



Antimicrobial Properties of Pineapple Peel Extract in Beef Skin Gelatin-Based Meatball Products

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Abstract

The aim of this study was to determine the microbiological parameters of meatballs prepared with beef skin gelatin extracted with pineapple peel after various storage periods. A laboratory approach to experiments and descriptive analysis were used. Beef skin was pretreated with pineapple peel extract for 12, 24, 30 and 40 hours before the gelatin extraction. The gelatin produced was subjected to Fourier Transform Infrared (FTIR) spectroscopy and amino acid analysis which were used to characterize it, and then used in meatball products. The meatballs were kept for 14 and 21 days at 10°C and then microbiological tests were conducted to assess Total Plate Count (TPC), Gram staining and biochemical tests. The FTIR analysis showed amide characteristic functional groups that are associated with the formation of gelatin, and the amino acid profiling also showed that glycine and proline are the main components. Bacterial count was higher with the longer storage time for all pretreatment groups as revealed by the TPC results. The 24-hour pretreatment group had the lowest count of bacteria after 14 days (4.4×10^4) and the lowest count after 21 days (100×10^5) was recorded in the 30-hour pretreatment group. Microbiological examination showed Gram-negative bacteria (*Neisseria* sp. and *Escherichia coli*) and Gram-positive bacteria (*Staphylococcus aureus*). The results showed that meatballs with pineapple peel extract-pretreated beef skin gelatin were still detectable microbial contamination during a long storage period. Further controlled investigations are needed to establish its antimicrobial effectiveness.

Introduction

Meatballs are one of the most widely consumed meat-based food products in Indonesia and have considerable potential for further development as value-added food products. However, due to their relatively high moisture and nutrient content, meatballs are susceptible to spoilage caused by microbial growth during storage. Microbial growth can not only reduce organoleptic quality, including aroma, color, texture, and taste, but can also affect food safety (Anumudu et al., 2024; Francis et al., 2012). Therefore, efforts are needed to maintain the microbiological quality of meatballs during storage through the use of safe preservatives derived from natural sources (Mia et al., 2025; Afidah et al., 2025; Jaworska et al., 2021; Teshome et al., 2022; Elhassaneen et al., 2023).

Gelatin is a promising ingredient for the development of meatball products. Gelatin can be obtained from beef hides through a series of processing steps and has the ability to improve the textural characteristics, water-holding capacity, and functional properties of food products

(Wulandari & Yuliatmo, 2018; Reji et al., 2025; Zhang et al., 2023). The utilization of beef skin-derived gelatin also provides added value to livestock industry by-products and supports the concept of a sustainable livestock industry through the more optimal utilization of animal by-products. Nevertheless, the use of gelatin in food products does not directly ensure microbiological stability; therefore, additional approaches are required to control microbial growth (Ying et al., 2025; Xu et al., 2025; Mahamud et al., 2023; Yarahmadi et al., 2024).

One natural source with potential antimicrobial activity is pineapple peel (*Ananas comosus*). Pineapple peel is an abundant agro-industrial by-product, but its utilization remains relatively limited. Pineapple peel contains various bioactive compounds, including phenolic compounds, flavonoids, and other components that may exhibit antioxidant and antimicrobial activities (Dewi & Simamora, 2023; Polania et al., 2023; Meena et al., 2022; Hashim et al., 2024). The utilization of pineapple peel extract as a natural ingredient in food products may provide an alternative approach to increasing the value of agricultural by-products while supporting the implementation of green technology and the circular economy.

The incorporation of pineapple peel extract into beef skin gelatin-based meatballs is expected to inhibit microbial growth during storage (Sarmast et al., 2023). This activity can be evaluated through the Total Plate Count (TPC) test as one of the parameters used to determine the total number of viable microorganisms growing in the product. Monitoring TPC during storage, for example on days 14 and 21, can provide information regarding the ability of pineapple peel extract to maintain the microbiological quality of meatballs.

