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## Fat Reduction in Improving The Quality Of Chicken Feet Gelatine for Functional Food Application

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### Abstract

Fat reduction has been performed on crude gelatin extracted from chicken feet (shank) through curing process using sodium hydroxide and acetic acid in various proportions. This study was conducted to evaluate the best fat reduction condition by the addition of acetic acid at concentrations of 0, 2.5 and 5% (v/v) with decantation time 0, 6 and 12 hours on crude gelatine from curing process at a ratio of 1% acetic acid; 1% acetic acid with 1% NaOH; 1% acetic acid and 3% NaOH and without curing (initial gelatine). The analysis was carried out to determine the composition of gelatin and their byproducts to total solids, total fat and fat binding capacity. The results showed that the variation in the ratio of the addition of acetic acid and decantation time affect the composition of fat and fat-binding ability. Based on fat binding capacity (FBC), treatment of chicken feet gelatin with curing of 1% acetic acid and 3% NaOH and continued by the addition of 5% (v/v) acetic acid with a 12-hour decantation time resulted in gelatin with the best FBC of 7.4 g/g, fat content 1.0248% and total solids 2.7575%.

**Keywords:** *Gelatine, fat reduction, decantation, acetic acid, fat binding capacity (FBC).*

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### 1. INTRODUCTION

Gelatin is a polypeptide derived from collagen by partial thermal hydrolysis. Gelatin is an important functional biopolymer that has been widely used in food, pharmaceutical, photography and technical products (Karim and Bhat, 2009). In food industry, gelatin is one of the water soluble polymers that can be utilized to improve stability and consistency of food, particularly in confections, low fat spreads, bake goods, and meat products. While in medical and pharmaceutical industry, it can be used to produce soft and hard capsules, wound dressing, and adsorbent pads. For non-food industry, gelatin is used in making film for photography (Karim and Bhat, 2009; Haug *et al.*, 2004).

Generally, gelatin is commercially produced from skins and skeletons of mammalian (bovine and porcine). For several reasons, there is still concern among the consumers to consume gelatin from bovine and porcine due to religious matters, mad cow disease (Bovine Spongiform Encephalopathy or BSE) and social reasons (Karim and Bhat, 2009; Sadowska *et al.*, 2003). The searching for new sources of gelatin to substitute the mammalian gelatin has lead several studies on different raw materials such as fish and poultry. However, fish gelatin has various disadvantages such as strong odor and possible allergic effect to consumers (Jamilah and Harvinder, 2002; Hamada *et al.*, 2001; Sakaguchi *et al.*, 1999) and is less stable than mammalian gelatin especially in terms of physicochemical properties such as low

viscosity and gelling properties (Gomez-Guillen *et al.*, 2011; Badii and Howell, 2006).

Gelatin from poultry by-products have been receiving some attention since the wastes generated during processing contains varying amount of protein where head, feet and skin are rich in collagenous protein (Lasekan *et al.*, 2013). Extraction of gelatin from chicken feet has been done by using alkaline treatment (Rahman and Jamlulail, 2012) and acid treatment (Sarbon *et al.*, 2013). Collagen extracted from chicken broiler feet was reported to have higher hydroxyproline and proline content and exhibited higher thermal stability (Lin and Liu, 2006).

However, a fairly high fat content in the chicken feet gelatin would affect gelatin appearance and its application as food additive in food products. The presence of fat in gelatin will interfere the functional properties of its binding capacity to active compound in food. Crude chicken feet gelatin produced from curing in acid and alkaline conditions need further fat reduction treatment by the addition of acetic acid with various concentration and decantation time. The addition of acetic acid was carried out as acetic acid is able to bind fat, while decantation time will affect entrapped fat content in emulsion. Fat reduction might possibly affect the quality of gelatin in functional food applications.

This study was conducted to investigate fat reduction treatment of crude chicken feet gelatin extracted from curing with combination of acid and alkaline by adding acetic acid in various concentrations and decantation time and its functional properties (fat binding capacity) in comparison with commercial bovine gelatin

## 2. MATERIAL AND METHODS

The main material used in this experiment was chicken feet obtained from local poultry butchery. The chemicals and solvents were analytical grade and purchased from Merck.

