Salminen and Weiss, 2014; Semenova, 2017). Previously, researchers described that the solubility of whey protein in beverage products could be enhanced upon forming soluble biopolymer complexes with pectin (Wagoner and Foegeding, 2017). Due to the strength of the electrostatic interactions, the biopolymer complexes will remain as soluble complexes or undergo associative phase systems becoming insoluble complexes, i.e., complex coacervations or precipitation.

Several researchers have previously characterized electrostatic complexes formed by proteins and polysaccharides, such as β-lactoglobulin-pectin, whey protein-pectin, pea protein-high methoxyl pectin, pea protein-gum Arabic, canola protein isolate-gelatin (Jones et al., 2009; Liu et al., 2009; Aryee and Nickerson, 2012; Salminen and Weiss, 2014; Lan et al., 2018). However, no research describes factors that impact the charge, size and emulsification properties of biopolymer particles formed by thermal treatment of *blondo* protein-pectin complexes. The researchers hypothesized that the initial pH, protein and pectin concentration would influence the properties and stability of the formed biopolymer complexes. Therefore, this study aimed to examine the effect of pH, protein and pectin concentration on the properties, stability and emulsification properties of *blondo* protein concentrate-pectin complexes.

In this experiment, researchers used *blondo* protein concentrate and pectin. Pectin was used since it has previously shown that pectin can form relatively stable biopolymer particles with other proteins (Jones et al., 2009; Salminen and Weiss, 2014; Lan et al., 2018). The heat treatment used in this experiment is expected to enhance the stabilization of biopolymer particles over the pH range and inhibit the dissociation of electrostatic interaction between particles when pH is altered. This information can produce particles with notably desirable characteristics and be applied to a food product.

MATERIALS AND METHOD

Material

Blondo obtained from Grubiku Group, Yogyakarta, was transformed into a protein concentrate with the following characteristics: 8.61% moisture, 71.24% protein and 0.57% fat. The other materials used are pectin, buffer phosphate pH 7, palm oil, sodium hydroxide, chloride acid, sodium azide, sodium dodecyl polyphosphate, Na_2CO_3, $CuSO_4$, Na.K.tartrat and folin ciocalteu reagent. All chemical reagents used are of analytical quality and were bought from a Yogyakarta distribution center.

Protein and pectin stock solution preparation

The preparation of protein and pectin solution is based on the procedure described by Patil and

Benjakul (2017). *Blondo* protein concentrate solution was prepared in a concentration of 0.5% to 1.5% (w/v); pectin solution was 0.1 to 0.35% (w/v). Pectin dissolved in buffer phosphate solutions at pH 7, stirred for 30 minutes at 60 °C using a hot plate magnetic stirrer at 1000 rpm. *Blondo* protein concentrate dissolved in buffer solutions at pH 11 and stirred for one hour at room temperature using a magnetic stirrer at 800 rpm. Both solutions were left to rest for 16 to 18 hours at 4 °C to ensure complete hydration. After incubation for 16 to 18 hours, the protein solutions were adjusted to pH 7 with 0.5 N chloride acid and ready to use.

Blondo protein concentrate-pectin complexes preparation

The preparation of protein and pectin complexation is based on procedure described by Oduse et al. (2017). *Blondo* protein concentrate-pectin complexes were prepared by mixing the appropriate mass of stock solutions to achieve the desired concentrations of each biopolymer solution. The mixed solutions were adjusted to a known pH (3 to 5) using 0.5 N chloride acid and stirred with a magnetic stirrer at 800 rpm at room temperature. Then, the mixed solutions were heat treated in a temperature-controlled water bath (set at 82±2 °C) for 20 minutes. The samples were unstirred during heat treatment. After the thermal treatment, solutions were removed from the water bath, allowed to cool at room temperature, and stored for two hours before analysis. The combination concentration of pectin and protein was used in the experiments to explain the effect of pH in forming complexes, respectively 0.3 wt% and 1 wt%. Concentration pectin used to investigate the effect of pectin in the formation of protein-pectin complexes are 0.1 wt% to 0.35 wt% and 1 wt% protein at pH 4, while 0.5 wt% to 1.5 wt% protein and 0.35% pectin at pH 4 used to investigate the effect of protein concentration in the formation of protein-pectin complexes.

Emulsion preparation

The emulsion was prepared according to the method developed by Pearce and Kinsela (1978) and Naik et al. (2012) with some modifications. The method by Albano and Nicoletti (2018) was used to prepare oil in water emulsions containing 10% palm oil (w/w) as the oil phase and 90% complexes or protein solutions as the aqueous phase (w/w). As much as 0.25% (w/w) sodium azide was added to prevent microbial growth. The emulsions were made by homogenizing the oil and aqueous phase using an IKA Ultra-Turrax T-25 Digital high shear mixer for 10,400 rpm at 5 minutes, then followed by ultra-sonication using an ultrasonic probe at 69% amplitude for 2 minutes with a probe immersed 1 cm below the emulsion surface.

