



Evaluation of Cooking Methods for Reducing Nitrite Content in Ham

Evaluasi Metode Pemasakan untuk Mengurangi Kandungan Nitrit Pada Ham

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Abstract. Nitrite is a common food additive used to enhance the safety and shelf life of processed meat products. However, recent concerns have emerged regarding its potential link to colorectal cancer in humans. Despite this, the effectiveness of different cooking methods in removing nitrite from processed meats remains unclear. This study aimed to identify cooking methods that effectively reduce nitrite content in ham. Three cooking methods—boiling, pan-frying, and microwave heating—were evaluated. Nitrite content in ham was measured using the diazotization-coupling reaction. The results showed that boiling significantly reduced the nitrite content in ham compared to the uncooked state. In contrast, pan-frying and microwave heating had no significant effect. Long-term storage also reduced nitrite levels, and combining storage with boiling had a synergistic effect in further reducing nitrite content. Additionally, soaking ham in condiments prior to cooking was found to be effective in lowering nitrite levels. Notably, soaking in grain vinegar resulted in a substantial reduction. Overall, the study demonstrated that boiling is an effective method for reducing nitrite content in ham. Furthermore, extended storage and pre-soaking in condiments, particularly grain vinegar, also contributed to nitrite reduction.

Keywords: food additive, nitrite, cooking methods

Abstrak. Nitrit merupakan zat aditif makanan umum yang digunakan untuk meningkatkan keamanan dan masa simpan produk daging olahan. Namun, kekhawatiran baru-baru ini muncul mengenai potensi kaitannya dengan kanker kolorektal pada manusia. Meskipun demikian, efektivitas berbagai metode pemasakan dalam menghilangkan nitrit dari daging olahan masih belum jelas. Penelitian ini bertujuan untuk mengidentifikasi metode pemasakan yang efektif mengurangi kandungan nitrit dalam ham. Tiga metode pemasakan – perebusan, penggorengan, dan pemanasan microwave – dievaluasi. Kandungan nitrit dalam ham diukur menggunakan reaksi diazosisasi-kopling. Hasil penelitian menunjukkan bahwa perebusan secara signifikan mengurangi kandungan nitrit dalam ham dibandingkan dengan keadaan tidak dimasak. Sebaliknya, penggorengan dan pemanasan microwave tidak memberikan efek yang signifikan. Penyimpanan jangka panjang juga mengurangi kadar nitrit, dan menggabungkan penyimpanan dengan perebusan memiliki efek sinergis dalam mengurangi kandungan nitrit lebih lanjut. Selain itu, merendam ham dalam bumbu sebelum dimasak terbukti efektif dalam menurunkan kadar nitrit. Terutama, perendaman dalam cuka biji-bijian menghasilkan penurunan yang substansial. Secara keseluruhan, penelitian ini menunjukkan bahwa perebusan merupakan metode yang efektif untuk mengurangi kandungan nitrit dalam ham. Lebih lanjut, penyimpanan yang lebih lama dan perendaman awal dalam bumbu, terutama cuka biji-bijian juga berkontribusi untuk penurunan nitrit.

Kata kunci: bahan tambahan makanan, nitrit, metode pemasakan

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INTRODUCTION

Food additives are widely used around the world to enhance food quality (Authority, Linares, Salvatore, Belmonte, & Ruiz, 2025). More than 1,000 food additives are approved and utilized globally, and they are classified into various categories, such as sweeteners, colorants, and preservatives, based on their intended purposes. Each additive has an established Acceptable Daily Intake (ADI), which indicates the amount considered safe for human health, and strict safety standards have been established in each country accordingly (Lalani et al., 2024; Inoue et al., 2025).

