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# THE USE OF VARIOUS HYDROCHLORIC ACID CONCENTRATION LEVELS ON THE RABBIT BONE GELATIN QUALITY

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**Abstract**
Rabbit meat production generates by-products in the form of bones which contain proteins, including collagen. Collagen can be hydrolyzed to produce gelatin. This study aimed to determine the impact of hydrochloric acid concentration on the quality of rabbit bone gelatin and identify the optimal concentration for producing high-quality gelatin. The research followed a Completely Randomized Design (CRD) with five hydrochloric acid concentration treatments (P1= 3%, P2=4%, P3=5%, P4=6%, P5=7%), each replicated four times. The analysis employed analysis of variance, followed by the Duncan Multiple Rank Test. The findings indicate that hydrochloric acid concentration did not affect moisture content, while it influenced yield, ash content, pH, and viscosity. The use of 6% hydrochloric acid produces the best-quality rabbit bone gelatin.
**Keywords: Ash content, gelatin, hydrochloric acid, moisture, yield**

# *PENGGUNAAN BERBAGAI TINGKAT KONSENTRASI ASAM KLORIDA TERHADAP KUALITAS GELATIN TULANG KELINCI*

***Abstrak***
*Produksi daging kelinci menghasilkan produk ikutan berupa tulang. Tulang menganndung protein diantaranya berupa kolagen. Kolagen dapat dihidrolisis menghasilkan gelatin. Tujuan penelitian ini untuk mengetahui pengaruh konsentrasi asam klorida terhadap kualitas gelatin tulang kelinci serta untuk mengetahui konsentrasi asam klorida yang menghasilkan kualitas gelatin terbaik. Rancangan penelitian yang dilakukan adalah Rancangan Acak Lengkap (RAL) dengan lima perlakuan konsentrasi asam klorida (P1= 3%, P2=4%, P3=5%, P4=6%, P5=7%) masing-masing diulang empat kali. Analisis menggunakan sidik ragam, dilanjutkan dengan uji Duncan. Hasil penelitian menunjukkan bahwan pengaruh konsentrasi asam klorida tidak mempengaruhi kadar air, tetapi konsentrasi asam klorida memberikan pengaruh terhadap rendemen, kadar abu, pH, dan viskositas. Pemakaian asam klorida 6% menghasilkan mutu gelatin tulang kelinci terbaik.*
***Kata kunci: Asam klorida, gelatin, kadar abu, kadar air, rendemen***

## INTRODUCTION

The estimated world production of rabbit meat ranges from 1.2 to 1.8 million tons with major contributors being China, Italy, Spain, Egypt, and France, respectively (FAO, 2013). Indonesia, particularly Java Island, owns local rabbit breeds, likely descendants of the Nederland dwarf race (Sarwono, 2008), which contributed to the Indonesian rabbit meat industry. In 2021, Indonesia was estimated to produce 490 tonnes of rabbit meat, increasing from 460 tonnes in 2020 (Makmun et al., 2022).

During meat production, bones are generated as by-products. The increasing slaughter of rabbits leads to a surplus of bones, so they can be used further to increase the value. Gelatin, a processed product using bones as raw material, is in high demand in Indonesia, leading to substantial gelatin imports. According to data from the Central Statistics Agency (2023), Indonesian gelatin imports were estimated to be 2,705,179 kg in 2022, 1,891,100 kg in 2021, and 1,290,306 kg in 2020, indicating a steady increase every year. Rabbit bones, rich in collagen (24%), offer a valuable resource for gelatin production (Nurrachmawati, 2015). Utilizing rabbit bones as raw material for gelatin is expected to increase their selling value.