Turbidity analysis

This analysis was prepared according to the method developed by Ibrahim et al. (2019) with some modifications. The turbidity of solutions was analyzed using a Thermo Scientific Genesys 10S UV-Vis spectrophotometer at 600 nm, using cuvettes with a path length of 1 cm. Before the measurements, samples were vortexed 15 seconds. Distilled water was used as a blank reference. The results of measurements were obtained from triplicate measurements. Turbidity value is calculated using Equation 1.

$$\text{Turbidity} = \frac{(2.303 \text{ x absorbance})}{(\text{Cuvette length in cm})} \quad (1)$$

Particle size and charge analysis

This analysis was prepared according to the method developed by Kori et al. (2021) with some modifications. Particle size and charge measurements were determined using dynamic light scattering (Zetasizer Nano, Malvern Instruments). Solutions were diluted 5x using buffer phosphate solution at the same pH as the samples to avoid multiple scattering effects and changes in pH. The particle size data is reported as the volume-based mean diameter, while the particle charge data is described as the zeta potential value. The results of measurements were obtained from the average of the fourth measurement.

Emulsion activity index (EAI) and emulsion stability index (ESI)

This analysis was prepared according to the method developed by Aizawa (2014) with some modifications. As much as 50 µl of the emulsion was quickly carried out from the bottom of the glass at the first emulsion after preparation and at 60 minutes. It respectively mixed with 5 ml of 0.1% (w/v) sodium dodecyl sulfate (SDS) solutions. After that, the solution

is then mixed by the vortex. The absorbance of the mixture was measured at 500 nm by Thermo Scientific Genesys 10S UV-Vis spectrophotometer using cuvettes with a path length of 1 cm. As much as 0.1% (w/v) SDS solution was used as a blank reference. The results of measurements were obtained from duplo measurements. EAI and ESI were calculated based on Equations 2 and 3.

$$ESI = \frac{A_0 \times \Delta t}{\Delta A} \quad (2)$$

$$EAI = \frac{2T\ (A_{60} \times D)}{(1-V) \times C \times 1000} \quad (3)$$

Where, A_0 and A_{60} are the absorbance of the sample emulsions measured at 0 and 60 minutes, Δt is 60 minutes, ΔA is A_0 to A_{60}, C is protein concentration (g ml^{-1}), V is oil fraction from emulsions and D is the dilution factor.

Heat stability analysis

This analysis was prepared according to the method developed by Jourdain et al. (2008) with some modifications. Ten milliliters of emulsion samples were transferred into a test tube, tightly sealed with a plastic cap, then heated in a temperature-controlled water bath (set at 85±2 °C) for 15 minutes. These emulsions were removed from the water bath and allowed to cool at room temperature until their temperature was 30 °C; then, these emulsions were centrifuged for 10 minutes, 2000 rpm at room temperature (27 °C). Some emulsions are separated into an opaque layer (cream) at the top and a slightly turbid or transparent layer (serum) at the bottom. The total height of the emulsions in the tubes (He) and the serum layer (Hs) height were measured. Heat stability was calculated based on Equation 4.

$$\text{Heat stability } (\%) = \frac{Hs}{He} \times 100 \quad (4)$$

Statistics analysis

All research was carried out on two prepared samples, and findings are expressed as the mean standard deviation. Differences between the tests were discovered using an ANOVA and a post hoc Tukey test with a 95% confidence level. Statistics analysis was performed using Minitab 16.0 software.

RESULTS AND DISCUSSION

Effect of pH on turbidity of protein-pectin complexes formation

The turbidity is one of the main factors that indicate the formation of *blondo* protein concentrate-pectin complex. pH significantly affected the turbidity value ($p < 0.05$) in forming *blondo* protein concentrate-pectin complex. The turbidity of unheated *blondo* protein concentrate was very high at pH 4 but decreased at pH 3 and 5 (Figure 1). This occurs because pH 4 is expected as the isoelectric point (pI) of *blondo* protein concentrate based on protein solubility analysis (data not shown). Protein at pI had zero net charge, which causes self-aggregation due to the relatively weak electrostatic repulsion between the protein molecules (Jones et al., 2009; Salminen and Weiss, 2014). Self-aggregation of proteins could cause the formation of large particles that cause increased turbidity. Based on T-test analysis, heating could increase the turbidity of the protein solution. The heated protein solution had a higher turbidity value 2.07 to 2.23 times higher than the unheated one. This indicates that heat could increase protein aggregation due to increased hydrophobic interactions and disulfide bonds (Dickinson, 1998; Jones et al., 2009).

