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Physico Chemical Properties of Whey Protein and Gelatine Biopolymer Using Tea Leaf Extract as Crosslink Materials

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ABSTRACT

The purpose of this research was to extract tea leaf phenols using Microwave Assisted Extraction (MAE) method at 3 levels of microwave power (high, medium high and medium) and investigated the influence of physco chemical properties of whey protein and gelatine biopolymer using tea leaf extract as crosslink materials at different concentration (5%, 10% and 15% (v/v)). MAE method gave significantly effect on phenolic content. High level power of MAE gave higher phenolic content of tea leaves extracts. Tea leaves extracts as crosslinked agent of biopolymer gave highly significant effect on the stability of the emulsion, the emulsion activity and foaming power. SDS-PAGE protein profile showed increase molecular weight with the addition of tea leaf extract, it can be presumed presence crosslinked both on whey protein or gelatine.

Key words: Tea leaf extract, crosslink materials, protein whey, gelatine, physico chemical.

INTRODUCTION

Whey and gelatin are both byproduct of cheese and leather industry , respectively. The most abundant of protein in Bovine Whey are β-lactoglobulin and β-lactalbumin, contain two and four disulphide bond, respectively (Farrel et al., 2004). Gelatin is a protein-based biopolymers form of collagen the skin and bones of animals (Gomez-Guillen *et al*., 2010). The functionality need be improved using chemical and physical methods for modifying whey protein and gelatin to be a biopolymer.

Whey protein was modified by transglutaminase to obtain crosslink bonds which can increase the viscosity and gelation properties. Transglutaminase catalyzes the reaction of protein crosslink by acyl transferase mechanism involving protein-bound glutaminyl residues (acyl donor) and primary amines (acyl acceptors), including the ε-amino group of lysine residues in certain proteins. The covalent cross-linking of proteins catalyzed by transglutaminase can cause dramatic changes in the size, conformation, stability, and other properties of proteins (Truong *et al*., 2004). Transglutaminase formed intramolecular crosslinks at low gelatin concentrations and both intra- and intermolecular crosslinks at higher concentrations (Hernandez-Balada, 2009), produce triple helix more, but less swell in water and have a stronger physical characteristics (Chiou *et al*., 2006). Glutaraldehyde was used in crosslink process for improving the stability of the mechanical properties of gelatin. Glutaraldehyde is used at certain levels can cause in toxicity, so needs to be considered to use it (Kim et al., 2007). The price of enzymes was so expensive, so it needs to find out another alternative that can be used as safe and cheap crosslink agent. It can be plant polyphenols.

Tea leaves are one of the plant that contain of polyphenols, so it can be used as crosslink agent in whey protein dan gelatin. The extraction of green tea by MAE (*Microwave Assisted Extraction*) method can produced polyphenols (Quan *et al.*, 2006). MAE is an extraction method using microwaves to accelerate the extraction by heating the solvent quickly and efficiently (Jain, et al., 2009). Microwave energy causes the movement of molecular ion migration and dipole rotation. The rapid movement causes friction eventually produce heat energy in the material so that the cell wall material and tissue will be damaged, and phenolic compounds extracted (Delazar et al., 2012). Tea leaf were withered, cut, and grinded until had small size. Polyphenols of tea leaf will be broken on heating 80 °C (Quan et al., 2006). The ratio of solvent used in the extraction various. Olive leaf were extracted using solvent with a ratio of 50% ethanol, 80% methanol, acetone and water (Rafiee *et al.*, 2011). Extraction can be done by microwave, and add 50 ml solvent (Xiao et al., 2008). The best results in the study using the extraction method of MAE in optimal conditions with a ratio of liquid: solid is 33: 1 (mL / g) (Zhang et al., 2011).

Phenolic compounds possessing one or more aromatic rings bearing a hydroxyl substituent, phenolic compounds may be oxidized in alkaline solution to corresponding quinones (Rawel et al., 2005). The quinones may react with cysteine, lysine, methionine and tryptophan residues in protein (Rawel et al., 2002, 2004). Polyphenol in greean tea could covalently bind to the amino acid residues in proteins by meas of its autooxidation (Ishii et al, 2008; Mochizuki et al 2002). Crosslink formation in proteins can be caused by a reaction of polyphenols in oxidation conditions with amino acid groups of the peptide side chains. Phenolic acids interact with the side chain amino or sulfhydryl groups of the polypeptide to form covalent bonds with CN or CS phenol ring (Strauss dan Gibson, 2004). Gelatin Crosslink with polyphenols can produce a stable gel gelatin. Tea polyphenols have a strong ability to interact with milk proteins and stable on heating (Wu et al., 2013). Crosslink polyphenol with protein gel will get results biopolymer with a greater mechanical strength, reduce swelling and decrease the amount of free amino acids (Staruss and Gibson, 2004). Polymerization can be formed with crosslink in intra- and intermolecular protein.