### Experimental Design and Analysis

This experiment was conducted using crude gelatin extracted from chicken feet (shank) through curing process with variation of acetic acid and sodium hydroxide concentration of no NaOH and 1% acetic acid; 1% NaOH and 1% acetic acid; 3% NaOH and

1% acetic acid, and initial gelatin without alkali/acid treatment, respectively, following by the addition of acetic acid at 0, 2.5% and 5% with decantation time of 0, 6 and 12 hours.

### Gelatin extraction from chicken feet

Chicken feet was cleaned in running water tap and steamed for 20 minutes in order to remove skins from the bones, then soaked (curing) for 20 hours each in acetic acid 1%; NaOH 1% and acetic acid 1% and NaOH 3% and acetic acid 1% and without the addition of acid or alkali. The mixtures were then drained and washed in running tap until pH neutral. The extraction was then performed gradually in a water bath at 60 °C for 2 hours with the addition of distilled water in the ratio of 2: 1, then extracted twice at 100 °C for 1 hour. Finally, the extract was then filtered to obtain crude gelatine for further fat reduction process.

### Fat Reduction of Gelatin

Fat reduction was performed on crude gelatin according to experimental design in which the addition of acetic acid were 0, 2.5 and 5% (v/v) respectively at decantation time of 0, 6 and 12 hours. Fat separation was carried out manually. Gelatin solution was stored in plastic coated tray and cooled at room temperature then stored at 40 °C for 3-4 days and dried in oven at 50 °C for 48 hours. Then, the dried matter was grounded and sieved at 80 mesh as low-fat gelatin powder.

### Proximate Analysis

The proximate composition of the gelatin was determined using AOAC (2000) procedures. Moisture content was determined by gravimetric method. Fat content was determined by the Soxhlet method with hexane as a solvent extraction system. The protein content was determined by estimating its total nitrogen content by Kjeldahl method. A factor of 5.4 was used to convert the nitrogen value to protein.

### Fat Binding Capacity

Fat-binding capacity was measured by a partially modified method of Zhang *et al.*<sup>14</sup> 0,5 gram of gelatin was added with 5 mL of soybean oil, then homogenized using vortex and held at room temperature for 1 hour. The gelatin solutions were then centrifuged at 4000

rpm for 30 minutes. The upper phases were removed and the centrifuge tubes were drained for 30 min on a filter paper after tilting to a 45° angle. Their capacities were calculated as the weight of gelatin before and after adsorbing fat, respectively, and expressed as grams of fat/grams of gelatine before binding.

### 3. RESULT AND DISCUSSION

#### Physicochemical properties of gelatin

The physicochemical properties of gelatin from chicken feet (shank) are shown in Table 1. Chicken feet was extracted by alkali and acid treatment to weaken the collagen structure, solubilize the non-collagen proteins and hydrolyse some of the peptide bonds and maintain the consistency of the collagen fibres (Sarbon *et al.*, 2013). Proximate analysis conducted on gelatin as parameter in ensuring the removal of fat, mineral and hydrolysis processes are performed efficiently (Muyonga *et al.*, 2004), as well for determining the composition of proteins and amino acids that affect the gel bloom strength and gelling effects (Gomez-Guillen *et al.*, 2011).

**Table 1.** Physicochemical properties of gelatin from chicken feet

Composition	Gelatin from chicken feet
Moisture (%)	8.05
Ash (%)	3.7
Fat (%)	5.56
Protein (%)	87.15
Gel Strength (g/Bloom)	300

Gelatin from chicken feet contained protein as the major component (87.15%), followed by moisture (8.05%), fat (5.56%) and ash (3.7%). Gelatin mainly consist of most protein and water, but the presence of ash, lipid and other impurity at very low level are very important for the quality of gelatin (Jones, 1977).