The hydrolysis process plays a crucial role in determining gelatin quantity and quality. Based on the production process at the initial stage of collagen treatment, gelatin is divided into two types: the acid process, which produces type A gelatin, and the alkaline process, which produces type B gelatin (Alipal et al., 2021). The acidic collagen hydrolysis process uses sulfuric acid, phosphoric acid, and especially hydrochloric acid (HCl) (Rapika et al., 2016). The acidic process takes less time and produces more collagen as it changes triple-helix collagen fibers into single chains. In contrast, alkaline solutions can only produce double chains, which result in less hydrolyzed collagen than acidic solutions within the same soaking duration (Schrieber and Gareis, 2007).

Increasing hydrochloric acid concentration and soaking time accelerate collagen hydrolysis rate and results in over-dissolution (Rapika et al., 2016), leading to reduced gelatin yield (Ockerman and Hansen, 2000). Hydrochloric acid that is too low also causes a low yield because the gelatin is still tightly bound to bone tissue, so the extraction process is less effective (Khirzin et al., 2019). The collagen hydrolysis stage must be carefully managed (in terms of time and concentration), otherwise, the collagen will dissolve completely in the solvent, causing a decrease in the gelatin produced (Gumilar and Pratama, 2018).

Key parameters affecting gelation quality include moisture content, ash content, and yield. Moisture content influences gelatin's shelf life (Ulfah, 2011). According to SNI 1995, gelatin should have a maximum of 16% water content, and a maximum of 3.25% ash content. Higher acid concentration causes more H+ ions in the soaking solution, which hydrolyzes more collagen, increases hydrophobic groups on the protein molecule surface, and decreases molecule size, thereby reducing the water storage capacity (Yasin et al., 2017). Ash content indicates the mineral content in a material (Huda et al., 2013). Reducing the calcium content in gelatin decreases ash content (Mad-Ali et al., 2016).

Previous research on duck bone gelatin suggests that a 5% hydrochloric acid concentration gives the best results, with a yield value of 6.24%, moisture content of 13.43%, and ash content of 13.42% (Khirzin et al., 2019). Research on skipjack tuna bone gelatin with a 5% hydrochloric acid concentration produced the highest yield, namely 16.25%, with a moisture content value of 9.75% (Singkuku et al., 2017). Meanwhile, other research conducted by Juliasti et al. (2015) on sheep's leg bone waste gelatin showed that a hydrochloric acid concentration of 6% produced the highest yield (14.69%), with 0.96% moisture content and 2.86% ash content.

Considering the scarcity of information on gelatin production from local rabbit bones using hydrochloric acid, this research aims to determine the physicochemical quality of gelatin from rabbit bone and to determine the best HCl concentration.

## MATERIALS AND METHODS

### Material

The primary ingredients include 4.3 kg of local rabbit bones and 37% hydrochloric acid (Merck, Germany). The tools used were a bucket, knife, cutting board, pan, gas stove, 500 ml beaker glass, label, aluminum foil, pH meter (HI 2211 Benchtop pH meter, Hana Instrument), digital scale (Ohaus), water bath (Julabo), stirring rod, sieve, plastic pan, plastic, mortar and pestle, analytical balance (Sartorius BSA224S), porcelain cup, crucible tongs, drying oven (Julabo), desiccator, and furnace (Thermo Scientific).

### Gelatin-Making Method

The gelatin production process followed the method outlined by Ockerman and Hansen (2000). The initial step was degreasing, which was done by boiling the bones in water for 30 minutes. After draining, the bones were cut into 3 cm pieces to widen the surface. Each unit, weighing 100 grams/unit, was put into a breaker glass. Cleaned raw materials were soaked in HCl solution with 3%, 4%, 5%, 6% and 7% concentration. The bone-to-solution ratio was 1:3, covered with aluminum foil, and soaked for 24 hours to eliminate calcium content and mineral salts, rendering the rabbit bones soft or referred to as ossein. The ossein was washed with running water until the pH became neutral. After neutralization, extraction was conducted using a water bath at 80ºC for 7 hours. The extraction solutions were filtered with a cloth, and the concentrated liquid gelatins obtained were dried at 50ºC for 48 hours in a drying oven. Post-drying, it was grounded and analyzed to determine the quality of the gelatin.