Tea leaf extract would be added in whey protein and gelatin bioplymer can increase physical and chemical stability, so it could increase biopolymer quality if use this modification biopolymer as food additif.

MATERIAL AND METHODS

Plant and protein materials

Green tea leaf were obtained from Malang, East java, Indonesia. Green tea was dried at 50°C for 24 hours, dried tea were ground to pass a 80 mesh screen and stored at -20°C before experiments. Dried green tea leaf was extracted and analyzed on phenolic and chemical structure.

Whey protein isolate (Merck) and Gelatine (Merck)

Chemicals

NaN_3 (Merck) (to retard spoilage), NaOH (Merck) and CH_3COOH (Merck), Gallic acid (Merck), Folin reagen (Merck), sodium carbonate, KBr powder, soy bean oil, polyacrylamide gel, sodium dedocyl sulfate (Merck), Tris-HCl (Merck), EDTA (Merck), b-mercaptoetanol (Merck), bromphenol blue (Merck).

Extraction of green tea Phenolic by MAE

Extraction of green tea according to the method of Quan et al. (2006) with a slight modification. Experiment were used a domestic microwave oven (Sharp Model R - 222Y (S)) which used level power high, medium high, and medium. Green tea was dried at 50°C for 24 hours, dried tea were ground to pass a 80 mesh screen and stored at -20°C before experiments. 3 g of dried tea (Liang and Xu, 2001), then mixed with 100 ml solvents (aquades) for 90 minutes (Pan et al., 2003). The solution was radiated in microwave oven which used level power high, medium high, and medium (one minute radiation and two minutes off) to keep temperature not rise above 80°C (Quan et al., 2006). The infusions were let to cool down to room temperature, filtered and stored in refrigerator at 4°C for determine total polyphenols and chemical structure.

Measurement of phenolic total of green tea extraction

Total polyphenol content (TPC) was

determined by Folin-Ciocalteu reagent method as described by Maung and Chamba (2012). Saturated sodium carbonate (Merck) was prepared and stored. Making solutions of Gallic acid standards (25 ppm, 50 ppm, 100 ppm, 150 ppm, 200 ppm) were prepared freshly each time at room temperature. Mixing of 0.5 ml of Gallic acid or 1:10 diluted sample, 4.5 ml of distilled water, 0.2 ml of Folin reagent (Merck) and 0.5 ml of saturated sodium carbonate were prepared for standard curve and sample analysis. The T-1900 UV spectrophotometer was used to determine the absorbance of each sample at 725 nm using 1 cm^3 cuvette. The results were determined by calibration curve using gallic acid as standard. Total polyphenols content was calculated from absorption value and linear regress equation using acid gallic as standard. Results was shown as ppm GE (Gallic acid Equivalent)

Determination of chemical structure – fourier transform infrared spectroscopy (FTIR)

Chemical structure analyzed by method as described by Moela et al. (2009). The UV-Vis spectrum was recorded at room temperature on a GBS UV/VIS 920. One milligram of green tea extraction dried in a vacuum desiccator was ground and mixed thoroughly with 200 mg of oven-dried KBr powder of analytical regent (Merck, DAC, USP). The powder was placed in a die and compressed into a transparent disk.

Preparation of crosslinked whey protein and gelatine using phenolic of green tea as crosslink agent

Crosslink modification according to the method of Strauss and Gibson (2004) with a slight modification. The whey protein/gelatin was hydrated at room temperature by solution in water containing 0,01% NaN_3 (Merck) (to retard spoilage) then mixed at 40°C for 2 h. Phenolic of green tea was added in accordance with treatment (5%, 10% and 15% (v/v)). Solutions of the phenolics were mixed with those of protein whey/ gelatin in various proportions and adjusted to the desired pH. Most cross-linking reactions were carried out at pH 8. The protein whey/gelatine polyphenol solutions were exposed to oxygen at 40°C. Either oxygen was bubbled through the solution for 1 h. The remainder was aged for 24 h at room temperature, then kept for a further 24 h at 10°C, and returned to room temperature.

Determination of emultion stability and emulsion activity

Emulsion activity index (EAI) was determined as describe by Zheng and Jiang (2014) with a slight modification and emulsion stability index (ESI) of whey protein and gelatin samples was determined as describe by Nagarajan et al (2012). Soy bean oil (5 ml), whey protein and gelatin solution (15 ml) were homogenised using a homogeniser for 1 min. Emulsions were pipetted out at 0 and 10 min and 100-fold diluted with 0.1% SDS. The mixture was mixed thoroughly for 10 s using a vortex mixer. The resulting dispersion was measured using a spectrophotometer. EAI and ESI were calculated by the following formulae:

$$EAI\ (m^2/g) = (2 \times 2{,}303 \times A \times DF) / IxC$$

Where; A= A_{500}, DF= dilute factor (100), I= path length of cuvette (m), x=oil volume fraction, C= sample concentration

$$ESI\ (\%) = A_{10}/A_0 \times 100$$

Where; A_0= A_{500} at time of 0 menit ,A_{10}= A_{500} at time of 10 menit

Determination of microscopy emulsion

Microscopy emulsion of whey protein and gelatin samples were analyzed as describe by Surh et al. (2007) with a slight modification. Soy bean oil (5 ml), whey protein and gelatin solution (15 ml) were homogenised using a homogeniser for 1 min. The emulsion were viewed with an Olympus light microscope 100x to know the approximate droplet of the emulsions.

