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Characteristics of Demineralized Gelatin from Lizardfish (*Saurida* spp.) Scales Using NaOH-NaCl Solution

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Abstract

Demineralization is required to reduce the ash content of gelatin. Previous studies have confirmed the high quality of gelatin was produced after demineralized using combination of NaCl and aqueous NaOH. The purpose of this study was to determine the effect of NaCl on the properties of gelatin from Lizardfish (Saurida spp.) scales. The cleaned scales was demineralized using a mixture of NaOH 0.5% and NaCl (0- 0.8%), at various temperatures (45, 65, 86oC) and time (30, 60, 90 and 150 minutes). The obtained samples was determined by ash content, pH, the lightness and moisture content. Addition of NaCl reduced the ash, pH and moisture content but increased the whiteness. Gelatin physical properties increased inline with NaCl concentration. Extended demineralized period gave a positive effect on ash, pH and moisture content but opposite effect was observed in the lightness and physical properties. Extended demineralization periods in high temperature reduced gelatin quality. The highest gel strength was found at demineralization for 150 min at 65oC using a combination of NaOH 0.5% and NaCl 0.8%. At this condition, the gelatin has 10%, 1,7%, 248 bloom, and 7,1 cP of moisture content, ash, gel strength and viscosity, respectively, which fulfil SNI 06.3735.1995.

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INTRODUCTION

Gelatin includes as an essential supporting product for food and pharmaceutical industries as a stabilizer, gelling agent, and emulsifying agent. This polypeptide is extracted from the animal's collagen tissue including bone, scale, and connective tissue. Gelatin is obtained by partial hydrolysis of collagen, in which the raw material is processed using acid or diluted alkaline to break the cross-linking bonding. The collapsed triple helix structure of collagen will form gelatin with heating treatment (Karim & Bhat, 2009).

The gelatin production comprises pretreatment, extraction and purification steps, in which each step determines the resulting gelatin characteristics (Badii & Howell, 2006). Some attempts were made in the pretreatment stage, including the elimination of calcium (demineralization) (Sha et al., 2014), removal of non-collagen proteins (deproteinizing) (Ahmad & Benjakul, 2011), and removal of fat (defatting) (Badii & Howell, 2006). According to Sha et al. (2014), the processing of fish scales into gelatin requires the demineralization process which removes minerals on the scales. After the demineralization process, the remaining collagen is extracted.

Approximately 95% of commercial gelatin is obtained from mammals, especially pig and cow skin, while the remain part is obtained from cattle and pig bones (Sabbaghpour et al., 2014). The prohibition of some religions in consuming food containing pigs or cattle led to the need for an

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alternative source of gelatin that is acceptable for the public. One of the alternatives is gelatin that comes from fish (Silva et al., 2014) by using the by-product of the fish industry. As much as 50 -70% of the weight of fish is scales and fish bones (Zhang et al., 2012). Fish scale is reported to have high collagen content, such as in sea-bass (51,4%), mackerel (49,8%) and bullhead shark (50,1%) (dry weight) (Karim & Bhat, 2009). Utilization of by-product of fish processing into sources of gelatin has been reported previously such as using the scale of leather catfish (Ardekani et al., 2013), red tilapia fish (Jamilah et al., 2011) and sea-bass (Sae-leaw & Benjakul, 2015).

Lizardfish is one of the raw materials for salted fish (Riyadi, 2006). In 2010 this fish production reached 18,830 tons, in 2011 reached 21,663 tons and in 2012 reached 20,441 tons (Directorat General, 2013). With its highly consumed, it is necessary to consider the utilization of lizardfish waste. One part of lizardfish waste that can be utilized is the scales. Wangtueai & Noomhorm (2009) reported that lizardfish scales have a high protein content of 38.9%.

Demineralization of fish scales using 0.51% NaOH solution for 3.1 hours at 30°C resulted in an optimum yield of 10.7% with a gel strength of 252 bloom and a viscosity of 7,5 cP (Wangtueai & Noomhorm, 2009). Although in general, the characteristics of gelatin meet the standard, however, the ash content is still higher than the required ash content of 2% (JECFA, 2003). Akagunduz et al. (2014) reported the use of NaCl in the demineralization process of sea bream scales help the demineralization process by binding Ca and P ions and form $CaCl_3$ and PCl_3. The combination treatment of NaCl 5 g/100 g for 30 min and followed by 4 g/L NaOH for 1 hour resulted in gelatin from sea beam with the ash content of 0.57% that meets gelatin standard (Akagunduz et al., 2014). Montero and Go'mez-Guille`n (2000) reported that the combination of NaCl and aqueous NaOH in gelatin extraction resulted in high quality and soluble gelatin. The addition of NaCl in the demineralization process can increase the gelatin yield on the extraction of fish scales from 13% to 16% (Gimenez, 2005). Moreover, the use of NaOH improved the rheological properties of gelatin (Montero and Go'mez-Guille`n, 2000). The use of a combination of NaOH and NaCl on the demineralization of lizardfish has not been conducted, hence there is no information regarding the gelatin characteristics. Therefore, this study aims to study the characteristics of lizardfish gelatin in various conditions of demineralization reaction using a combination of NaOH and NaCl solution.

MATERIALS AND METHOD

Material

Lizardfish scales were obtained from the local fish market in Cilacap. Pure NaOH, pellets and NaCl were obtained from Merck.