**Yield**

Yield was calculated as the ratio of the dry weight of the produced gelatin to the weight of the rabbit skin used. The yield calculation method used the AOAC (2019) method formula:

$$\text{Yield (\%)} = \frac{Gelatin\ Weight}{Rabbit\ Skin\ Weight} \times 100\%$$

**Chemical Quality**

Chemical quality was determined by analyzing rabbit bone gelatin's water and ash content using the AOAC method (2019).

**Physical Quality**

The physical quality of rabbit bone gelatin was tested based on its pH value and viscosity, following the method specified by BSI 757.

**Statistical Analysis**

This research adopted a completely randomized design (CRD) with a unidirectional pattern, consisted of 3% (P1), 4% (P2), 5% (P3), 6% (P4), and 7% (P5) HCl concentration treatment, each repeated three times. The effects of HCl concentration treatments on yield, moisture content, ash content, pH, and viscosity were analyzed with analysis of variance, and further testing was done using the Duncan Multi Rank Test (DMRT) to determine the differences between each treatment.

## RESULTS AND DISCUSSION

**Effect of Hydrochloric Acid Concentration Treatment on Yield**

Gelatin yield, defined as the ratio of dry gelatin weight to fresh bone weight, was produced using varying hydrochloric acid concentrations (3-7%). The average yield value produced in this study ranged from 2.40 ± 0.25% to 3.92 ± 0.40%, as presented in Table 1.

This study revealed a positive correlation between hydrochloric acid concentration and gelatin yield. The yield increased with rising hydrochloric acid concentration, reaching its peak at 6%, and declining at 7%. This increase in yield indicates increased denaturation of bone collagen (Roy et al., 2022), a crucial step in gelatin formation through hydrolysis. Analysis of variance demonstrated significant (P<0.05) in the effects of different hydrochloric acid concentrations (3, 4, 5, 6, and 7%) on gelatin yield. Subsequent DMRT comparisons revealed that the use of 6% hydrochloric acid (P4) produced a significantly higher yield (P<0.05) compared to 3% hydrochloric acid (P1), 4% (P2), and 7% (P5). No significant difference was observed compared to the use of 5% hydrochloric acid (P3). Treatment 3% (P1), 4% (P2), and 7% (P5) did not exhibit significant differences (P>0.05) among themselves.

The observed yield is closely related to the collagen conversion into gelatin. Sasmitaloka et al. (2017) stated that increased yield influenced by a higher amount of collagen converted into gelatin. Acid will dissolve the calcium salts in the bone matrix, causing the collagen that binds the calcium in the bones to be released (Huda et al., 2013). According to Ridhay et al. (2016), this process releases collagen in the bones, facilitating its conversion into gelatin as the triple helix coils break into mono helixes. Proper acid quantity is required for appropriate collagen conversion, as insufficient acid inhibits the collagen triple helix from being converted into a mono helix. Excessive acid not only alters the mono helix structure but also continues the conversion until the amino acids are reduced, resulting in decreased gelatin production (Gumilar and Pratama, 2018).