Determination of foaming properties

Foam expansion (FE) of whey protein and gelatin sample solutions were determined as described by Zheng and Jiang (2014) with a slight modification. Added 50 ml whey protein and gelatin solution was transferred into 100 ml cylinders. The solution was homogenised at a speed of 13,400 rpm for 1 min at room temperature. The sample was allowed to stand for 0 and 60 min. FE were calculated using the following equations:

$$FE\ (\%) = V_{T/}Vo \times 100$$

Where; VT =total volume after whipping, Vo = the original volume before whipping

Electrophoretic analysis

Electrophoretic analysis was determined as described by Hernandez-Balada et al. (2009) Inter-protein crosslinking was evaluated by polyacrylamide gel electrophoresis in sodium dodecyl sulfate (SDS–PAGE) using precast gradient gels (4–15%). Gels were calibrated using the broad range (BRM) SDS calibration standard that contains a mixture of nine proteins ranging in size from 6.5 to 200 kDa. Samples (approximately 0.5 mg) of protein dissolved in sample buffer (10 mMTris–HCl at pH 8.0 containing 1 mM EDTA, 25 mg/ml SDS, 50 ll/ml b-mercaptoethanol and 0.1 ll/ml bromphenol blue) (Merck) were heated at 40°C for 4 h. Gels were stained with Coomassie Blue.

RESULT AND DISCUSSION

Phenolic extraction of green tea leave

Phenolic content of green tea extract

The level of microwave power on green tea leaves extraction gave a difference significant effect ($P < 0.05$) on phenolic content. This is indicated that higher microwave lever power produce higher electromagnetic wave, higher electromagnetic wave gave better absorption of microwave energy. Higher extraction of phenolic by MAE could be attributed to better absorption of microwave energy. Microwave energy causes molecular movement by means of ion migration and dipole rotation. The rapid movement produces friction that ultimately produce heat energy in the green leaves cell wall so that the cell wall material and tissue will be damaged, and phenolic compounds extracted. Higher level of microwave power increases temperature inside grean leaf tea leaves cell, resulting in breaking the cell walls and releasing phenolic in to the surrounding solvent. (Delazar et al., 2012; Nayak et al., 2015).

The phenolic content-on Table 1 showed that , the highest results phenolic content 0.45 mg / ml of green tea leaves extract was high level of microwave power. Extraction of green tea used high level of microwave power did not damage phenolic content of green tea leaves. Rusak et al. (2008) describes the results of research total epigallocatechin ranges from 0.43 mg / ml for the extraction of the tea leaves using a solvent distilled water. The highest polyphenol content of green tea leaves from China amounted to 204.58 mg / g and green tea leaves from Myanmar amounted to 152.5 mg / g. Green tea contains 30-40% of fluid extract of polyphenols, while black tea contains only 3-10% (Bruno et al., 2008).

Table 1: Phenolic content of green tea extract

Microwave level power	Phenolic content (mg/g)
Medium	0,37 ±0,011[a]
Medium High	0,42±0,015[ab]
High	0,45±0,04[b]

Different uppercase letters in the same column indicated significant effect ($P < 0.05$)

Table 2: Functional properties of whey protein and gelatine biopolymers crosslink tea leaf extract

Protein types	Green Tea Extract (%)	Emulsion Stability (%)	Emulsion Activity (m^2/g)	Foaming (%)
Whey Protein	5	29.04±0.45[a]	44.79±0.87[a]	175±1.00[a]
	10	31.78±0.78[b]	45.36±0.80[ab]	193±1.00[b]
	15	25.90±0.74[c]	46.52±0.99[b]	199.67±0.58[c]
Gelatine	5	49.11±0.46[d]	95.56±0.79[c]	169±1.00[c]
	10	95.89±0.44[e]	86.12±0.39[d]	177.00±1.00[d]
	15	23.84±0.88[f]	74.98±0.06[e]	177.00±1.00[e]

Different uppercase letters in the same column indicated highly significant effect ($P < 0.01$)

Another research describe that extraction of green tea leaves using a microwave with a power of 90 W to 900 W, total phenolic will decrease if using power more than 450 W. The the penolic content 4.68 mg / ml at 450 W power and decreases with the increase in power (Handayani et al., 2014). This can be caused by damage to the phenolic extracts of the tea leaves. Kusumaningrum (2008) explains his research that the tea is heated in an autoclave at a temperature of 120ºC, occur epimerisasi of (-) - EGCG into (-) - GCG and catechin levels decreased by 24%. Catechins can decrease dramatically to 50% when heated for 2 hours.

