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Development of Collagen Peptide Raw Materials from Milkfish Scale Waste (Chanos Chanos) as a Bioactive Emulsifier

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Abstract--- Collagen peptides produced from milkfish (*Chanos chanos*) scales can be an alternative to mammalian collagen. Besides the high price of mammalian collagen, it also risks spreading infectious diseases like Bovine Spongiform Encephalopathy (BSE) and Transmissible spongiform encephalopathies (TSEs). This study aims to identify the effect of acetic acid and pepsin concentrations in optimizing the extraction of collagen peptides from milkfish scales due to waste and their application as a bioactive emulsifier. This study used an experimental design using a completely randomized design (CRD) with a 3 x 2 factorial pattern with three replications. The first factor is three levels of acetic acid concentration, and the second is three levels of pepsin concentration. The results of this study on the proximate analysis of collagen peptides showed that the results of the moisture content test, ash content test, protein content test, fat content test, and pH test met the requirements for collagen quality. In testing the quality of the emulsifier, all groups met the criteria for homogeneity, viscosity, and percent transmittance, where a concentration of 0.5 M with a variation of pepsin 0.10%, a concentration of 0.75 M with a variation of 0.05% pepsin, and a concentration of 0.75 M with a variation of pepsin 0.10% produced the most stable collagen in terms of Creaming Index and Measurement of visible layer boundaries during storage. Conclusion: The concentration of 0.75 M acetic acid with a variation of pepsin 0.10% produced the most stable collagen and fulfilled the requirements for homogeneity, viscosity, and percent transmittance.

Keywords--- milkfish scales, collagen, emulsifying agent.

Introduction

Collagen is an essential protein in connective tissue, widely used as a raw material for medicine. Most collagen comes from cows and pigs at risk of spreading infectious animal diseases, so it is necessary to find alternative sources of raw materials. Domestic production of collagen itself is still not optimal. In 2003, Indonesia still imported

as much as 6200 tons of collagen (Nurhidayah et al., 2019), so efforts are needed to be self-sufficient in medicinal raw materials, especially collagen production. Recently, collagen produced from milkfish (*Chanos chanos*) scales can be an alternative to replace mammalian sources.

Karawang Regency is a milkfish-producing center and supply chain for the Bekasi, Bandung, and Jakarta areas (Widria et al., 2016). The presto milkfish business actors produce an average of 1,000-2,000 packages per day (KKP RI, 2012). The processing of milkfish produces waste in the form of skins, scales, and bones, which have the potential to pollute the environment. Efforts are needed to process this waste into a valuable commodity, namely as a raw material for collagen. Besides the high price of mammalian collagen, there is also a risk of spreading infectious diseases, such as Bovine Spongiform Encephalopathy (BSE) (Mahboob, 2015) and Transmissible spongiform encephalopathies (TSEs) (Jafari et al., 2020).

Collagen is the most abundant structural protein in the body's connective tissue. Collagen bioactivity is primarily medicinal (Jafari et al., 2020). Collagen can be obtained from fish scales, which mostly contain type I collagen and hydroxyapatite, $\text{Ca}_{10}(\text{OH})_2(\text{PO}_4)_6$ (Romadhon et al., 2019; Noomhorm et al., 2014).

Collagen peptides are collagen with a molecular weight of 0.3-8 kDa (Sibilla et al., 2015). Collagen peptides have bioactivity, including those for skin and bones (Zdzieblik et al., 2017), antioxidants (Ketnawa et al., 2016), and antimicrobials (Ennaas et al., 2016). Collagen isolation from milkfish scales can be performed by extraction using acetic acid (Romadhon et al., 2019; Wahyu, 2018; Paudi et al., 2020) or enzymatic extraction (Manikkam et al., 2016). Collagen yield can be increased by using pepsin during extraction (Veeruraj et al., 2013; Jamilah et al., 2013). This enzyme increases its solubility in acidic media (León-López et al., 2019). There are several studies regarding the potential of collagen from fish scales as a bioactive emulsifier (León-López et al., 2019; Kumar et al., 2019; de Castro Santana et al., 2012). We see that previous methods rarely determine the combination of acetic acid and pepsin levels in the extraction process, so an in-depth study is needed regarding the optimal combination for extracting collagen peptides from milkfish scales.