Demineralization and Extraction

The demineralization procedure was performed using a modified Limpisophon method (Weng et al., 2014). The washed fish scales (400 g) were soaked with 0.5% NaOH solution with the addition of various NaCl concentrations (0, 0.2, 0.5, and 0.8%). The demineralization process was carried out at temperatures of 45°, 65°, 85°C and for 30, 60, 90 and 150 minutes. The demineralized fish scales were then extracted using aquadest with ratio 1:2 of scales-Aquadest (g/ml). The extraction process was carried out at 80°C for 3 hours. The solution was filtered with filter paper and then concentrated with the evaporator. The concentrated gelatin liquid obtained from evaporator was poured into an aluminium pan to be dried in an oven at 50°C for 24 hours. The dried film is then milled into powder which was subject to the analys of its properties.

Analysis of Results

Water Content (AOAC, 2005)

Porcelain cups that have been dried at 105°C for 1 hour after cooled down to room temperature was weighed. The sample (5 g) was placed on the porcelain cups and fed into an oven at temperature 105°C until it reaches a constant weight. Record the sample weight after reaching a constant weight.

Ash content (AOAC, 2005)

The cup to be used was dried for 30 minutes at 100-105°C. After that, it was cooled in the desiccator for 30 minutes then weighed (B1). The sample (5 g) was placed in the cup, then burned over Bunsen until it was not smoky. Then, it was inserted into a furnace and burned at 400°C until grey ash was obtained or the sample weight was

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fixed. Then the furnace temperature was increased to 550°C for 12-24 hours. The sample was cooled in a desiccator for 30 minutes then weighed (B2).

$$\text{Ash content} = B2 / B1 \times 100\% \quad (1)$$

pH (British Standard 757, 1975)

The sample (0.2 g) was dissolved into 20 ml of water at 25°C and homogenized with a magnetic stirrer. The degree of acidity was measured at room temperature by pH meters.

Viscosity (British Standard 757, 1975)

Viscosity of gelatin solution (6.67%, w/w) was measured using Brookfield Viscometer, LVF model, spindle no. 4. Measurements were made at 60°C at 60 rpm. The viscosity value is expressed in centipoise (cPs).

The strength of the gel (British Standard 757, 1975)

The gelatin solution (6.67%, w/w) was stirred using a magnetic stirrer until homogeneous and then heated to 60°C for 15 minutes. Pour the solution into a bottle 58-60 mm in diameter, 85 mm in height, close the bottle and let stand for 2 minutes. The solution was incubated at 10°C for 16-18 hours, then measured using TA-XT plus texture analyzer at a probe velocity of 0.5 mm/sec with a depth of 4 mm. The gel strength is expressed in units of bloom.

Melting point (Suryaningrum & Utomo, 2002)

A homogeneous concentration of gelatin solution (6.67%, w/w) was incubated at 10°C for 17 ± 2 hours. The melting point was determined by heating the gelatin gel after incubation in the water heater. The metal round indicator was placed on top of the gelatin. The temperature when the indicator falls to the bottom of the gelatin gel corresponded with the melting point temperature.

Gel point (Suryaningrum & Utomo, 2002)

Gelatin solution (6.67%, w/w) was placed in a thermometer-reaction tube. The ice is placed around the outside of the test tube. The gel point is the temperature when the gelatin solution begins to gel.

Lightness

Lightness was determined using Minolta CR 300 Chromameter. The observations were performed using the Hunter notation system characterized by three colour notations L, a *, and b *. The colour notation L represents the brightness (light) that has a value from 0 (black) to 100 (white). The value of L denotes the reflected light resulting in a red-green mixed chromatic colour, with + a (positive) from 0 to + 60 for the red value -a (negative) from 0 to -6 for green. Notation b denotes a blue-yellow mixed chromatic colour, with a value of + b (positive) from 0 to +60, for the yellow colour of the -b (negative) value from 0 to -60 for blue (Rhim et al. 1999).

RESULTS AND DISCUSSION

International standards for gelatin set lightness, moisture content, ash content and other physical properties as characteristics which determine quality of gelatin. SNI 06.3735.1995 for quality and determination of gelatin requires colourless gelatin, with a maximum water content of 16% and maximum ash content of 3.25%. Europe Pharmaceutical and USFCC require a maximum water content of 15%. While the ash content required by US FCC and USP are 3 and 2%, respectively (Schrieber & Gareis, 2007). In this study, the addition of NaCl in demineralization process using NaOH for gelatin from lizardfish scales was expected to help reduce ash content. Mineral content in gelatin needs to be reduced because its presence reduces the quality and physical characteristics of gelatin.

Effect of NaCl concentration

Figure 1 shows the negative effect of addition NaCl on moisture content. This is probably due to the increasing concentration of NaCl affected on the increasing amount of ash removed from the gelatin. As a result the gelatin size shrunk and the water content decreased. The highest water content was shown in the sample without the addition of NaCl. This sample also has the highest minerals content but the lowest lightness compared to the other samples. According to Sha et al. (2014), the demineralization process was required in preparation gelatin to remove of the minerals on the scales and left only remaining collagen. Akagunduz et al. (2014) reported that calcium and phosphate are common minerals found in fish scales. High calcium causes turbid on gelatin solution (Haris, 2008). Combination of NaCl and NaOH was used to prepare non-collagen proteins of sea bream gelatin (Akagunduz et al.,