Extraction can be done using MAE methods which it is an extraction method utilizes microwaves to accelerate the extraction by heating the solvent to quickly and efficiently (Jain et al., 2009), can also use the method DMME (Domestic Microwave maceration Extraction) which is an extraction process by maceration / soaking with radiation in a household microwave oven (Agnes, Widjaja, Ayucitra and Indraswati, 2013).

Polyphenols of green tea leaves would be damaged if it heated at temperatures above 80 °C, so it requires the right temperature when the extraction of the tea leaves so that the content of phenolic in it are not reduced.

FTIR of green tea leaves extract

Figure 1 showed the results of functional groups observation of green tea leaf extract according to treatment level of microwave power.

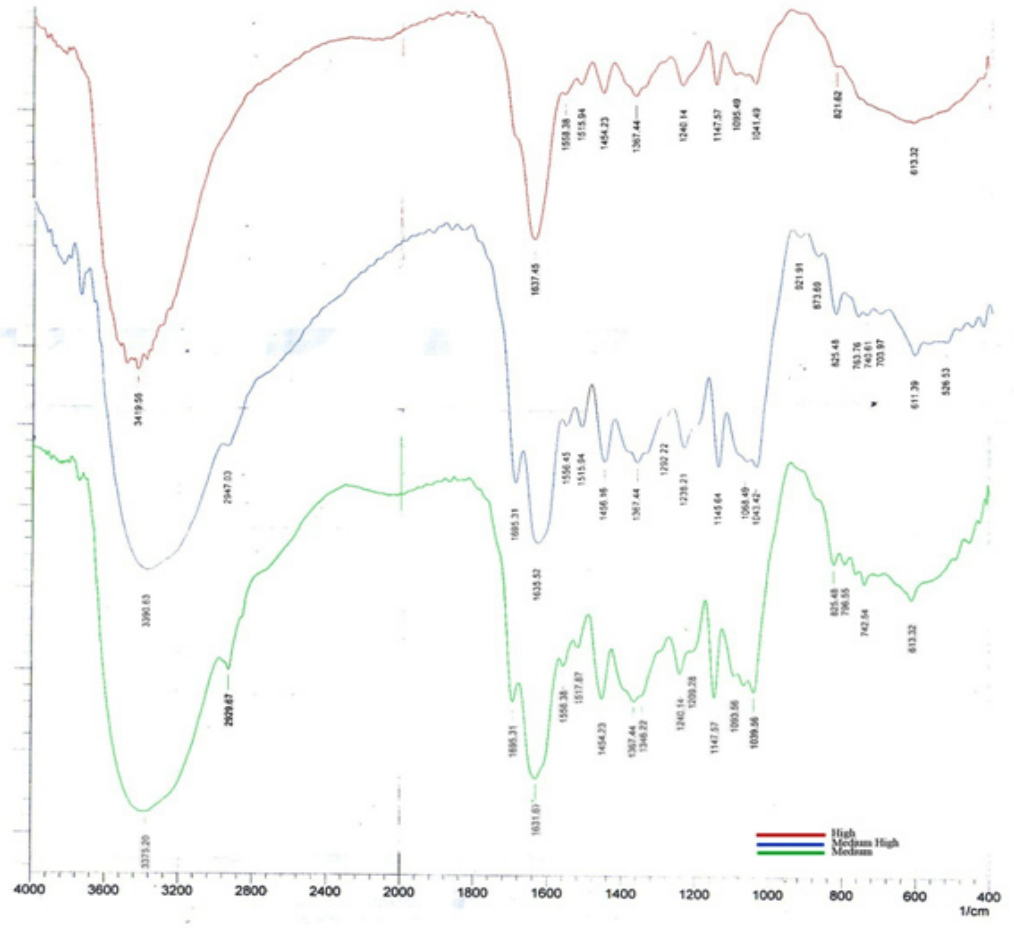

Fig. 1: Functional groups of green tea leaf extract to treatment level of microwave power

Based on the image above can be seen that tea leaves extract were extracted with domestic microwave at several level of microwave power has almost the same functional group. The result of green tea leaves extract , using medium level of microwave power, wave number of FTIR spectra at 3375,2cm^{-1}, 1517,87-1631,67cm^{-1}, 1240,14-1039,56cm^{-1} were assigned to OH, C=C, C-O, respectively . The result of green tea leaves extract , using medium high level of microwave power, wave number of FTIR spectra at 3390,63 cm^{-1}, 1635,52-1515,94 cm^{-1}, 1292,22-1043,42 cm^{-1} were assigned to OH, C=C, C-O, respectively. The result of green tea leaves extract , using high level of microwave power, wave number of FTIR spectra at 3419,56 cm^{-1}, 1637,45-1515,94cm^{-1}, 1147,57-1095,49 cm^{-1} were assigned to OH, C=C, C-O, respectively. The resulting absorbance values on the research were in accordance with the value of the absorption of catechins in recent research, it was indicated that the extract of tea leaves are extracted using a different level of microwave power containing catechins similar with recent research.