This study aims to identify the effect of acetic acid and pepsin concentrations in optimizing the extraction of collagen peptides from milkfish scales due to waste and its application as a bioactive emulsifier. This study applied a completely randomized design (CRD) by comparing the concentrations of acetic acid and pepsin to the yield of collagen peptides, proximate analysis of collagen peptides, pH test, and bioactive emulsifier activity.

Material and Method

Research Methodology

The research used an experimental design, applying a completely randomized design (CRD) with a factorial pattern of 3 x 2 with three replications. The first factor uses three levels of acetic acid concentration, and the second one uses three levels of pepsin concentration. The potential of the isolated collagen was examined against the yield of collagen, proximate analysis of collagen (water content test, ash content test, protein content test, and fat content test), pH test, and examination of emulsifiers, including emulsion viscosity test, emulsion stability test, and layer boundary test during storage.

Materials

Ingredients: waste milkfish scales, acetic acid, NaCl, Bromocresol green, concentrated HCl, sodium sulfate. Tools: Analytical Balance (Mettler Toledo), Freeze Dryer (Magnum XL), pH Meter (Hanna), Brookfield Viscometer (Amatex DVI Digital), Furnace (Muffle Furnace), Oven (IKA)

Pretreated Fish Scales (Chuaychan et al., 2015)

The pre-treatment procedure used a modification of the method by Chuaychan et al. (2015). Before extraction, fish scales went through three stages: deproteinization using 1 M NaOH solution, demineralization using 0.5 M Na₂EDTA, and hydrolysis in 0.5 M acetic acid solution with a ratio of 1:15 (w/v).

Collagen Peptide Extraction Process (Mahboob, 2015)

The extraction process refers to modifying the Mahboob method (2015). Fish scales were macerated at four °C with a ratio of 0.25 M, 0.5 M, and 0.75 M for 48 hours. Then, pepsin 0.05%, 0.10%, and 0.15%. The solution was centrifuged at 8000 rpm for 30 minutes until white lumps formed, then filtered and dried using a freeze dryer—calculated yield of collagen peptides.

Proximate Analysis (Pamungkas et al., 2018)

Proximate analysis of collagen peptides was conducted for water, ash, protein, and fat content.

Measurement of Creaming Index and Viscosity (Ermawati et al., 2017; Hartayanie et al., 2014)

Weigh 1 g of collagen peptide, dissolve it in 50 ml of distilled water, and then homogenize it. The emulsion was measured for Creaming Index and its viscosity. Creaming Index analysis is calculated using the formula: $CI = \frac{HS}{HE} \times 100 \%$

Description: CI = Creaming Index; HS = clear layer height (ml); HE = initial emulsion height before centrifugation (ml).

Measurement of visible layer boundaries during storage

This method is a modification of Rocha et al. (2020). The emulsion was measured for the precise solution's height limit and the cream visible during the seven days of observation. The Emulsifying Activity (EA) value was calculated using the following formula:

$$EA = \frac{\text{formed layer of cream(ml)}}{\text{Total emulsion(ml)}} \times 100 \%$$

Percent Transmittance Measurement (%T)

The percent transmittance test used a UV-Vis spectrophotometer and aqua dest as a blank. Transmittance is expressed in percent. $\%T = I/I_0 \times 100$.

Homogeneity testing

The emulsion was tested for its homogeneity by smearing it on a piece of glass and observing its homogeneity.

Conductivity measurement

The emulsion is put in a beaker and connected to an electric current circuit. If it ignites, it is an oil-in-water emulsion.

Statistical analysis

Analysis of quality data from a factorial Completely Randomized Design (CRD) including the concentration of acetic acid using a ratio of 0.25 M acetic acid concentration; 0.5 M; and 0.75 M and pepsin concentration 0,05% w/v, 0,10% w/v, dan 0,15% w/v. All treatments were repeated three times. Each experimental unit was examined for the results of collagen yield, proximate collagen analysis, and emulsifier tests. ANOVA was used to analyze the data obtained, and if there was a significant difference, we continued with Duncan's test at a 95% confidence level.

