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The Effects of Acetic Acid Concentration and Extraction Temperature on Physical and Chemical Properties of Pigskin Gelatin

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Abstract

This research was aimed to study the influence of acetic acid concentration and extraction temperature on physical and chemical properties of pigskin gelatin. The experiment used Completely Randomized Design (CRD) with two factors and three replicates of treatment. The first factor was concentration of acetic acid solution consisted of 3 levels (2, 4 and 6 %). The second factor was extraction temperature consisted of 3 levels (50, 55 and 60 ^{0}C). The result showed that interaction of acetic acid and extraction temperature had no significant effect (P>0.05) on the gel strength, viscocity, protein content and pH value of pigskin gelatin. Pigskin gelatin with acetic acid concentration 2, 4 and 6 % and extraction temperature up to 60^{0}C had similar characteristics to the commercial gelatin. The optimum production was obtained from 4 % acetic acid and temperature 55^{0}C such as gel strength 134.22 g/Bloom; viscocity.7.16 cP; protein content 88.56 % and pH value 5.21.

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Keywords: Pigskin, Gelatin, Extraction temperature, Acetic acid

INTRODUCTION

Gelatin is a hydrocolloid product obtained by hydrolyzing collagen protein found in skin, bone, and connective tissue [14]. Sobral [20] explained that 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. Its functional properties depend on processing conditions as well as the raw material. The quality of gelatin depends on its physichochemical properties, rheological properties and manufacturing method. Gelatin has been applied within the food as a gelling agent, thickener, emulsifier, pharmaceutical, medical, cosmetic and photographic industries because of its uniqe functional [1,7,8,9,11,16,17].

Extraction of gelatin from goat skin has been reported by Said *et al*., [18], gelatin from chicken legs skin [24], pig skin [20], [21], gelatin from fish skin [4], [5], [12] [16], and salmon [3]. The extraction conditions (temperature and time)

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can affect the polypeptide chain length and functional properties of gelatin [10] and concentration of acetic acid solution also affects the amount of soluble collagen extraction process [25]. Acetic acid concentration of 3.5% significantly affect the physical characteristics of skin gelatin chicken legs [24]. The different physical properties of gelatins are related not only to the amino acid composition but also to the relative α-chain, β- or γ- component, and higher molecular weight aggregate contents and to the presence of lower-molecular-weight protein fragments [6]. The process of gelatin production required a curing step to improve quality of gelatin [17]. Curing materials from the group of acids have been widely applied in gelatin production, particularly from the skin and bones of fish [8]. Lee *et al.* [11] mixed pigskin gelatin with gellan to obtain composite film for packing or coating materials. However, effects of alkali process from pigskin was limited information. The aim of the research was aimed to study the effect of concentration acetic acid solution and temperature extraction on physical and chemical properties of pigskin gelatin.

MATERIALS AND METHODS

Materials

Two hundred (200) g pigskin were used as a raw material and acetic acid (CH_3COOH 0.5M) as a curing material.

Preparation of gelatins

The skin used for gelatine extraction were obtained from pig at 7 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 cut into small pieces (approximately 2x2 cm). Gelatine was prepared by the acid extraction method [15]. Acetic acid 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 24 hours. After soaked, samples 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 50^0C, second step at 55^0C and then at 60^0C. Solubilized gelatin was separated from residual skin fragments by filtration through a nylon filter. The extracted gelatin was concentrated at 70^0C for 5 hours and it was stored in the refrigerator 5-10^0C for 30 minutes, and dried at 60^0C 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

Gel Strength

Gel strength was determined with 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 60^0C to dissolve the particles. Solution in the container Ø5 cm and height 6 cm was stored at 5^0C for 16-18 hours. Gelatin was placed at the bootom 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 = constant 0.07 [11,13,18]

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 Gomez.

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. 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 [2]

pH determination

The pH of gelatin was determined using a pH metre with The British Standard Method then the solution was measured with a Hanna Instrument 1270 pH electrode Scew type.

Experimental Design and Data Analysis

The experiment were determined by analysis of Completely Randomized Design [23] with two factors and three replicates of treatments. The first factor was concentration of acetic acid solution with 3 levels (2, 4 and 6 percents). The second factor was extraction temperature consisting of 3 levels (50, 55 and 60 degrees of Celcius). The significant differences of the average were determined using Duncan's new multiple range test.

RESULTS AND DISCUSSIONS

Gel strength

Gel strength of gelatin is very important on physical properties of gelatin. Gel strength of pigskin gelatin was presented in Table 1. Statistical analysis indicated that the interaction between acetic acid concentration and extraction temperature had no significant effect ($P>0.05$) but that the extraction temperature had significant effect ($P<0.05$) on pigskin gelatin. Duncan test results showed the value of gel strength increased with increasing extraction temperature. Ulfah [24] and Said [18] reported that gel strength formation occurs due to the development of gelatin molecules during heating. Heat will open up the bonds of the gelatin molecules and free flowing liquids initially be trapped inside the structure, forming a viscous gel. Gel strength values from pigskin gelatin was ranged 130.04 - 136.09 g Bloom, that in line with the criteria of ISO 75-300 g Bloom [18]. 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. Said [18]) reported that the gel formation of a stable condition that ability of a free chain to form a lot of crosslinking. Pigskin gelatin gel strength obtained from the treatment of the highest concentrations of acetic acid 4% and extraction temperature 55^0 C.