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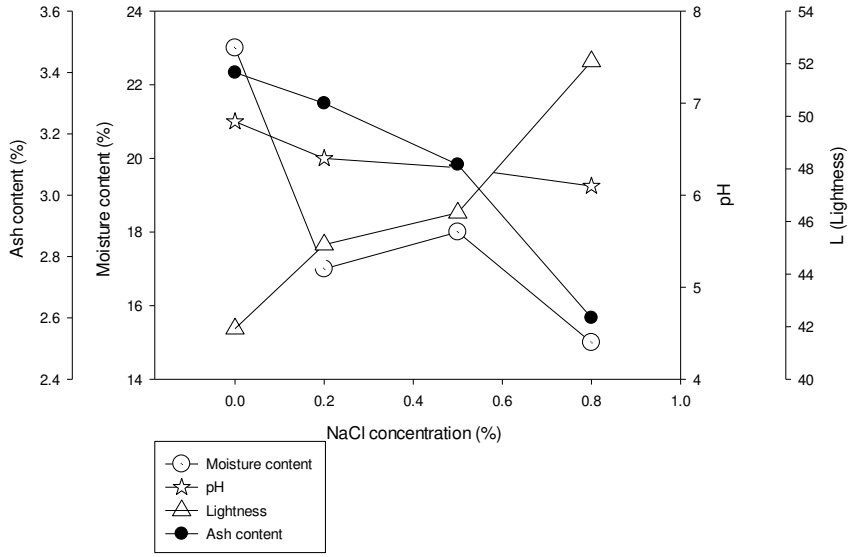

Figure 1. The effects of NaCl concentration of demineralization on moisture content, ash content, pH and lighness in demineralization process using NaOH.

2014). Ash content of lizardfish is up to 43.2% (wet weight) (Wangtueai & Noomhorm, 2009).

Figure 1 shows the lowest ash content of 1.7% was found in the addition of 0.8% NaCl. This condition is still above the standard specified by JECFA (2003) which requires no more than 2% ash content. Sanaei et al. (2013) reported that mineral content of catfish gelatin is still high due to the short immersion time and the low concentration of submersion solution. Alfaro et al. (2013) emphasize the high mineral content in gelatin will cause dark spots on the application in meat products.

According to Gimenez et al. (2004), the addition of NaCl facilitates the removal of Ca^{2+} from scales based on the proportion of added NaCl, without causing the NaCl to left in gelatin. Aquilina et al. (2004) reported the addition of NaCl helps to keep gelatin proteins from being twisted. This condition allows the impurities in the gelatin structure to be remove easily.

The standard colour of gelatin in accordance to SNI 06-3735-1995 is colourless or pale yellowish. Colors on gelatin can be influenced by raw materials as well as the manufacturing process. Gelatin in this study was obtained from natural collagen hydrolysis found in lizardfish scale. The colour of gelatin was strongly influenced by lizardfish collagen which is the source of the gelatin (Wangtueai & Noomhorm, 2009).

Figure 1 shows the lightness value of gelatin colour proportional to concetration of NaCl. This is probably because the addition of NaCl helps in binding the minerals in the scales. The more minerals removal from the scales, the lighter the colour of gelatin. This result is supported by Silva et al. (2014) which shows a similar relationship between degree of whiteness and ash content of gelatin of *Rachycentron canadum* fish.

Acidity is one of the parameters in quality standard of gelatin. In this study, gelatin was processed using NaOH hence the obtained gelatin can be categorized as type B. The pH standard for this type of gelatin is 5-7.5 (GMIA, 2012). The pH value of gelatin was closely related to the presence of acids or bases absorbed in the gelatin, in which the presence of acids or bases trapped in gelatin affect the viscosity or gel strength (Chancharern et al., 2016).

Figure 1 shows the highest pH value was shown by the sample without the addition of NaCl (6.8) and the lowest pH was at 0.5% NaCl (6.1). The pH value of all samples tends to be neutral because, after the demineralization process, the extraction process of gelatin was conducted at neutral pH (pH 6-7). When the extraction was carried out on the base condition, the peptide bond in collagen was degraded easier resulting in a shortening of the peptide chain (Weng et al., 2014). At strong acidic pH values (pH 3) or too high (pH 9) the viscosity and gelatin gel strength become lower. The peptide bonds in collagen is degraded easier under acidic conditions or bases conditions causing the shortening of the peptide chain. The shortening chain decreases viscosity and gel strength (Weng et

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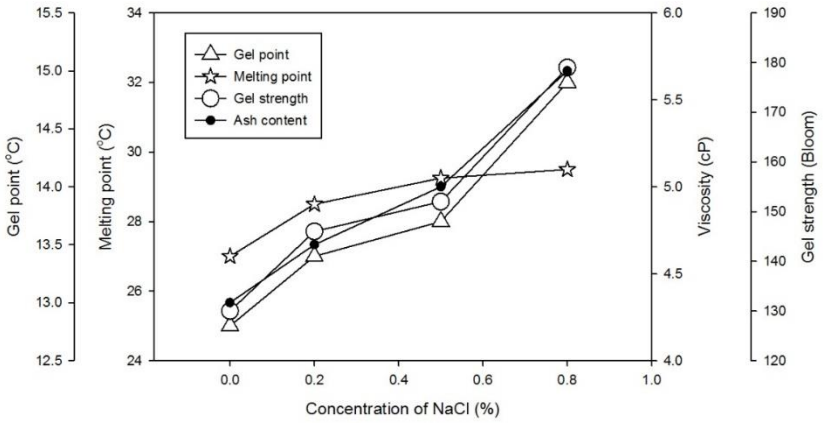

Figure 2. The effects of NaCl concentration of demineralization on gel point, melting point, viscosity and gel strength of gelatin.

al., 2014, Chancharern et al., 2016). In this study, the extraction was carried out using aquadest at 80° C to keep the peptide chain in the length and obtain high gelatin convert.

Melting point, gel point, and gel strength are among the important physical properties of gelatin. Gelatin as a thermoreversible gel will melt when the temperature increases at a certain point which known as the melting point. While the temperature at which gelatin begins to form gel is known as the gel point (Karim and Bath, 2009). Gel strength is an important feature of gelatin which shows the ability to convert liquid into solids or turn the solution into a reversible gel. The effect of the addition of NaCl to the physical properties of gelatin presented in Figure 2.