Result and Discussion

Production of collagen peptides

Waste milkfish scales are washed under running water and dried in the sun for 2-3 days. The dried milkfish scales are then sent to the process: Deproteinization, Demineralization, and Hydrolysis. Deproteinization aims to remove non-collagen proteins found in milkfish scales (Kurniasih & Kartika, 2011). Demineralization is a mineral separation stage that aims to remove inorganic compounds contained in fish scale waste. The demineralization process is carried out by soaking fish scale waste in a solution of Na2EDTA with a ratio of 10% (w/v) for 24 hours at 4°C. According to Maulana et al. (2022), in the demineralization process, minerals are lost from a material by adding a low concentration of HCl solution. The hydrolysis process was carried out by immersing it in a 0.5 M acetic acid solution with a ratio of 10% (w/v) for 48 hours at 4°C.

Fish scales were extracted by maceration at 4°C with a concentration of acetic acid consisting of a ratio of 0.25, 0.50, and 0.75 M, with variations of pepsin 0.05%, 0.10%, and 0.15%. Extraction variations were made to determine which variation in acetic acid concentration produced the most excellent collagen. Then the solution was centrifuged until a white precipitate formed in the form of wet collagen, filtered with filter paper, and then the residue was dried using a freeze dryer.

The collagen extraction method with acid and pepsin can produce collagen peptides with different yields and characteristics depending on the source of the raw material and the process conditions used (Regenstein & Zhou 2007). The yield of collagen varies from 2.20% to 7.55%. The lowest yield value is obtained at a concentration of 0.75 with a variation of pepsin 0.05%, namely with a yield of 2.20%, while the highest yield value is 7.55%, obtained at a concentration of 0.50 with a variation of pepsin 0.05%. The higher the yield, the higher the economic

value. The yield is low because, in an acetic acid solution, the concentration of H^+ ions is less than the concentration of H^- ions, so the yield produced is inferior; besides, acetic acid is a weak acid, so the concentration of H^+ ions is lower (Astana, 2016).

Based on the results of research by Paudi et al. (2020), showed that the high yield can be caused by the concentration of acetic acid given. The higher the concentration of acetic acid, the higher the collagen yield. The amount of dry collagen produced will be significant, but the aesthetic value created will be lower. The high yield of collagen isolation is due to the use of the pepsin enzyme in the extraction process. Collagen is more effectively isolated through enzymatic extraction (Manikkam et al., 2016). Collagen yield can be increased by using the enzyme pepsin during extraction (Veeruraj et al., 2013; Jamilah et al., 2013).

Collagen Peptide Moisture Test Results

Testing the water content aims to provide a minimum limit or range regarding the amount of water content in the material (Depkes RI, 2000). The results of determining the water content of collagen show an average water content ranging from 0.40% to 3.0%, the lowest water content is 0.40%, which is observed at a concentration of 0.25 with a pepsin variation of 0.10%. The highest is 3.03% in have by a concentration of 0.75, with a pepsin deviation of 0.05%. From the collagen water content test results above, all values meet the collagen quality requirements set by the National Standardization Agency (2014) $\leq 12\%$. The water content in collagen will affect its shelf life because it is closely related to the metabolic activities that occur while the collagen is stored, such as enzyme activity, microbial activity, and chemical activity, namely rancidity and non-enzymatic reactions that cause changes in organoleptic properties. And value quality. The higher the water content in collagen, the lower the age of collagen, but conversely, if the water content is low, the shelf life of collagen will be longer (Noorman, 1988).

Percent Results of Collagen Peptide Ash Content

Determination of ash content aims to determine the quality of processing, determine the type of material used, and, as a parameter, determine the nutritional value of food and beverages. This makes ash content an essential parameter in maintaining the quality of food and beverage products, according to the Indonesian National Standard (SNI) 01-2891-1992 and AOAC. The ash content was determined by placing a dry collagen sample in an ashing cup. Then burned on an electric stove until there was no smoke and placed in an ashing furnace at a temperature of 60°C for 1 hour. Then the cup was put in a desiccator, and the ash content was weighed. The results of the ash content of collagen peptides show an average ash content ranging from 0.01%-0.36%, the lowest ash content is 0.014%, which is observed at a concentration of 0.25 M with a pepsin variation of 0.05%, and the highest is 0.36%, owned by the concentration of 0.25 M with a pepsin variation of 0.10%.

