THE EFFECT OF NITRITE USE IN MEAT PRODUCTS ON HUMAN HEALTH

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Abstract: Because of their high nutritional value, meat and processed meat products play a crucial role in human dietary habits, offering a wide variety of options in today's market. Consumers seek these products to meet specific sensory standards, including appearance, color, flavour, and aroma, while also prioritizing safety. This has led the meat industry to rely on various additives, with nitrites being extensively used in meat processing. Nitrites, classified as preservatives, exhibit antimicrobial effects by impeding the proliferation of microorganisms, specifically the bacterium *Clostridium botulinum*. From a technological perspective, nitrites are pivotal for color enhancement and stability, prevention of fat oxidation, and contribution to the aroma in meat products. Despite these positive contributions, nitrites can have adverse effects on consumer health. Their reaction with proteins at elevated temperatures results in the formation of *N*-nitrosamines, known for their carcinogenic and mutagenic qualities. According to a recent classification by the World Health Organization, processed meat has been categorized as carcinogenic. It has been found that consuming 50g of processed meat daily can lead to an 18% higher risk of developing colon cancer. Nitrites, viewed from a health standpoint, can be toxic, causing the conversion of hemoglobin into methemoglobin, leading to methemoglobinemia and potential fatality with elevated methemoglobin levels.
Adhering to legal regulations regarding the permissible levels of added nitrites and nitrates in processed meat products is crucial to mitigate the risk of carcinogenic N-nitroso compound formation.
Keywords: nitrites, N-nitroso, N-nitrosamines, N-nitrosamides, toxicity, carcinogenicity, mutagenicity

1. INTRODUCTION

Aside from consuming meat itself, people also include various meat products in their diets. In the processing of meat, nitrites are one of the most commonly used additives, following table salt (Vossen et al., 2012). The addition of nitrites during meat processing initiates a reaction between nitrites and meat proteins, resulting in the formation of potentially harmful carcinogenic compounds known as *N*-nitrosamines through the nitrosation reaction (Silovska Nikolova & Belichovska, 2021). Xie et al. (2023) points out that *N*-nitrosoamines are very dangerous compounds because of their carcinogenicity and teratogenicity. Nitrosamines' toxicity and carcinogenic properties have been well studied and detailed in a large number of published publications (Flores и сор., 2019; Jo et al., 2020; Silovska Nikolova & Belichovska, 2022).
Consuming high amounts of nitrites and nitrates can be potentially lethal for humans. Methemoglobinemia, a condition where the blood contains an elevated level of methemoglobin, is one of the most common consequences of acute toxicity in humans resulting from the ingestion of high doses of nitrites and nitrates (Santamaria, 2006).

2. TOXICITY OF NITRITES

Nitrites are soluble in water and exhibit high reactivity, particularly at lower pH values. Lewis (1989) characterizes nitrites as a potential human poison when present in food. In humans, excessive intake can lead to alterations in motor activity, coma, a decrease in blood pressure, dilation of arteries and veins, gastric distress, nausea, vomiting, diarrhea, methemoglobinemia in the blood, and eye irritation. It is important to note that these toxic effects are primarily associated with the surpassing of recommended amounts, and, to a lesser extent, they impact the physiological state of the human organism.
In 1998, in the United States of America, nitrites were officially classified as toxic to reproduction and development (Pavlinić Prokurica et al., 2010).
Nitrates and nitrites possess the potential for toxicity in humans when used improperly. Consequently, stringent regulations have been established to govern and monitor their utilization in the meat industry.

Acute toxicity

In the early decades of the last century, there were instances where significant quantities of nitrites were added to meat products. Unfortunately, this practice led to the death of consumers due to intoxication (Honikel, 2008).

In fact, nitrites are many times more hazardous in the short term than nitrates. According to Schuddeboom (1993), the proven lethal oral dose range for nitrates in humans is 80–800 mg/kg body mass, or 32–250 mg/kg body mass for nitrite. Acute nitrite poisoning, which is frequently the consequence of consuming too much nitrite, can cause methemoglobinemia, a serious adverse effect marked by cyanosis. Notably, because nitrites prevent oxygen from reaching hemoglobin, infants younger than three months of age are more susceptible to this illness than older kids and adults WHO (2007).