Nitrite is a food additive primarily used as a color retention agent in processed meat products (Crowe, Elliott, & Green, 2020; Stos et al., 2024). Myoglobin is normally present in fresh meat (purple-red in color), and it binds with oxygen during meat processing to become oxymyoglobin (bright red). Over time, oxymyoglobin oxidizes to metmyoglobin (brown), and during heating in the production of ham and other processed meats, it further transforms into metmyochromogen (brown), reducing visual appeal and consumer value (Tushar, Rahman, & Hashem, 2023). Nitric oxide from nitrites combines with the iron in both myoglobin (Fe^{2+}) and metmyoglobin (Fe^{3+}) to produce a cured pink color through the formation of nitrosyl-myoglobin. This bright red complex is the basis of the distinct color of cured meats. Although highly unstable, nitrosyl-myoglobin transforms into a stable, eye-catching, reddish-pink pigment (nitrosohemochrome) during heat treatment (Shikil et al., 2022). Thus, nitrites play a key role in giving cured meats their characteristic pink color and savory flavor. In addition, nitrite inhibits the growth of harmful bacteria such as *Clostridium botulinum*, helping to prevent spoilage. As such, nitrite is considered an important additive in processed meat products (Nikolova & Belichovska, 2024).

To regulate the use of nitrite and nitrate in meat products, many countries have implemented specific directives and regulations. The European Union and its member countries have imposed strict limits on both treatment levels and residual content in various meat products. China, the world's largest meat producer, also widely consumes cured meat as a traditional food and has established similar limits in its food additive standards (Maximum addition amount (sodium (potassium) nitrite) ≤ 0.5 g/kg). Likewise, countries such as Canada (Residual nitrite level $< 7 \times 10^{-4}$), Korea and Japan (Residual nitrite level $< 7 \times 10^{-5}$) have imposed restrictions on the input and residual levels of nitrites and nitrates in meat products (Olesen, Duedahl-Olesen, Christensen, & Fagt, 2023; Zhang et al., 2023).

Despite these regulations, many studies have reported various health effects associated with food additives (Giacintucci, 2025; Sultana et al., 2023; Romanos-Nanclares et al., 2025; Landrigan & Straif, 2021). Recent research has linked processed meat consumption to an increased risk of colorectal cancer (CRC) (Chazelas et al., 2022; de Andrade Júnior et al., 2021; Olesen et al., 2023). Individuals who consume large amounts of processed meat were found to have an 18% higher risk compared to those who consume less (Farvid et al., 2021; Kim et al., 2019). Furthermore, nitrite and nitrate in processed meats have been identified as potential direct carcinogens (Hord & Hays, 2025; Picetti et al., 2022). Therefore, although food additives provide many benefits, it is essential to consider their effects on human health.

Recently, it has been reported that the effect of boiling time on the concentration of nitrite in sausages (Rakhmina et al., 2020). The effect of boiling on nitrites in processed meat appears to be beneficial. However, detailed cooking methods have not been explored. Currently, there are no established methods for removing nitrite from processed meat products. Therefore, the purpose of this study was to explore the most effective cooking methods for reducing nitrite content in processed meats.

METHOD

MATERIALS

This study used sliced loin ham purchased from Nippon Ham Co., Ltd. (Japan). Sulfanilamide and N-(1-naphthyl)ethylenediamine used for the diazotization-coupling reaction, and other reagents were obtained from Nacalai Tesque, Inc.

EQUIPMENT

The equipment used in this study included IH cooking heater (boiling and pan-frying) and microwave ovens (Panasonic Holdings Corp., Japan).

RESEARCH DESIGN

For boiling, water five times the weight of the ham was brought to a boil using the IH cooker and maintained at 80–100 °C. Ham samples were then heated for either 1 or 3 min.

For pan-frying, 10 mL of canola oil was added to a frying pan and heated on medium heat (80–100 °C) using the IH cooker. Ham samples were then cooked for either 1 or 3 minutes.

For microwave heating, samples were processed at two different wattages: 170 W for 30 s and 500 W for 30 s. For long-term storage, the ham was unsealed and stored in a refrigerator (4–8 °C) for one week before experimentation.

To assess the effects of soaking in various condiments, ham samples were immersed in 20 mL of one of the

following: plain (no additive), water, cooking sake, or grain vinegar. Each sample was soaked for 1, 5, or 10 minutes. After each cooking or treatment procedure, the residual nitrite content in the ham was measured using the diazotization-coupling reaction. For all procedures, two sampling points were taken per test, and each procedure was repeated three times.

