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Comparison of Microwave and Oven Extraction Methods on the Quality of Pork, Beef and Duck Gelatine

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Abstract

Gelatine is a biopolymer that is made from the partial hydrolysis of collagen, a fibrous protein that is found in the skin, bones, and connective tissues of animals. The choice and use of the extraction procedure are critical for producing high-quality gelatine. As a response, the goal of this study is to determine the impact of microwave and oven extraction methods on the quality of pork, beef, and duck gelatine using moisture content, ash content, pH value, and gel strength as factors. Microwave and oven extraction methods were used to remove bone gelatine from pork, beef, and duck. Gelatine was placed in a plastic bag and vacuum sealed for the microwave extraction. The extract was then coated in plastic and microwaved for 10 minutes at a wavelength of 360 nm before being filtered. The oven extraction was done by weighing and adding aquadest in a 1:4 ratio, then baking for 24 hours at 70 °C. After that, it was filtered and gelatine was obtained. Based on characteristics such as water content, ash content, pH value, and gel strength, the results showed that microwave and oven extraction methods had a significant difference ($p<0.005$) in gelatine quality. The microwave extraction method yielded the following results: water content 9.567%, ash content 2.026%, pH value 5.467, and gel strength 268.733 blooms. The oven extraction method yielded the following gelatine test results: water content 3.677%, ash content 2.864%, pH value 4.667, and gel strength 226.631 bloom. The results showed that utilizing a microwave to extract pork, beef, and duck gelatine generated the best grade of gelatine that could be used in subsequent studies.

Keywords: gelatine; microwave; oven

1. Introduction

One of the countries that imports gelatine is Indonesia, with 5 million kg of gelatine imported annually, gelatine is already the most imported product in Indonesia. Because the majority of the fundamental ingredients are sourced from pork, gelatine is often imported from non-Muslim countries, which are indifferent about the product's halal certification [1]. Pork is used as a raw material for gelatine in 44.9 % of all gelatine manufacturing around the world [2]. To avoid or reduce the usage of non-halal items by Muslims in Indonesia, a new alternative to pig gelatine is required [3].

One option is to use gelatine made from duck bone, sheepskin, fish skin, or chicken bone [4,5]. Beef and poultry bones are calcium and collagen protein-rich by-products of the livestock industry. To manufacture good gelatine, you need a good production technique as well as good gelatine. In the manufacturing of gelatine, both traditional and microwave methods are used [6,7].

This study will compare the traditional method and microwave for gelatine extraction of pork, beef, and duck bones to the bone immersion procedure employing a 4 percent hydrochloric acid (HCl) solution and a 24-hour immersion time. The study's main goal is to see how the microwave and oven extraction methods affect the quality of pig, beef, and duck gelatine based on moisture content, ash content, pH value, and gel strength parameters.

2. Experimental

Materials

The chemicals used in this research were purchased from Merck (Darmstadt, Germany) such as 4% HCl. Pork, beef, and duck bones were purchased from the local market in Malang, Indonesia.

Methods

Sample preparation

A total of 6 kg of pork, beef, and duck bones were cleaned by boiling them for 1 hour at 70 °C. Then it was rinsed and cut into 2 cm pieces.

Gelatine Isolation

Soaking Pork, Beef, and Duck Bone

250 g of dry bone were soaked in 4 % HCl for 24 hours at a 1:4 (w/v) sample weight to solvent volume ratio. During the soaking process, the bones were stirred, then placed on a sieve lined with filter paper and cleaned with water. The procedure was repeated three times.

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Extraction of Gelatine

Two methods of extraction were used, namely the microwave (Hento) and the oven (Memmert) method. Placing gelatine in a polyethylene bag and packing it with a vacuum packaging technique was used for the microwave procedure. The extract was then wrapped with plastic and microwaved for 10 minutes at a wavelength of 360 nm before being filtered. For the oven method, gelatine was weighed, aquadest was added in a 1:4 ratio, and the mixture was baked for 24 hours at 70 °C. The gelatin is then obtained after filtering.