Catechins have important functional group located in the catchment area 500-1900cm^{-1}. Shifting group of numbers in the component may vary due to many factors that influence. The process of heating the tea leaves may cause a shift in numbers (Chen et al., 2006). Maoela *et al.* (2009) describes that infrared spectrophotometer can determine cathecin of green tea extract, the wave number of FTIR spectra at 3400-3100 cm^{-} , 1.600 cm^{-1} , 1150-1010 cm^{-1} were assigned to OH, C=C, C-O. Ramos-Tejada *et al.* (2002) describe that cathecin can determine by using infrared spectrophotometer and generating absorption region which is not much different.

Based on the results of the first step research, it can be determined that the extraction treatment using level high of microwave power in the extraction of tea leaf extract is the best treatment. So, this treatment was continued to second step research. The best results of green tea leaf extract is used as an crosslink ingredient whey protein and gelatin of biopolymer to improve the physico chemical properties of these biopolymers. Crosslink ingredient of green tea leaves extract expected to replace other crosslink materials such as glutaraldehyde which cause the toxin if its use is not controlled and transglutaminase that are quite expensive.

Crosslink ingredient of green tea extract expected can be sae crosslink ingredient, because naturally derived from plants and affordable prices. So if doing crosslink with whey protein and gelatin can produce food additives that have good functional properties.

Whey protein and gelatine biopolymers crosslinked using phenolic green tea leaves extract

Results of a step II research, using tea leaves extract as crosslink materials at different concentration (5%, 10% and 15% (v/v)) of the whey proteins and gelatin can be seen in Table 2.

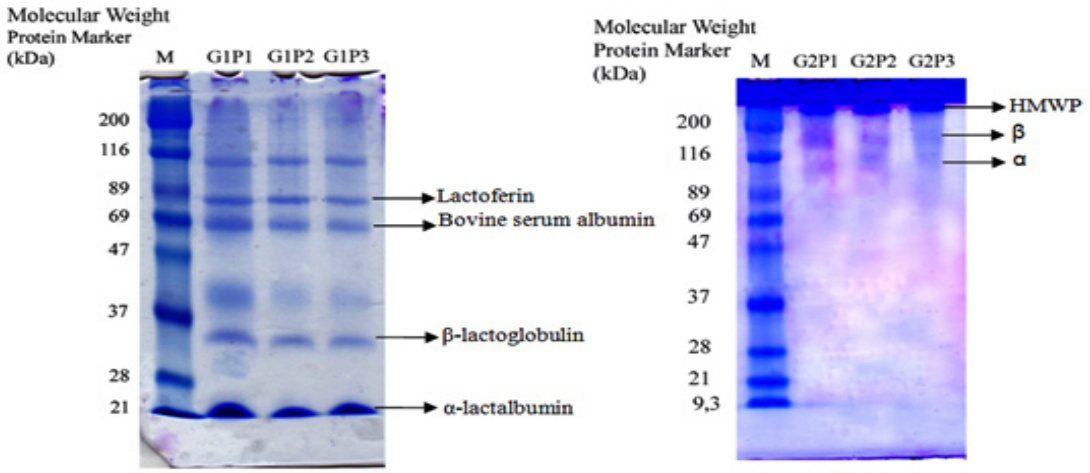

Fig. 2: Electropherogram of Whey Protein and Gelatine Crosslinked Biopolymers Protein pattern

Different uppercase letters in the same column indicated highly significant effect (P < 0.01).

Emulsion Stability

The results of variance analysis showed that type of protein gave a difference highly significant effect (P <0.01) on emulsion stability of biopolymer (Table 2). Addition of green leaves tea extract as treatment on protein types gave a difference highly significant effect (P <0.01) on emulsion stability of whey protein and gelatine crosslinked.

The average emulsion stability of whey protein and gelatin biopolymer crosslinked with addition extract green tea 5%, 10% dan 15% improved emulsion stability. Nagarajan *et al.* (2012) explained that emulsion stability of gelatin increased with increasing concentration of gelatin is added. Biopolymer gelatin has a long chain and rigid which can improve the stability of the emulsion. Crosslinked gelatin with green tea leaves extract can improve the stability of the emulsion. This is consistent with the findings that the cows gelatine crosslinked produce emulsions value higher than the pure gelatin. This is supported by Prommajak and Ravivan, (2013) states that pure beef gelatin emulsion stability from 30.00 to 37.36%. Nagarajan et al. (2012) explains that the emulsion stability of squid gelatine has 12.67 -26.55%. The best emulsion of fish gelatine can be used as food additif for ice cream, yogurt or other milk product (Prommajak dan Ravivan, 2013).

