THE EFFECTS OF ANIMAL AGE AND ACETIC ACID CONCENTRATION ON PIGSKIN GELATIN CHARACTERISTICS

M. Sompie[1,2], S. Triatmojo[1], A. Pertiwiningrum[1] and Y. Pranoto[3]

[1]Faculty of Animal Science, Gadjah Mada University,
Jl. Fauna 3 Bulaksumur Yogyakarta 55281 - Indonesia
[2]Permanent Address: Faculty of Animal Science, Sam Ratulangi University,
Jl Kampus Kleak, Manado 95115 - Indonesia
[3]Faculty of Agricultural Technology, Gadjah Mada University
Jl. Flora Bulaksumur Yogyakarta 55281 - Indonesia
Corresponding E-mail: sompiemeity@yahoo.com

Received June 18, 2012; Accepted August 21, 2012

ABSTRAK

Penelitian ini bertujuan untuk mengetahui pengaruh umur potong ternak dan konsentrasi larutan asam asetat terhadap karakteristik gelatin kulit babi. Penelitian ini menggunakan Rancangan Acak Lengkap pola faktorial 3x3. Faktor pertama yaitu umur potong ternak babi (5, 7 dan 9 bulan), faktor kedua adalah konsentrasi larutan asam asetat (2, 4 dan 6%). Bubuk gelatin kulit babi yang dihasilkan dianalisis rendemen, kekuatan gel, viskositas, kadar protein, dan nilai pH. Hasil penelitian menunjukkan bahwa tidak ada interaksi antara umur potong dengan rendemen, kekuatan gel, viskositas, kadar protein dan pH, namun terdapat interaksi atara umur potong dan konsentrasi larutan asam asetat.dengan rendemen, kekuatan gel, viskositas, kadar protein dan pH gelatin kulit babi. Kesimpulan penelitian ini adalah gelatin kulit babi umur 5,7 dan 9 bulan dengan konsentrasi larutan asam asetat 2,4 dan 6% memiliki karakteristik yang baik dan hampir sama dengan gelatin komersial, tetapi produksi gelatin optimum adalah kombinasi gelatin kulit babi 7 bulan dan 2% asam asetat.

Kata kunci : gelatin, kulit, babi, umur potong, konsentrasi asam asetat

ABSTRACT

This research was aimed to study the influence of animal age and concentration of the acetic acid solution on physical and chemical properties of pigskin gelatin. The experiment used Completely Randomized Design (CRD) with two factors. The first factor was animal age consisted of 3 levels (5, 7 and 9 months). The second factor was concentration of acetic acid solution consisted of 3 levels (2, 4 and 6 percents). The result showed that animal age had significant effect (P<0.01) on the yields, gel strength, viscosity, protein content and pH value but interaction of animal age and concentration of acetic acid had no significant effect (P>0.05) on the yields, gel strength, viscosity, protein content and pH value. It was concluded that pigskin gelatin from ages of 5, 7 and 9 months and acetic acid concentration of 2, 4 and 6% had similar characteristics to the commercial gelatin, but the optimum production of gelatin was combination of pigskin gelatin from 7 months and of 2% acetic acid.

Keywords: pig, skin gelatin, animal age, acetic acid concentration

INTRODUCTION

Gelatin is a denaturalized protein derived from collagen and is important functional biopolymer that has a very broad application in many industrial fields. The quality of gelatin depends on its physichochemical properties, rheological properties and manufacturing method. Gelatin has been applied within the food, pharmaceutical, medical, cosmetic and photographic industries because of its unique functional (Shakila *et al.*, 2012; Nunez-Flores *et al.*, 2012; Karim and Bath, 2008; Shuxian *et al.*, 2008; Jamilah and Harvinder, 2002). Gelatin is a protein of animal origin, that can be obtained from collagen by acidic or alkaline hydrolysis. Its functional properties depend on processing conditions as well as the raw material (Sobral and

Habitante, 2001). The use of gelatin to form edible films or coatings were very well studied in the 1960's, which resulted in many patents being filed, mainly in the pharmaceutical area (Antoniewski *et al.*, 2007; Park *et al*., 2008)