The increasing of NaCl concentration gave a positive effect on all the physical properties. Processing the gelatin with NaCl is expected to expose the structures of collagen. This condition favors for extracted high molecular weight polymers. The high molecular weight gelatin melts at higher temperatures than the low molecular weight gelatin melting points. The increasing amount of high molecular weight gelatin causes the melting point and gel point increases. In addition, the melting point of gelatin is also affected by the pH, the magnitude of the gelatin molecule, the temperature of the measurement and the concentration of gelatin in the solution (Mariod & Fadul, 2013).

Figure 2 shows the effect of adding NaCl to gel strength. The addition of 0.8% NaCl provides the greatest gel strength of 248 bloom. While gelatin with the addition of 0% NaCl has the least gel strength of 130 bloom. The low of gel strength in gelatin without the addition of NaCl is probably due to the high impurities found in the scale. These cause shorter amino acid chain in the gelatin, hence less hydrogen bonds will form. This low gel strength lead to low gel point (Bower et al., 2006). This condition supports the data of the lowest gel point obtained on demineralized gelatin without NaCl.

The standard for gelatin gel strength is 50-300 bloom (GMIA, 2001). It indicates the hardness, stiffness, robustness and gel compressibility at a certain temperature. The strength of gelatin gel is influenced by the amino acid condition of its constituents, especially the length of amino acid chain (Hasan, 2007). This property also associates with proline and hydroxyproline amino acids (Nhari et al., 2012). Hydroxyproline has a major role in stabilizing collagen triple helix through hydrogen bonding of its hydroxyl groups (Jridi et al., 2014). The high proportions of proline and hydroxyproline affect high affinity gelatin toward water. As a result gelatin is incapable to form helical coils such as usually found in proteins in general (Hasan, 2007).

The increasing gel strength with the addition of NaCl could be related to differences in the molecular weight distribution. NaCl contributes on the formation of high molecular weight polymers which having better stability interactions in forming the more triple helix structures. As a result of the gel strength also increases with increasing NaCl concentration (Gimenez et al., 2005, Du et al., 2013).

The viscosity of a fluid is an inhibitory force caused by friction between fluid molecules to resist the fluid movement. The standard of gelatin viscosity is range of 1.5 - 7.5 cP (GMIA, 2001).

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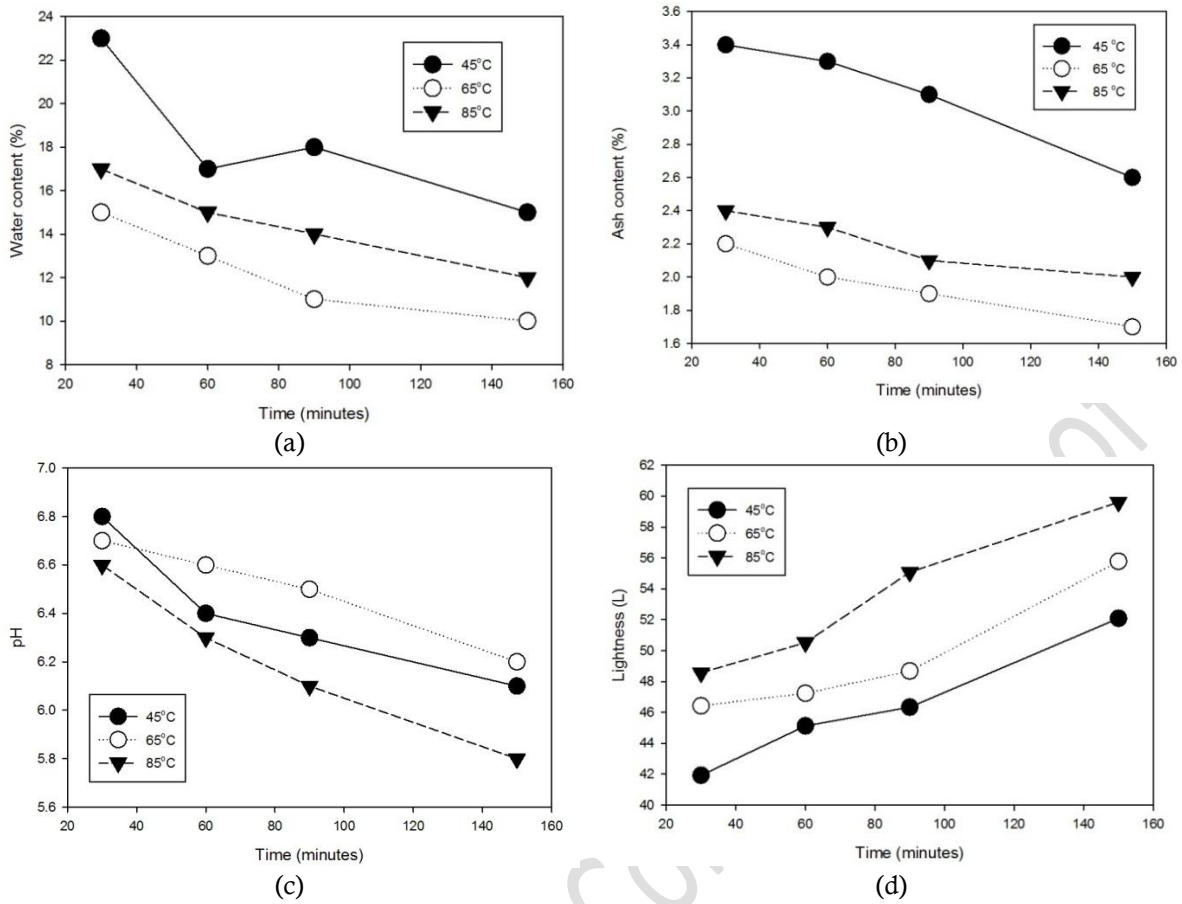

