INTISARI

Bahan tambahan pangan emulsifier sangat dibutuhkan oleh industri pangan untuk membentuk tekstur produk pangan emulsi yang stabil. Pada umumnya emulsifier berupa biopolimer yang terabsorpsi secara cepat ke permukaan droplet sehingga terbentuk lapisan yang dapat melindungi droplet dari kerusakan. Gelatin adalah bioplomer *amphiphilic* yang diperoleh dari hidrolisis kolagen dalam kulit, jaringan otot dan tulang hewan. Gelatin dari proses asam berpotensi sebagai emulsifier dalam sistem emulsi minyak dalam air karena dapat bermuatan kation pada kisaran pH yang lebih luas daripada emulsifier dari protein lainnya. Hal ini mendukung dikembangkannya penelitian mengenai gelatin sebagai emulsifier dalam sistem emulsi minyak dalam air. Penelitian ini bertujuan untuk (1) mengetahui pengaruh waktu perendaman, konsentrasi dan jenis asam dalam isolasi gelatin dari kulit ikan nila (*Oreochromis niloticus*) terhadap rendemen dan karakteristik mutu kekuatan gel, (2) mengetahui pengaruh perlakuan asam asetat dan asam sitrat dalam isolasi gelatin kulit ikan nila (*Oreochromis niloticus*) terhadap karakteristik emulsi, (3) mengetahui pengaruh berat molekul dan komposisi asam amino dari gelatin kulit ikan nila (*Oreocromis niloticus*) terhadap sifat fungsionalnya sebagai emulsifier dalam sistem emulsi minyak dalam air.

Penelitian ini terdiri dari 3 tahap, yaitu (1) isolasi gelatin dari kulit ikan nila (*Oreochromis nilotichus*) dengan perlakuan waktu perendaman, konsentrasi dan jenis asam terhadap rendemen dan karakteristik mutu kekuatan gel, (2) karakteristik emulsi gelatin dari kulit ikan nila (*Oreochromis niloticus*) dengan proses asam asetat dan asam sitrat dan (3) fraksinasi ekstrak gelatin dari kulit ikan nila (*Oreochromis niloticus*) terhadap sifat fungsionalnya sebagai emulsifier dalam sistem emulsi minyak dalam air.

Isolasi gelatin dari kulit ikan nila (*Oreochromis nilotichus*) dengan perlakuan waktu perendaman selama 1, 2, 3, 4 dan 5 jam dalam larutan asam sitrat dan asam asetat (0; 0.05 ; 0,10; 0,15; 0,20; 0,25) M dengan rasio kulit dan larutan asam (1:8 b/v) diseleksi berdasarkan rendemen yang tinggi dengan karakteristik mutu kekuatan gel. Gelatin dari perlakuan kedua jenis asam terpilih selanjutnya dikarakterisasi berat molekulnya dengan SDS - PAGE dan nonSDS - PAGE, spektra FTIR dan morfologi molekul dengan SEM (*Scanning Electron Microscopy).* Fraksinasi ekstrak gelatin dari kedua jenis asam yang terpilih dilakukan dengan menggunakan kantong dialisis *Molecule weight cut off* (MWCO) 100 kDa yang dilanjutkan dengan pemekatan vacuum evaporator pada suhu ± 40^{o}C dan tekanan 25 mbar. Karakteristik emulsi terhadap gelatin dari perlakuan kedua jenis asam terpilih dan fraksinya meliputi ; sifat emulsi (*Activity Emulsion Index* / EAI dan *Emulsion Stability Index* /ESI), ukuran droplet emulsi, zeta potensial, pH emulsi, viskositas emulsi, pengamatan visual emulsi dengan *microscope optic* serta komposisi asam amino dan nilai HLB (*Hydrophilic–Lipophilic Balance*).

Hasil penelitian.menunjukkan gelatin dari kulit ikan nila (*Oreochromis niloticus*) dari proses asam setat 0,10 M selama 2 jam (GAs) dan asam sitrat

0,05M selama 1 jam (GSi) menghasilkan rendemen tertinggi yaitu 25,33 % dan 21,9 % dengan kekuatan gel sebesar 346,16 g bloom dan 144,21 g bloom. Gelatin GAs dan GSi masing-masing memiliki berat molekul *native* sebesar > 260 kDa dan 260 kDa, dengan distribusi berat molekul GAs dan GSi sebesar 35,56 - 240,43 kDa dan 22,1 - 143,22 kDa. Viskositas dan kadar abu pada gelatin GAs sebesar 7,5 cps dan 0,94% sedangkan pada gelatin GSi sebesar 4,75 cps.dan 0,78%.