**Table 1**. The Influence of Various HCl Concentrations on the Rabbit Skin Gelatin Quality

| Treatment Variables | Treatments | | | | |
|---|---|---|---|---|---|
| | $P_1$ | $P_2$ | $P_3$ | $P_4$ | $P_5$ |
| Yield (%) | 3.16±0.32 $^{ab}$ | 2.95±0.29 $^{a}$ | 3.33±0.32 $^{bc}$ | 3.92±0.40 $^{c}$ | 2.40±0.25 $^{a}$ |
| Moisture (%) | 11.20±0.31 $^{a}$ | 9.70±0.26 $^{a}$ | 9.74±0.86 $^{a}$ | 10.23 ± 0.89 $^{a}$ | 10.52±0.62 $^{a}$ |
| Ash (%) | 10.48±0.61 $^{a}$ | 8.32±0.81 $^{b}$ | 5.59±0.55 $^{c}$ | 4.70±0.07 $^{c}$ | 4.63±0.26 $^{c}$ |
| pH | 4.15±0.16 $^{a}$ | 4.18±0.12 $^{a}$ | 3.97±0.05 $^{ab}$ | 3.75±0.17 $^{b}$ | 3.85±0.23 $^{ab}$ |
| Viscosity (mP) | 7.29±0.66 $^{a}$ | 7.51±0.60 $^{a}$ | 11.18±1.09 $^{c}$ | 10.63±0.32 $^{bc}$ | 8.71±0.73 $^{a}$ |

**Note**: Different superscripts in the horizontal rows indicate significant differences (P<0.05).

**Effect of Hydrochloric Acid Concentration on Moisture Content in Rabbit Bone Gelatin**

Moisture content is a critical parameter in food ingredients, as it determines acceptability, freshness, appearance, texture, taste, and the overall quality and durability of the food product. Table 1 illustrates the average moisture content of rabbit bone gelatin produced using 3-7% hydrochloric acid. The observed moisture content in this study ranged from 9.70 ± 0.26 - 11.20 ± 0.31%. The gelatin's moisture content adheres to the standards in Indonesia, based on the Gelatin Manufacture of Europe standard, with a maximum moisture content of 15% (GME, 2017).

The variations in hydrochloric acid concentrations did not significantly affect (P>0.05) the moisture content of the produced gelatin. This result implies that treatment with different hydrochloric acid concentrations produces comparable moisture content values. In an acid-based soaking process, both free water and bound water are released due to protein denaturation induced by acid influence. Protein denaturation, characterized by changes in protein polypeptide bonds, leads to the subsequent release of water bound to the protein. The uniformity in water evaporation between treatments is caused by the consistent application of heat in a drying oven at the same temperature and duration. Sartika (2009) found that moisture content is influenced by water loss during the drying process. This is also supported by Rapika et al. (2015) who stated that the lengthy drying process (48 hours) for bone gelatin contributes to the low moisture content produced as the water content evaporates.

**Effect of Hydrochloric Acid Concentration on Ash Content in Rabbit Bone Gelatin**

Ash content represents the concentration of inorganic substances that are not burned in the organic substance combustion process, such as calcium, potassium, sodium, iron, magnesium and manganese. Table 1 shows the average ash content values for rabbit bone gelatin produced using 3-7% hydrochloric acid. The ash content produced in this study ranged from 4.63 ± 0.26% - 10.48 ± 0.61%. The highest ash content value was observed at a 3% hydrochloric acid concentration, while the lowest was produced at a 7% hydrochloric acid concentration. The ash content in this study was above the standard maximum limit (3.25%) set by SNI No. 06.3735 1995, and 2% based on GME (2017). The high ash content is attributed to the bone's inherent mineral-rich composition, reaching up to 40%, primarily due to the presence of calcium salts which requires additional processing at the demineralization stage (Aewsiri et al., 2008).

The variations in P3, P4, and P5 hydrochloric acid concentration were not significantly different (P>0.05), whereas these treatments were significantly different (P<0.05) compared to P1 and P2. This outcome suggests that higher hydrochloric acid concentration correlates with lower ash content, as increased acid concentration intensifies the dissolution of calcium content in rabbit bones during the demineralization process (Gumilar and Pratama 2018). The demineralization stage involves a reaction between hydrochloric acid and calcium phosphate, a key component of the bone structure compound. This reaction results in the production of calcium salts, which dissolve and soften the bones, and the dissolution of calcium into $Ca_{2+}$ ions, which will dissolve in the solvent (Huda et al., 2013).