The acute toxic effect of nitrites is attributed to their capacity to oxidize oxyhemoglobin into methemoglobin. This transformation impairs the ability of methemoglobin to bind and transport oxygen through the bloodstream to all cells in the human body (Watson, 1993; Santamaria, 2006).

Hemoglobin consists of a protein component (globin), four prosthetic groups known as heme, and contains iron. Its primary function is the transportation of oxygen. When hemoglobin binds with oxygen, it forms red oxyhemoglobin, which subsequently breaks down, releasing oxygen to the tissues (Hill, 1991). Hemoglobin undergoes a reaction with ingested nitrites, leading to the oxidation of the Fe_2+ ion to Fe_3+, resulting in the formation of methemoglobin. Methemoglobin is unable to effectively bind to oxygen. When the level of methemoglobin is below 10% of the total hemoglobin, poisoning may be asymptomatic. However, if it surpasses 10%, it can cause cyanosis, characterized by a blue discoloration of the skin and lips (Hill, 1991). Indeed, if a substantial percentage of hemoglobin is replaced by methemoglobin, it can lead to severe consequences, including a coma and, in extreme cases, death. The inability of methemoglobin to efficiently bind and transport oxygen can result in a critical reduction of oxygen supply to vital tissues and organs, leading to life-threatening conditions (Hill, 1991).

Vombergar et al. (1989) state that just 1 gram of sodium nitrite ($NaNO_2$) has the capability to readily convert 1.885 grams of hemoglobin into methemoglobin. The conversion of hemoglobin to methemoglobin is described by the chemical reaction illustrated in Figure 1(Kroupova et al., 2005; Fakhre и Qader, 2013; Gassara et al., 2016).

Figure 1. Representation of the chemical reaction converting hemoglobin to methemoglobin

$$4HbO_2 + 4NO_2^- + 4H^+ \rightarrow 4Hb^+ + 4NO_3^- + O_2 + 2H_2O$$

Aquanno et al. (1981) point out that the lethal dose of orally ingested nitrites in humans is from 2 to 9 g. The first signs of oral intoxication develop from 15 to 45 minutes after ingestion.

Matteucci et al. (2008) point out an instance of acute nitrite intoxication in Italy involving a forty-year-old mother and her son. This incident occurred after the consumption of turkey contaminated with elevated nitrite levels, ranging from 6000 to 10000 mg/kg.

In order to establish safety standards, acceptable daily intakes for nitrites and nitrates have been determined based on studies of their toxic effects. According to the Joint FAO/WHO Expert Committee on Food Additives (JECFA), the acceptable daily intake for nitrates is set at 3.7 mg per kg of body mass. In contrast, the acceptable daily intake for nitrites is much lower, at 0.07 mg per kg of body mass (SCF,1995). In 2008, these quantities for acceptable daily intake were also approved by the European Food Safety Authority (EFSA, 2008).

Indeed, from the information provided, it can be inferred that nitrates have lower toxicity for the human body compared to nitrites. Consequently, nitrates and nitrites have distinct effects on human health. Thomson (2004) points out that the main toxicity of nitrates is due to their endogenous conversion to nitrites.

3. MUTAGENICITY AND CARCINOGENICITY

Over sixty years ago, two pivotal discoveries significantly contributed to the exploration of the formation and etiology of carcinogenic *N*-nitrosamine compounds. In 1956, British scientists made a noteworthy announcement about the identification of the carcinogenic compound *N*-nitrosodimethylamine (NDMA) in experimental animals. A few years later, in 1962, Norwegian researchers observed that sheep, which had consumed fishmeal treated with nitrites, experienced fatalities attributed to the presence of N-nitrosamines. The introduced nitrites in the fishmeal reacted with the amines found in the fish meat, leading to the formation of lethal doses of *N*-nitrosodiethylamine (NDEA) and *N*-nitrosodimethylamine (NDMA) (Preussmann, 1984; Rački et al., 2010).