Research to compare different sources of gelatin as potential materials for the manufacture of biodegradable or edible films have been carried out previously (Chiou *et al.*, 2008; Gómez-Estaca *et al.*, 2009; Sobral *et al.*, 2001) but to our knowledge there is no published research comparing the physical and mechanical properties of films manufactured from the three primary commercial types of gelatin available on the market today (beef skin, pork skin and fish skin gelatins). Most commercial gelatins are currently sourced from beef bone and hide, different species of fish (Nunez-Flores *et al*., 2012; Gomez-Estaca *et al*., 2011). Recently, scientists has been doing many research of gelatin from goat skin (Said *et al.*, 2011b), from chicken legs skin (Ulfa et al., 2011), from pig skin (Sobral *et al*., 2001), and gelatin from tilapia fish skin (Pranoto *et al*., 2006).

The process of gelatin production required a curing step to improve quality of gelatin (Said *et al.*, 2011a). Curing materials from the group of acids have been widely applied in gelatin production, particularly from the skin and bones of fish (Gudmundsson, 1997; Jamilah and Harvinder, 2002; Kasankala *et al*., 2007). The application of the curing time and concentration of acetic acid has published, acetic acid 3.5 % had significant effect on physic properties of gelatin chicken legs skin (Ulfah, 2011). Lee *et al.* (2004) mixed pigskin gelatin with gellan to obtain composite film for packing or coating materials. However, information about further effects of animal age on the alkali process from pigskin had been limited. Thus, the research was aimed to study the effect of animal age and concentration of acetic acid on the characteristics of pigskin gelatin.

MATERIALS AND METHODS

Materials

Five hundred g pigskin were used as a raw material and acetic acid (CH_3COOH) as a curing material. Preparation of raw materials. were used as a treatment. The skin used for gelatine extraction were obtained from pig at 5, 7 and 9 months. The pigskin were weighed and washed in running water for 5 minutes and continued with the process of neutralization in a solution of HCOOH (pH~7). Pigskin without hair and meat attached was cut into small pieces (approximately 2x2 cm).

Preparation of gelatins

Gelatine was prepared by the acid extraction method (Ockerman and Hansen, 2000). Acetic acid (CH_3COOH 0.5M) concentrations of 2%, 4% and 6% (v/v) were used as a treatments. The raw material were soaked at different concentrations of acetic acid solution 2%, 4% and 6% in accordance with the treatment for 48 hours. After soaked they were neutralized to pH~6, weighed and extracted. The extraction process were performed on three steps (each step for 3 hours), the first step at 55^{o}C, second step at 60 ^{o}C and then at 65^{o}C. Solubilized gelatin was separated from residual skin fragments by filtration through a nylon filter. The extracted gelatin was concentrated at 70 ^{o}C for 5 hours and it was stored in the refrigerator 5-10^{o}C for 30 minutes, then dried at 60 ^{o}C for 24-36 hours until the gelatin sheet solid. Gelatin sheets were milled and packaged in vacuum plastic and stored in a desiccator for subsequent process.

Method of Analysis

Yield

The yield obtained from dry weight ratio of raw material and the weight of the extracted pigskin multiplied by 100% (AOAC, 2005).

Gel Strength

Gel strength was determined by a Universal Testing Machine (Zwick/Z.0,5). Gelatin solution 6.67% w/v (6.67 grams to 100 ml distilled water) was heated at 60^{o}C to dissolve the particles. Solution in the container Ø5 cm and height 6 cm was stored at 5^{o}C for 16-18 hours. Gelatin was placed at the bottom of the plunger (Ø=13mm). Measurement was conducted at the temperature of 10^{o}C and the speed 10 mm/min as deep as 4 mm was used as plunger. The value of gel strength (g Bloom) was calculated using the formula = 20 + 2,86 x 10^{-3}D, where D = F/G x 980; F = height chart before fracture; G = constant (0.07) (Muyonga *et al*., 2004; Liu *et al*, 2008., Said *et al.*, 2011b).

Viscosity

Viscosity was measured by gelatin powder dissolved in distilled water at a temperature of 40°C with a solution concentration of 6.67%. The values was measured by Stromer Viscosimeter Behlin CSR-10. It was obtained by expressed in centipoise according to the method of Gomez (Guillen-Guillen *et al*., 2002).