From the collagen ash content test results above, all values met the collagen quality requirements set by the National Standardization Agency (2014) SNI, namely $\leq 1\%$. Ash content is related to the minerals of a material. The minerals contained in a material can be of two kinds of salt: organic salts and inorganic salts (Sudarmadji, 2010). The high ash content is caused by there is still a lot of mineral content in the sample and can be minimized through demineralization in the early stages of extraction (Alhana et al., 2015). The resulting dry collagen contains minerals because, before deproteinization, mineral separation was not carried out. When tested for ash content, the minerals contained in collagen will not disappear but will also turn into ashes, thereby increasing the amount of ash (Astawan & Aviana, 2003).

Collagen Fat Levels Test Results

Fat content is determined by oxidizing organic compounds at 105°C and weighing the remaining substances after the combustion process. The reflux process is carried out until the solution is clear and the solvent in the fat flask is clear. The fat flask containing the extracted fat is heated in an oven at 105°C until the weight is constant, then placed in a desiccator and weighed. The results of the ash content of collagen peptides show an average ash content ranging from 0.86% to 1.86%, and the lowest fat content is 0.86%, which is associated with an acetic acid concentration of 0.75 with a pepsin variation of 0.05%. The highest is 1.86 %, owned by an acetic acid concentration of 0.25 acetic acid with a pepsin variation of 0.05%. The fat content test does not yet have an Indonesian National Standard, so it is not sure how much fat content meets the requirements of this test. The high-fat content indicates the need to optimize the skin preparation process to remove fat from the fish skin to improve the quality of the collagen produced. The presence of fat and other minerals would interfere with the effectiveness of collagen in its application in various products (Shon et al., 2011).

Test results for collagen protein levels

This analysis is carried out to determine the total protein content, specific protein content in a mixture, protein content during the process of protein isolation and purification, non-protein nitrogen, amino acid composition, and the nutritional value of a protein product. Testing for protein levels was obtained by making a standard series of bovine serum albumin, then measuring the protein levels of milkfish scale collagen samples, then using UV-VIS spectrophotometry at a wavelength of 595 nm. The following is a picture of the standard series of Bovine Serum Albumin:

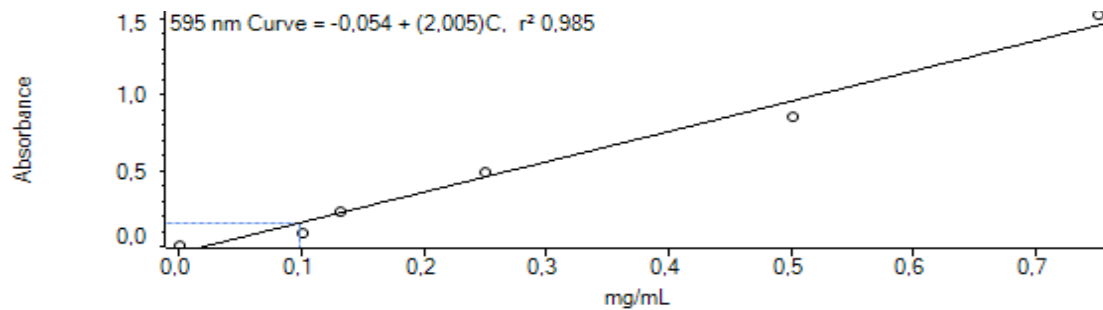


Figure 1. Standard series of Bovine Serum Albumin

The lowest average yield of collagen peptide protein levels was obtained with a 0.5 M acetic acid concentration and 0.10% pepsin, with a variation of 68.62%, and the highest at 0.75 M acetic acid concentration with 0.10% pepsin, with a variation of 83.25%. High protein and low water content in collagen indicate that the extraction method used effectively extracts collagen from milkfish scales (Wang et al., 2013). From the test results for collagen protein content at a concentration of 0.75 M acetic acid with a variation of pepsin 0.10%, 83.25% fulfilled the collagen quality requirements set by the National Standardization Agency (2014), namely $\geq 75\%$.