Figure 3. Effect of time and temperature of demineralization on (a) moisture content, (b) ash content, (c) pH and (d) Lightness (L)

Figure 2 shows the addition of 0% NaCl having the lowest viscosity of 4.6 cP, while the highest viscosity (5.1 cP) obtain in the addition of 0.8% NaCl. The viscosity of gelatin is influenced by many factors including amino acid composition, molecular weight and its distribution (Jakhar et al., 2012). The low viscosity of demineralization without the addition of NaCl could be related to the high ash content as shown in Figure 1. The presence of minerals, especially those associated with the reactive groups such as OH, COOH, and NH_2 groups have significant influence in gel characteristics, such as gel strength, melting point, and viscosity. Moreover, the low viscosity value could also influenced by the distribution of gelatin molecules in the solution as well as the weight of the gelatin molecule. When the molecule of gelatin binds to the minerals, it causes the less of the molecule bind with the solvent. This condition results in the lower viscosity (Haris, 2008).

Effect of Temperature and Time Demineralization

From the results obtained in Section III.1, a 0.8% NaCl concentration was used to study the effect of temperature and demineralization time on water content, ash content, pH, and white degree, as shown in Figure 3. In general, time affected pH, moisture content and ash content negatively, but reverse trend was observed for lightness.

Water content is an important parameter of a food product due to closely related to the shelf life. In addition, low water content keeps the gelatin become non-sticky (Widyasari & Rawdkuen, 2014). The standard gelatin of water content set by FAO is 18%. Figure 3a describes that the increasing temperature and time decrease the water content. Since the optimum condition of demineralization was at 65°C, a further increase in demineralization temperature would increase water content. The lowest water content (11%) was obtained at 65°C

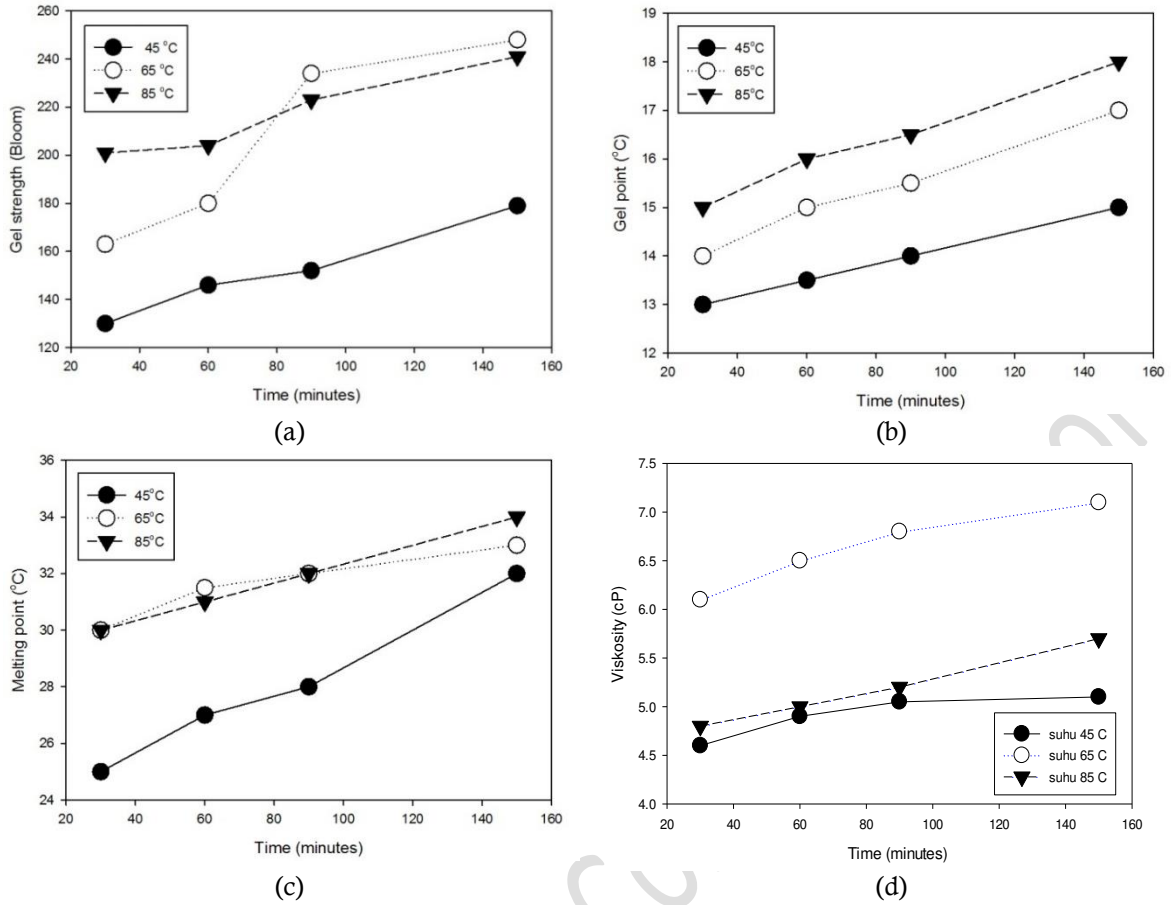

Figure 4. Effect of time and temperature of demineralization on (a) gel strength, (b) gel point, (c) melting point, and (d) viscosity

for 90 minutes. The high moisture content of gelatin reduces gel strength, viscosity, gel point and gelatin melting point although the decrease in physical properties is insignificant (Haris, 2008).