RESEARCH PROCEDURS

Research Procedurs

Residual nitrite content was measured using the diazotization-coupling reaction (Ishiwata et al., 1975). Ham samples were finely chopped and ground using a mortar and pestle. A 5 g portion of the ground sample was collected and mixed with approximately 40 mL of hot water (80 °C), 5 mL of 0.5 N NaOH, and 5 mL of 12% zinc sulfate solution. The mixture was heated at 80 °C for 20 minutes. After cooling to room temperature in cold water, 10 mL of ammonium acetate buffer solution was added, and the total volume was adjusted to 100 mL with purified water. The solution was then filtered and used as the test solution. A 10 mL aliquot of the test solution and a 10 mL standard nitrite solution were each mixed with 1 mL of sulfanilamide solution and allowed to react for 10 minutes. Then, 1 mL of N-(1-naphthyl)ethylenediamine solution was added, and the mixture was allowed to react for another 20 minutes to develop color. The resulting solution was used as the measurement sample. The absorbances of the measurement and standard solutions were determined using a spectrophotometer (Jasco V-630 BIO, JASCO Corporation) at a wavelength of 540 nm. Residual nitrite content in ham (ppm) was calculated following the method described by Ishiwata et al. (1975).

Analysis Methods

Residual nitrite content in ham (ppm) and reduction rates (%) were expressed as mean \pm standard deviation. Statistical analysis was performed using IBM SPSS Statistics version 24. A t-test was used to determine the significance of differences between the means of two datasets.

RESULT AND DISCUSSION

1. Differences in Nitrite Reduction by Cooking Methods

Three common cooking methods—boiling, microwave heating, and pan-frying—were evaluated to identify their ability to reduce nitrite content in ham. Boiling for 1 minute significantly decreased nitrite content (9.7 ± 1.2 ppm) compared to pre-cooked ham (20.3 ± 2.8 ppm) ($p < 0.01$) (Figure 1.). Moreover, the nitrite-reducing effect was time-dependent: boiling for 3 minutes reduced the nitrite content to approximately 70% of that in pre-cooked ham (Figure 2.). In contrast, microwave heating (Figure 3.) and pan-frying (Figure 4.) did not significantly reduce nitrite content compared to pre-cooked ham.

In our experiments, boiling sliced ham for as little as 1 minute removed approximately 50% of the residual nitrite. Previous studies have shown that water can permeate sliced ham within one minute (Martuscelli et al., 2015). For this reason, nitrites in the ham were removed within a short period. Extending the boiling time to 3 minutes further reduced nitrite levels in a time-dependent manner. However, the additional reduction between 1 and 3 minutes was relatively small, indicating that boiling beyond 3 minutes may not be necessary. In contrast, microwave and pan-frying treatments did not reduce the nitrite content. Surprisingly, pan-frying even showed a tendency for nitrite levels to increase. Previous studies have reported that nitrites can be generated during heating (Shabbir, Raza, Anjum, Khan, & Suleria, 2015; Shakil et al., 2022), which aligns with our findings. Our results also suggest that nitrite is not easily degraded by short-term heating alone.