Based on the above considerations, research entitled “Antimicrobial Properties of Pineapple Peel Extract in Beef Skin Gelatin-Based Meatball Products” is important to evaluate the potential of pineapple peel extract as a natural ingredient for inhibiting microbial growth in beef skin gelatin-based meatballs. This study is expected to provide scientific information on the utilization of pineapple peel and beef skin by-products to produce value-added food products, while supporting the development of safer, functional, and sustainable food products.

Methods

Research Design and Location

The present study was conducted using an experimental method in the laboratory with descriptive analysis to determine the characteristics of beef skin gelatin produced by the use of pineapple peel extract (PPE) before the gelatin was used in meatballs processing and to determine the microbial contamination present in meatballs products. The research was carried out from February until August 2024 at the Microbiology and Biotechnology Laboratory (Farm Lingsar) and Animal Product Processing Technology Laboratory (APPT) in Faculty of Animal Science, Universitas Mataram, Indonesia, and Microbiology and Animal Health Laboratory (MHL) in Faculty of Animal Science, Universitas Hasanuddin, Indonesia. The study consisted of two main stages: characterization of gelatin obtained from pineapple peel extract-pretreated beef skin and microbiological evaluation of meatball products during refrigerated storage.

Preparation of Gelatin and Experimental Treatments

The primary material used for the production of gelatin was beef skin while pineapple peel extract was used as pretreatment agent for facilitating the collagen processing before the gelatin extraction. The results of the experiments were categorized into four groups, namely: A12 (soaking for 12 hours), A24 (soaking for 24 hours), A30 (soaking for 30 hours) and A40 (soaking for 40 hours). After the pretreatment, the beef skin was processed to yield gelatin and used in meatball products.

The meatball samples were evaluated after 14 and 21 days of storage at 10°C in order to study the changes in microbial populations during the storage period. Bacterial counts were compared between the pretreatment groups and potential differences related to long storage periods were noted during these observation periods.

Characterization of Beef Skin Gelatin

The gelatin extracted with pineapple peel extract pretreatment was characterized by Fourier Transform Infrared (FTIR) spectroscopy and amino acid profiling. The functional groups responsible for the formation of gelatin were identified by FTIR analysis of the spectra in the range of 4,000–500 cm⁻¹. Structural characteristics related to collagen denaturation and gelatin formation were described by analyzing the characteristic amide regions (Amide A, I, II, III).

The amino acid profile was used to determine the composition of the gelatin produced. Glycine, proline, arginine, alanine, glutamic acid and other amino acids detected were included in the examination. The amino acid content that is recorded was analyzed descriptively for information of the chemical composition of the gelatin produced by pineapple peel extract pretreatment.

Total Plate Count Analysis

The meatball products were analysed for their microbial load by Total Plate Count (TPC) using pour plate technique. Samples of meatballs from each of the pretreatment groups were observed after 14 and 21 days of storage at 10°C. A sample of the meatballs was taken for laboratory analysis and was 1 g in mass.

The method used for the analysis was the growth of bacteria in microbiological growth media and counting of the colonies to quantify the total bacteria. The recorded TPC values were arranged based on the pretreatment time and storage time. The differences in bacterial counts of the four pretreatment groups and differences in bacterial counts between the two storage periods were described using these measurements.

Study of the isolation and identification of bacteria. Isolation and Identification of bacteria.

Bacterial identification of stored meatball samples was carried out to determine the type of microorganisms present in meatballs which may be a cause of microbial contamination. The bacterial culture, Gram staining and biochemical tests were performed. The bacterial cultivation was carried out on the culture media which are listed in the laboratory procedures such as Nutrient Agar and Endo Agar.

Gram staining was conducted to determine the Gram positive and Gram negative bacterial isolates by the staining properties and the microscopic morphology. The biochemical tests performed were those of methyl red (MR), Voges–Proskauer (VP), Triple Sugar Iron Agar (TSIA), sulfide-indole-motility (SIM) and carbohydrate utilization glucose and maltose.