The moisture content in gelatin from chicken feet is still met the requirement value of national standardization (SNI), which is the prescribed limit of 16% (SNI, 1995). The percentage of moisture content in gelatin affected by drying time, humidity, storage room and type of packaging used (Ockerman and Hansen, 1988). Low moisture content will

increase the shelf-life of gelatin, as well as affect the rheological properties such as elasticity and product viscosity. Meanwhile high moisture content in gelatin will induce damaging due to sticky effects of gelatin (Rahman and Jamalulail, 2012).

The ash content of gelatin from chicken feet were slightly higher compared with recommended value of 2.6% (SNI, 1995). High ash content could be possibly caused by a high level of bone residue and indicated the presence of inorganic salts which might be generated during the pretreatment with either alkali or acid (Lassoued *et al.*, 2014).

Gel strength of gelatin is essential on physical properties of gelatin to determine the gelatin quality by measuring the hardness, stiffness, firmness and compressibility of the gel at a particular temperature (Gudmundsson and Hafsteinsson, 1997). Gel strength is a function of complex interactions determined by amino acid composition and the ratio of  $\alpha$ -chain and the amount of  $\beta$ -component (Gomez-Guillen, 2011). The presence of hydroxyproline could increase the gel strength since it stabilizes the hydrogen bonds between water molecules and free hydroxyl groups of amino acids in gelatin (Arnesen and Gildberg, 2002). Gel strength of gelatin from chicken feet is still in the range of Gelatin Manufacturers Institute of America (GMIA) standard (50-300 g/bloom) and is suitable to be applied in edible film, food ingredient and soft and hard capsules for supplement formulation (GNIA, 2006).

However, the fat content in gelatin from chicken feet is slightly higher than the recommended value which should not exceed 5% (SNI, 1995). Fat content in chicken meat and chicken feet is relatively higher than bovine skin which are 10.9% and 5.2% respectively (Purnomo, 1992). The difference in fat content is greatly influenced by physicochemical properties and various methods of production, including types of tissue and animal species (Gómez-Guillén and Montore, 2001). Therefore, further treatment is required to reduce fat content in gelatin since high level of fat could affect the functional properties of gelatin and increase the rancidity.

#### Effect of The Addition of Acetic Acid on Gelatin Composition

Gelatin is derived from collagen by thermohydrolysis. The conversion of collagen into soluble gelatin can be achieved by heating

the collagen in either acid or alkali. Gelatin processing has three steps, alkali pretreatment, acid pretreatment and hot water extraction. The alkali and acid treatment removes non-collagenous protein and the sample swells in the acid solution. The hot water extraction uses thermohydrolysis to solubilize gelatin which is then separated (Jamilah and Hrvinder, 2002). During the extraction process acid and alkali will cleavage protein structures until it finally dissolves and is followed by the release of fatty molecules that were bound (Zeugolis *et al.*, 2008; Wang *et al.*, 2008). The extraction process (temperature, time, and pH) can influence the length of the polypeptide chains, and the functional and biological properties of the gelatin.

In this experiment, chicken feet gelatin was obtained through curing process for 20 hours by acid and alkali treatment to undermine the crosslinked collagen molecules (Kim *et al.*, 2008) and continued by several extraction in water bath to produce sticky suspension, colloidal white and odorless of gelatin. Since fat content in crude gelatin is still quite high, the further fat reduction is required by adding acetic acid in various concentration and decantation time to obtain low-fat gelatin from chicken feet. An additional acid process using acetic acid was chosen since it is known that acetic acid molecule can cleave hydrogen bonds and become associated with the carboxyl group of the peptide bond (Gustavson, 1956). The effect of acetic acid addition to precipitate fat in gelatin were investigated in total solids, fat and fat-binding capacity.