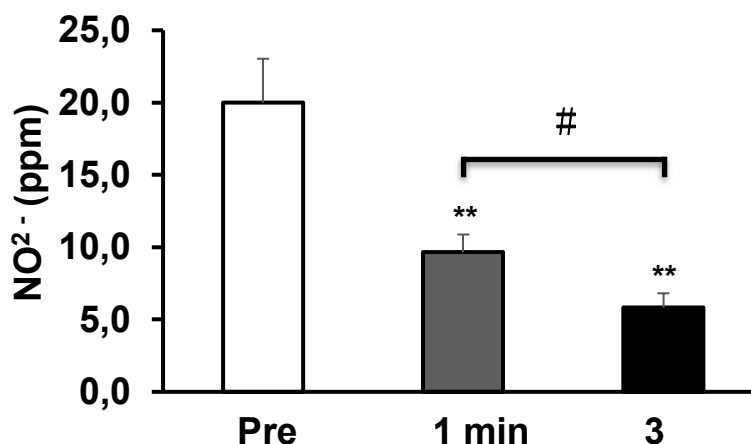


Figure 1. Nitrite content result of boiling cooking method

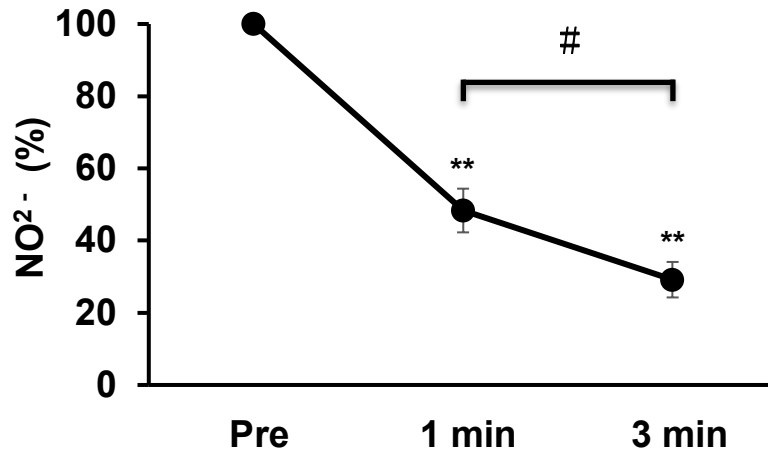


Figure 2. Graph of reduction in nitrite content using boiling cooking method

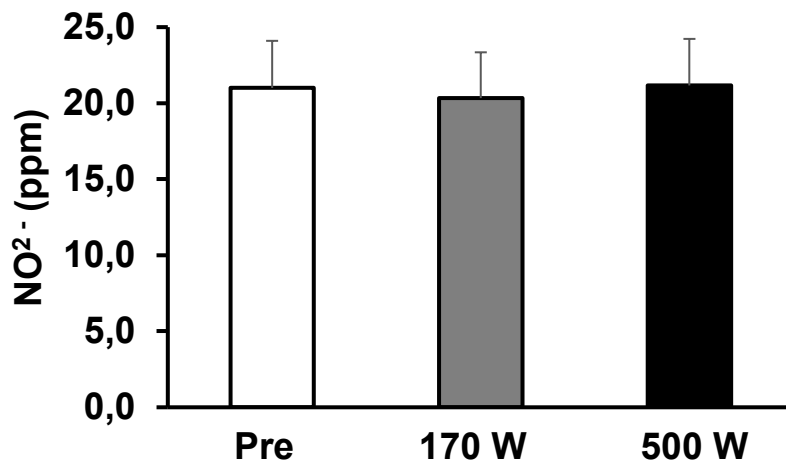


Figure 3. Nitrite content result of microwave heating cooking method

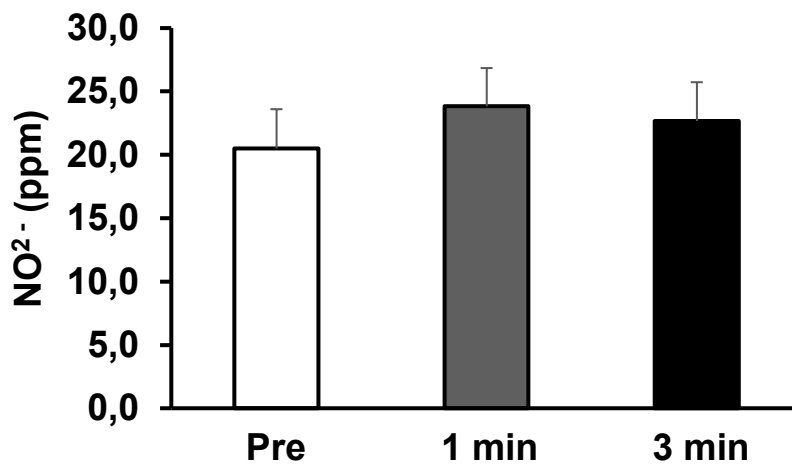


Figure 4. Nitrite content result of pan-frying cooking method

2. Changes in Nitrite Content Due To Long-Term Storage

Previous studies have suggested that nitrite levels in meat may decrease over time during storage (Huang et al., 2020). In this study, long-term storage of ham decreased nitrite content by approximately 30% compared to pre-storage levels ($p < 0.01$) (Figure 5.). Furthermore, combining long-term storage with subsequent boiling resulted in a synergistic decrease in nitrite content compared to boiling alone ($p < 0.05$) (Figure 5.).