The identification results were evaluated descriptively in terms of the morphological, Gram staining and biochemical reactions recorded. The bacterial groups detected in the meatballs during storage were described using these examinations.

Data Analysis

Analysis of the laboratory findings was done descriptively based on the duration of application of pretreatment and storage periods. FTIR spectra were analyzed to describe the functional groups related to the gelatin formation and the amino acid profiling results were used in characterization of the gelatin composition.

The total Plate Count values between the four pretreatment treatments were compared at 14 and 21 days of storage. The comparison was made on the basis of the observed differences in the numbers of bacteria and changes which occurred during the storage period. The results of identification of bacteria were summarized based on morphological characteristics, gram staining and biochemical tests. The interpretation was confined to the descriptive findings discussed in the study. Microbial differences were not considered to be statistically significant unless supported by microbially inferential statistical analysis.

Results and Discussion

Quality of Beef Skin Gelatin Pretreated with Pineapple Peel

FTIR of Beef Skin Gelatin Pretreated with Pineapple Peel

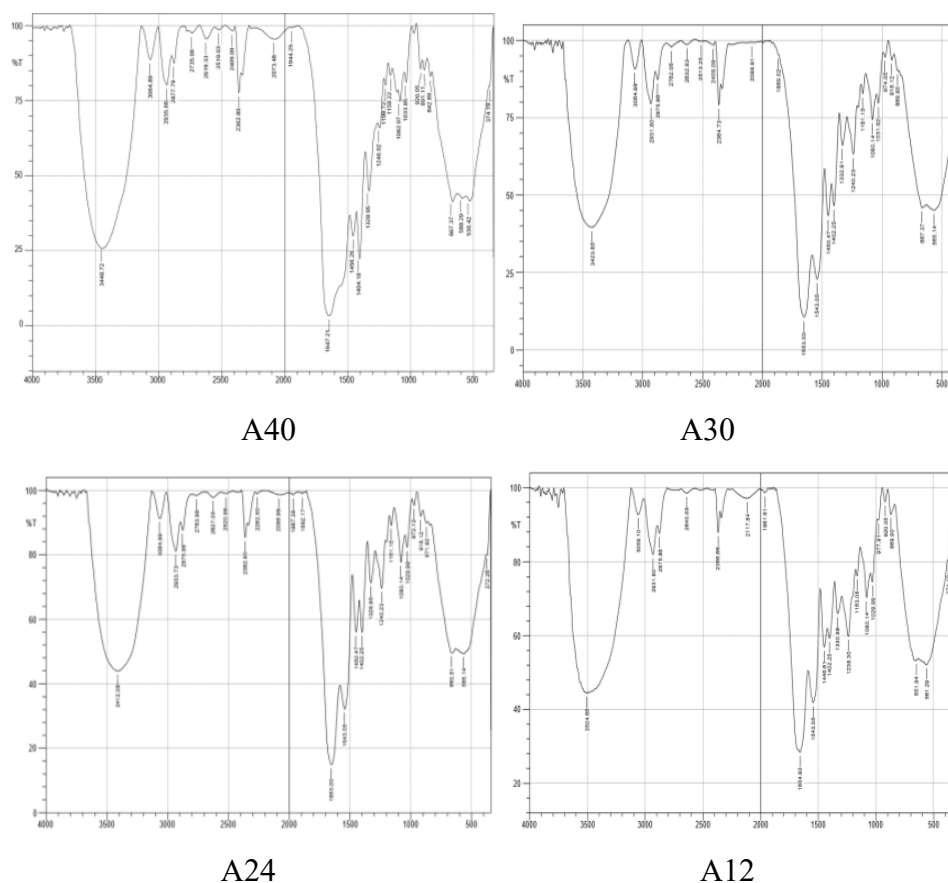


Figure 3. FTIR of beef skin gelatin pretreated with pineapple peel.