The most widely known collagen type I collagen consists of three polypeptide α chains. Type I collagen is most abundant in soft parts of the body, such as skin and tendons, and complex aspects of the body, such as bones, teeth, and connective tissue (Cardoso et al., 2014). In a study conducted by Massod et al. (2018). Protein is in the collagen content, where glycine and proline are essential components of collagen. The protein levels obtained in this study were higher than those in collagen made from milkfish scales in the study of Nurhidayah et al. (2019), which has an average protein content of 49%; besides that, Pamungkas et al. (2018) had an average protein content of 69%, whereas in the research by Suptijah et al. (2018), which has a moderate protein content of 58%.

Collagen pH test

The pH test results were obtained by dissolving 1 g of the sample in 50 ml of distilled water and stirring until homogeneous. Then the pH of the solution was measured using a pH meter. The collagen peptide pH test results ranged from 4.50 to 5.80, the lowest pH was 4.5 at a concentration of 0.25 M acetic acid with a variation of 0.10% pepsin, and the highest pH was 5.8 at a concentration of 0.50 M acetic acid with a variation of 0.10% pepsin. The higher the concentration of the solution, the lower the pH. This is because acetic acid diffuses more in the fish scale tissue, so in the washing process, more acid is left on the scales than at low concentrations. The pH value of collagen is related to the process being carried out. According to Lehninger (1982), denatured proteins will be damaged not only by heat but by the influence of pH, which is a change in the protein's main structure of the peptide chain. If the protein is denatured, the arrangement of the polypeptide chain bonds is disrupted, and the protein molecule unfolds into a random structure and then coagulates, resulting in a lower amount of extracted collagen (Rusli, 2004).

The results of the analysis of the pH value show that collagen with enzymatic extraction has the best value for testing the pH value of 6.5, whereas the SNI for dry collagen for the pH value is 6.5 - 8. At the pH value of collagen, milkfish scales have an acidic pH. This is because the solvent used in collagen uses acetic acid, so the pH of collagen tends to be acidic. At the optimum pH level, enzymes can catalyze reactions faster than at other pH levels. The pepsin enzyme in the collagen extraction process functions as a protein catalyst. It is known to be most active at acidic pH. pH will work optimally on the substrate compatible with the enzyme (Poedjiadi & Supriyanti, 2005).

Emulsion Percent Transmittance (%T) Measurement.

The emulsifier test was carried out by homogenizing the collagen solution and using Ultra Turrax T25 at 8,000 rpm for 1 minute. Then add 50 ml of palm oil. After the addition of oil, the speed of homogenization was increased to 14,000 rpm for 3 minutes. The measurement of transmittance percentage (%T) aims to find out whether there is a light penetrates through the sample being tested, which indicates if there is a light that penetrates, it means that there is still separation in the emulsion. In other words, the emulsion is not mixed homogeneously. If there is no light, the emulsion is translucent, which is a sign that the emulsion does not transmit light. In other words, it is thoroughly mixed and homogeneous (Bhati & Nagrajan, 2012).

The average yield of the percent transmittance of the collagen peptide emulsion at each concentration was 0.0%. This was due to the homogeneous mixing of the emulsion between water and oil with the addition of milkfish scales collagen powder, where 0.0% here indicates that the emulsion mixture cannot be penetrated by UV-VIS light, so the transmittance obtained is 0.0%. In comparison, this test uses absorbance in the form of aquadest, where the aquadest itself has a transmittance of 100-110%. The percent transmittance test (%T) is used to measure the clarity of a solution or dispersion system qualitatively. Transmittance values close to 100% indicate clear and transparent dispersions with droplet sizes reaching nanometers (Bhati & Nagrajan, 2012).

Emulsion Homogeneity Test

The emulsion formed from collagen is tested for its homogeneity by placing it on a piece of glass and then looking at its composition. The results were homogeneous based on the results of the homogeneity test of the emulsion originating from milkfish scale waste. The nine samples looked homogeneous during the examination; on the glass slide, no coarse grains were visible, and the color was even. Among the good physical properties of the emulsion is that the emulsion must remain homogeneous from the time of shaking in the container until the time of pouring from the container (Aulton & Cooper, 2002).