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Nitrite has a growth-inhibitory effect on obligate anaerobic Gram-positive bacilli, such as *Clostridium botulinum*, but lactic acid bacteria can grow even in the presence of nitrite. It has also been reported that fermentation of processed meat products with lactic acid bacteria decreases nitrite content. This mechanism involves several factors, such as chemical reactions between lactic acid produced by the bacteria and nitrite, as well as enzymatic degradation of nitrite (L. Huang et al., 2020). In addition, lactic acid lowers the pH of processed meat, which can cause nitrites to be released into the air as nitrogen gas (Y.-y. Huang et al., 2021). In this study, storing ham under refrigeration for one week led to a reduction in nitrite content. Lactic acid bacteria are capable of growing even under refrigerated conditions (Kawahara, Nakamura, & Sakagami, 1999), suggesting that the reduction was due to their activity. Furthermore, boiling the stored ham reduced nitrite content even further, indicating that combining storage and boiling can enhance nitrite removal.

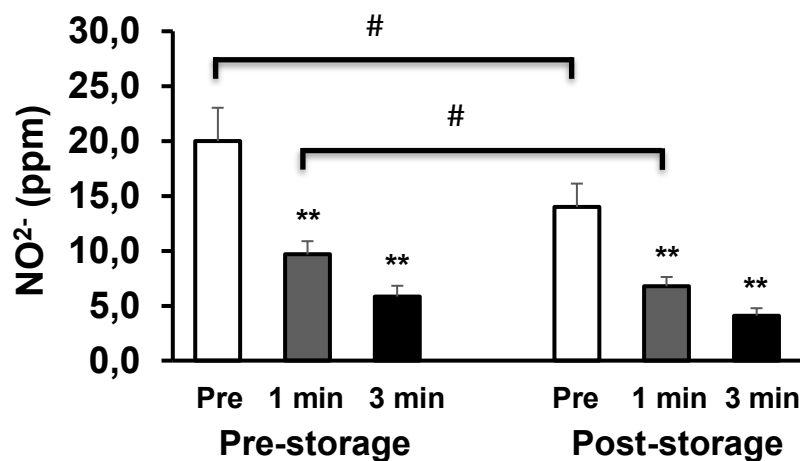


Figure 5. Result of nitrite content on long-term storage treatment and boiling cooking method

3. Effect of Pre-Soaling in Condiments on Nitrite Removal

The ham samples were soaked in various condiments—tap water, cooking sake, and grain vinegar. All treatments significantly reduced nitrite content compared to untreated samples ($p < 0.01$) (Figure 6.). Among the tested condiments, grain vinegar was the most effective in reducing nitrite levels. The effect was also time-dependent: soaking for 10 minutes reduced nitrite content to approximately 40% of that in untreated ham (Figure 7.). These results suggest that soaking ham in acidic solutions, especially grain vinegar, is an effective method for nitrite reduction. The reason why soaking in vinegar reduced nitrite is thought to be because it was released into the air as nitrogen gas.