Drying of Gelatine Solution

The gelatine solution was concentrated by freeze drying (Gea Lr-600) for 24 hours until the water content was around 25–35 %. In addition, the gelatine was baked in a 60 °C oven for 24 hours until it was dry, and then pulverized.

Gelatine Quality Test

Yield

The amount of yield can be obtained by the formula [8]:

$$Yield = \frac{weight\ of\ dry\ matter\ gelatin}{fresh\ material\ weight}\ x\ 100\%$$

Water Content

As much as 0.5 g of gelatine sample was put in the moisture analyzer, then the device was closed and waited for the analysis process to complete. Heating is carried out to a temperature of 110 ^{o}C [9].

Ash Content

A total of 0.5 g of gelatine sample was put in a porcelain dish that had been weighed, then put in a furnace at 600^{o}C for 6 hours, or until the sample turned white. The sample remaining in the cup is weighed as the final weight. Ash content can be calculated using the formula [8]:

$$Ash\ Level = \frac{(d-a)}{(b-a)}\ x\ 100\%$$

Where:
a = constant weight of empty cup
b = weight of the cup + sample before drying
d = weight of the cup + sample after drying

pH Test

At 80^{o}C, 0.2 g of sample was dissolved in 20 mL of distilled water and homogenized. Measured the degree of acidity at room temperature with a pH meter [9].

Gel Strength

To make a gelatine solution with a concentration of 6.67 % (w/w), dissolve 3.335 g of gelatine in 50 mL of warmed distilled water. The solution was poured into Standard Bloom Jars (bottles with a diameter of 58–60 mm and a height of 85 mm), then sealed and set aside for 2 minutes. Then it was incubated for 2 hours at 10 °C [10].

Gel strength then measured with texture analyzer. This tool uses a probe with an area of 0.1923 cm^{3}. Gel strength was measured using the formula [10]:

Gel strength (dyne/cm3) = F/A x 980
Gel strength (bloom) = 20 + (2.98 x 10^{-3}) x D

Where:
F = height of curve
A = constant (16)
D = gel strength (dyne/cm3)

Data Analysis

The data analysis used was the two-way ANOVA method of analysis of variance. The statistical test used is the Post-hoc Test, namely LSD.

3. Results and Discussion

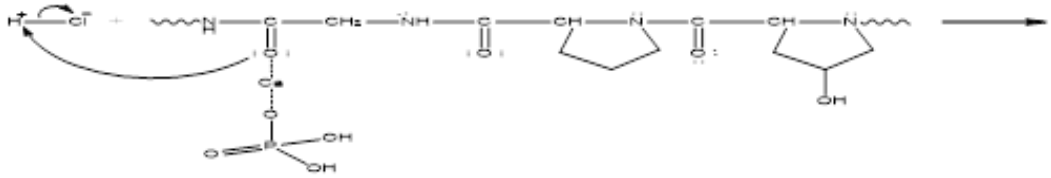
Sample Preparation

This study uses dried pork, beef, and duck bones as raw materials, with the goal of not extracting the meat adhering to the bones during the extraction process. Furthermore, the bone size reduction was completed; the bone size reduction seeks to increase the surface area of the bone so that the reaction can occur more quickly and maximally throughout the immersion and extraction procedure. Furthermore, the smaller bone size is intended to aid in the homogeneity of bone with its solvent during the soaking phase [9].

Gelatine Isolation

Soaking Pork, Beef, and Duck Bone

Bone immersion with a 4% concentration of HCl as a solvent. Because HCl can dissolve collagen fibers in a shorter extraction time without harming the quality of the gelatine produced, it was chosen as the solvent [11]. Stirring is done during the immersion procedure to maximize the interaction between the solvent and the sample and hence speed up the demineralization process. The purpose of demineralization is to remove calcium salts and other salts present in the bone so that gelatine is obtained, which contains collagen in it [12]. During the immersion process, the reaction proceeds in four steps, as follows:



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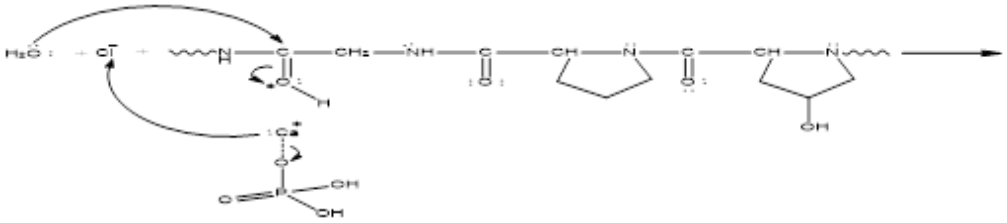

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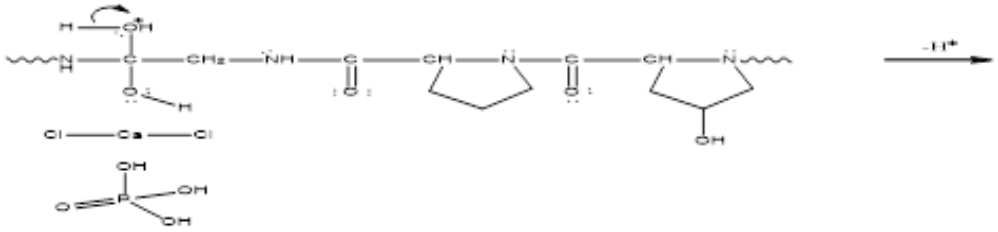

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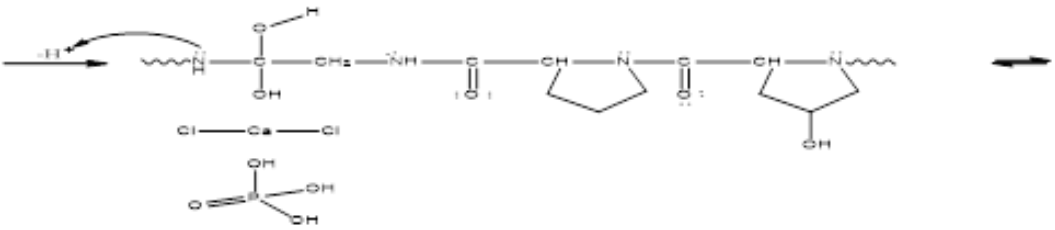

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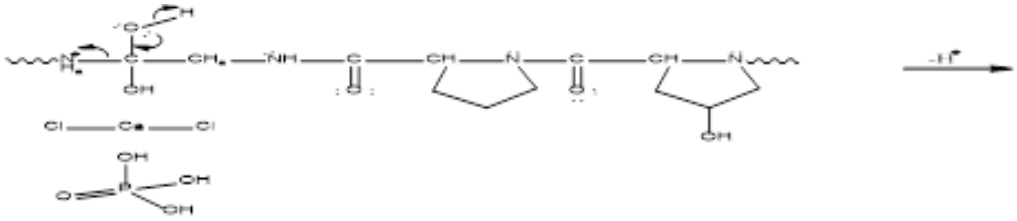

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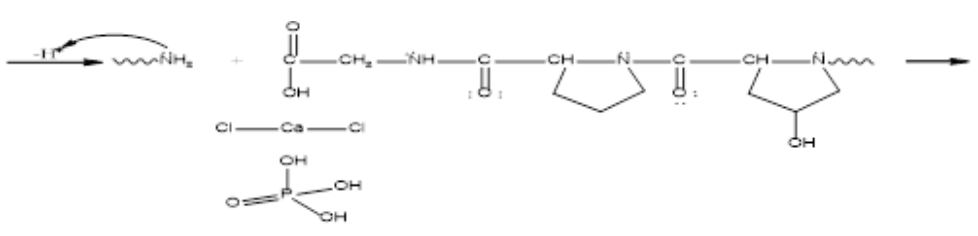