The effect of pH on a turbidity of heat-treated *blondo* concentrate protein-pectin complex solutions differed from protein alone. The heat-treated *blondo* concentrate protein-pectin complex solutions had the lowest turbidity at pH 4, which showed that relatively small complexes were formed (Figure 1). At pH 3 to 4, the turbidity of complex heated solutions decreased compared to a turbidity of protein heated alone. It probably indicated that adding pectin could reduce the self-aggregation of proteins. It was probably due to the blocking of hydrophobic binding sites on the surface of the globular protein in *blondo* of protein concentrate by the bulky carbohydrate moiety (Dickinson, 1998). Meanwhile, in pH 4.5 to 5 turbidity of complex heated solutions increased compared to complexes at pH 4 and it wasn't significantly affected compared to protein heated alone. It was probably that adding the pectin in the solution could not prevent the aggregation

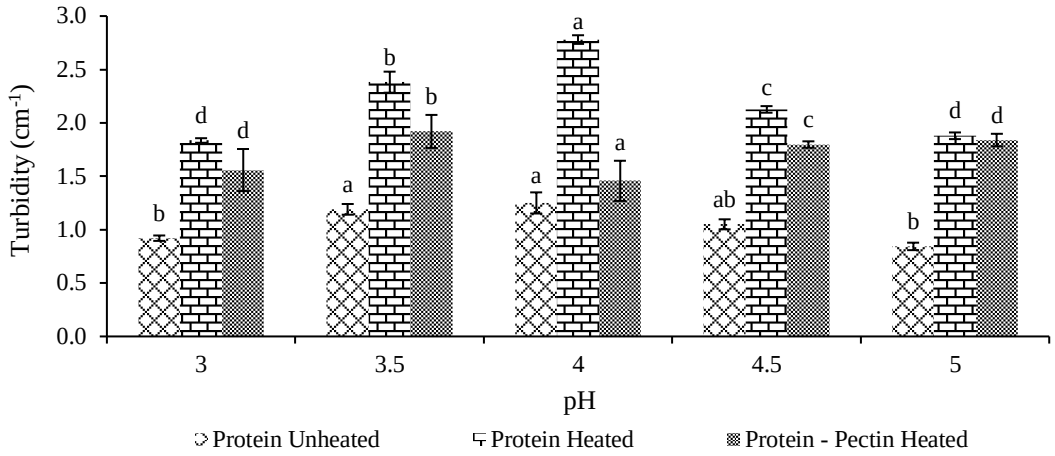

Figure 1. Effect of pH on turbidity (at 600 nm) of *blondo* protein and *blondo* protein-pectin solutions containing 1 wt% protein to 0.3% pectin mixture and 1 wt% protein. Heated at 82±2 °C for 20 minutes

between protein molecules because in this pH, both components had a higher charge, so it could decrease the electrostatic interaction between protein and pectin and increase the particle size of complexes were formed.

Effect of pH on particle size of *blondo* protein-pectin complexes formation

Figure 2 shows the effect of pH on the particle size of protein-pectin complexes formation. The particle size measurements of *blondo* protein-pectin complexes obtained from this experiment had the same pattern as the turbidity of protein-pectin complexes. At pH 3 to 4, the particle size of protein-pectin complexes decreased. It was probably due to the ability of pectin to prevent the self-aggregation of protein molecules that could increase the particle size of complexes that were formed through blocking of hydrophobic binding sites on the surface of the globular protein in *blondo* of protein concentrate by the bulky carbohydrate moiety (Dickinson, 1998).

On the other hand, at pH 4.5 to 5, the particle size of protein-pectin complexes increased. It was because the ability of pectin molecules to prevent the aggregation in protein molecules is decreased. Both components had a higher charge in this pH, so it could decrease the electrostatic interaction between protein and pectin, and increase the particle size of complexes that were formed.

Effect of pH on zeta potential of *blondo* protein-pectin complexes

Figure 3 shows the effect of pH on the zeta potential of protein-pectin complexes. Based on these experiments, the isoelectric points of *blondo*