In 1978, scientific findings confirmed that fried bacon contains a notable quantity of a specific nitrosamine called *N*-nitrosopyrrolidine. This compound is formed when a sufficient amount of secondary amines is present and subjected to high temperatures (T > 130°C) and an appropriate pH value during the cooking process (De Mey et al., 2017). Following this finding, there was scrutiny regarding the utilization of nitrites in meat products, even though they have beneficial technological effects.

In 2015, a working group from the International Agency for Research on Cancer (IARC), comprising 22 scientists from 10 countries, categorized processed meat as carcinogenic and red meat as potentially carcinogenic. The initial conclusions were based on evidence linking the consumption of processed meat to the increased risk of colorectal cancer, stomach cancer, pancreatic cancer, and prostate cancer (IARC Working group, 2018).

Formation of *N* - nitroso compounds

Bodily secretions, including amines and amides, are naturally occurring substances found both as food ingredients and metabolites within the human body. When these substances react with nitrites, they have the potential to generate carcinogenic *N*-nitroso compounds, which can be categorized into two groups Karolyi (2003): Nitrosamines, which necessitate metabolic activation to become reactive, pose a risk of causing mutations or cancers when in an unstable state. On the other hand, nitrosoamides are formed in acidic environments, exhibit instability, and rapidly break down, potentially causing damage to the organs in which they originated.

Residual nitrites can undergo a reaction with free amino acids and amines in cured meat products or in the human stomach after consuming meat cooked at high temperatures, resulting in the formation of N-nitrosamines. Extensive research has been conducted on the metabolism of nitrates in the human body, which involves several metabolic changes and ultimately leads to their elimination through saliva, intestines, and bile (EFSA, 2008).

Bartholomew and Hill (1984) point out that, upon ingestion, nitrates are swiftly absorbed in the upper small intestine of humans. Once absorbed, these nitrates undergo rapid transportation through the bloodstream and are salivary glands selectively release this substance, and it may also be secreted by other exocrine glands.

Lundberg et al. (1994) highlight that about a quarter of ingested nitrates are excreteд through saliva. Additionally, specific bacteria present in the mouth contribute to the conversion of approximately 20% of nitrates into nitrites (Lundberg et al., 2008).

Lundberg et al. (1994) indicate that, in a healthy adult, approximately 5-7% of ingested nitrites arise from the reduction of nitrates to nitrites, a process facilitated by saliva. Subsequently, in the stomach, nitrites undergo rapid transformation into nitrogen oxides, primarily converting into nitrogen monoxide. Bartholomew and Hill (1984) state that the majority of the nitrate that is taken in by the digestive system is eventually eliminated through urine. However, a considerable portion is also retained through selective renal reabsorption and salivary recirculation. Apart from being ingested, nitrates can also be produced within the human body, deriving from nitric oxide as a precursor. Within mammals, L-arginine acts as the primary endogenous supplier of nitrate. This crucial amino acid, found in almost all cell types within the mammalian body, serves as the biological precursor for nitric oxide (Lundberg et al., 2009).

The excretion of nitrites from the human body is mainly carried out through the urinary tract an dtakes place approximately 4-6 hours after the consumption of the meal, while almost all ingested nitrites are excreted within 24 hours. Approximately 75% of total nitrates are excreted in the urine (through the kidneys), while the remaining amount is excreted through saliva and sweat (Gassara et al., 2016).

N-nitrosoamines are fairly thermostable compounds (Institute of Food Technologists 1972). Nitrosamines are generated through a sequence of reactions involving nitrites and secondary amines. Primary amines undergo rapid degradation into alcohol and N_2, whereas tertiary amines remain unreactive and do not lead to nitrosamine formation.