**Effect of Hydrochloric Acid Concentration on pH in Rabbit Bone Gelatin**

pH value is a critical chemical property of gelatin, as it influences other gelatin characteristics and determines its subsequent applications (Hasdar and Rahmawati, 2016). Table 1 presents the average gelatin pH value resulting from this research. The observed pH ranged from 3.75 ± 0.17 to 4.18 ± 0.12. The highest pH value was recorded in the P2 hydrochloric acid treatment, while the lowest value was in the P4 treatment. The average pH of rabbit bone gelatin across most treatments adhered to the pH requirements for type A gelatin outlined by the Gelatin Manufacturers Institute of America (GMIA, 2012), which falls between the range of 3.8 - 5.5.

Gelatin processed by P1, P2, P3, and P5 acid concentration did not exhibit significant differences (P>0.05), while P4 displayed lower pH (P<0.05) compared to P1 and P2. No significant difference (P>0.05) was observed between P3 and P5. Gelatin characteristics, including the pH value of the gelatin solution, are influenced by the extraction process (Alfaro et al., 2013). During swelling, the collagen molecule's amino acid bond structure opens, encapsulating the curing agent or acid between the bonds. This trapped acid remains undissolved during the neutralization process, and therefore, it will directly affect the pH value in the final gelatin product (Agustin and Sompie, 2015). A lower concentration and shorter soaking time result in a more favorable pH value, as the hydrochloric acid utilized during soaking, avoids absorption into the collagen fibril network. Consequently, during the skin-washing process, the hydrochloric acid is easily removed from the skin, ensuring an optimal pH value (Rapika et al., 2016). As the hydrochloric acid concentration in the solution increases, the heightened hydrochloric acid concentration prompts an increase in H+ ions, leading to enhanced penetration into the gelatin, resulting in a decreased pH level (Gumilar and Pratama, 2018).

**Effect of Hydrochloric Acid Concentration on Viscosity in Rabbit Bone Gelatin**

Viscosity, a pivotal physical property, is consistently measured to assess gelatin quality. The gelatin viscosity greatly influences gel properties, particularly the gel formation point and melting point (Hasdar and Rahmawati, 2016). Table 1 outlines the viscosity values of gelatin from rabbit bones with various hydrochloric acid treatments. The viscosity ranged from 7.29 ± 0.66 to 11.18 ± 1.09, exceeding the viscosity values of gelatin from other bone sources, such as poultry (4.96 - 9.8); pork (5.0 to 7.28); and fish (7.70) (Rigueto et al., 2022).

P1, P2, and P5 treatments were not significantly different ($P>0.05$), while P3 showed significant difference ($P<0.05$) compared to P1, P2, and P5. The rise in viscosity is influenced by the molecular structure of amino acids that constitute the gelatin protein. High viscosity results from factors, such as molecular weight, chain length of gelatin amino acids, and the complete breakdown of collagen chains (Rapika et al., 2016). Collagen chain breakdown is influenced by the amount of hydrochloric acid in the dehairing process. Inadequate hydrochloric acid inhibits complete collagen breakdown, while higher HCl concentration induces structural changes in collagen, leading to various viscosity values. Increasing HCl concentration may also cause the opening of amino acid chains and their structures, resulting in shorter chains and decreased viscosity (Juliasti et al., 2015). Consequently, higher acid concentrations yield gelatin structures with lower relative molecular masses, causing a decrease in viscosity. Conversely, higher molecular masses in gelatin correspond to higher viscosity values (da Silva et al., 2022).

## CONCLUSION

The various hydrochloric acid concentrations did not impact the moisture content, but significantly affects the yield, ash content, pH and viscosity of rabbit bone gelatin. Optimal results were achieved with 6% hydrochloric acid, producing the best rabbit bone gelatin with an average 3.92 ± 0.40% yield value, 10.23 ± 0.89% for moisture content, 4.70 ± 0.07% for ash content, 3 .75±0.17 for pH, and 10.63±0.32 mP for viscosity.

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