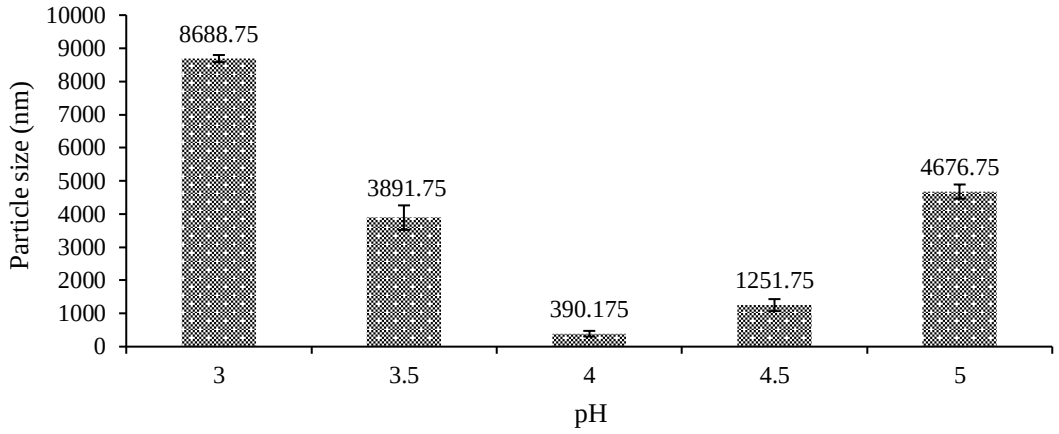

Figure 2. Effect of pH on the particle size of *blondo* protein and *blondo* protein-pectin solutions containing 1 wt% protein to 0.3% pectin mixture and 1 wt% protein. Heated at 82±2 °C for 20 minutes

protein solution were at pH 3.5. The zeta potential of protein above the isoelectric pH point was positive and negative at below the point. pH significantly affected the zeta potential value ($P < 0.05$) in forming *blondo* protein concentrate-pectin complex. Based on the T-test analysis, the formation of protein-pectin complexes could increase the zeta potential value compared to the protein alone at the same pH range. It is probably the cause of the addition of pectin molecules which was the negatively charged polysaccharide that could increase the zeta potential value of complexes formed. At pH 4, the electrostatic interaction between protein and pectin resulted in the formation of soluble complexes with highly negatively charged than the protein alone (Figure 3) (Jones et al., 2009). Soluble protein-polysaccharide complexes are produced when both the biopolymers carry a net negative charge. In this case, the attraction implicates positively charged local patches on the protein interacting with the anionic polysaccharide (Dickinson, 1998).

The zeta potential of complexes showed the lowest value at pH 3. This pH was relatively close to the pKa of pectin (pKa 2.5 to 3.0), which could be the protonation of carboxyl groups of pectin that decreased the negative charge (Bengoechea et al., 2011). Charge neutralization could occur in behalf of this phenomenon, leading to the bridging effects of complexes formed that could increase particle size (Jones et al., 2009; Oduse et al., 2017). Turgeon et al. (2007) also stated that if the pH is reduced too far below the isoelectric point, extensive complex formation occurs, leading to larger particle size and weakly charged complexes are formed. Low zeta potential values could result in the formation of aggregates between particles, thereby affecting particle size.

Effect of pH on EAI and ESI of *blondo* protein-pectin complexes formation

EAI is a parameter to measure a protein's ability to act as an emulsifier. EAI value also shows a particle's ability to reduce the surface tension in the oil and water interface area. pH significantly affected the EAI value ($P < 0.05$) in forming *blondo* protein concentrate-pectin complex.

Figure 4a shows the effect of pH on EAI values on the formation of the *blondo* protein concentrate-pectin complexes. *Blondo* protein concentrate-pectin complexes formed at pH 3; 3.5; 4 and 4.5 could increase the EAI value respectively, 59%; 146.88%; 169.63% and 21.02% compared to EAI values of the native protein. However, the *blondo* protein concentrate-pectin complexes formed at pH 5 could decrease the EAI value by 60.62% compared to the EAI value of the native protein. The highest EAI value was obtained in complexes formed at pH 4 (47.67 m^2 g^{-1}), while the lowest EAI value was at pH 5 (7.08 m^2 g^{-1}). The most significant increase in EAI values in complexes formed at pH 4 could be caused by its smallest particle size due to electrostatic interactions between *blondo* protein concentrate and pectin molecules that could prevent the self-aggregation of proteins.