Emulsion stability of whey protein nested on the addition of green tea leaves extract 5%, 10%, and 15% have an average value of 29.04 ± 0.45, 31.78 ± 0.78, 25.90 ± 0.7 respectively, gelatin has an average value 49.11 ± 0.46, 95.89 ± 0.44, 23.84 ± 0.88 respectively, the optimal stability emulsion contained in whey proteins and gelatine on the addition of tea leaf extract 10%.

Crosslink bonds were formed in each treatment gives different results on the emulsion stability the. The values of emulsion stability increased on the addition of green tea leaves extract 10% in both types of protein. The value of emulsion stability decreased with the addition of tea leaf extract 15%. It is explained that the optimal addition of green tea leaves extract was 10%. Based on these results, it can be explained that the crosslinking of biopolymer improved the stability of whey protein and gelatine biopolymers. This is supported by the opinion of Li et al. (2009) explains that transglutaminase casein crosslinked can increase emulsion stability compared with pure casein. Casein crosslinking using transglutaminase gave higher emulsion stability compared with unmodified casein crosslink at the same concentration of protein.

Interaction between phenols of green tea leaves extract with gelatin and whey protein ~~can~~ formed biopolymer which improves the stability emulsion, so expected whey protein and gelatine crosslinked can be used as food additives that can improve the functional properties of the product.

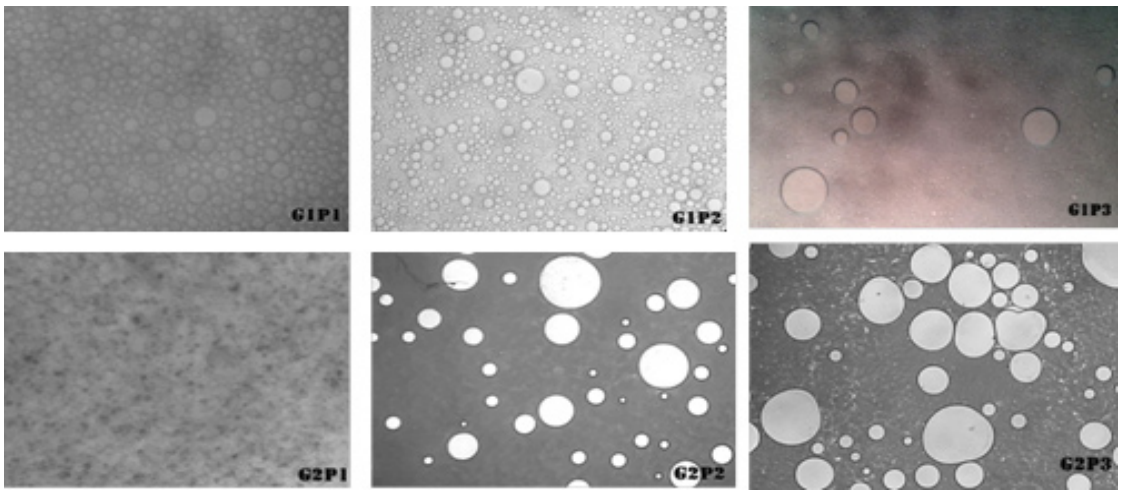

Fig. 3: Microscopic emulsion of whey protein and gelatine crosslinked

The emulsion stability has an important role to determine the properties of emulsions in food emulsion systems. Protein is the main food emulsions of food, such as increasing the froth, emulsifying, gelling and water binding. The protein in food is absorbed on the surface of the liquid between the liquid and gas phases, thereby stabilizing the structure of food (Ibanoglu and Karatas, 2000).

Emulsion Activity

The results of variance analysis showed that treatment of protein gave a difference highly significant effect (P <0.01) on emulsion activity of biopolymer. Addition of green tea leaves extract as treatment on protein types gave a difference highly significant effect (P <0.01) on emulsion activity of whey protein and gelatine crosslinked.

The average emulsion activity of whey protein and gelatin biopolymer crosslinked with addition green tea leaves extract at 5%, 10% dan 15% improved emulsion activity. The result of emulsion activity can seen at Table 2.

Crosslinked gelatin with tea leaves extract can improve the activity of the emulsion. This is according to Li et al. (2009), which explains that the crosslinked casein emulsion increases the activity compared to pure casein. Crosslinked casein has the highest emulsion activity index 1.42 m^2/g at a concentration of 0.03% protein, pure casein has the highest emulsion activity index of 1.35 m^2/g at a concentration of 0.02% protein. Li et al. (2009) the presence of hydrogen peroxide and ferulic acid causes increased activity of emulsions and emulsion stability of casein crosslinked. This is consistent with the research which showed that the higher crosslink agent is added, the emulsion activity in whey protein also higher.

Emulsion activity of whey protein nested on the addition of tea leaf extract 5%, 10%, and 15% have an average value of 44,79 ± 0,87, 45,36 ± 0,80, 46,52 ± 0,99 , respectively, gelatin has an average value 95,56 ± 0,79, 86,12 ± 0,39, 74,98 ± 0,06, respectively, the optimal emulsion activity contained in whey proteins on the addition of tea leaf extract 15% and gelatine on the addition of tea leaf extract 5%.