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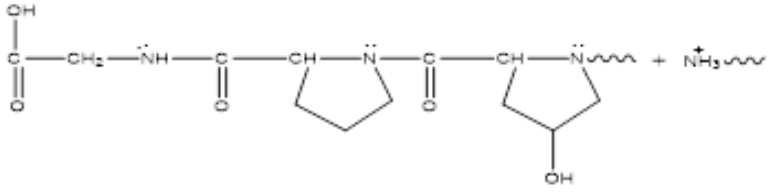


+ CaCl2 + H3(PO4)2

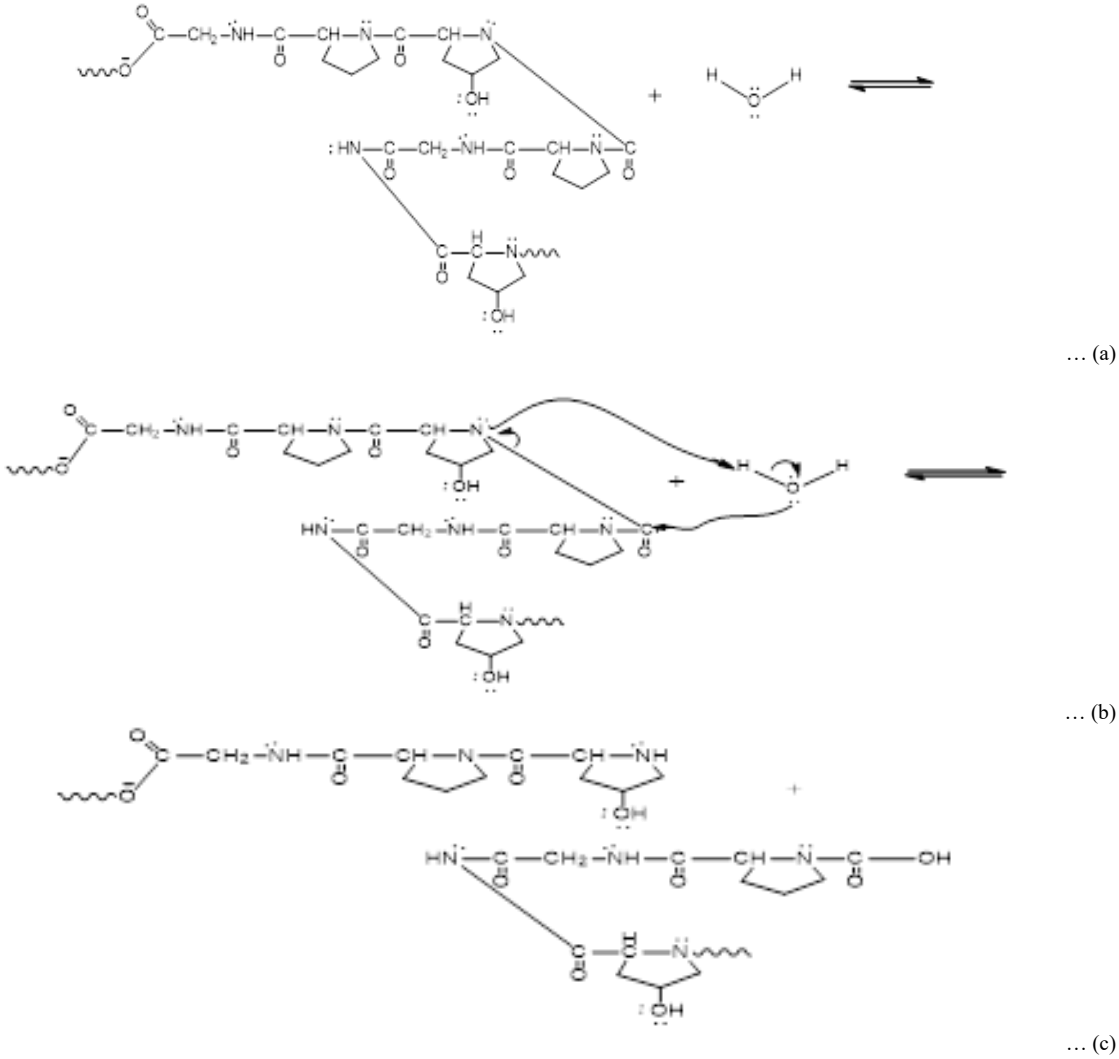
… (7)

