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EFFECT OF TEMPERATURE AND EXTRACTION TIME ON THE CHARACTERISTICS OF PIGSKIN GELATIN

Meity SOMPIE, Siswosubroto SURTIJONO, Meilani Rita TINANGON

Sam Ratulangi University, Jl Kampus Unsrat Kleak Manado 95115, Manado, Indonesia

Corresponding author email: meitysompie@yahoo.com

Abstract

The quality of gelatin depends on its physichochemical properties and manufacturing method. The process of gelatin required the extraction step to improve the quality of gelatin. The aimed of this study was to research the effect of temperature extraction and extraction time on the characteristics of gelatin produced from pigskin. This study used Completely Randomized Design (CRD) with two factors 3x3 and three replicates of treatments. The first factor was temperature extraction with 3 levels (50^oC, 60^oC and 70^oC). The second factor was extraction time consisting of 3 levels (3 hours, 6 hours and 9 hours). The result showed that the interaction of the extraction temperature and extraction time had significant effect ($P<0.05$) to content of protein, gel strength, yield and viscocity from gelatin of pigskin. The highest amino acid content was glycine. It was concluded that the use of extraction temperature 60^0C and time of extraction 6 hours was the best characteristics of pigskin gelatin.

Key words*: extraction, gelatin, pigskin, temperature.*

INTRODUCTION

Gelatin is a denaturalized protein that is derived from collagen and is an important functional biopolymer that has a very broad application in many industrial fields (Cho et al., 2004). Gelatin is a protein of animal origin, that can be obtained from collagen by acidic or alkaline hydrolysis (Said et al., 2011; Ward, 1977). The typical characteristics collagen protein include containing at least 33% amino acid glycine and 22% proline (Karim and Bath, 2008). The functional properties of gelatin are divided into two types. The first properties relate to gel formation processes (gel strength, gel formation time, melting temperature, viscosity, thickness, texture and water content) and the second nature is related to the properties of the gelatin surface shape and stabilization of emulsions, colloid protection, foam stability, film shape andadhesion cohesion. Gel strength is a very important physical property of gelatin, depending on the hydrogen bonds between water molecules and the free hydroxyl group of amino acid groups, the size of the protein chain, concentration and molecular weight distribution of gelatin (Said et al., 2011). Its functional properties depend on processing conditions as well as the raw material (Sobral and Habitante, 2001). Animal age also affect of the protein content of skin collagen, increasing the animal age, protein collagen and fibrous collagen growing stronger (Krochta et al., 1997; Ockerman and Hansen, 2000; Swatland, 1984). Gelatin production required a extraction step to improve quality of gelatine (Sompie et al., 2015). Good quality of gelatin is formed with a low extraction temperature, because at this temperature less hydrolysis of polypeptide chains occurs (Phillips and Williams, 2000). Furthermore, it was stated that at a temperature of 50^oC the extraction process can separate the structure of collagen, changing helical shape and hydrolyzed some covalent bonds. High quality gelatin is obtained from low extraction temperatures, but high extraction temperatures will increase yield (Ockerman and Hansen 2000). Yield produced from a gelatin is strongly influenced by the extraction process on collagen protein (Kasankala et al., 2007, Zhou and Joe, 2005).

The effect of extraction time to produce gelatin from pigskin was limited information. Thus, this research was conducted to study the effect of combination between temperature and extraction time on characteristics of pigskin gelatin.

MATERIALS AND METHODS

Materials: Three thousand grams of pigskin were used as a raw material, acetic acid solution and stilled water.

Procedures: Gelatine was prepared by the acid extraction method (Ockerman and Hansen, 2000). Acetic acid (CH_3COOH 0.5M) then diluted again with water in of 2%, 4% and 6% (v/v) were used as a treatments. The pigskin was soaked on acetic acid solution 24 hours. After soaked, samples were neutralized to pH 6, weighed and extracted on water bath for 3 hours, 6 hours and 9 hours at 50^0C, 60^0C and 70^0C. Solubilised gelatin was separated from residual skin fragments by filtration through a nylon filter. The extracted gelatin was concentrated at 70^0C for 6 hours and it was stored in the refrigerator 5^0C for 30 minutes, then dried at 60^0C for 24-36 hours until the solution dries and forms a gelatin sheet. Gelatin sheets were milled and packaged in vacuum plastic and stored in a desiccator for subsequent process.