Note: A40 = soaking beef skin with pineapple peel for 40 hours; A30 = soaking beef skin with pineapple peel for 30 hours; A24 = soaking beef skin with pineapple peel for 24 hours; A12 = soaking beef skin with pineapple peel for 12 hours.

The functional groups of goat-skin gelatin were observed from 4000 cm^{-1} to 500 cm^{-1} (Figure 3). Gelatin from skin and beef skin pretreated with pineapple peel showed differences at soaking durations of 40, 30, 24, and 12 hours, detected at a wavenumber of 3562.52 cm^{-1} , whereas gelatin from skin and goat skin aged 2 and 3 years did not show differences. However, pineapple-peel pretreatment (3566.38 cm^{-1}) showed a difference from acetic acid (3564.45 cm^{-1}).

Amide A in skin and goat-skin gelatin occurred at 3566.38–2854.65 cm^{-1} . This result was not very different from the findings of Muyonga et al. (2004), who reported that the Amide A

region was identified at wavenumbers of 3600–2300 cm^{-1} . N–H stretching at the Amide A peak indicates the presence of hydrogen bonds. The Amide A wavenumber of 3562.52–2875.86 cm^{-1} in one-year-old material treated with pineapple peel extract approached the vibration range reported by Kaewruang et al. (2013), who stated that wavenumbers of 3400–3440 cm^{-1} indicate molecular denaturation of gelatin. Gelatin polypeptides have two terminal groups: a carboxyl group at the right end and an amino group at the left end. These two ends allow gelatin to form hydrogen bonds (Aprilyani et al., 2013). Hydrogen-bond formation in gelatin may enable free-radical capture and thus contribute to antioxidant properties (Li et al., 2014).

Amide I in skin and goat-skin gelatin was described as involving hydrogen bonds of carboxylic acid with C–O–H bonds. Figure 24 shows wavenumbers of 2358.94–2126.56 cm^{-1} in one-year-old material pretreated with pineapple peel extract. Ogwok et al. (2008) stated that Amide I absorption is used to analyze the secondary structure of proteins. Amide I also represents the stretching vibration of C=C bonds (1695.43–1519.91 cm^{-1}) in two-year-old material pretreated with pineapple peel extract. The observed wavenumber range was described as meeting the gelatin-coil structure, consistent with Ogwok et al. (2008), who reported a characteristic gelatin-coil absorption peak around 1633 cm^{-1} .

Amide II in skin and goat-skin gelatin, with a C–N stretching peak, was attributed to N–H vibration (Ogwok et al., 2008). Material aged 1, 2, and 3 years and pretreated with pineapple peel extract had the same wavenumber value of 1452.40 cm^{-1} , while acetic-acid pretreatment had a value of 1456.24 cm^{-1} .

Amide III was detected at wavenumbers of 1080.14–669.30 cm^{-1} in skin and goat-skin gelatin, with C–OH stretching at 1080–1028.06 cm^{-1} . N–O–C stretching under pineapple-peel-extract pretreatment tended to be lower (698.23–673.16 cm^{-1}) than under acetic-acid pretreatment (704.02–669.30 cm^{-1}). The change was attributed to the loss of the collagen triple-helix structure during denaturation into gelatin (Zhu et al., 2019).

Amino Acid Profile of Beef Skin Gelatin Pretreated with Pineapple Peel

Table 1. Amino acid profile of beef skin gelatin pretreated with pineapple peel

No.	Peak Name	Amount
1	L-Histidine	5.947
2	L-Serine	42.473
3	L-Arginine	29.633
4	Glycine	1188.284
5	L-Aspartic Acid	118.337
6	L-Glutamic Acid	206.666
7	L-Threonine	15.402
8	L-Alanine	247.467
9	L-Proline	215.103
10	AABA	135.903
11	L-Lysine	47.688
12	L-Tyrosine	3.346
18	L-Valine	26.212
13	L-Isoleucine	10.921
14	L-Leucine	34.770
15	L-Phenylalanine	15.704

The amino acid content of gelatin is characteristic of gelatin. The presence of glycine, proline, and hydroxyproline in skin and goat-skin gelatin indicates its characteristic amino acid composition. According to Kadler et al. (2007), the primary structure of collagen consists of a repeating Gly-X-Y sequence, in which X and Y are commonly proline and hydroxyproline. Gelatin is a derivative of collagen (Said et al., 2011).