Viscosity

The average viscosity of pigskin gelatin is displayed in Table 2. Statistical analysis indicated that the level of extraction temperature 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. Table 2 shows that the concentration of acetic acid had no significant effect on the viscosity of the gelatin. In order words, the higher acetic acid concentration cause decreased viscosity. This is because the curing material has been breaking the peptide bonds of amino acids into short-chain molecule so that its viscosity decrease [17]. Furthermore, Ulfah [24] explained that viscosity is affected by molecular weight and amino acid chain length. Increased concentrations of acetic acid in the gelatin production process can reduce the viscosity, therefore 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 was ranged 6.37 to 7.28 cP. It values is included in the ISO range 2.0 to 7.5 cP [18].

Protein Content

Gelatin is the collagen protein, a group derived from the structural proteins and extracellular matrix and produced in large quantities [17]. The average protein content of pigskin gelatin was presented in Table 3. Statistical analysis indicated that concentration of acetic acid had highly significant effect (P<0.01) on protein content of pigskin gelatin, whereas the extraction temperature and the interaction between these two different factors had not significant effect (P> 0.05) on levels of protein gelatin. Duncan test results showed that protein content of gelatin from pigskin had decreased with increasing the acetic acid content. Decreased levels of protein at high acid concentrations caused by acetic acid to hydrolyze the peptide bond is stronger so that there will be a loss of protein in pig skin when washing. According to Ulfah [24], the concentration of acetic acid solution resulted in the termination of high hydrogen bonds and the opening of the coil structure of collagen in excess so that some amino acids extracted and separated from the collagen and carried away by water washing, so that the protein content obtained is low. Protein content from pigskin gelatin ranged 86.03 to 89.22 %. That it was not different with commercial gelatin 89. 63% [17].

pH Value

The pH of gelatin is very important on chemical properties because its can affect the properties of gelatin others to determined the subsequent application of gelatin. The pH value of pigskin gelatin was presented in Table 4. The average of pH value is ranged between 5.03 to 5.41. Statistical analysis indicated that interaction between concentration of acetic acid and extraction temperature had no significant effect (P>0.01) while the level concentration of acetic acid factor, gave had significant effect (P<0.05) on pH value of pigskin gelatin. This is because the raw materials that have been in curing skin with acetic acid before undergoing a process of neutralization and washing before further processing so that the acid molecules that are bound to skin protein amount is very small. Conditions in the range of neutral pH values indicate that the process of neutralizing and washing the raw material before the extraction process is 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, Ulfah [24] explained that the acid tends to produce a low pH value of gelatin. The pH value of pigskin in this experiment still in the pH range of normal by the standards of GMIA is 4.5 to 6.5 and not much different from the pH tilapia skin gelatin which is 5.0 [22]

Table 1. Gel strength (g/Bloom±Sd) of pigskin gelatin

Extraction(^{0}C)	Concentration of acetic acid (%)			Average
	2	4	6	
50	130.84±0.62	134.81±0.46	134.87±0.65	133.51±0.36^a
55	134.16±0.30	134.22±0.12	136.09±0.81	134.82±1.02^b
60	134.72±0.16	136.09 ±0.31	136.44±0.21	135.75±1.06^c
Average	133.24±0.50	135.04±1.04	135.80±0.29	

Different letters in the separate row indicated the significant differences (P<0.05)
Sd = Standard deviation

Table 2. Viscocity (cP±Sd) of pigskin gelatin

Extraction(^{0}C)	Concentration of acetic acid (%)			Average
	2	4	6	
50	6.51±0.13	6.42±0.21	6.37±0.36	6.43±0.13^a
55	7.20±0.07	7.16±0.07	7.01±0.01	7.12±0.29^b
60	7.28±0.14	7.28±0.06	7.12±0.18	7.23±0.03^b
Average	6.99±0.52	6.95±0.29	6.83±0.52	

Different letters in the separate row indicated the significant differences (P<0.05)
Sd = standard deviation

Table 3. Protein content (%±Sd) of pigskin gelatin

Extraction(^{0}C)	Concentration of acetic acid (%)			Average
	2	4	6	
50	86.70±0.37	86.18±0.05	86.03±0.37	86.30±0.33^a
55	88.68±0.66	88.56±0.59	87.26±0.11	88.17±0.36^b
60	89.22±0.26	89.05±0.22	87.23±0.13	88.52±0.30^b
Average	89.20±0,71^q	87.93±0.71^q	86.84±0.61^p	

Different letters in the separate row and column indicated the significant differences (P<0.05)
Sd = Standard deviation

Table 4. pH Value ± Sd of pigskin gelatin

Extraction(^{0}C)	Concentration of acetic acid (%)			Average
	2	4	6	
50	5.71±0.14	5.23±0.03	5.18±0.07	5.37±0.03^a
55	5.34±0.13	5.21±0.13	5.04±0.13	5.19±0.12^{ab}
60	5.32±0.11	5.26± 0.05	5.20±0.07	5.26±0.11^a
Average	5.46±0.13^q	5.23±0.08^p	5.14±0.07^p	

Different letters in the separate row and column indicated the significant differences (P<0.05)
Sd = Standard deviation

CONCLUSION

Pigskin gelatin with acetic acid concentration 2, 4 and 6 % and extraction temperature up to 60^{0}C had similar characteristics to the commercial gelatin. The optimum production was obtained from 4 % acetic acid and temperature 55^{0}C such as gel strength 134.22 g/Bloom; viscocity.7.16 cP; protein content 88.56 % and pH value 5.21

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