The experiment were determined by analysis of Completely Randomized Design (Steel and Torrie, 1991) with two factors and three replicates of treatments. The first factor was temperaturextraction (50, 60 and 70 degrees Celsius). The second factor was time of extraction consisting of 3 levels (3, 6 and 9 hours). The significant differences of the average were determined using Duncan's new multiple range test.

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

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 Gomez (Sompie et al., 2012).

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 distillated in Erlenmeyer disk and titrated with 0,099 N HCl until the color changed from blue to pink. Five point fifty five was used as the conversion factor of gelatin protein.

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.

Amino acid analysis (sample preparation):

1. Weigh the sample 60 mg
2. Add 4 ml of HCl 6 N
3. Reflux for 24 hours with a temperature of 120^oC
4. Cool to room temperature
5. Neutralize with NaOH 6 N (PH.7) volume of 10 ml
6. Filter with Wattman 0.2
7. Take 50 filtered samples
8. Add an OPA solution of 300 ul
9. Take 20 ulsamples with the syringe into the HPLC

The tool used is LC 10 SHIMADZU HPLC Column: LiChrospher 100 RP-C18 (5 um) Eluent A: 50 mM Natrium Acetate: THF: Methanol (96:2:2) Eluent B: 65% Methanol.

RESULTS AND DISCUSSIONS

Protein Content

Gelatin is a source of protein derived from large amounts of collagen and is a group of structural proteins originating from the extracellular matrix (Hidaka and Liu, 2002; Karim and Bhat, 2008).

Statistical analysis on Table 1 indicated that the interaction between temperature and time of extraction had highly significant effect ($P<0.01$) on the protein content of pigskin gelatin. Duncan test results showed that protein

content of gelatin from pigskin had a tended to increase with increasing temperature of extraction.The increase in levels of gelatin protein is related to changes in the amount of amino acid bonding structures that make up collagen proteins. According to Swatland (1984), age slaughter affects the content of collagen in the skin, increasing age increased collagen protein. Protein content ranged 86.14 to 90.24%. That it was not different with protein content from chicken leg skin ranged 83-90 % and commercial gelatin, 91, 63% (Said et al., 2011).

Table 1. The characteristics of pigskin gelatin

Parameters	Extraction time (hours)	extraction temperature (ºC) + Sd		
		50	60	70
Protein	3	89.04±0.57	90.24±0.16	90.04±0.07
content	6	88.50±0.27	88.30±0.77	88.03±0.47
(%)	9	88.16±0.03	87.14±0.63	86.14±0.23
Gel	3	78.10±0.62	78.30±0.22	79.91±0.12
strength	6	77.08±0.20	78.38±0.21	78.84±0.20
(g bloom)	9	77.20±0.02	78.20±0.32	78.24±0.02
Yield	3	12.87±0.21	13.67±0.12	13.85±0.37
(%)	6	12.06±0.13	13.27±0.27	14.53±0.17
	9	12.17±0.05	13 .07±0.32	14.87±0.17
Viscocity	3	8.21±0.01	9.24±0.01	9.27±0.01
(cP)	6	8.16±0.16	8.17±0.04	9.18±0.06
	9	8.93±0.07	8.06±0.07	8.86±0.07

Gel Strength

One of the functional properties of gelatin is gel strength (Bergo and Sobral, 2007; Schrieber and Gareis, 2007). The average of gel strength from pigskin gelatin is displayed in Table 1. Statistical analysis indicated the interaction between temperature and time of extraction had significant effect (P<0.05) on pigskin gelatin. The value of gel strength tended to increase with increasing level of extraction temperature. Gel formation occurs due to the development of gelatin molecules during the extraction process. The presence of hydroxyproline caused the stability of the hydrogen bonds between water molecules and free hydroxyl groups of amino acids in gelatin, it is very important for gel strength (Kolodziejska et al., 2003; Wang et al., 2008).The amino acid monomer chains from one another to the next combine to form a continuous three-dimensional structure and bind water to form a compact gel structure (Said et al.,2011). Gel strength values from pigskin gelatin were ranged 77.08 – 79.91 g Bloom, that in line with the criteria of ISO 75-300 g Bloom (Sompie et al., 2014). Bloom is the value used to determine the quality of gelatin.