The highest arginine content among the amino acids in skin and goat-skin gelatin was described as indicating antioxidant activity. This is supported by Nongonierma et al. (2013), who reported that free amino acids, including arginine (Arg) and tyrosine (Tyr), were identified as amino acids associated with inhibition of dipeptidyl peptidase IV (DPP-IV), inhibitory activity, and oxygen radical absorbance capacity (ORAC), indicating high antioxidant activity.

Sarboon et al. (2018) identified glycine and proline as gelatin amino acids that contribute to free-radical scavenging. Jiang et al. (2018) reported that leucine has strong antioxidant activity. Liu et al. (2016) suggested that tyrosine, tryptophan, and phenylalanine may also act as free-radical scavengers.

Microbial Identification in Beef Skin Gelatin-Based Meatballs Treated with Pineapple Peel Extract

Total Plate Count (TPC) of Beef Skin Gelatin-Based Meatballs Pretreated with Pineapple Peel The Total Plate Count (TPC) of beef skin gelatin-based meatballs pretreated with pineapple peel is presented in Table 2.

Table 2. Total Plate Count (TPC) of beef skin gelatin-based meatballs pretreated with pineapple peel.

Pretreatment (hours)	Storage duration: 14 days	Storage duration: 21 days
A40	6.7×10^4	148×10^5
A30	7×10^4	100×10^5
A24	4.4×10^4	124×10^5
A12	7.7×10^4	110×10^5

The source text states that fermentation duration in skin and goat skin had a highly significant effect ($P < 0.01$) on total bacteria (TPC). Goat age did not show a significant effect ($P > 0.05$) on total bacteria (TPC). Goat skin showed relatively rapid growth with fermentation durations of 24, 48, and 72 hours. Total bacteria in goat-skin gelatin-based meatballs ranged from 8.24 $\log_{10}$ CFU/ml to 9.67 $\log_{10}$ CFU/ml, and these values were reported as higher.

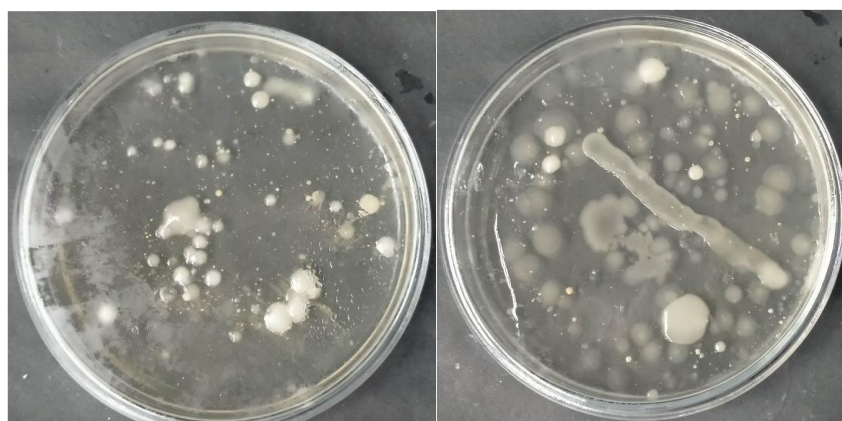


Figure 4. Microbial growth in meatballs based on gelatin pretreated with pineapple peel extract after 14 days of storage.