Hasil proses fraksinasi tersebut menunjukkan fraksi gelatin GAs *retentate* 100 kDa dan GSi *retentate* 100 kDa dengan distribusi berat molekul 13,6 - 140 kDa dan 107,5 - 142,23 kDa. Fraksi gelatin GAs *permeate* 100 kDa dan GSi *permeate* 100 kDa dengan distribusi berat molekul sebesar 15.27 - 61.1 kDa dan 12,5 - 82,9 kDa. Perubahan spektra FTIR 1650-1400 cm^{-1} menunjukkan adanya ikatan rantai α *helix* yang merupakan pecahan dari struktur sekunder molekul *triple helix* terlihat lebih tinggi pada fraksi GSi *retentate* 100 kDa dan GAs *retentate* 100 kDa daripada molekul GAs dan GSi *native*. Fraksi GSi *retentate* 100 kDa mengandung rantai α lebih banyak daripada GAs *retentate* 100 kDa. Fraksi GAs *retentate* 100 kDa memiliki puncak spektra FTIR 1091 cm^{-1} lebih tinggi yang menunjukkan sebagian besar ikatan rantai α telah terlepas menjadi rantai polipeptida..

Perubahan karakteristik emulsi gelatin GAs dan GSi dengan fraksinya diketahui dari sifat emulsi (*Activity Emulsion Index* /EAI dan *Emulsion Stability Index* /ESI), ukuran droplet emulsi, zeta potensial, pH emulsi, viskositas emulsi, pengamatan visual emulsi dengan *microscope optic* serta komposisi asam amino dan nilai HLB (*Hydrophilic– Lipophilic Balance*). Aktifitas emulsi gelatin GAs dan GSi tertinggi pada konsentrasi 0,5% yang semakin rendah dengan semakin besarnya konsentrasi. Namun sebaliknya pada stabilitas emulsi semakin meningkat dengan semakin besarnya konsentrasi. Stabilitas emulsi tertinggi pada GAs 3% (112,57 menit) dan GSi 2 % (79,37 menit). Ukuran droplet emulsi GAs dan GSi paling besar pada konsentrasi 3% dengan nilai zeta potensialnya yaitu 1809,3 nm (+12,74) dan 1785,2 nm (+14,42). Nilai pH emulsi gelatin GAs dan GSi semakin rendah dengan semakin besarnya konsentrasi. pH emulsi GAs 3% (5,15) dan GSi 3% (3,55) dengan titik isoelektrik pH 8,5, sehingga terjadi interaksi ionik yang besar pada emulsi menyebabkan zeta potensial dan viskositas emulsi lebih meningkat. Aktifitas emulsi fraksi gelatin GAs lebih besar daripada fraksi GSi dengan nilai tertinggi pada fraksi GAs *retentate*100 kDa sebesar 4,77 m^2/g dengan stabilitas emulsi 20,15 menit. Ukuran droplet emulsi dan zeta potensial yang dihasilkan paling besar pada fraksi GAs *retentate* 100 kDa dan GSi *retentate* 100 kDa masing-masing sebesar 1389 nm (+4,02) dan 1145 nm (+ 6,15).

Kandungan asam amino hidrofilik dan lipofilik gelatin GAs sebesar 30,37 % dan 49.20 % lebih besar daripada GSi sebesar 36,92 % dan 39,34 %, sedangkan fraksi GAs *retentate* 100 kDa sebesar 10,49 %.dan 9,26 % serta fraksi GSi *retentate* 100 kDa sebesar 10,95% dan 8,70%. Kandungan asam amino hidrofilik dan lipofilik fraksi GAs *permeate* 100 kDa sebesar 5,44 % dan 3,00 % sedangkan fraksi GSi *permeate* 100 kDa sebesar 3,60 % dan 1,92 %.