Fig. 1: The reaction during immersion process: [13]

(1) Ionization of HCl to H^+ and Cl^-, (2) Attack on the carbocation by the free electron pair of O atoms on H2O, (3) Deformation of OH cations with release of H^+, (4) Attack of the free electron pair on the NH group by H^+, (5) Release of H^+ from the nearest OH group, (6) Breaking the bonds of the C atom with the RNH_2 group, (7) Attack of the NH group by H^+

The Extraction of Gelatine from Pork, Beef, and Duck Bone

The goal of the extraction procedure is to turn collagen into gelatine [14]. For extraction, a microwave and an oven were used. More collagen is converted to gelatine when the hydrolysis time is extended [15]. In addition to the time, the temperature at the time of extraction is modified. Collagen cross-links and hydrogen bonds, which are a stabilizing element for collagen structure, can be destroyed by the gelatine extraction process at 55–70 °C [16]. The stabilizer bond is disrupted by a hydrolysis reaction, commonly known as cross linking.



… (a)

… (b)

… (c)

Fig. 2. The reaction of breaking the stabilizer bond (cross-linking): [12] (a) Reaction of collagen with water, (b) Mechanism of breaking the peptide bond by water, and (c) The triple helix structure becomes a single helix

Stirring was done occasionally during the extraction process to guarantee that the collagen is converted to its full potential and forms a single helix strand that can be dissolved in water and termed gelatine. The current stirring approach affects the collagen hydrolysis process because it expands the contact between the bone and the solvent and prevents clumping during the extraction phase [15]. Filtration, the final stage, eliminates impurities from the gelatine solution.

Gelatine Solution Concentration and Drying

The goal of gelatine solution concentration was to evaporate the solvent included in the gelatine solution, which is achieved by freeze-drying [17]. As a result of the packing process, the gelatine solution was packaged as a concentrated gelatine extract with a slightly chewy texture. The gelatine sheets were dried in an oven at 60 °C for 24 hours, yielding brownish yellow gelatine sheets with a distinct odo r.

(a) (b) (c)

Fig. 3. Various kinds of gelatine from animal bones: (a) Pork (b) Beef (c) Duck

Gelatine Quality Test

Yield

To determine the yield, the final step was to weigh the dried gelatine. If more yields are produced, the therapy will be more successful. The average yield, according to the research, is as follows:

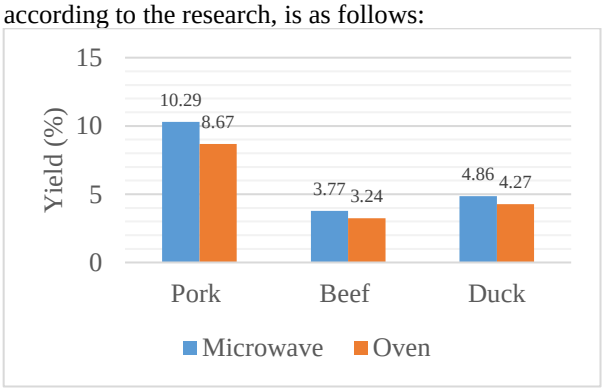


Fig. 4. Average yield of gelatine

The yield of gelatine produced by the microwave method and the yield produced by the oven method are shown in Figure 4. This is most likely due to the microwave method's greater success in eliminating hydrogen bonds between tropocollagen molecules that were not completely dissolved by acid during the immersion process (4 % HCl).