### Total Solids

Total solids of gelatin after fat reduction by acetic acid addition at various concentration and decantation time were given in Figure 1. The overall total solids are relatively low in the range of 2.305% to 7.153% due to the gelatin were still in the liquid form after acetic acid addition. Figure 1 showed that total solids were decreased by the increasing of sodium hydroxide and acetic acid concentration from curing process. The differences of total solids is mainly due to the differences in collagen content, as the higher degree of crosslinking via covalent bonds caused the decrease in solubility of collagen and might lead to a lower amount of gelatin total solids (Foegeding and Hultin, 1996). Acid is used to extract and solubilize the collagen rod while maintaining it

triple-helix configuration (Gómez-Guillén and Montore, 2001). There are three factors that greatly influence the swelling properties and solubilization of collagen, which are type of acid used, the ionic strength and the pH that the acid produces (Gómez-Guillén and Montore, 2001). Due to the hydrogen binding power of acid, the nonionized acid acts as a swelling agent to compete with peptide bonds and involved in the intermolecular linking of the protein chain (Asghar and Henrickson, 1982). Total solids were also influenced by increasing temperature and decreasing pH of extraction as well as alkali and acid concentrations due to opening of cross-links during swelling (Ninan and Jose, 2009).

### Fat Content

High fat content could be a hindrance to functional properties of gelatin. After the curing process, crude gelatin from chicken feet still contained high level of fat. The further addition of acetic acid was carried out to reduce fat content in gelatin. Acid is used to extract and solubilize the collagen rod while maintaining it triple-helix configuration (Gómez-Guillén and Montore, 2001). Due to the hydrogen binding power of acid, the nonionized acid acts as a swelling agent to compete with peptide bonds and involved in the intermolecular linking of the protein chain (Asghar and Henrickson, 1982).

It can be shown from Figure 2, that the addition of acetic acid could generally decrease fat by 98% from initial fat content of gelatin before treatment. Fat content after acetic acid addition were less than 2% and still in the range of SNI recommended value (SNI, 1995), indicating that there was an efficient removal of fat from chicken feet gelatin.

### Fat Binding Capacity (FBC)

Fat-binding capacity is functional property that is closely related to texture by the interaction between components such as oil and other components. Fat binding capacities of gelatin from chicken feet after acetic acid addition were given in Figure 3. Chicken feet gelatin with curing of 1% acetic acid and 3% NaOH and continued by the addition of 5% (v/v) acetic acid with a 12-hour decantation time has the best fat binding capacity of 7.40 g/g. This property is due to the presence of non-covalent bonds, such as hydrophobic, electrostatic and hydrogen bonding forces that

are involved in lipid-protein interactions (Lawal, 2004).

Fat-binding capacity depends on the degree of exposure of the hydrophobic residues inside gelatin (Jellouli *et al.*, 2011). The degree of exposure of the hydrophobic residues and the high amount of tyrosine were found

responsible for the high FBC (Ninan *et al.*, 2011). The fat binding capacity of chicken feet gelatin was higher than that of commercial bovine gelatin. Thus gelatin from chicken feet can be proposed as fat replacer and binding agent in food products.