Berdasarkan perhitungan keseimbangan jumlah komposisi asam amino hidrofilik dan lipofilik dalam molekul gelatin GAs dan GSi diketahui nilai HLB

(*hydrophile – lipophile balance)* GAs sebesar 32,25 dan GSi 30,45. Gelatin GAs dan GSi dapat berfungsi sebagai bahan emulsifier dalam sistem emulsi minyak dalam air dan sebagai pembentuk gel.

Nilai HLB fraksi GAs *retentate* 100 kDa dengan distribusi berat molekul 13,6 -140 kDa memiliki nilai HLB 8,15, sedangkan GSi *retentate* 100 kDa dengan distribusi berat molekul 107,5 – 142,23 kDa memiliki nilai HLB 8,28. Kedua fraksi tersebut dapat berfungsi sebagai emulsifier dalam sistem emulsi minyak dalam air. Selain itu, fraksi GAs *permeate* 100 kDa dengan distribusi berat molekul 15,27 – 61,10 kDa memiliki nilai HLB 3,67, sedangkan GSi *permeate* 100 dengan distribusi berat molekul 12,5 – 82,9 kDa memiliki nilai HLB 2,22. Kedua fraksi tersebut tidak dapat berfungsi sebagai emulsifier.

Kata kunci, gelatin, kulit, ikan, emulsi, HLB (*hydrophile–lipophile balance).*

ABSTRACT

As a food additive, an emulsifier is very important in food production to stabilize the emulsion texture. Generally, an emulsifier is biopolymers that rapidly adsorb to the surface of the emulsion droplets so that a protective membrane is formed to prevent droplets from aggregating with each other. Gelatin is an amphiphilic biopolymer which processed from hydrolysis of collagen that found in skin, connective tissue, and animal bone. Gelatin from acid treatment has a potention to be an emulsifier oil in water emulsion system because it could have cation charge in larger pH condition than other emulsifier from protein. This leads to development of research on gelatin as an emulsifier. The aim of this research was to investigate (1) the effect of soaking time, concentration and acid type in the isolation of gelatin from nila skin (*Oreochromis niloticus*) on the yield and the quality of gel strenght, (2) the effects of asetat acid and citric acid in the isolation of gelatin from nila fish (*Oreocromis niloticus*) skin on the emulsion characteristic, and (3) the effect of the molecule weight and amino acid content of amino acid from gelatin of nila fish (*Oreocromis niloticus*) skin on functional properties as an emulsifier oil in water emulsion system.

This research consists of three steps that were (1) the isolation of gelatin from nila fish (*Oreochromis nilotichus*) skin by treatment of soaking time, concentration and acid type on yield and quality characteristic of gel strenght, (2) emulsion characteristics of gelatin from nila fish (*Oreochromis niloticus*) skin by acetic acid and citric acid treatment, and (3) fractionation of extract gelatin from nila fish (*Oreochromis niloticus*) skin on functional properties as emulsifier in oil in water emulsion system..

The isolation of gelatin from nila fish (*Oreochromis nilotichus*) by treatment of soaking time (1, 2, 3, 4 and 5) hours in acetic acid and citric acid solution (0; 0.05 ; 0,10; 0,15; 0,20; 0,25) M with ratio of skin and acid solution (1:8 b/v) were selected based on high yield with its quality characteristics of gel strenght. The molecule weights of selected gelatin from both acid types treatment was characterized by SDS-PAGE and non-SDS-PAGE, FTIR spectra and molecule morphology with SEM. Fractionation of gelatin extract from both acid types treatment was selected by using dialysis membrane with molecule weight cut-off (MWCO) of 100 kDa and followed vacuum evaporator at ± 40^{o}C ; 25 mbar. The characteristics of gelatin emulsion from two types of acid treatment selected and its fractions include emulsion properties (Emulsion Activity Index / EAI and Emulsion Stability Index / ESI), size of droplet emulsion, zeta potential, pH of emulsion, viscosity of emulsion, visual of emulsion observation with optical microscope, amino acid composition and value of HLB (Hydrophilic-Lipophilic Balance).

The result of this research showed that the highest yield of gelatin were soaked into 0.10 M of acetic acid for 2 hours (GAs) and 0.05 M of citric acid for an hour (GSi) were 25.33 % and 21.9 % respectively, with the gel strength of 346.16 g bloom and 144.21 g bloom. Gelatin GAs dan GSi had native molecule weight of >260 kDa and 260 kDa respectively, with the molecule weight

distribution of GAs and GSi were 35.56 to 240.43 kDa and 22.1 to 143.22 kDa.. Viscosity and ash contents of GAs was 7.5 cps and 0.94 %, whereas in GSi was 4.75 cps and 0.78 %..