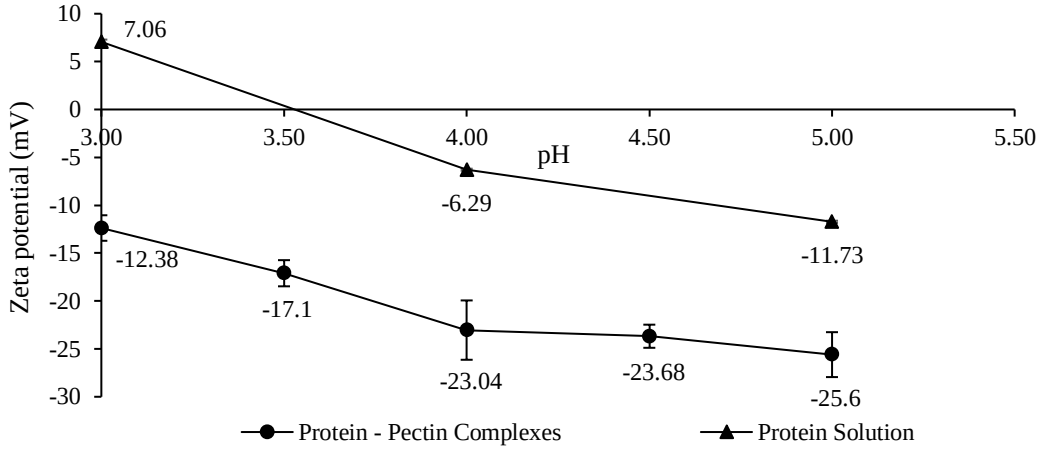

Figure 3. Effect of pH on the zeta potential of *blondo* protein and *blondo* protein-pectin solutions containing 1 wt% protein to 0.3% pectin mixture and 1 wt% protein. Heated at 82±2 °C for 20 minutes

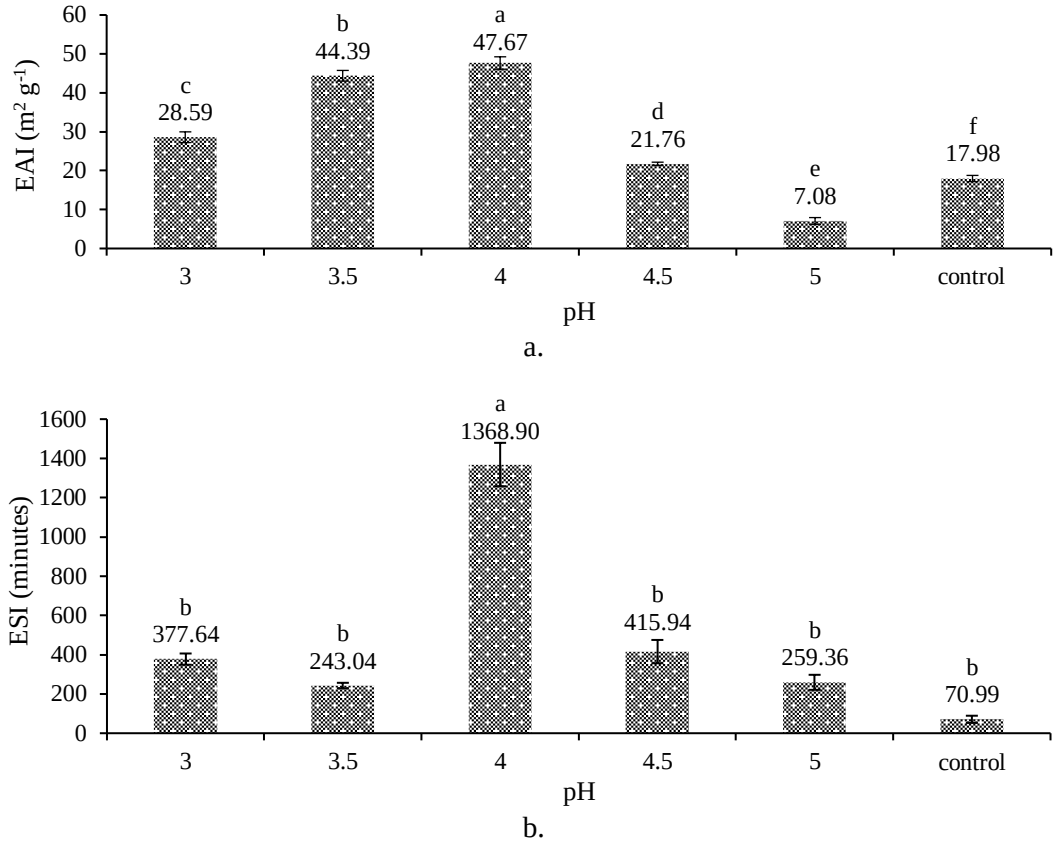

Figure 4. Effect of pH on the EAI and ESI of *blondo* protein and *blondo* protein-pectin solutions containing 1 wt% protein to 0.3% pectin mixture and 1 wt% protein. Heated at 82±2 °C for 20 minutes

Meanwhile, the complexes formed at pH 3 and 5 had a lower EAI value because they had large molecules (Figure 2), probably because of the self-aggregation of protein molecules at this pH. Complex with a small size would be easier to spread at the oil-water interface, so it had better emulsification activity than the native protein. On the other hand, aggregated proteins do not spread as quickly at the oil-water interface, and it had less efficient at emulsification activity (Oduse et al., 2017). Besides the particle size of complexes, the increased solubility of protein molecules due to the formation of protein-pectin complexes could probably enhance the EAI value in emulsions formed using protein-pectin complexes.