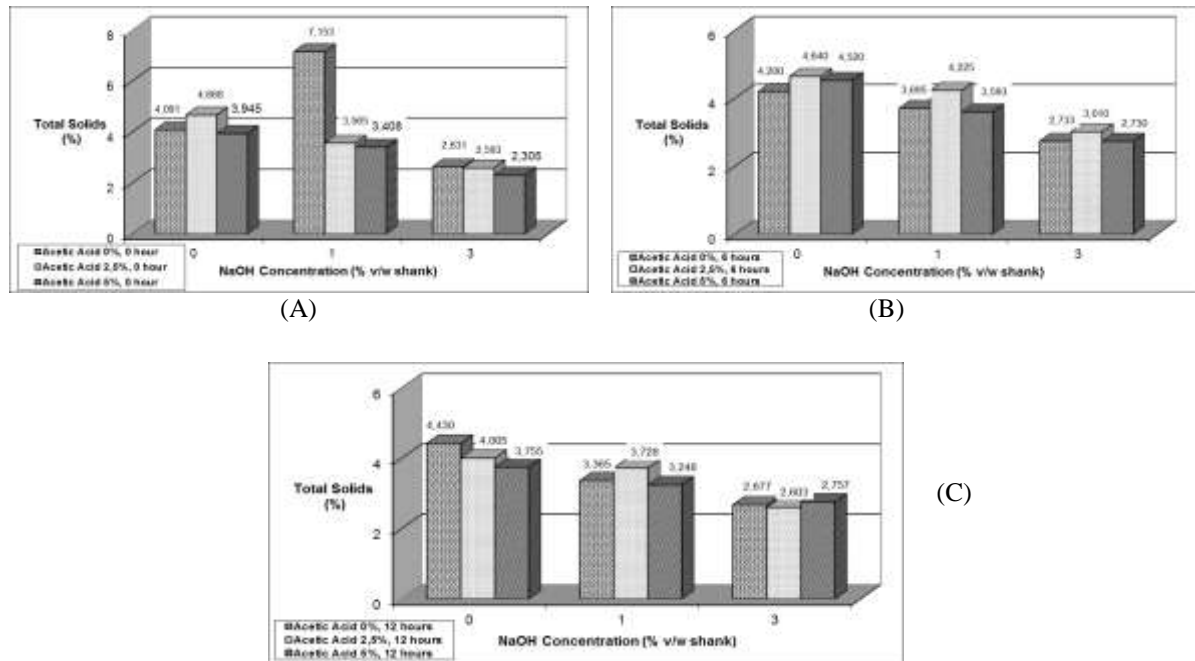


Figure 1. Effect of the addition of acetic acid to gelatin total solids at decantation time of 0 hours (A), 6 hours (B) and 12 hours (C).