Protein Content

FOSS Kjeltec 2200 was used to determine protein content. A total of 0.5 g of sample + ¼ bussino tablet + 12 ml H_2SO_4 was concentrated in the destruction of the tube FOSS at ± 410^0C for 1 hour. The results of destruction was distilled with thio-NaOH 40% + H_3BO_4 4% + BCGMR indicators. A total of 150 ml was destilated in Erlenmeyer disk and titrated with 0.099 N HCl until the color changed from blue to pink. Conversion factor for gelatin protein was 5.55. The protein content (%) was calculated using the formula (ml HCL – ml Blanko) x N HCL x 14.0008 x 100 x 5.55)/g sample x 1000 (AOAC, 2005).

pH Determination

The pH of gelatin was determined using a pH meter with The British Standard Method in Yang *et al.* (2005) then the solution was measured by a Hanna Instrument 1270 pH electrode Scew type.

Experimental Design and Data Analysis

The experiment were determined by analysis of Completely Randomized Design (Steel and Torrie, 1991) with two factors. The first factor was animal age consisted of 3 levels (5, 7 and 9 months). The second factor was concentration of acetic acid solution consisted of 3 levels (2, 4 and 6 percents). The differences between treatment were determined using Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

Yield

The yield is amount of dry gelatin produced from a number of raw materials with extraction process (Said *et al*., 2011a). Yield of pigskin gelatin were 10,22 to 12.67% (Table 1). Statistical analysis showed that the level of animal age and concentration of acetic acid gave highly significant effect (P<0.01), whereas the interaction between the two factors was not significant (P>0.05) on the yield of pigskin gelatin. Duncan test results showed that the yield of gelatin from pig skin have increased with the increasing of pig age as well as the concentration of acetic acid. The yield of gelatin tended to increase. The highest yield of pigskin gelatin were 12.36%. Swatland (1984) reported that the protein content of collagen in the skin of animals affected by age, increasing age of the animal, collagen protein will also grow and get stronger collagen fibers. Furthermore, Cole and McGill (1988) reported that the yield of a cow skin gelatin increased with the increasing of cattleage. Several authors have reported different gelatin yield, from the bones of Nile perch was 6-11 % (Muyonga *et al*., 2004), the skin of fish that ranged from 4 to 16 % (Binsi *et al*., 2009), the skin of goat was 6.32% (Said *et al*., 2011a) and from the chicken leg skin was 15 % (Taufik, 2011).

Gel Strength

Gel strength of gelatin is very important on physical properties of gelatin. The average gel strength of pigskin gelatin is displayed in Table 2. Statistical analysis indicated that the level of animal age gave highly significant effect (P<0.01) while the level concentration of acetic acid and their interaction had no significant effect (P>0.05) on pigskin gelatin. The value of gel strength increase with the increasing of animal age. Gel strength values from pigskin gelatin ranged 134.77-143.12 g Bloom, that in line with the criteria of ISO 75-300 g Bloom (Said *et al.*, 2011b). Arnesen and Gildberg (2002) reported that a high content of hydroxyproline caused the gel strength increased. The presence of hydroxyproline caused the stability of the hydrogen bonds between water molecules and free hydroxyl groups of amino acids in gelatin, that is very important for gel strength. Furthermore, Sims *et al.* (1997) reported that the gel formation of a stable condition has a free chain to form a lot of crosslinking. Pigskin gelatin gel strength obtained from the treatment of the highest concentrations of acetic acid 2% and at the animal age of 9 months.

Viscosity

The average viscosity of pigskin gelatin is displayed in Table 3. Statistical analysis indicated that the level of animal age gave highly significant effect (P<0.01) while the level

Table 1. Yieldof Pigskin Gelatin (%)

Animal Age (months)	Concentration of Acetic Acid (%±SD)			Average
	2	4	6	
5	10.22±0.25	10.74±0.75	11,01±0,43	10.65±0.43a
7	10.46±0.42	11.59±0.09	12.29±0,18	11.45±0.83b
9	12.08±0.17	12.34± 0.30	12.67 ±0,10	12.36±0.31c
Average	10.92±0.92p	11.55±0.71q	11.99±0.79r	

a,b,c: Different superscript at the same column indicate significantly different (P<0.05)
p,q,r: Different superscript at the same row indicate significantly different (P<0.05)
SD: Standard Deviation