Meanwhile, demineralized gelatin at 65°C for at least 90 minutes resulted in the gelatin which meets the standard of ash content (<2%). The longer demineralization time allows the more solvent enter the collagen matrix. This affect to more release of the non-collagen material trapped within the matrix (Alhana, 2015). Temperature has an influence in accelerating the demineralization process which is marked by the decreasing amount of ash content observed. When the demineralization is carried out at higher than its optimum temperature, less amount of the ash successfully removed. This is probably due to hydrolization of protein at high-temperature of demineralization (Chuaychan et al., 2016) and undergo a change in shape. Although the more water enters the matrix but the trapped impurity in the matrix is still high. As a result, high water content and ash content in high-temperature demineralization was observed.

Figure 3b shows the optimum temperature in this demineralization process is 65°C. The demineralization process performed at 65°C using 0.5% NaOH and 0.8% NaCl resulted in gelatin with the ash content of 1.7%. Wangtueai and Noomhorm (2009) reported ash content of gelatin from lizardfish scales is 2.33% in demineralization using only NaOH. This indicates that the use of NaCl in combination with NaOH in the demineralization process as performed in this study, could help to bind mineral content in the demineralization process and led to lower the ash content.

The pH of the gelatin solution affects other properties such as viscosity, solubility and gel strength, which will ultimately affect gelatin applications (Bower et al., 2006). Gelatin with a neutral pH will be stable, and its application is wider. Figure 3c shows that the pH value ranges from 5.8 to 6.8, meaning that the gelatin with various treatments in this study meets the GMIA standard that is 5-7.5. Temperature and time of demineralization were less significant in affecting the resultant pH value of gelatin due to washing

process which carried out after the demineralization process. The gelatin is washed first until the pH becomes neutral to remove the mineral content and then extraction process was conducted at neutral pH (Weng et al., 2014). Neutralization affects the final pH of collagen because the process reduced acid or base residues after immersion. A success neutralizing process results in a final pH close to neutral pH (Alhana et at., 2015). Taheri et al. (2009) reported the type and concentration of compounds used in the extraction procedure also influenced the final result of pH gelatin.

The standard colour of gelatin in accordance to SNI 06.03735.1995 is colourless. Figure 3d shows the value of L representing lightness which proportional to the rise in temperature and time. This occurs probably because the higher the temperature and the longer the demineralization time, the more impurities in the collagen were successfully removed. The lightness of gelatin colour depends on the impurities, especially the metal ions which emit the light. The presence of these impurities affect the colour of gelatin produced. Also, the colour of gelatin is also influenced by the raw materials, isolation method and the number of the extraction stages (Hasan, 2007).

Gelatin is a type of physical gel which has a physical interaction between chains through van der Waal interactions and hydrogen bonds in nature. However, since the bonding energy in gelatin is relatively weak then gelatin forms as a thermo reversible gel (Gilsenan & Ross-Murphy, 2000). This impacts on the characteristics of gelatin produced. The strength of the gel, gel point, melting point and viscosity are important characteristics that are taken into account in the standard of gelatin. Figure 4 shows demineralization performed up to 150 minutes tended to increase melting point, gel point, and gelatin viscosity. Increasing demineralization temperature improves the physical characteristics of gelatin. However, demineralization at 85°C decreased viscosity. The gel strength decreased at high temperature and more than 90 minutes demineralization.

The gel strength obtained in this study was in the range of 130-248 bloom with the highest gelatin gel strength obtained at demineralization at 65°C for 150 min. The same gel strength trends are reported by Hanjabam et al. (2015) on the gelatin extracted from the Unicorn Leatherjacket fish scales. Based on its gel strength, gelatin is differentiated into gelatin with low gel strength (<150), moderate (150-220) and high (220-300) (Rosli & Sarbon, 2015). This means that the gelatin strength value obtained from this study meets commercial gelatin standards, although the preferred gel strength is 250-260 Bloom (Hanjabam et al., 2015). With this result, the gelatin obtained in this study was a moderate-high gel strength.

However, the strength of this gel is still lower than the gel strength of lizardfish scale reported by Wangtueai & Noomhorm (2009), which has 268 Bloom of gel strength. This difference could be due to differences in amino acid content. Proline and hydroxyproline are the amino acids responsible for the stability of the triple-helix collagen structure as a result of hydrogen bonding between water-free molecules and hydroxyl groups of hydroxyproline in gelatin. Low hydroxyproline content will cause in lower gel strength (Rosli & Sarbon, 2015). This high gel strength also contributes to the high melting point and gel point value (Rosli & Sarbon, 2015). This result is in accordance with the study of Hanjabam et al. (2015) which reported an increased gel strength followed by an increase in melting point.

The combination of long demineralization time and high temperature leads to decreased viscosity. High temperature could further hydrolysis of collagen and breaks the chain of amino acids into smaller units which decrease the viscosity (Wulandari et al., 2013). According to Montero and Gomez-Guillen (2000), the treatment of proteins at high temperatures causes the denatured protein to lose its triple helical structure which forms gelatin. Gelatin of *Megrim* has the highest viscosity at 60°C of demineralization temperature. The process of demineralization at high temperatures causes a decrease in the number of inter-chain covalent resulting in low viscosity (Montero & Gomez-Guillen, 2000).