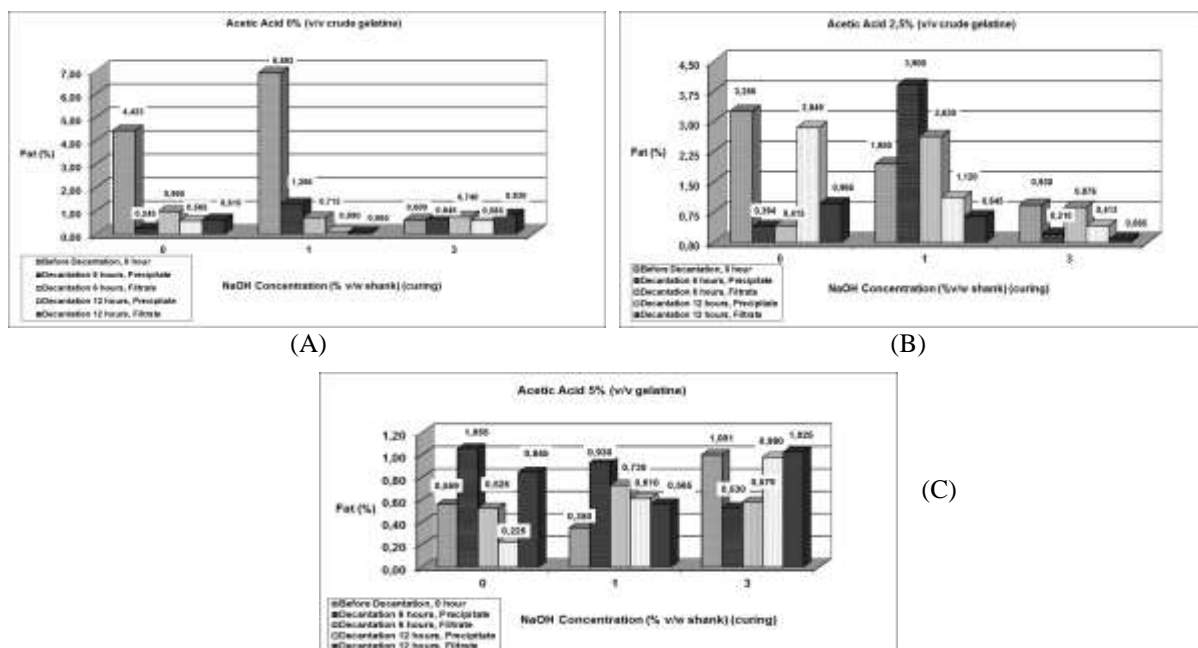
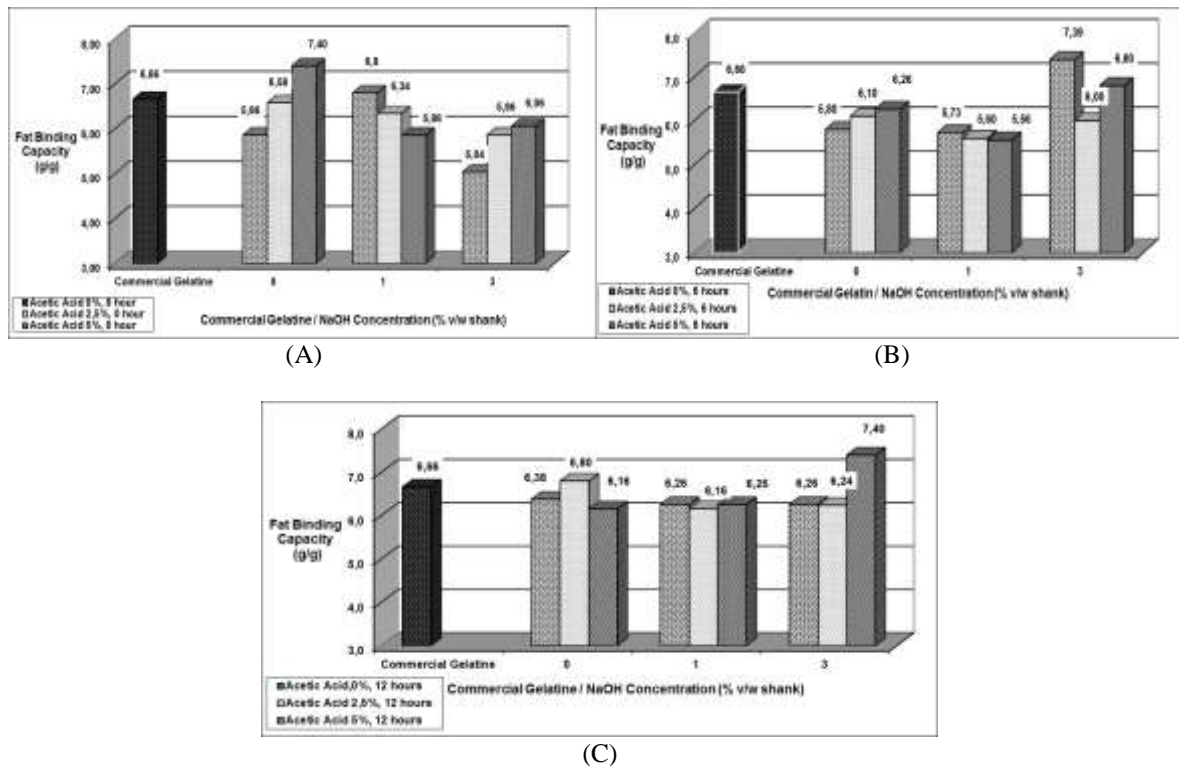


Figure 2. Effect of the addition of acetic acid at concentration 0% (A), 2.5% (B) and 5% (C) to fat content of gelatin precipitate and filtrate at variation of decantation time.







**Figure 3.** Effect of the addition of acetic acid at decantation time of 0 hour (A), 6 hours (B) and 12 hours (C) to oil binding capacity in comparison with commercial gelatin.

**4. CONCLUSIONS**

Gelatin extracted from chicken feet which is further defatted after curing by 5% acetic acid at decantation time 12 hours has the highest fat binding capacity 7.4 g/g, fat content 1.0248% and total solids 2.7575% respectively. The variation in the ratio of the addition of acetic acid and decantation time affect the composition of fat and fat-binding ability. From overall results of its physicochemical properties and functional properties (fat binding capacity), low-fat gelatin from chicken feet can be proposed as alternative gelatin in functional food application.

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