Table 2. Gel Gtrength of Pigskin Gelatin (g/Bloom)

Animal Age (months)	Concentration of Acetic Acid (%±SD)			Average
	2	4	6	
5	134.84±0.62	134.81±0.56	134.77±0.65	134.81±0.56a
7	138.16±0.40	136.02±0.02	137.09±0.81	137.09±1,03b
9	143.12±0.17	142.09 ±0.91	140.44±0.21	141.88±1.06c
Average	138.71±2.50	137.64±4.04	137.43±0.29	

Different superscript at the same column indicate significantly different (P<0.05)
SD: Standard Deviation

Table 3. Viscocity of Pigskin Gelatin (cP)

Animal Age (months)	Concentration of Acetic Acid (%±SD)			Average
	2	4	6	
5	6.47±0.38	6.41±0.21	6.52±0.13	6.47±0.23a
7	7.21±0.01	7.18±0.07	7.04±0.07	7.14±0.09b
9	7.22±0.19	7.16±0.06	7.08±0.14	7.15±0.13b
Average	6.97±0.42	6.91±0.39	6.88±0.29	

Different superscript at the same column indicate significantly different (P<0.05)
SD: Standard Deviation

concentration of acetic acid and their interaction had no significant effect (P>0.05) on pigskin gelatin. Table 3 shows that the concentration of acetic acid had no significant effect on the viscosity of the gelatin. The higher concentration of acetic acid caused the decreasing of viscosity. This is because the curing material has been broken the peptide bonds of amino acids into short-chain molecule so that its viscosity decrease. This is because the viscosity of gelatin is directly proportional to the gel strength that was not significantly different among the treatments (Astawan *et al*, 2002). Furthermore, Ulfah (2011) explained that viscosity is affected by molecular

Table 4. Protein Content of Pigskin Gelatin (%)

Animal Age (months)	Concentration of Acetic Acid (%±SD)			Average
	2	4	6	
5	89.70±0.57	89.18±0.07	88.93±0.57	89.27±0.53a
7	90.68±0.77	90.56±0.69	90.26±0.11	90.49±0.56b
9	91.22±0.16	91.05±0.32	90.23±0.15	90.83±0.50b
Average	90.53±0.82q	90.26±0.92q	89.79±0.71p	

a,b,c: Different superscript at the same column indicate significantly different (P<0.05)
p,q : Different superscript at the same row indicate significantly different (P<0.05)
SD: Standard Deviation

Table 5. pH Value of Pigskin Gelatin

Animal age (months)	Concentration of Acetic Acid (%±SD)			Average
	2	4	6	
5	5.41±0.14	5.25±0.04	5.19±0.09	5.27±0.04a
7	5.36±0.15	5.21±0.13	5.03±0.14	5.20±0.13ab
9	5.18±0.13	5.28± 0.07	5.20±0.09	5.22±0.13a
Average	5.32±0.13r	5.25±0.08q	5.14±0.09p	

a,b,c: Different superscript at the same column indicate significantly different (P<0.05)
p,q : Different superscript at the same row indicate significantly different (P<0.05)
SD: Standard Deviation

weight and amino acid chain length. Increased concentrations of acetic acid in the gelatin production process can reduce the viscosity. This is because the curing material has broken the peptide bonds of amino acids into short-chain molecule so that its viscosity decrease. Viscosity values of pigskin gelatin was ranged from 6.41 to 7.22 cP. These values were included in the ISO ranged from 2.0 to 7.5 cP (Ulfah *et al*., 2011).

Protein Content

Gelatin is the collagen protein, a group derived from the structural proteins and extracellular matrix and produced in large quantities (Karim and Bath, 2008). The average protein content of pigskin gelatin is presented in Table 4. Statistical analysis indicated that the differences in level of animal age had highly significant effect (P<0.01) on protein content of pigskin gelatin, whereas the concentration of CH_3COOH and the interaction between these two different factors had not significant effect (P> 0.05) on levels of protein gelatin. Duncan's test results showed that protein content of gelatin from pigskin had increased with the increasing of pig age. According to Swatland (1984), slaughtered age affected the content of collagen in the skin, and the increasing of age increased collagen protein. Protein content of pigskin gelatin ranged 89.18 to 91.22 %. It was not different from the commercial gelatin that had protein content of 91. 63% (Said *et al*., 2011b).