ESI is one of the main parameters used to measure a protein's ability to stabilise the emulsion. pH significantly affected the ESI value ($P < 0.05$) in the formation of blondo protein concentrate-pectin complex. Figure 4b shows the effect of pH on ESI values on the formation of the *blondo* protein concentrate-pectin complexes. *Blondo* protein concentrate-pectin complexes were formed at pH 3; 3.5; 4; 4.5 and 5 could increase the ESI value compared to the ESI values of the native protein. The highest ESI value was obtained in complexes formed at pH 4, while the lowest ESI value was at pH 5.

Complexes formed at pH 4 had the best ESI value because of the electrostatic interaction between protein and pectin, which resulted in the formation of soluble complexes with highly negatively charged. Complexes with high negative charge could increase the electrostatic repulsion between emulsion droplets (Figure 3). Besides that, the highest ESI value in pH 4 was probably because of the effect of the steric barrier with the presence of pectin (Jourdain et al., 2008). Meanwhile, the complexes formed at pH 5 had the lowest ESI value because they had large molecules (Figure 2), probably because of the self-aggregation of protein molecules. Aggregated proteins do not adsorb as quickly at the oil-water interface. This phenomenon leads to the presence of un-adsorbed aggregated

protein-pectin, which results in depletion flocculation that could decrease the stabilization of the emulsion (Euston and Hirst, 1999; Oduse et al., 2017). Thus, pH 4 was chosen as the pH used in forming protein-pectin complexes because it has the best emulsion properties such as EAI and ESI value.

Effect of pectin-*blondo* protein concentration ratio on turbidity of protein-pectin complexes formation

After knowing that pH 4 had the best influence on the formation of protein-pectin complexes, researchers investigated the effect of pectin and protein concentration on formation of complexes. Pectin-*blondo* protein concentration ratio is critical in forming the *blondo* concentrate protein-pectin complex. The presence of pectin in the solution will affect the ability of the pectin to bind to proteins to prevent self-aggregation. The turbidity of heat-treated protein-pectin solutions was lower when compared to protein solutions heated without pectin (4.93% to 68.78%) (Table 1). The highest reduction in turbidity was reached in a heat-treated protein-pectin solution at concentration ratio of 0.70 (68.78%), while the lowest decrease at concentration ratio of 0.10 (4.93%) (Table 1). The negative charge on pectin-protein could not immerse the protein molecule at a low ratio of pectin. As a result, weakly charged complexes probably were formed. This leads to reduced electrostatic repulsion, increased bridging effect between molecule complexes, and increased particle size, so turbidity increases (Jourdain et al., 2008; Jones et al., 2009).

The turbidity of heat-treated *blondo* protein concentrate-pectin mixed solutions increased along with increasing protein concentration, which indicated that large complexes were formed that strongly scattered light. It was probably because of the higher protein aggregation due to increased protein concentration (Jones et al., 2010). This increase in turbidity may be associated with an increased size of complex particles. On the other hand, highly charged complexes probably formed at a high ratio of pectin-protein concentration. Thus, it could prevent the self-aggregation of protein molecules by cause of blocking hydrophobic interaction between proteins. The increased turbidity in ratio 0.23 is probably a result of the higher protein concentration used in this ratio. A possible justification for this condition is that heating led to some protein aggregation, which reduced the number of exposed cationic groups on the protein's surface to interact with the negatively charged pectin molecules (Jones et al., 2010).

Effect of pectin-*blondo* protein concentration ratio on EAI and ESI protein-pectin complexes

The ratio of pectin-protein concentration significantly affected the EAI value ($P < 0.05$) of *blondo* protein concentrate-pectin complex. Figure 4 shows the effect of protein and pectin concentrations used to form protein-pectin complexes on EAI values. The EAI value was increased along with the higher pectin-protein ratio of concentration used. *Blondo* protein concentrate-pectin complexes could increase the EAI value 3.8 to 9.3 times higher compared to the EAI values of the native protein. The EAI of *blondo* protein concentrate-pectin mixed solutions increased along with increasing pectin-protein concentration ratio. The highest EAI value was obtained in complexes formed at a pectin-protein ratio of 0.70, while the lowest EAI value was at ratio of 0.1 (Table 1). The 0.1 pectin-

Table 1. Effect of pectin-protein ratio in the turbidity, EAI, and ESI of complexes solutions containing 0.5 to 1 wt% protein and 0.1 to 0.35 wt% pectin mixture

Protein concentration (wt%)	Pectin concentration (wt%)	Ratio pectin/ protein	Turbidity	EAI (m^2 g^{-1})	ESI (minute)	Heat stability (%)
1	0.00	0.00	2.87	17.98±0.80	70.99±18.26	38.00±2.30
	0.10	0.10	2.64	68.32±0.55	508.65±72.64	79.88±0.45
	0.15	0.15	2.30	70.93±0.96	676.41±67.93	82.50±0.45
	0.20	0.20	2.17	71.48±2.91	805.82±32.81	83.07±1.01
	0.25	0.25	2.08	72.56±3.12	1,104.74±47.43	85.11±0.44
	0.30	0.30	1.46	75.91±0.22	1,368.90±42.98	86.59±0.14
	0.35	0.35	1.27	77.44±1.83	2,063.35±69.28	88.98±0.34
0.5	0.35	0.70	0.87	163.18±1.47	4,637.32±20.89	90.34±0.86