In general, high viscosity values result in higher melting rates and gel formation than low viscosity gelatin. The formation of gel and its melting is influenced by the amount of hydrogen bond that is formed. Gelatin with a small amount of hydrogen bond form a gel at a lower temperature. Amiruldin (2007) reported the gel point gelatin of tuna scales is influenced by the amount of amino acid. The gel point is lower if the number of amino acids is lower which affect to less hydrogen bonding in gelatin. Furthermore, Montero and Gomez-Guillen (2000) reported the type and composition

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of amino acids also affect the physical characteristics of gelatin.

CONCLUSION

Demineralization of lizardfish scales using a combination of NaOH and NaCl (0-0.8%) decreased ash content, pH and water content of the gelatin. The use of NaCl increased the lightness of gelatin. The physical properties of gelatin increased with the addition of NaCl. The longer demineralization period decreased the ash, pH and moisture content. The opposite effect was observed in lightness and the physical properties of gelatin. Long-term demineralization at high temperatures led to a decrease gelatin quality. The highest gel strength was obtained in the demineralization treatment using NaOH combined with 0.8% NaCl for 150 min at 65°C. In this condition, water content, ash content, gel strength and gelatin viscosity were 10%, 1.7%, 248 bloom, and 7,1 which fulfilled SNI 06.3735.1995.

REFERENCES

Ahmad, M., Benjakul, S. 2011. Characteristics of gelatin from the skin of unicorn leatherjacket (Aluterus monoceros) as influenced by acid pretreatment and extraction time. Food Hydrocolloids. 25(3) : 381-388.

Akagündüz, Y., Mosquera, M., Giménez, B., Alemán, A., Montero, P., Gómez-Guillén, M.C. 2014. Sea bream bones and scales as a source of gelatin and ACE inhibitory peptides. LWT-Food Science and Technology. 55(2) : 579-585.

Alhana, A., Suptijah, P., Tarman, K. 2015. Extraction and characterization of collagen from sea cucumber flesh. Jurnal Pengolahan Hasil Perikanan Indonesia. 18(2).

Amiruldin, M. 2007. Preparation of gelation and characteristic determination of gelation from tuna fish (*Thunnus albacares*). [SKRIPSI] IPB , Bogor.

AOAC. 2005. Latimer JW, Horwitz W, editors. Official methods of analysis

Aquilina, A., Müller, D., Farrugia, C., Sinagra, E. 2004. The Effect of Sodium Chloride on Type-Based Differences in Gelatin Desolvation Behaviour. International Meeting on Pharmaceutics, Biopharmaceutics and Pharmaceutical Technology, Nuremberg, Jerman. http://staff.um.edu.mt/cfar2/Publications/Abstract11.pdf access on 5 September 2016

Ardekani, V.S., Mahmoodani, F., See, S.F., Yusop, S.M., Babji, A.S. 2013. Processing optimization and characterization of gelatin from catfish (*Clarias gariepinus*) skin. Sains Malaysiana. 42(12):1697-1705.

Badii, F., Howell, N.K. 2006. Fish gelatin: structure, gelling properties and interaction with egg albumen proteins. Food Hydrocolloids. 20(5) : 630-640.

Bower C.K., Avena-Bustillos R.J., Olsen C.W., Mchugh T.H., and Bechtel P.J. (2006), *Characterization of fish-skin gelatin gels and films containing the antimicrobial enzyme lysozyme*. J Food Sci. ;71:141–145.

Chancharern, P., Laohakunjit, N., Kerdchoechuen, O., Thumthanaruk, B. 2016. Extraction of type A and type B gelatin from jellyfish *(Lobonema smithii).* International Food Research Journal. 23(1) : 419-424.

Chuaychan, S., Benjakul, S., Nuthong, P. 2016. Element distribution and morphology of spotted golden goatfish fish scales as affected by demineralisation. Food chemistry. 197 : 814-820.

Da Trindade Alfaro, A., Fonseca, G.G. and Prentice-Hernández, C. 2013. Enhancement of functional properties of wami tilapia *(Oreochromis urolepis hornorum)* skin gelatin at different pH values. Food and Bioprocess Technology. 6(8) : 2118-2127.

Directorat General. 2013. Capture fisheries statistics of Indonesia 2007-2012. Ditjen Perikanan Tangkap. Kementerian Kelautan dan Perikanan Republik Indonesia

Du, L., Khiari, Z., Pietrasik, Z., Betti, M. 2013 Physicochemical and functional properties of gelatins extracted from turkey and chicken heads. Poultry Science. 92 : 2463-2474.

Giménez, B., Turnay, J., Lizarbe, M.A., Montero, P., Gómez-Guillén, M.C. 2005. Use of lactic acid for extraction of fish skin gelatin. Food hydrocolloids. 19(6) : 941-950.

IPB Document Corrected Proof

Gilsenan, P.M., Ross-Murphy, S.B. 2000. Viscoelasticity of thermoreversible gelatin gels from mammalian and piscine collagens. Journal of Rheology. 44(4) : 871-883.

GMIA, G.H. 2012. Gelatin Manufacturers Institute of America. New York.

Hanjabam, M.D., Kannaiyan, S.K., Kamei, G., Jakhar, J.K., Chouksey, M.K., Gudipati, V. 2015. Optimisation of gelatin extraction from Unicorn leatherjacket *(Aluterus monoceros)* skin waste: response surface approach. Journal of food science and technology. 52(2) : 976-983.

Haris, M. A. 2008. Uutilization of waste of fish bone of Nila *(oreochromis niloticus)* as gelatin and the effect of storage period at room temperature. [SKRIPSI] IPB , Bogor.

Harriyati, P. 2006. Analysis food safety policy of farming product at Pantura at Central Java and DIY [Dissertation] Universitas Diponegoro. Semarang.