pH

The pH value of gelatin is very important on chemical properties because it can affect the properties of gelatin to determine the subsequent application of gelatin. The average pH value of pigskin gelatin was ranged between 5.03 to 5.41. Statistical analysis indicated that interaction between level of animal age and concentration of acetic acid had no significant effect (P>0.01), while every single factor had significant effect (P<0.05) on pH value of pigskin gelatin. Conditions in the range of neutral pH values indicated that the process of neutralizing and washing the raw material before the extraction process was running perfectly so that contamination can be minimized. Therefore, the neutralization process plays an important role to neutralize the remnants after acid or alkaline immersion. Furthermore, Hinterwaldner (1977) explained that acid tends to produce a low pH value of gelatin. The pH value of gelatin was produced is still in the pH range of normal by the standards of GMIA was 4.5 to 6.5 and not much different from the pH tilapia skin gelatin which is 5.0 (Stongchotikunpan *et al.*, 2007).

CONCLUSIONS

Pigskin gelatin from ages of 5, 7 and 9 months and curing concentration acetic acid 2, 4 and 6% extracted had similar characteristics to the commercial gelatin, but the optimum production was combination of pigskin gelatin from 7 months and 2% acetic acid.

REFERENCES

Antoniewski, M. N., S. A. Barringer, C. L. Knipe and H. N. Zerby. 2007. Effect of a gelatin coating on the shelf life of fresh meat. J. Food Sci. 72 : E382-387.

AOAC. 2005. Official Methods of Analyses Association (18th Ed), Official Analytical Chemists. Association Analytical Chemist, Washington, D.C.

Arnesen, J. A. and A. Gildberg. 2002. Preparation and characterization gelatin from the skin of Harp seal (Phoca groendlandica). J. Bioresource Techn. 82:191-194.

Astawan, M., P. Hariyadi and A. Mulyani. 2002. Analisis sifat reologi gelatin dari kulit ikan cucut. J. Teknologi dan Industri Pangan 13:38-46

Binsi, P. K., B. A. Shamasundar., A . O. Dileep., F. Badii and N. K. Howell. 2009. Rheological and functional properties of gelatin from the skin of bigeye snapper (*Priacanthus hamrur*) fish influence of gelatin on the gel-forming ability of fishmince. Food Hydrocolloids, 23(1):132 -145

Chiou, B, R. J., P.J. Avena-Bustillo., H. Bethel, R. Jafri, S.H. Narayan, G. Imam. and W. J. Orts. 2008. Cold water fish gelatin films: Effects of cross-linking on thermal, mechanical, barrier, and biodegradation properties. European Polimer J. 44:3748-3753.

Cole, C.G.B. and A.E.J. McGill. 1988. The properties of gelatins derived by alkali and enzimic conditioning of bovine hide from animals of various ages. J. Food Sci. Leather Technology Chemistry. 72: 159-164

Gomez-Estaca, J., A.L. de Lacey., M.E. Lopez-Caballeno., M.C. Golmez-Guillen and P. Montero. 2009. Antimicrobial activity of composite edible films based on fish gelatin and chitosan incorporated with clove essential oils. J. Aquatic Food Product Technology. 18 : 46 – 52

Gomes-Guillen, M.C., J. Turney., M.D. Fernandez Diaz., N. Ulmo., M.A. Lizarbe and P. Montero. 2002. Structural and physical properties of gelatin extracted from different marine species. Food Hydrocolloids. 16(1): 25-34

Gudmundsson, M. 2002. Rheological properties of fish gelatin. J. Food. Sci. 67(6):2172-2176.

Hinterwaldner, R. 1997. Raw material. In Ward, A.G and Court. Editors: The Science and Technology of Gelatin. Academic Press. New York.

Jamilah, E. and K.G. Harvinder. 2002. Properties gelatins from skins of fish-blacktilapia (Oreochrommmis mossambicus) and red tilapia (Oreochrommmis nilotica). Food Chem. 77 :81-84

Karim, A.A and R. Bhat. 2008. Fish gelatin: properties, challenges, and prospects as an alternative to mammalian gelatins. Food Hydrocolloids. 23(3): 563 - 576 .