The higher the tea leaves extract is added to the whey protein increased the activity of the emulsion, but the higher the tea leaf extract is added to the gelatin decreased emulsion activity. Tea leaf extract was added to whey protein and gelatin have different values emulsion activity. Li et al. (2009) explains that the casein emulsion activity index increases with the addition of protein concentration. Casein crosslinked has the highest emulsion activity index (1.42 m^2/g) at a concentration of 0.03%, while casein protein unmodification has the highest emulsion activity index of 1.35 m^2/g in protein concentration of 0.02%

The value of emulsion activity increased due to low molecular mass crosslinked. Emulsion activity decreased was associated with the high molecular mass crosslinked protein. High molecular weight of protein or biopolymer will be more easily and effectively on the stability and activity of emulsion. However, if the molecular weight exceeds a certain value, will disrupt the emulsion stability (Tang et al., 2005). This is consistent with the observation that the molecular weight of gelatin were higher by electrophoresis testing affect the activity of the emulsion decreases with increasing crosslink agent added.

Bao *et al*. (2011) explained that crosslink is a requirement for microencapsulation. Enkcapsulan have ability to form a stable emulsion before drying. Sodium caseinate with sodium caseinate crosslinked have different emulsifying properties. Activity emulsion sodium caseinate obtained at 0 min (P0), 30 minutes (P30), 60 minutes (P60), 90 minutes (P90), 180 minutes (P180), 300 minutes (P300) and 420 minutes (P420), especially P30 reaches maximum activity at 20.82 m^2/g. Activity of P60 showed decreasing and P420 emulsion has fallen to 17.04 m^2/g.

Foaming Power

The results of variance analysis showed that treatment of protein gave a difference highly significant effect (P <0.01) on foaming power of biopolymer. Addition of green tea leaves extract as treatment on protein types gave a difference highly significant effect (P <0.01) on foaming power of whey protein and gelatine crosslinked. The average foaming power of whey protein and gelatin

biopolymer crosslinked with addition extract green tea 5%, 10% dan 15% improved foaming power. The result of foaming power shown at Table 2.

Crosslinked gelatin with green tea leaves extract improveed the foaming power. The higher foaming power generated to explain that the network of crosslinked protein formed is stronger, it caused the foam is formed. Protein crosslinked more resistant denatured. This is consistent with Ali et al. (2010), which explains that the protein modification can improve the functional properties of proteins such as foaming.

Foaming power of whey protein nested on the addition of green tea leaves extract 5%, 10%, and 15% have an average value of 44,79 ± 0,87, 45,36 ± 0,80, 46,52 ± 0,99 , respectively, gelatin has an average value 95,56 ± 0,79, 86,12 ± 0,39, 74,98 ± 0,06, respectively, the optimal Foaming power contained in whey proteins on the addition of tea leave extract 15% and gelatine on the addition of green tea leaves extract 10% and 15%.

Foaming power played an important role in making some food. The ability power foaming of biopolymers was important in the manufacture of food. The stability of the foam is determined 15 minutes after mixing. Protein of soy crosslinked improve foaming power (Zheng dan Jiang, 2014). This is consistent with research that crosslink can increase foaming power. The higher the crosslink agent is added then also increase the foaming power on whey protein and the gelatine.

Panga fish skin gelatine foam has a power of 1.13 and 0.71 froth stability, emulsion stability from 34.2 to 44.6%. Average ability foaming power of panga fish skin gelatine Thailand 1.13 ± 0.24, higher than the bovine bone gelatin were 1.03 ± 0.32. Fish gelatine foam showed a better power at pH 9 and 10 while beef gelatin has a better at pH 4. Fish gelatin foaming power 1.3 times the initial gelatine solution at pH 5 to 9. Beef gelatin foam has a power of 1.47 times at pH 5. Average of gelatin foam stability 0.71 ± 0.16 times higher compared with 0.64 ± 0:13 beef gelatin. Foam power of fish gelatine has a maximum stability at pH 3-6 and decreases foaming power as rising pH. Foam power of cow gelatine has maximum stability at pH 7 and pH decreased to lower than 5 (Prommajak and Ravivan, 2013).

Foaming power of soy whey protein decreases when approaching the isoelectric pH due to increased aggregation. The treatment I WPI 1% with a pressure of 300 MPa for 15 minutes in 50 mM phosphate pH 7 generate foaming power and foam stability the highest (Ibanoglu and Karatas, 2000). Foaming power has an important role to determine the properties of emulsions in food emulsion systems. Testing foaming power is one important requirement in the manufacture of food (Zheng and Jiang, 2014).

Protein pattern using SDS PAGE

The results of SDS PAGE from 6 samples of whey protein and gelatin crosslinked can be seen in Figure 2. Based on the curve equation y = -1,239x + 2.356 with R^2 = 0.963.

