https://doi.org/10.17113/ftb.63.01.25.8744 review

Nitrite Effects and Nonthermal Technology for Reducing or Replacing Its Content in Meat Products

Running title: Nonthermal technology for nitrite reduction

Bruna Fernandes Andrade[1], Lorrany Ramos do Carmo[1], Marcelo Stefani Tanaka[1], Robledo de Almeida Torres Filho[2], Alcinéia de Lemos Souza Ramos[1] and Eduardo Mendes Ramos[1*]

[1]Department of Food Science, School of Agricultural Sciences of Lavras, Federal University of Lavras, 35690-900, Lavras, Minas Gerais, Brazil
[2]Institute of Exact and Technological Sciences, Federal University of Viçosa, Forestry Campus, 35690-000, Florestal, Minas Gerais, Brazil

Received: 17 June 2024
Accepted: 15 February 2025

SUMMARY

Nitrite and nitrate salts are preservatives that act as antimicrobial (bacteriostatic and bactericidal activity) and antioxidant agents during the processing of meat products and confer sensory characteristics to meat (by generating and maintaining colours and flavours). Nitrite is mainly applied as a preservative to prevent the growth *Clostridium botulinum* and production of its toxins. However, nitrite and nitrate are also associated with the production of N-nitroso compounds, such as carcinogenic N-nitrosamines, which may cause adverse health effects. Therefore, the health risks of these preservatives must be weighed against the need to prevent foodborne pathogens from infecting food, especially spores of *C. botulinum*. In this review, we discuss the advantages and disadvantages of using nonthermal technologies as a strategy for partially or totally replacing nitrite in meat products, especially regarding antimicrobial efficacy and N-nitrosamine formation. Methods such as high-pressure processing, pulsed electric fields, and cold plasma have been studied for these purposes,

*Corresponding author:
Phone: +553538291403
Fax: +553538291401
E-mail: emramos@ufla.br.

but these technologies can alter foods' sensory properties and stability. Nevertheless, irradiation at lower doses has great potential as a tool for reformulating cured meat products, contributing to the reduction of the residual nitrite and preformed N-nitrosamines while ensuring microbiological safety without significant quality changes in the products.

Keywords: nitrosamines; *Clostridium botulinum*; irradiation; plasma; high pressure

INTRODUCTION

Curing salts, such as nitrite (NO^{2-}) or nitrate (NO^{3-}), are food additives that must be included in the formulations of cured meat products. Although classified as antimicrobial preservatives, curing salts perform a variety of activities in the meat matrix, such as maintaining the colour and flavour (taste and aroma) characteristics of meat and acting as an antioxidant agent to ensure greater physicochemical stability (*1,2*). The use of these additives ensures that the sensory quality and stability of meat products are maintained throughout their shelf life, which is important for customer acceptance and satisfaction. Most cured products are ready-to-eat (RTE) or have not undergone heat treatment before being consumed. For this reason, ensuring the microbiological safety of these products is a major concern in the food industry. The use of additives, such as curing salts, is even more important in meat products with high pH (~6.5), as well as products with added mechanically separated meat, products stored at room temperature, and products handled after processing or subjected to a variable cold chain; this is a particular concern in countries near the tropics, with high temperatures most of the year, and where food is transported over great distances (*3,4*).

The minimum amount of nitrite needed to generate sensory characteristics and maintain microbiological safety is variable. In general, 50 to 75 mg/kg nitrite is needed to generate the characteristic cured colour, while a residual concentration of nitrite between 40 and 80 mg/kg is needed to effectively control microbes (*5,6*). As nitrites and nitrates are highly reactive, they react with different constituents of the meat and the added ingredients; these reactions are favoured by thermal processes, such as cooking. Therefore, the amount of nitrite or nitrate (hereafter referred to as nitrite/nitrate) added to the formulations is greater than the amount found in the final product and continues to decrease during storage (*1*). The amount of nitrite/nitrate that is permitted in the final product varies with the legislation of each country. In Brazil, the maximum residual concentrations of nitrite (E249 and E250) and nitrate (E251 and E252) permitted in meat products are 300 and 150 mg/kg, respectively (*7*). In Europe, through Regulation (EU) 2023/2108 (*8*), the maximum of ingoing nitrite allowed was reduced from 150 mg/kg to 80 mg/kg in non-heat-treated cured meat products,

and from 100 mg/kg to 50 mg/kg in heat-treated meat products. Furthermore, depending on the product, the maximum residual nitrite amount during the entire shelf life may not exceed 25-45 mg/kg. The Codex Committee on Food Additives (CCFA/FAO/WHO) has set a maximum limit of 80 mg/kg nitrite in meat products (*9*).