Water content

This test was carried out because gelatine is a form of hydrocolloid material that is soluble in water and may absorb large volumes of water [18]. Based on the outcomes of this investigation, the average value of gelatine water content was calculated:

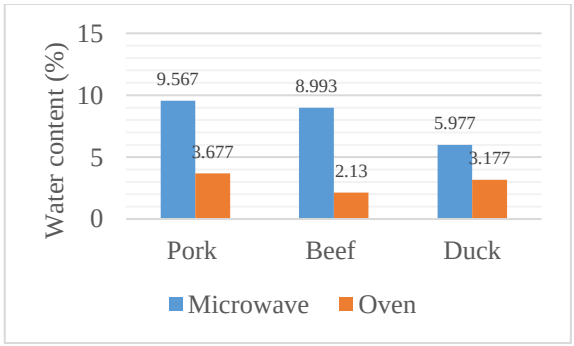


Fig. 5. The average value of gelatine water content

In the microwave, pork bones had the highest moisture content of 9.567 % and duck bones had the lowest of 5.977 %, but in the oven, pork bones had the highest moisture content of 3.677 % and beef bones had the lowest of 2.13 %. This amount still meets the SNI 1995 criterion [19] with a maximum water content of 16 %.

According to the results of statistical tests in table 1, the significant value reached is less than 0.0001 ($p<0.005$), showing that the procedure has a significant effect on the quality of gelatine water content.

Tests of Between-Subjects Effects

Dependent Variable: Moisture Content %

Table 1: Two way ANOVA test results gelatine water content

Source	Type III Sum of Squares	df	Mean Square	F
Corrected Model	147.008a	5	29.402	437,668
Intercept	561,795	1	561,795	8362.813
Sample	12,552	2	6.276	93.422
Method	120.953	1	120.953	1800,493
Sample * Method	13,503	2	6.752	100,503
Error	.806	12	.067	
Total	709,609	18		
Corrected Total	147,814	17		

Ash Level

The average value of gelatine ash content in this study is as follows:

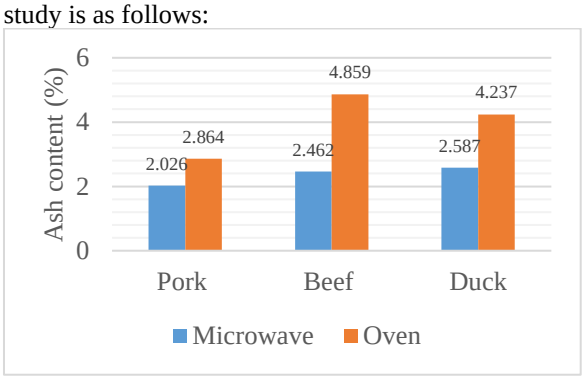


Fig. 6. Average value of gelatine ash content

In the microwave, duck bones had the highest ash level at 2.587 % and pork bones had the lowest at 2.026 %, but in the oven, beef bones had the highest ash content at 4.859 % and pork bones had the lowest at 2.864 %. With a maximum ash concentration of 3.25, the ash content measured using the microwave method is still within SNI 1995 standards. In the oven technique that meets the SNI 1995 standard limitations, only pork bones exceed the maximum limits imposed by SNI 1995, which are 4.237 % and 4.859 %, respectively.

The significant value obtained is smaller than the significant value obtained based on the results of statistical tests in Table 2.

Tests of Between-Subjects Effects

Dependent Variable: Ash Content %

Table 2. Two way ANOVA test results gelatine ash content

Source	Type III Sum of Squares	df	Mean Square	F
Corrected Model	19,748a	5	3.950	41,989
Intercept	177.081	1	177.081	1882,556
Method	13.010	1	13.010	138,313
Sample	4.366	2	2.183	23,209
Method * Sample	2,372	2	1.186	12,608
Error	1,129	12	.094	
Total	197,958	18		
Corrected Total	20,877	17		

pH value

The average pH value obtained in this study is as follows:

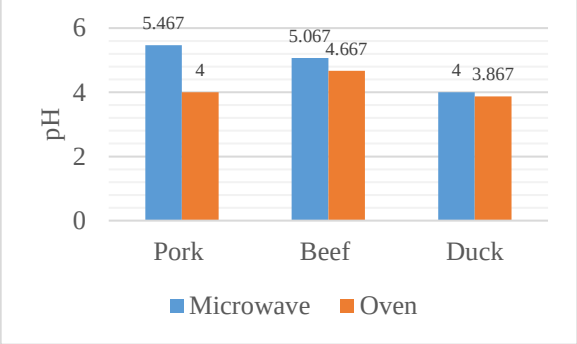


Fig. 7. Average pH value of gelatine

In the microwave method, the maximum pH value was 5.467 on pork bone and the lowest was 4 on duck bone, whereas in the oven method, the highest pH value was 4.667 on beef bone and the lowest was 3.867 on duck bone. This result is within the 3.8-5.5 range established by the Gelatine Manufactures Association of Asia Pacific (GMAP) (2004) for type A gelatine [20].