Emulsion Conductivity Measurement Test

Identification of the most convincing type of emulsion can be obtained from conductivity testing. The preparations that have been made are then connected to the multimeter. If the needle moves on the tool, it conducts electricity; then the outer phase is water, or the emulsion type is oil in water (O/A). In this study, the results obtained were from an emulsion type (A/M). The results of the six pepsin concentrations and variations show a conductivity measurement value of 0 A at each voltmeter current of 2V, 6V, and 10V. This indicates that the milkfish scale collagen emulsion cannot conduct electric current, so the ammeter reading from each voltmeter is 0. A. Based on these results, it can be concluded that the emulsion is of the A/M type. It is known that water has electrical conductivity due to the presence of ions in the water, while oil does not. The type of emulsion that is formed is an O/M emulsion because it cannot conduct electricity (Toha et al., 2020). As the outer phase, water contains the electrolyte needed to conduct electric current. The O/M emulsion, whose external phase is oil, will function as an insulator so that there is no deviation in the ammeter (Voigt, 1995).

Creaming Index (CI) Measurement

14 ml of sample was put into a centrifuge tube, then centrifuged at 4,500 rpm for 5 minutes. Centrifugation was carried out at room temperature, and then the volume of cream formed was measured. The following is the average gain in emulsion stability:

Table 1
Average Results of *Creaming Index (CI) Measurement*

No	Sample	Average Results CI (%) $\pm$ SD
1	A0,25P0,05	1,53 $\pm$ 0,13*
2	A0,25P0,10	1,63 $\pm$ 0,04
3	A0,25P0,15	1,62 $\pm$ 0,05
4	A0,50P0,05	2,26 $\pm$ 0,40
5	A0,50P0,10	2,28 $\pm$ 0,26**
6	A0,50P0,15	2,27 $\pm$ 0,20

No	Sample	Average Results CI (%) $\pm$ SD
7	A0,75P0,05	2,28 $\pm$ 0,26**
8	A0,75P0,10	2,28 $\pm$ 0,26**
9	A0,75P0,15	2,75 $\pm$ 0,75

Information: * = Lowest
 ** = Highest

The table above shows the results of emulsion stability based on the creaming index, which is lowest at a concentration of 0.25 M with a variation of 0.05% pepsin of 1.53. The highest was at a concentration of 0.5 M with a variation of 0.10% pepsin of 2.28; at a concentration of 0.75 M with a variation of pepsin of 0.05% was 2.28. A concentration of 0.75 M with a variation of pepsin of 0.10% was 2.28. Based on statistical processing, there was a significant difference in emulsion stability for each group ($p < 0.05$), with good emulsion stability, namely $> 2\%$ (Dybowska, 2011). Emulsion stability in this study was better than the results obtained from Dokic et al. (2012) of 2% and Anwar et al. (2017) of 2.1%. According to Lachman et al. (2008), an emulsion can be stable if it can return to its initial state or can be redispersed (reversible) with regular shaking after the temperature is disturbed. Based on this, an emulsion that has experienced precipitation and phase separation can be said to be stable if it can be redispersed after shaking. (reversible). The purpose of carrying out an emulsion stability test is to determine whether the emulsion phase is separated (Suryani et al., 2015).

Emulsion Viscosity Test

The results of making the emulsion using fish scale collagen were then measured for viscosity using a Brookfield viscometer. The correct rotation spindle was selected with a speed of 100 rpm until measurement stability was achieved. The following is a table of average emulsion viscosity test results:

Table 1
Average Viscosity Results

No	Sample	Average Viscosity Results (cP) $\pm$ SD
1	A0,25P0,05	2300,5 $\pm$ 15,50
2	A0,25P0,10	2550,4 $\pm$ 10,55**
3	A0,25P0,15	2420,5 $\pm$ 9, 50
4	A0,50P0,05	2372,2 $\pm$ 10,05
5	A0,50P0,10	2275,5 $\pm$ 7,12
6	A0,50P0,15	2350 $\pm$ 8,05
7	A0,75P0,05	2250,0 $\pm$ 7,50
8	A0,75P0,10	2250,3 $\pm$ 6,42*
9	A0,75P0,15	2320 $\pm$ 5,40

Information: * = Lowest
 ** = Highest

The table above shows the test results ranging from 2250-2550 cP, and the lowest viscosity value is 2250 cP, which is obtained at a concentration of 0.75 with a variation of pepsin 0.10%. The highest is 2550 cP, which is obtained at a concentration of 0.25 with a variation of pepsin 0.10 %. The higher the viscosity, the more stable the emulsion (Fatimah, 2005). Based on the normality and normal distribution tests, it was found that the viscosity of collagen data was not normal, so the Kruskal-Wallis test was continued. Based on the results of statistical processing, there was no significant difference in viscosity between groups ($p \geq 0.05$).