Hasan. 2007. Study of extraction on gelation type B of cow skin. [SKRIPSI] . IPB. Bogor.

Jakhar, J.K., Pal, A.K., Reddy, D.A., Sahu, N.P., Venkateshwarlu, G., Vardia, H.K. 2012. Fatty acids composition of some selected Indian fishes. African Journal of Basic Applied Science. 4 : 155-160.

Jamilah, B., Tan, K.W., Hartina, M.U., Azizah, A. 2011. Gelatins from three cultured freshwater fish skins obtained by liming process. Food hydrocolloids. 25(5) : 1256-1260.

Joint FAO/WHO Expert Committee on Food Additives (JECFA). 2003. Summary and conclusions of the sixty-first meeting on food additives. Rome. June 10–19, 2003. www.who.int/pcs/jecfa/jecfa.htm. Accessed February 23, 2014.

Jridi, M., Hajji, S., Ayed, H.B., Lassoued, I., Mbarek, A., Kammoun, M., Souissi, N., Nasri, M. 2014. Physical, structural, antioxidant and antimicrobial properties of gelatin–chitosan composite edible films. International journal of biological macromolecules. 67 : 373-379.

Karim, A.A., Bhat, R. 2009. Fish gelatin: properties, challenges, and prospects as an alternative to mammalian gelatins. Food hydrocolloids. 23(3):563-576.

Mariod, A.A., Fadul, H. 2013. Gelatin, source, extraction and industrial applications. Acta Scientiarum Polonorum Technologia Alimentaria. 12(2):135-147.

Montero, P., Gómez-Guillén, M.C. 2000. Extracting conditions for megrim *(Lepidorhombus boscii)* skin collagen affect functional properties of the resulting gelatin. Journal of Food Science. 65(3):434-438.

Nhari, R.M.H.R., Ismail, A., Man, C., Yaakob, B. 2012. Analytical methods for gelatin differentiation from bovine and porcine origins and food products. Journal of food science. 77(1).

Rhim, J.W., Wu, Y., Weller, C.L., Schnepf, M. 1999. Physical characteristics of a composite film of soy protein isolate and propyleneglycol alginate. Journal of food science. 64(1):149-152.

Rosli, N., Sarbon, N.M. 2015. Physicochemical and structural properties of Asian Swamp Eel *(Monopterus albus)* skin gelatin as compared to bovine gelatin. International Food Research Journal. 22(2).

Sabbaghpour, S., Motamedzadegan, A. 2014. Investing the effects of preprocessing conditions on qualitative characteristics of skin gelatin of the Iranian Beluga. European Online Journal of Natural and Social Sciences. 3(3):758.

Sae-Leaw, T., Benjakul, S. 2015. Physico-chemical properties and fishy odour of gelatin from seabass *(Lates calcarifer)* skin stored in ice. Food Bioscience. 10:59-68.

Sanaei, A.V., Mahmoodani, F., See, S.F., Yusop, S.M., Babji, A.S. 2013. Optimization of gelatin extraction and physico-chemical properties of catfish *(Clarias gariepinus)* bone gelatin. International Food Research Journal. 20(1).

Sha, X.M., Tu, Z.C., Liu, W., Wang, H., Shi, Y., Huang, T., Man, Z.Z. 2014. Effect of ammonium sulfate fractional precipitation on gel strength and characteristics of gelatin from bighead carp *(Hypophthalmichthys nobilis) scale*. Food Hydrocolloids. 36:173-180.

Silva, R.S., Bandeira, S.F., Pinto, L.A. 2014. Characteristics and chemical composition of skins gelatin from cobia *(Rachycentron*

JBAT Document Correction Proof

canadum). LWT-Food Science and Technology. 57(2):580-585.

SNI. 063735. 1995. Standard and determination of gelation. Dewan Standarisasi Mutu Pangan Jakarta

Standard, B. 1975. 757. Methods for sampling and testing gelatine (Physical and Chemical Methods). 3rd ed. London: British Standards Institution.

Suryaningrum, T.D., Utomo, B.S.B. 2002. Guidelines of determination of seaweed and its derivative products. Jakarta: Pusat Riset Pengolahan Produk dan Sosial Ekonomi Perikanan dan Kelautan.

Taheri, A., Kenari, A.M.A., Gildberg, A., Behnam, S. 2009. Extraction and physicochemical characterization of greater lizardfish *(Saurida tumbil)* skin and bone gelatin. Journal of Food Science. 74 (3):160-165

Wangtueai, S., Noomhorm, A. 2009. Processing optimization and characterization of gelatin from lizardfish *(Saurida spp.)* scales. LWT-Food Science and Technology. 42(4):825-834.

Weng, W., Tang, L., Wang, B., Chen, J., Su, W., Osako, K., Tanaka, M. 2014. Antioxidant properties of fractions isolated from blue shark *(Prionace glauca)* skin gelatin hydrolysates. Journal of Functional Foods. 11:342-351.

Widyasari, R., Rawdkuen, S. 2014. Extraction and characterization of gelatin from chicken feet by acid and ultrasound assisted extraction. Food Applied Bioscience Journal. 2(2):83-95.

Wulandari, Supriadi, A., Purwanto, B. 2013. The Effect of Defatting and Extraction Temerature on the Physical Poperties of Snakehead Fish Bone Gelatin *(Channa Striata)*. Fishtech journal. Indonesia. 2(1):38-45.

Zhang, H.Y., Tehrany, E.A., Kahn, C.J.F., Ponçot, M., Linder, M., Cleymand, F. 2012. Effects of nanoliposomes based on soya, rapeseed and fish lecithins on chitosan thin films designed for tissue engineering. Carbohydrate polymers. 88(2):618-627.

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