Tests of Between-Subjects Effects

Dependent Variable: pH

Table 3. Two way ANOVA test results gelatine pH value

Source	Type III Sum of Squares	df	Mean Square	F
Corrected Model	6.551a	5	1,310	58,960
Intercept	366.302	1	366.302	16483,600
Sample	3.058	2	1,529	68,800
Method	2,000	1	2,000	90,000
Sample * Method	1.493	2	.747	33,600
Error	.267	12	.022	
Total	373120	18		
Corrected Total	6.818	17		

Gel Strength

The average value of gel strength in this study is as follows:

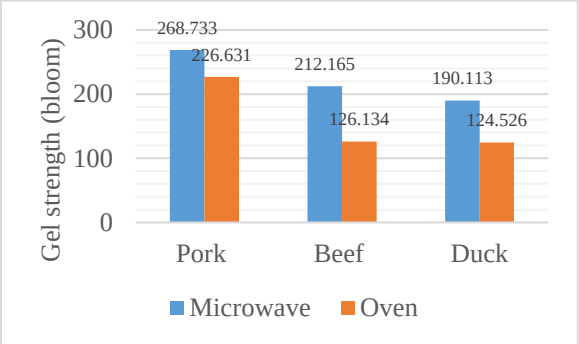


Fig. 8. The average value of gelatine gel strength (bloom)

The greatest gel strength value was 268.733 blooms on pig bone and the lowest was 190.113 blooms on duck bone while using the microwave method. The highest gel strength value was 226.631 blooms on pork bone and the lowest was 124.526 blooms on beef bone while using the oven procedure. This value is still below the British Standards 1975 and GMAP 2004 bloom limit of 50-300 [20].

According to the results of statistical tests in table 4, the significant value reached is less than 0.0001 ($p<0.005$), indicating that the technique has a significant effect on the quality of gelatine gel strength.

Tests of Between-Subjects Effects

Dependent Variable: Gel Strength

Table 4. Two way ANOVA test results gelatine gel strength

Source	Type III Sum of Squares	df	Mean Square	F
Corrected Model	49215.481a	5	9843.096	58,980
Intercept	659816,345	1	659816,345	3953,620
Method	18851.124	1	18851.124	112,956
Sample	28885.558	2	14442.779	86,541
Method * Sample	1478,799	2	739,400	4.430
Error	2002.670	12	166,889	
Total	711034.496	18		
Corrected Total	51218.151	17		

4. Conclusions

Microwave and oven procedures had a substantial effect (p0.005) on the quality of gelatine based on criteria such as water content, ash content, pH value, and gel strength, according to studies. The best quality gelatine test results on each parameter, namely water content 9.567 percent, ash content 2.026 percent, pH value 5.467, and gel strength 268.733 blooms, were obtained utilizing the microwave method.

5. Conflicts of interest

The authors declare there are no conflicts of interest.

6. References

[1] Nirwandar, S. Halal Gelatine and its Business Opportunity in Indonesia. International Journal of Halal Research. 2020 June 30; 2(1):50-57.

[2] Bahar, A., Rusijono, R., Kusumawati, N. Extraction and characterization of the base halal gelatine based on bovine bone. Adv. Eng. Res. Proc. Natl. Semin. Chem. 2018 September; 171(9):46-49.

[3] Rakhmanova, A., Khan, Z. A., Sharif, R., Lv, X. Meeting the requirements of halal gelatine: A mini review. MOJ Food Proc. Technol. 2018 November 22. 6: 477-482.

[4] Khirzin, M. H., Hilmi, M. Study of Gelatine from Duck Bone As An Alternative Sources of Halal Gelatine. Jurnal Sains Natural. 2020 January. 10(1):1-5.