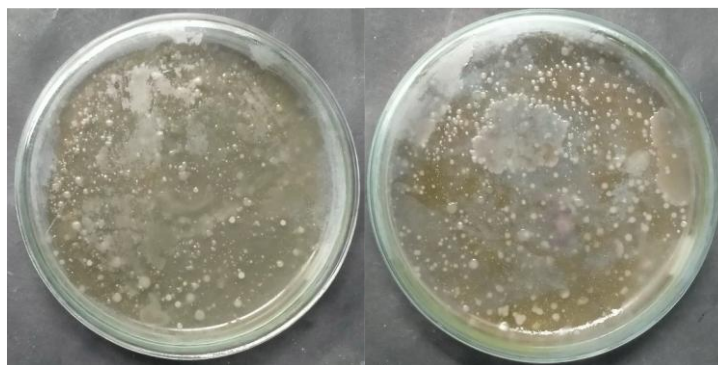


Figure 5. Microbial growth in meatballs based on gelatin pretreated with pineapple peel extract after 21 days of storage.

The difference in total population indicated that bacterial growth increased with longer storage (21 days). These conditions may still allow microorganisms to grow because sufficient nutrients are available for microbial cell growth and development (Naharia et al., 2018). This is consistent with Yang et al. (2018). Each microbial cell can synthesize its own body constituents from simple substances found in its environment for growth (Irianto et al., 2010). A suitable environment, including adequate nutrient availability, increases bacterial productivity during fermentation and affects the number of microorganisms produced.

Bacterial Identification in Meatballs

The identification results for gelatin-based meatballs stored for 21 days showed Gram-negative bacteria, namely *Neisseria* sp. and *E. coli*, and Gram-positive bacteria, namely *Staphylococcus aureus*.

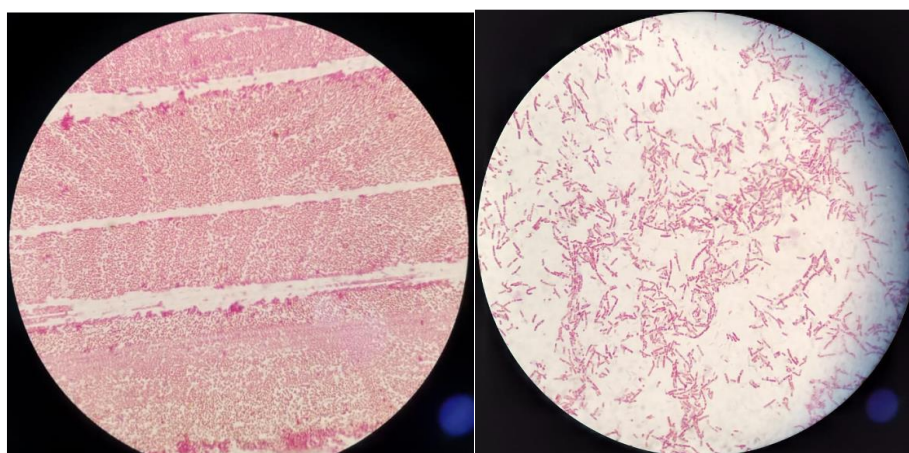


Figure 2. Neisseria sp. (Gram-negative) in gelatin-based meatballs stored for 21 days at 10°C.

Neisseria sp. is a Gram-negative, coccus-shaped bacterium with a diameter of 0.6–1.0 μm . These organisms are usually seen in pairs, with the adjacent sides flattened. Pili, hair-like filamentous appendages extending several micrometers from the cell surface, play a role in attachment.