MW (kDa): Molecular Weight (kilodalton), M: Marker, a: whey protein, b: gelatine, HMWP: High Molecular Weight Protein, 1 (G1P1): whey protein with addition of green tea leaves extract 5%, 2 (G1P2): whey protein with addition of green tea leaves extract 10%, 3 (G1P3): whey protein with addition of green tea leaves extract 15%, 1 (G2P1): gelatine with addition of tea leaves extract 5%, 2 (G2P2): gelatine with addition of tea leaves extract 10%, 3 (G2P3): gelatine with addition of tea leaves extract 15%

Electropherogram above showed that all the samples consist of components, among others, b-lactoglobulin (MW about 18 kDa) and a-lactalbumin (MW about 14 kDa) and bands vague to bovine serum albumin (MW about 66 kDa) and lactoferrin (MW approximately 86 kDa). MW on the above crosslinked whey protein increased.

Whey protein crosslink green tea leaves extract increases MW of protein pattern. This indicated that phenolic in green tea leaves extract whey protein plays a role in the formation of crosslinked, so it can improve mw of whey protein. This is according to Li et al. (2009) explains that crosslink can be shown to increase with the advent MW or other proteins in addition to observation. SDS

PAGE can indicate crosslink bonds in proteins. The Results showed that the conformational changes of different soy protein will improve the properties of the emulsion (Zheng and Jiang, 2014).

Based on the curve equation y = -1,522x + 2,460 dengan R^2= 0,946. Molecular weight of gelatin crosslinked can be obtained from the linear formula. The molecular weight of the crosslinked gelatin increased compared to pure gelatin. This is consistent with Azira et al. (2012) explained that pure gelatine from cattle have approximately 110 kDa dan135 BM kDa.

The result of phenolic gelatine crosslinked Electropherogram was compared (Hernandez-Balada *et al.*, 2009) with gelatin crosslink transglutaminase, MW of gelatin crosslinked transglutaminase bigger than phenolic gelatine crosslinked. It can be seen, that great MW did not entry in gelatine gel. Gelatin protein profile analysis using SDS PAGE is difficult because MW of gelatin is too large, so we need a proper preparation for the test. Hernandez-Balada et al. (2009) explains that gelatine with a large polymerization can not seem to gel and can be seen by chromatography. Band of gelatine crosslink using transglutaminase is not apparent on SDS PAGE gel because gelatin crosslinked increase MW.

Polymer was formed intermolecular of protein detected using electrophoresis chromatography method. Polymer of protein were characterized by a high molecular weight band on gel electrophoresis, whereas the method of chromatography, polymer formation shown by the fractions with a retention time which is much lower than the original protein (Li et al., 2009).

Microscopic of Emulsions of Polymers Crosslinked Gelatin and whey proteins

Microscopic of emulsion to determine particle emulsion whey protein and gelatine biopolymers crosslinked. Observations were made using a microscope, before it was performed the preparation of whey protein and gelatine with soybean oil. The result of the particle size distribution of whey protein and gelatine crosslinked using a microscope can be seen in Fig 3.

(G1P1): whey protein with addition of green tea leaves extract 5%, 2 (G1P2): whey protein with addition of green tea leaves extract 10%, 3 (G1P3): whey protein with addition of green tea leaves extract 15%, 1 (G2P1): gelatine with addition of tea leaves extract 5%, 2 (G2P2): gelatine with addition of tea leaves extract 10%, 3 (G2P3): gelatine with addition of tea leaves extract 15%

Fig 3. Showed the distribution of particles in the emulsion of whey protein and gelatine biopolymer crosslinked using a microscope with a magnification of 100 x above shows the existence of a big drop in Figure 3 for the treatment G1P1, G1P2, G1P3, G2P2 and G2P3. Large droplets more numerous in gelatin with G2P2 and G2P3 treatment. More green tea leaves extract was added in gelatine caused more large droplets are produced. This is consistent with recent research (Dickinson and Lopez, 2001; Lobo, 2002) which explains that the gelatin often produce relatively large size of the droplets during homogenization so that the necessary modifications to improve the effectiveness as an emulsifier. It can be concluded that the stability of the emulsion better in gelatin with addition of 10% extracts does not give effect to the particle size distribution of emulsion when viewed in the microscope.

Droplet were caused due to mixing of oil and samples were less prevalent. This large droplets due to the relatively low surface activity of fish gelatin with globular proteins such as β-lactoglobulin (Surh *et al.*, 2006).

CONCLUSION

MAE method gave significantly effect on phenolic content. High level power of MAE gave higher phenolic content of tea leaves extracts. Tea leaves extracts as crosslinked agent of biopolymer gave highly significant effect on the stability of the emulsion, the emulsion activity and foaming power. SDS-PAGE protein profile showed increase molecular weight with the addition of tea leaf extract, it can be presumed presence crosslinked both on whey protein or gelatine.

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