For the formation of nitrosamines in meat products, specific conditions must be met, including:

- Nitrosamine formation heavily relies on the presence of amines. Although fresh meat contains only trace amounts of amines, higher concentrations can be detected in substances like creatine, creatinine, free amino acids such as proline and hydroxyproline, and specific decarboxylated products derived from these amino acids. (Honikel, 2008);
- Stable nitrosamines are exclusively formed from secondary amines. Primary amines undergo immediate decomposition into alcohols and nitrogen, and tertiary amines remain non-reactive (Andrée et al., 2010). It's important to mention that the majority of amines found in meat are primary amines originating from α-amino acids (Honikel, 2008);
- In order to inhibit the production of nitric oxide, it is imperative to maintain a sufficiently low pH level or ensure the presence of iron ions (Honikel, 2008). Under low pH conditions, nitrosating agents like protonated nitric acid ($H_2NO_2^+$), nitrogen trioxide (N_2O_3), or nitrosyl halides (NOX) are generated. Concurrently, the concentration of unprotonated amines tends to decrease (Andrée et al., 2010).

The nitrosation reaction leading to the formation of nitrosamines is directly influenced by several factors, including the quantities of amines and added nitrites, pH level, temperature, redox potential, and the presence of other chemical compounds or agents. Besides being added directly, nitrites can also be produced indirectly through enzymatic reduction from nitrates (commonly found in plant-based products) during processing or the addition of nitrates to food, presenting an indirect risk of harmful nitrosamine formation.

For most secondary amines, the optimal pH value for nitrosation falls within the range of 2.5 to 3.5. As the pH value increases (within the range of pH 5-6.5), the reaction tends to slow down. The formation of nitrosamines is a time-consuming process. (Walker, 1990; Hui, 1992).

N- nitrosamines are commonly found in processed meat products primarily as a result of the addition of nitrites and nitrates during their processing. The added nitrates undergo reduction to nitrites, facilitated by the enzyme nitrate reductase found in many bacteria. Subsequently, during the stages of production, storage, or processing, nitrites engage in chemical reactions with amines, leading to the formation of *N*-nitrosoamines (Walker, 1990).

N- nitrosamines are frequently found in cured meat products, particularly in the durable ones, as nitrates or nitrites (in the form of nitrate salt or nitrite salt) are often added during processing. The reduction of nitrates to nitrites is facilitated by the nitrate-reductase enzyme, which is prevalent in many bacteria. De Mey et al., (2017) states that Following that, secondary amines react chemically with nitrites, which takes place in the production process, heat treatment, or storage, resulting in the creation of nitrosaminesThe highest levels of nitrosamine formation typically occur in meat products that have undergone curing and are subsequently exposed to high temperatures, especially exceeding 130°C, as seen in the case of fried cured bacon (Honikel, 2008; Honikel, 2010; Vuković, 2012).

Recently, there has been a growing trend to minimize the use of nitrates and nitrites in the canning and processing of meat products. A combination of 40 ppm sodium nitrite and 2600 mg/kg sorbate has demonstrated effectiveness in significantly reducing the formation of *N*-nitrosoamines in canned meat products, bringing levels down from nearly 100 mg/kg to less than 5µg/kg. In the United States and other countries, to prevent the formation of N-nitroso compounds, the addition of 500 mg/kg of sodium ascorbate to meat products is recommended. (Cassens, 1990; Schuddeboom, 1993). The formation of *N*-nitrosoamines can be mitigated by employing lower temperatures during the heat treatment process, particularly those below 160°C. Methods such as pasteurization or sterilization, as well as gamma irradiation, have been shown to be effective in reducing the occurrence of *N*-nitrosoamines (De Mey et al., 2017).

4. CONCLUSION

Adhering to legal regulations regarding the permissible levels of added nitrites and nitrates in processed meat products is crucial to mitigate the risk of carcinogenic N-nitroso compound formation. Continuous monitoring of the added nitrite levels in meat products is essential, primarily as a measure to safeguard consumer health. There is a growing trend in advocating for initiatives that aim to either decrease the allowable amounts of added nitrites in meat products or explore alternatives such as natural sources of nitrites. These efforts underscore a collective commitment to enhancing the safety of meat products and promoting healthier food processing practices.

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