Note: Complexes were heated at 82±2 °C for 20 minutes

protein ratio of concentration had the greatest turbidity, which indicated that large complexes formed that strongly scattered light (Table 1). It is probably caused by charge neutralization and bridging effects. At a 0.1 pectin-protein concentration ratio, the negative charge on pectin could not saturate the protein molecule. This eventually increases the bridging effect between molecule complexes that promote large-size complexes formed. Large complex particles had lower emulsification abilities; thus, the EAI value could be decreased (Jourdain et al., 2008; Jones et al., 2009; Oduse et al., 2017). The best EAI value occurred at the pectin-protein concentration ratio of 0.70. It was probably caused by the lowest turbidity value, which indicated that small complexes were formed (Figure 4a). Complexes with smaller sizes also enhance the ESI better because they could easily to adsorbs in the oil-water interface rather than larger particle-size complexes.

The ratio of pectin-protein concentration significantly affected the ESI value ($P < 0.05$) of *blondo* protein concentrate-pectin complex. Figure 4b shows the effect of the pectin-protein ratio of concentration on ESI values to form protein-pectin complexes. The ESI value was increased along with the higher pectin-protein ratio of concentration used. The ESI of *blondo* protein concentrate-pectin mixed solutions increased along with increasing pectin-protein. The highest ESI value was obtained in complexes formed at a pectin-protein ratio of concentration 0.70, the lowest ESI value was at a pectin-protein ratio of 0.10. The emulsion formed using *blondo* protein concentrate-pectin complexes formed in 0.70 pectin-protein ratio concentration had the most excellent ESI values. It was probably caused by the electrostatic interaction of protein and pectin, which produce complexes with a higher charge. It could enhance the stabilization of emulsion due to the electrostatic repulsion between droplets of emulsion. The presence of pectin at a higher concentration also could increase viscosity (data not showed) and steric barrier that could increase the stabilization of emulsion (Jourdain et al., 2008; Liu and Zhong, 2013; Setiowati et al., 2017).

The 0.1 pectin-protein concentration ratio showed the poorest emulsion stabilization. It was probably due to that charge neutralization that resulted in *blondo* protein concentrate-pectin complexes with weakly charged. This reduces the strength of the stabilizing electrostatic repulsion between the complexes-coated droplet (Jourdain et al., 2008). Other factors that caused this phenomenon are probably due that the pectin concentration used was insufficient. It will promote the bridging flocculation between droplets of emulsion. Bridging flocculation occurs when pectin molecules adsorb simultaneously on more than one droplet and promote flocculation between droplets of emulsion (Hogg, 2013). The poorest emulsion stabilization in the ratio pectin-protein 0.1 is also probably due to the higher amount of protein aggregation due to the increased protein concentration used (Jones et al., 2010). Aggregated proteins do not adsorb as quickly at the oil-water interface. This leads to the presence of un-adsorbed aggregated protein, which results in depletion flocculation that could decrease the stabilization of the emulsion (Euston and Hirst,1999; Oduse et al., 2017).

Effect of ratio of pectin and protein on the heat stability of emulsion formed with protein-pectin complexes

Heat stability is one of the main parameters that can be used to measure the ability of a protein to stabilize emulsion after heat treatment. The pectin and protein concentration ratio significantly affected the heat stability value ($P < 0.05$) of the emulsion stabilized by *blondo* protein concentrate-pectin complexes. Table 1 also shows the effect of protein and pectin concentrations used to form protein-pectin complexes on heat stability values. All the emulsion samples produced using *blondo* protein concentrate-pectin complexes had better heat stability values than emulsion produced with only *blondo* protein concentrate.