Viscosity can affect physical stability if there is a drastic change. Suppose the dispersed phase is less dense than the continuous phase. In that case, it causes upward creaming (Martin, 1993), which means that if the viscosity drops, the molecules in the preparation (dispersed phase) become less dense and can reduce their physical stability because it can cause the emulsion molecules or globules to break down. Move up.

Measurement of visible layer boundaries during storage

The 10 ml measuring cup is filled with the emulsion sample to 10 ml of the measuring cup volume. The measuring cup is closed and placed in the room. On each day of observation for seven days, the clear visible solution and cream

height limit or the Emulsifying Activity (EA) value was measured. The following is the result of the layer boundary test for each treatment group:

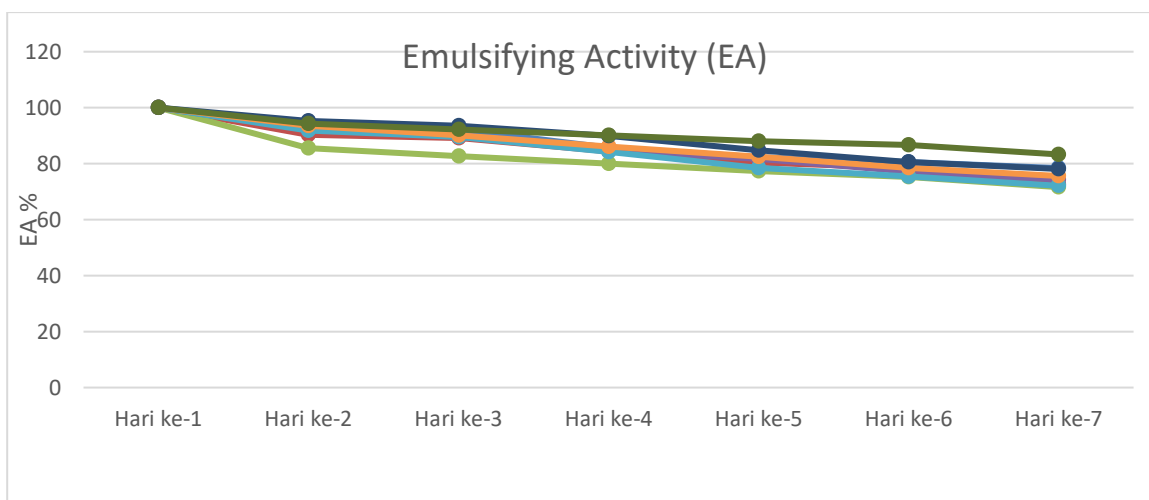


Figure 2. Layer Boundary Diagram

The figure above shows that increasing the concentration of acetic acid and pepsin in the process of isolating collagen peptides affects the stability of the emulsion during storage; the higher the concentrations of acetic acid and pepsin in the process of isolating collagen peptides, the more stable the emulsion is obtained. It can be seen in the graph that the emulsion with isolated collagen peptides using a concentration of 0.75 M acetic acid and 0.10 w/v Pepsin had the highest stability among the other treatment groups. The purpose of conducting an emulsion layer boundary test is to determine the existence of an emulsion phase layer boundary (Suryani et al., 2015). It is suspected that the role of collagen peptides is to increase the viscosity of the emulsion through the thickening of the continuous phase, thereby increasing the stability of the emulsion. The smaller the droplet size of the emulsion produced from the emulsifying collagen, the slower the movement of the oil droplets, and flocculation and coalescence are less likely to form (Sun & Gunasekaran, 2009; Saari et al., 2016).

Conclusion

The results of collagen isolation in all treatment groups had emulsifying abilities. The concentration of 0.75 M acetic acid with a variation of pepsin 0.10% produced the most stable collagen and fulfilled the requirements for homogeneity, viscosity, and percent transmittance.

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