Kasankala, L.M., Y. Xue, Y. Weilong, S.D. Hong and Q. He. 2007. Optimization of gelatin extraction from grass carp (*Catenopharyngodon idella*) fish skin by response surface methodology. Bioresource Tech. 98: 3338-3343

Lee. K.Y., J.Y. Shim and H. G Lee. 2004. Mechanical properties of gellan and gelatin composites film. Carbohydrate Polimer. 56(2) : 251-254.

Liu, H.Y., J. Han and S.D. Guo. 2008. Characteristics of the geltin extracted from channel catfilsh (*Ictalurus punctus*) head bones. Food Sci. and Tech. 43: 313 - 317

Muyonga, J. H., C.G.B. Cole. and K.G. Duodu. 2004. Extration and physic-chemical characterization of Nile perch (*Lates niloticus*) skin and bone gelatin. Food

Hydrocolloids. 18 : 581-592.
Ockerman, H. W and C.L. Hansen. 2000. Animal by product processing and utilization. CRC Press, USA.
Park, J.W., W.S. Whiteside and S.Y. Cho. 2008. Mechanical and water vapor barrier properties of extruded and heat-pressed gelatin films. LWT. 41 : 692-700.
Pranoto, Y., M.L. Chong and H. J. Park. 2006. Characterizations of fish gelatin films added with gellan and x-carrageenan. J. Food. Sci and Tech. 40 : 766-774.
Said, M.I., S. Triatmojo, Y. Erwanto and A. Fudholi. 2011a. Karakteristik gelatin kulit kambing yang diproduksi melalui proses asam basa. J. Agritech. 31(3) : 0216-0455.
Said, M.I., S. Triatmojo, Y. Erwanto and A. Fudholi. 2011b. Gelatin Properties of Goat Skin Produced by Calcium Hydroxide as Curing Material. Media Peternakan. 34(3): 184-189
Shakila, R.J., E. Jeevithan, A. Varatharajakumar, G. Jeyasekaran and D. Sukumar. 2012. Comparation of the properties of multi composite fish gelatin films with that of mammalian gelatin films. Food Chemistry 135 : 2260-2267.
Shuxian, H., L. Laihao, Y. Xianging, C. Jianwei, S. Honh, B. Qi and J. He. 2008. The characteristics of gelatin extracted from sturgeon (*Acipenser baeri*) skin using various pretreatments. Food Chem. 115 : 124-128.
Sims, T. J., A.J. Bailey and D.S. Field. 1997. The chemical basis of molecular weight differences in gelatins. The Imaging Sci. J. 45:171-177
Sobral, P.J.A. and A.M.Q.B. Habitante. 2001. Phase transitions of pigskin gelatin. Food Hidrocolloids. 15 : 377-382.
Sobral, P.J.A., F.C. Menegalli, M.D. Hubinger and M.A. Roques. 2001. Mechanical. Water vapor barrier and thermal properties of gelatin based edible film. Food Hidrocolloids. 15 : 423 – 432.
Stongchotikunpan, P., J. Tattiyakul and P. Supaphol. 2007. Extraction and electrospinning of gelatin from fish skin. J. Biological Macromolecules. 42: 247-255
Steel, R. G. D. and J. H. Torrie. 1991. Principles and Procedures of Statistics. McGraw-Hill Book Co. Inc. New York.
Swatland, H.J. 1984. Structure and development of meat animals. Prentice-Hall Inc, Englewood Cliffs, New Yersey.
Taufik, M. 2011. Kajian potensi kulit kaki ayam broiler sebagai bahan baku gelatin dan aplikasinya dalam edible film antibakteri. Disertation. Universitas Gadjah Mada.
Ulfah, M. 2011. Pengaruh konsentrasi larutan asam asetat dan lama waktu perendaman terhadap sifat-sifat gelatin ceker ayam. J. Agritech. 31(3):161-167.
Yang. H., Y. Wang, P. Zhou and J.M. Regenstein. 2008. Effects of alkaline and acid pretreatment on the physical properties and nanostructure of the gelatin from channel catfish skins. Food Hydrocolloids. 22:1541-1550