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) has set the acceptable daily intake (ADI) for nitrite at 0–0.06 mg/kg body weight/day (Casoni, Badiu, & Frențiu, 2019). Recent reports (Bouvard et al., 2015) indicate that consuming 50 g/day of processed meat or 100 g/day of red meat raises the risk of colorectal cancer by approximately 18%. The ham used in this study contained about 20 ppm of nitrite. Consuming 50 g of such ham would result in an intake of about 1 mg nitrite. For a 50 kg person, this intake is close to the ADI (3.0 mg/50 kg body weight). Moreover, nitrite is present in many other foods, and cumulative exposure may exceed the ADI—especially in populations with high processed meat intake (Cheng et al., 2021). Boiling reduced nitrite content by two-thirds, which is expected to help reduce cancer risk. Processed meats are also high in phosphorus, a known risk factor for kidney disease. Previous studies have shown that boiling can reduce phosphorus content by around 30% (Uenishi et al., 2023; Milešević et al., 2022). Therefore, boiling processed meat before consumption may improve food safety. However, boiling may also reduce umami compounds such as glutamic acid. Further research is needed to identify

and quantify changes in these flavor components. In addition, international discussion of ADI thresholds for nitrite should continue in light of new findings.

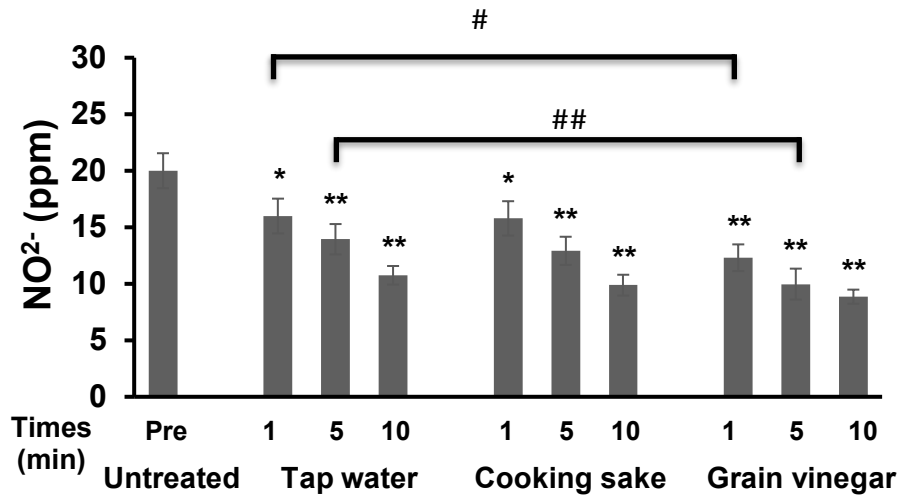


Figure 6. Results of nitrite content for various condiments soaking treatments

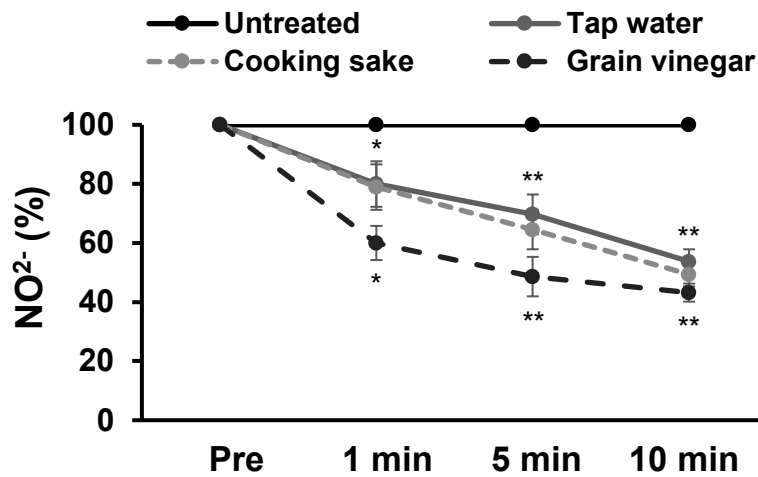


Figure 7. Graph of the decrease in nitrite content for various condiments soaking treatments

CONCLUSION

This study aimed to identify cooking methods that effectively reduce nitrite content in ham. The results of this study clearly demonstrate that boiling is an effective method for reducing nitrite content in ham. Nitrite removal can be further enhanced through long-term refrigeration and soaking in acidic solutions such as grain vinegar. These findings offer practical strategies for minimizing dietary nitrite intake from processed meats. Going forward, it will be important to extend this research to other processed meat products, such as sausages.

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Conflict of Interest Statements: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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