Yield

Yield is the amount of dry gelatin produced from a number of skin raw materials in a clean state through an extraction process (Giménez et al., 2005).Statistical analysis showed that the interaction between temperature and time of extraction gave highly significant effect (P<0.01) on the yield of pigskin gelatin. Duncan test results showed that the yield of gelatin from pigskin have increase with increasing temperature and time of extraction. The yield of gelatin tended to increase. The higher yield of pigskin gelatin was 14.53%.The higher of yield value indicates that the production process becomes more efficient.

Viscosity

The average viscosity of pigskin gelatin is displayed in Table 1. Statistical analysis indicated that the interaction between temperature and time of extraction had significant effect (P<0.05) on pigskin gelatin. The value of viscosity tended to decrease at the time of extraction acid increased. In order words, the higher extraction time, the viscosity was tended to decrease. This is because the viscosity of gelatin is directly proportional to the gel strength that was not significantly different between treatments (Astawan et al 2002). Furthermore, Sompie et al. (2015) explained that viscosity is affected by molecular weight and the length of amino acid chain.Increased concentrations of acetic acid in the gelatin production process can reduce the viscosity The curing material has been breaking the peptide bonds of amino acids into short-chain molecule so that its viscosity decrease. Viscosity values from pigskin gelatin were ranged 8.06 to 9.24 cP. It values is included in the ISO range 2.0 to 9.5 cP.

Amino acid profile

Amino acids are organic compounds that have a carboxyl functional group (-COOH) and amine (-NH2) in amphoteric solutions (Giménezetal, 2005). The amino acid profile of

pigskin gelatin is shown on Table 2 and Table 3.

Table 2. Amino acid profile from pigskin gelatin (ppm)

No	Amino Acid	Pigskin Gelatin (ppm)
1	Aspartic	53.11
2	Glutamic	124.19
3	Serine	37.67
4	Hidroxyproline	42.91
5	Proline	41.10
6	Histidine	-
7	Glycine	123.81
8	Threonine	-
9	Arginine	72.24
10	Alanine	63.91
11	Tyrosine	-
12	Methionine	7.65
13	Valine	13.22
14	Phenylalanine	9.60
15	Isoleucine	-
16	Leucine	17.06
17	Lysine	21.77

Table 3. Amino acid profile from pigskin gelatin (%)

No	Aminoacid	Pigskin Gelatin (%)
1	Aspartic	5.75
2	Glutamic	10.95
3	Serine	5.21
4	Hidroxyproline	1.44
5	Proline	1.36
6	Histidine	-
7	Glycine	37.65
8	Threonine	-
9	Arginine	7.01
10	Alanine	13.05
11	Tyrosine	-
12	Methionine	0.56
13	Valine	3.56
14	Phenylalanine	1.90
15	Isoleucine	-
16	Leucine	3.66
17	Lysine	1.42

Based on Tabel 2 and Table 3 indicated that the dominant amino acid profile in gelatin from pigskin was glycine (123.81 ppm). The amino acid glycine can be synthesized in several ways, namely through transaminases from glyoxylates, glutamate and alanine and from choline and serine through the reaction of serine hydroxy metal transferase (Pearson and Dutson, 1992). The amino acid glycine on each position of the three amino acid chains of collagen protein is an absolute requirement for the formation of collagen fibrils during its formation process (Gautieri et al., 2008; Gelse et al., 2003). Gelatin is a type of protein extracted from collagen so that it generally has an amino acid composition resembling collagen (Giménez et al., 2005; Nemati et al., 2003).

Curing has changed due to denaturation of skin collagen proteins and some certain amino acids change chemically (Pearson and Dutson, 1992; Said et al., 2011). Typical properties of collagen protein are high levels of amino acids glycine, proline and hydroxyproline, while amino acids from aromatic and sulfur groups are present in small amounts (Wolf, 2003). Acidic solution is able to convert collagen fibers from triple helix to monohelix whereas alkaline solution is only able to convert into bihelix (Kołodziejska et al., 2003; Said et al., 2011).

CONCLUSIONS

It was concluded that the use of extraction temperature 60^0C and time of extraction 6 hours was the best characteristics of pigskin gelatin.

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