The heat stability value was increased along with the higher pectin-protein ratio concentration. The highest heat stability value was obtained in emulsion produced by complexes formed at pectin-protein ratio of concentration 0.7, while the lowest heat stability value was at 0.1 (Table 1). The highest heat stabilizing ability of *blondo* protein concentrate-pectin complexes formed at 0.7 pectin-protein ratio of concentration was probably due to the steric barrier as an effect of pectin that had electrostatic interaction with protein. During heating, a steric barrier is expected to prevent the aggregation of *blondo* protein concentrate and hence prevent the

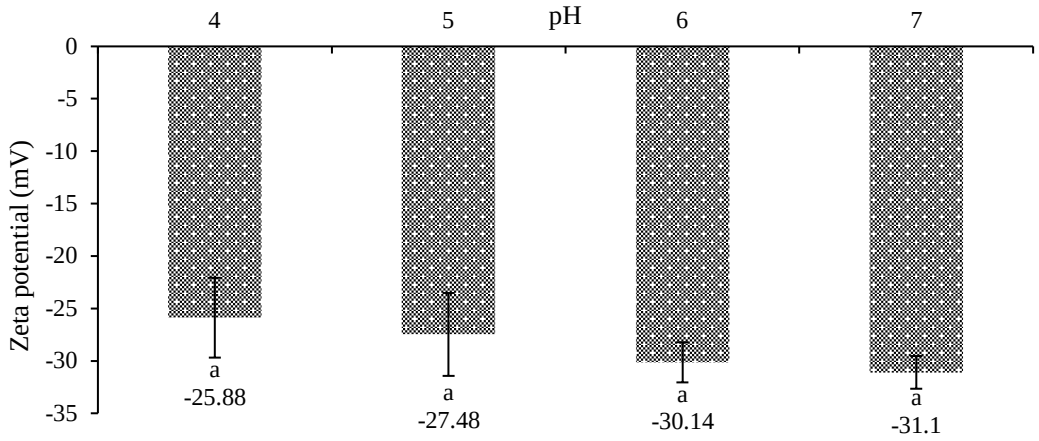

Figure 5. pH stability of heat treated (82±2 °C, 20 minutes) complexes solutions with ratio pectin-protein concentration 0.7 (containing 0.5 wt% protein and 0.35 wt% pectin mixture) formed at pH 4

aggregation of emulsion droplets (Setiowati et al., 2016). According to Dickinson (1998), the advantage consequence of complexation between protein and pectin is enhanced protection of self-aggregation protein during heating. This protective effect is probably mainly due to blocking the hydrophobic binding sites on the surface of the globular protein by the bulky pectin fraction.

On the other hand, the heat stability value was decreased along with the lower pectin-protein ratio of concentration used (Table 1). It was probably because of the higher protein aggregation due to increased protein concentration (Jones et al., 2010). Aggregated proteins that did not adsorb at the oil-water interface led to un-adsorbed aggregated proteins (Oduse et al., 2017). During heating, hydrophobic interaction between protein molecules had increased and increased aggregation between adsorbed and un-adsorbed protein molecules (Dickinson, 1998; Kim et al., 2002; Setiowati et al., 2016). It probably could make the protein accumulate on the surface of oil droplets. These protein aggregates will then act as a glue between oil droplets leading to the formation of oil droplet aggregates (Euston and Hirst, 1999; Setiowati et al., 2016).

Based on the results of the experiments obtained, pH 4 and ratio of pectin-protein concentration 0.7 was chosen as the pH and pectin-protein ratio of concentration used in the formation of protein-pectin complexes because it had the best emulsion properties among all.

pH stability of heat-treated *blondo* protein concentrate-pectin complexes

These experiments aimed to examine the effect of pH on heat-treated *blondo* protein concentrate-pectin complexes that were formed. Complexes that were formed using a pectin-protein ratio of concentration 0.7 containing 0.5 wt% protein and 0.35 wt% pectin at pH 4 followed by heat treatment at 82±2 °C had chosen to test the pH stability. Complexes formed using the best condition obtained from these experiments had a zeta potential value and particle size, respectively -25.88 mV and 192.92 nm. Figure 5 shows the effect of pH in complexes obtained from 0.5 wt% protein and 0.35 wt% pectin in pH 4. pH did not significantly affect the zeta potential value ($P < 0.05$). It indicated that the complexes formed stay stable across a range of pH values (pH 4 to7). It is probably mainly due to the effect of heat treatment during the formation of complexes that could form irreversibly aggregates that could not detach with a pH change. Jones et al. (2009) stated that heat-treated complexes would be more stable under environmental conditions such as pH changes.

CONCLUSIONS

This research proved that the formation of insoluble or soluble complexes is primarily pH. Electrostatic interactions were mostly responsible for the synthesis of *blondo* protein concentrate and pectin. This does not imply, however, that proteins and pectin must possess opposing

net charges for the interaction to occur. At pH 4, when electrostatic contact between two molecules is not excessive, soluble protein-polysaccharide complexes with a substantially negative charge and relatively tiny particle size are generated. These soluble compounds generated at pH 4 can potentially improve EAI, ESI and heat stability. The EAI, ESI, and heat stability values increased with the larger pectin-protein concentration ratio. Complexes produced with a pectin to protein ratio of 0.7 and comprising 0.5 wt% protein and 0.35 wt% pectin at pH 4 had the best zeta potential value and particle size, which were -25.88 mV and 192 nm, respectively.

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