The result of fractionation process shows molecule weight distribution of gelatin fraction GAs retentate 100 kDa and GSi retentate 100 kDa were 13.6 to 140 kDa and 107,5 to 142,23 kDa. Molecule weight distribution of gelatin fraction GAs permeate 100 kDa and GSi permeate 100 kDa were 15.27 to 61.1 kDa and 12.5 to 82.9 kDa. The FTIR spectra change from 1650 to 1400 cm^{-1} was indicating the presence of α helix chain bonds which was fractions of the secondary structure of triple helix molecule. These spectra in GSi retentate 100 kDa fraction and GAs retentate 100 kDa was higher than the GAs and GSi native molecule. GSi retentate 100 kDa fraction contains more α chains than GAs retentate 100 kDa. GAs retentate 100 kDa fraction has higher peak in spectra of FTIR 1091 cm^{-1} which shows most α chain bonds have been released into polypeptide chains.

Changes in the characteristics of GAs and GSi gelatin emulsions with their fractions are known from the emulsion properties (Activity Emulsion Index /AEI and Emulsion Stability Index / ESI), size of droplet emulsion, zeta potential, pH of emulsion, viscosity of emulsion, visual emulsion observation with optical microscope, composition of amino acids and HLB values (Hydrophilic-Lipophilic Balance). The highest activity of GAs and GSi gelatin emulsions at 0.5% concentration was decreased by increasing concentration. On the contrary, the emulsion stability increased with increasing concentration. The highest emulsion stability at GAs 3% (112.57 min) and GSi 2% (79.37 min). The size of droplet emulsion gelatin GAs and GSi was greatest at 3% concentration with the potential zeta value of 1809.3 nm (+12.74) and 1785.2 nm (+14.42). pH emulsion from gelatin GAs and GSi were lower by concentration increasing. pH of emulsion gelatin GAs 3% (5.15) and GSi 3% (3.55) with the isoelectric point of pH 8.5 which result a high of ionic interaction on the emulsion was causing zeta of potential and the viscosity of emulsion increase. The activity of GAs gelatin fraction emulsion was greater than the GSi fraction with the highest value in GAs retentate 100 kDa fraction of 4.77 m^2 / g with emulsion stability of 20.15 min. The largest of size droplet emulsion and zeta potensial produced in fractions of GAs retentate 100 kDa and GSi retentate 100 kDa were 1389 nm (+ 4,02) and 1145 nm (+ 6,15), respectively.

The content of hydrophilic and lipophilic amino acids of GAs gelatin is 30.37 % and 49.20 % which was greater than GSi of 36.92 % and 39.34 %, while the fraction of GAs retentate 100 kDa of 10,49 % and 9.26 % and the fraction GSi retentate 100 kDa of 10,95 % and 8,70 %. The content of hydrophilic amino acid and lipophilic fraction of GAs permeate 100 kDa were 5,44 % and 3,00 % while the fraction of GSi permeate 100 kDa was 3.60 % and 1.92%.

Based on calculation for the balance of hydrophilic and lipophilic amino acids in gelatin molecule, gelatin GAs had value of HLB (hydrophile-lipophile balance) was 32.25 and gelatin GSi 30,45. It shows that gelatin GAs and GSi gelatin could as emulsifiers oil in water emulsion system and gelling agent.

The HLB for fraction of GAs retentate 100 kDa with molecule weight distribution of 13.6 - 140 kDa had HLB value of 8.15, whereas GSi retentate 100

kDa with molecule weight distribution of 107.5 to 142.23 kDa had HLB value of 8.28. Both fractions could be as emulsifiers oil in water emulsion system. In addition, the fraction of GAs permeate 100 kDa with molecule weight distribution of 15.27 - 61.10 kDa had a HLB value of 3.67, whereas GSi permeate 100 kDa fraction with molecule weight distribution of 12.5 to 82.9 kDa had a HLB value of 2.22. It means that both fractions could not function as emulsifiers.

Key words : gelatin, skin, fish, emulsion, HLB (hydrophile-lipophile balance)