[5] Setyawaty, R., Triliandari, M. Gelatine Production from Skin of Chicken Leg using A Variety of Naoh Concentration. Jurnal Ilmu dan Teknologi Hasil Ternak (JITEK). 2018 October 28. 13(2):126-132.

[6] Kim, T. K., Ham, Y. K., Shin, D. M., Kim, H. W., Jang, H. W., Kim, Y. B., & Choi, Y. S. Extraction of crude gelatine from duck skin: effects of heating methods on gelatine yield. Poultry science. 2019 September. 99(1):590-596.

[7] Park J, Choe J, Kim H, Hwang K, Song, D, Yeo E, Kim H, Choi Y, Lee S, Kim C. Effects of Various Extraction Methods on Quality Characteristics of Duck Feet Gelatine. Korean J. Food Sci. An. 2013 April; 33(2):162-169.

[8] Gruber, L., Seidl, L., Zanetti, M., Schnabel, T. Calorifoc Value and Ash Content of Extracted Birch Bark. Forests. 2021 September 7. 10: 2-9.

[9] Association of Official Agricultural Chemist. Official Methods of Analysis of The Association of Official Analytical Chemist. Inc. Washington, DC. 1995.

[10] Junianto, Azzahra, H.Y., Misbahul, A., Taqiyyuddiin, N. Fish Bone Gelatine. Asian Journal of Fisheries and Aquatic Research. 2021 June 3. 12(4):36-44.

[11] Yeh, M.Y., Zhao, J.Y., Hsieh, Y.R., *et al*. Reverse Thermo-responsive Hydrogels Prepared from pluronic F127 and Gelatine Composite Materials. Royal Society of Chemistry. 2017 April 18. 7 : 2152-2157.

[12] Kadarani, D.K and Jannah, A. Optimization Chicken Bones Gelatine Extraction Using Hydrochloric Acid Immersion and Multi-Stage Thermal Treatment. Alchemy: Journal of Chemistry. 2022 March 8. 10 (1) : 1-7.

[13] Silviana, N.R., Junianto. 2021. Review Article: The Utilization of Fish Bone for Gelatine. Global Scientific Journals. 2021 November. 9(11):261-269.

[14] Ni'mah, N. Pengaruh Konsentrasi HCl terhadap Proses Demineralisasi pada Produksi Gelatine dari Tulang Ayam Broiler (Gallus domesticus). Thesis. Malang. Fakultas Farmasi UIN Malana Malik Ibrahim. 2017

[15] Liu, T., Dai, H., Ma, L., *et al*. Structure of Hyla Rabbit Skin Gelatine As Affected by Microwave-Assisted Extraction. International Journal of Food Properties. 2019 September 19. 22 (1) : 1594-1607.

[16] British Standard 757. Sampling and Testing of Gelatine. In Imeson. 1992. Thickening And Gelling Agents for Food. New York: Academic Press. 1975.

[17] Jafari, H., Lista, A., Siekapen, M. M., Ghaffari-Bohlouli, P., Nie, L., Alimoradi, H., & Shavandi, A. Fish collagen: extraction, characterization, and applications for biomaterials engineering. Polymers. 2020 September 28; 12(10):22-30.

[18] Abdullah, M.S.P., Noordin, M.I., Ismail, S.I.M., Nyamathulla, S., Jasamai, M., Wai, L.K., Mustapha, N.M, Shamsuddin, A.F. Physicochemical Evaluation and Spectroscopic Characterisation of Gelatine from Shank and Toes of Gallus dometicus. Sains Malaysiana. 2016. 45(3):435-449.

[19] SNI (Standar Nasional Indonesia). 06.3735. Mutu dan Cara Uji Gelatine. Dewan Standarisasi Nasional. Jakarta; 1995

[20] Website information GMAP (Gelatine Manufactures Association of Asia Pacific). Gelatine. Gelatine Food Science.; [updated 2004; cited 2022 May 17]. Available from http://www.gelatine.co.za/gltn1.html.