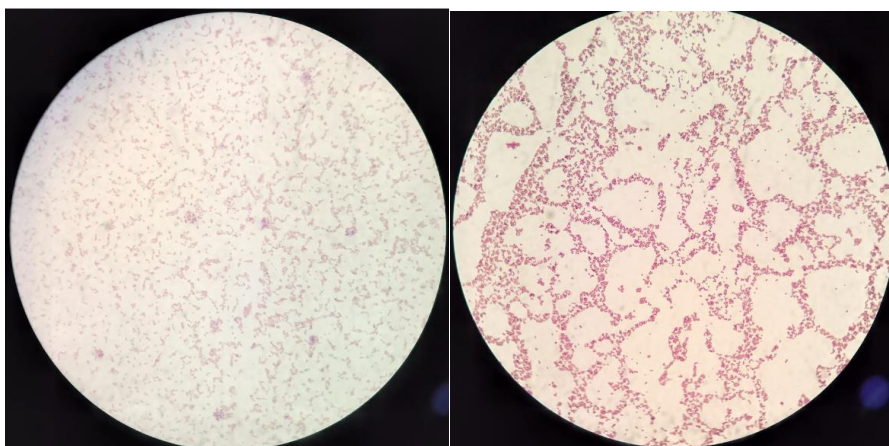


Figure 3. Escherichia coli and Gram-positive bacteria in gelatin-based meatballs stored for 14 days at 10°C.

Escherichia coli is a bacterial species found in humans. Most *E. coli* are harmless, but some, such as *E. coli* O157:H7, can cause serious food poisoning in humans, including bloody diarrhea, due to the exotoxin known as verotoxin.

Structural Characteristics and Functional Properties of Pineapple Peel-Pretreated Gelatin

The FTIR and amino acid results indicate that the pretreatment using pineapple peel extract can be included in the process of producing new gelatin from beef skin without significantly altering the structure of collagen-derived proteins. Ahmad et al. (2021) showed that enzymatic pretreatment affects gelatin yield, molecular organization, viscosity and gel strength. Based on the results, they suggest that gelatin cannot always be extracted from appropriate fish protein which is suitable for functional performance, because protein will undergo extensive degradation may affect the gelatin properties. Likewise, Li et al. (2025) showed that different processing conditions for bovine hides had an impact on the gelatin composition and the physicochemical properties, suggesting that processing conditions could be used to alter the properties of gelatin for food applications.

The present study shows that glycine is predominant and proline present as observed in collagen derived gelatin. But these amino acids are mainly used to support the structural and functional properties and not as a proof of antimicrobial activity itself. The pineapple peel is rich in phenolic and other functional compounds, indicating its potential as a source of bioactive ingredients (Nordin et al., 2023). However, their detection in the original plant does not guarantee their extraction into gelatin. Vargas-Serna et al. (2022) demonstrated that the extraction process and extraction solvent used can affect the recovery of phenolics from pineapple peel, and Polanía et al. (2022) demonstrated that the processing conditions can affect the recovery of phenolics and the antioxidant activity. The antimicrobial activity of pineapple derived gelatin indicated in these studies, is dependent not only on the material used in the pretreatment process, but also on the recovery and stability of the bioactive agents, as well as their availability.

An antimicrobial potential of pineapple peel extract in meat products. Antimicrobial potential of pineapple peel extract in meat products

However, the fact that the meatball samples contained microbial contamination throughout the storage period suggests that the formulation of gelatin investigated did not fully inhibit the growth of bacteria during storage. This is different from what was found in pineapple peel

extract (which is not effective against the antimicrobial activity). Jatav et al. (2022) showed that pineapple peel extracts exhibited inhibitory activity against a number of food-borne bacteria and enhanced the antibacterial activity when these extracts were combined with chitosan film. They found that the composition and concentration of the extracts and the type of delivery matrix are critical for antimicrobial effect. Because direct incorporation into antimicrobial films would not allow for the same exposure of microorganisms to the active compounds from pineapples, pretreating beef skin before gelatin extraction might not provide the same exposure of the microorganisms to the active compounds in pineapples.

Bai et al. (2025) also noted that the interactions between phenolic compounds and food proteins or other macromolecules could impact their antimicrobial activity. Such interactions are applicable to the case of meatballs which contain gelatin, as in this case the availability of active substances may be changed by the complex protein matrix. The importance of food matrix characteristics, microbial population, and application conditions on natural antimicrobial performance in processed meat has also been mentioned by Papadochristopoulos et al. (2021). Thus, the contamination observed was not to be viewed as evidence of the biological inactivity of the pineapple peel extract, but as an indication that microbial growth was still possible under the conditions investigated.

Microbial Growth and Storage Stability of Gelatin Based Meatballs

The bacterial counts after storage indicated the significance of storage conditions on the microbiological stability of the gelatin based meatballs. Li et al. (2021) showed that the bacterial community in meatballs could be altered during refrigerated storage, and thermal treatment could affect the growth of bacteria and deterioration of products. This confirms the notion that bacterial levels are affected by the processed history and storage conditions and not just a single functional ingredient. Similarly, Zhu et al. (2022) found that the meat spoilage process was a complex ecological process with interactions among the bacteria and the environment, so the increase or decrease of microbial growth was not solely due to the length of the gelatine pre-treatment.

One important aspect of these findings is the storage temperature. However, psychrotrophic microorganisms have been known to grow in animal derived foods at refrigerated temperatures as reported by Oh and Lee (2024) and thus the use of refrigerated temperatures does not necessarily prevent microbial deterioration. Therefore, storage at 10°C should not be taken as implying that it will be adequate protection against bacterial growth for long-term storage. Similarly, Olvera-Aguirre et al. (2023) reported that plant-based preservatives might contribute to the protection against microbial degradation in meat products, depending on the type and the amount of bioactivity and the food matrix. The present study has therefore been interpreted with prudence between the various pretreatment groups due to differences in the results obtained and the absence of statistically significant antimicrobial differences or the mechanisms involved in the growth of bacteria.

Future Preservation Strategies and Implications for Food Safety

The identification of Gram-positive and Gram-negative bacteria is an important matter of concern when it comes to microbial contamination and preservation of gelatin-based meat products. But morphological and biochemical results should be considered in the light of the resolution of the identification methods employed. The authors of Moura-Alves et al. (2023) stated that for effective antimicrobial applications in meat preservation, microbial safety, product stability, and compatibility of active ingredients with the food system must be taken into account. Furthermore, Zhang et al. (2025) highlighted the significance of antimicrobial

delivery systems and controlled exposure in enhancing microbial inhibition, suggesting that antimicrobial agents without these additional factors may not ensure preservation.

The results also indicate that using pineapple peel extract-pretreated gelatin could not be classified as an antimicrobial preservative but rather as a functional ingredient. A systematic review by Karbowiak et al. (2023) showed that the combination of microbial control measures was more effective in inhibiting spoilage and pathogenic microorganisms than using microbial control measures separately. Biopreservatives could also be used in conjunction with other preservation methods to enhance meat safety and storage stability, as noted by Rodríguez-Marca et al. (2025). In the meantime, Ahmadi et al. (2024) highlighted the need for phytochemical characterization, optimization of extraction, antimicrobial evaluation, and safety analysis in the development of plant-based food preservatives. In this regard, future studies should involve the use of untreated control formulations, identification of the microbes, quantified bioactive compounds, and storage comparison to see if the use of pineapple peel-derived gelatin can offer any measurable antimicrobial benefits other than the regular functional properties.

Conclusion

This study demonstrated that beef skin gelatin produced through pineapple peel extract pretreatment exhibited characteristic gelatin functional groups, as confirmed by FTIR analysis, and contained amino acids predominantly represented by glycine and proline. Microbiological examination revealed that bacterial populations in gelatin-based meatballs increased with prolonged storage at 10°C. Among the pretreatment groups, the lowest Total Plate Count (TPC) after 14 days was observed in the 24-hour pretreatment group (4.4×10^4), while the 30-hour pretreatment group showed the lowest bacterial count after 21 days (100×10^5). Bacterial examination also reported the presence of *Neisseria* sp., *Escherichia coli*, and *Staphylococcus aureus*, indicating microbial contamination during storage. These findings suggest that pineapple peel extract-pretreated beef skin gelatin did not prevent bacterial growth under the investigated storage conditions. However, its specific antimicrobial effectiveness cannot be conclusively established without an appropriate control group. Further studies incorporating untreated controls, standardized microbial testing, and additional storage conditions are recommended to evaluate its potential application in improving the microbiological stability of meatball products.

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