The nitrite and nitrates present in the human body can be exogenous in origin (when ingested through food, such as vegetables, water, and meat products), or endogenous, such as nitrite and nitrates present in human saliva. According to Milkowski (*10*), 5 % of dietary nitrite and nitrate originates from meat products, and the remainder originates from other foods, such as vegetables. However, nitrites and nitrates differ significantly in their toxicity and permissible levels. The lethal dose of nitrite is approximately 300 mg/kg body mass, whereas that of nitrate is higher, at around 800 mg/kg body mass (*9*). The ingestion of nitrite may harm the human body and cause disorders, such as blood disorder-related methemoglobinemia, which results in a lack of oxygen in the cells (*4*). Nitrates, on the other hand, are generally considered less toxic Nitrates, on the other hand, are generally considered less toxic because first needs to be converted into nitric in the body (*4*). Despite these concerns, the consumption of nitrite and nitrate in meat products is not a concern due to the low available levels and the amount of meat products generally ingested. The potential health risks associated with the use of these additives mainly results from the generation of N-nitroso compounds (NOCs), especially N-nitrosamines; these compounds exhibit mutagenic and genotoxic properties and therefore have a strong oncogenic effect in humans, particularly with respect to colorectal cancer (*11,12*). NOCs can induce cancer in approximately 40 different animal species, including higher primates, and are carcinogenic in multiple organs in animals (*13*). Due to these concerns, the International Agency for Research on Cancer (IARC) concluded that ingested nitrates or nitrites are probable carcinogens to humans under conditions favouring endogenous nitrosation; thus, cured meat products were classified as a group 1 carcinogens (*11*).

N-Nitrosamines are ubiquitous and are found in drinking water, animal or vegetable foods and drinks (*14*), in addition to cigarettes, pesticides, and cosmetics. However, despite the ubiquity of N-nitrosamines in common environments, individuals argue that curing salts should be banned due to the potential presence of these compounds and the high levels of residual nitrite in meat products. Nevertheless, it is the responsibility of the meat industry to reduce or eliminate the risk of its products, as the scientific community is increasingly focused on designing better strategies to completely or partially replace conventional nitrites in meat products.

Although there is an undeniable need to reduce or remove curing salts from meat formulations, this task is challenging because curing salts additives have multiple purposes. In general, potential nitrite substitutes include the use of natural food dyes to obtain a "cured colour"

similar to that of cured products (*15,16*); biopreservation techniques involving the use of bacteriocin, essential oils and nitrate-free plant extracts to guarantee microbiological safety (*17-19*); and the use of "natural curing agents", which replace nitrite with plant extracts that contain relatively high levels of nitrate (*20,21*). However, the use of these techniques involves the partial reduction of nitrite or requires additional procedures to generated all the effects (sensory, antimicrobial, and antioxidant) conferred by curing salts (*22*). Replacing synthetic nitrites with “natural” nitrite sources is not superior to utilizing synthetic nitrite salts and is performed to attain a clean label on the product. Moreover, the labelling and advertising of these products as "naturally cured" can mislead the consumer, as the elimination of N-nitrosamines is not confirmed; the residual nitrite (and possible N-nitrosamine content) of "naturally cured" products is similar (or even greater) to that of products cured with synthetic nitrite or nitrate.

Recently, researchers have suggested the use of S-nitrosothiols (RSNO), which is derived from amino acids or peptides (such as L-cysteine, glutathione, and N-acetyl-L-cysteine), as oxide nitric ($NO^{\bullet}$) carriers for use as total curing salt substitutes in meat products (*23-26*). Researchers have demonstrated that these carriers show high potential, can provide desirable sensorial and microbiological properties through mechanisms similar to those of nitrite, and can reduce the production of N-nitrosamines. However, research on the use of RSNO in meat products remains in early stages, and there is very little information about its application at the industrial scale. Andrade *et al.* (*22*) reported that for nitrite concentrations of 100–200 mg/kg, which are commonly used in cured cooked hams, equimolar amounts of RSNO (S-nitroso-N-acetylcysteine or S-nitroso-N-acetylcysteine ethyl ester) could achieve the same antioxidant effect, instrumental colour and volatile profile characteristic of these products, but producing lower amounts of residual nitrite.

The replacement of additives tends to depend on whether the characteristics of the food product can be maintained in some other way, which is challenging. Replacing nitrite/nitrate in the meat processing industry is complex because the food characteristics (composition, whole, or reconstituted) and processes applied (cooking, fermentation, drying, and smoking) are different. Thus, a proposed alternative to nitrite/nitrate should reduce the amount of N-nitrosamines while maintaining the sensory aspects of product; above all, the nitrite/nitrate alternative should exert effective activity against foodborne bacteria, especially *C. botulinum*, which produces a lethal toxin. In this context, emerging nonthermal technologies have revolutionized the food processing sector, as they increase the shelf life of food and retain the quality, such as nutritional, freshness, and sensory attributes, of food products (*27*). These technologies have great potential in reducing the health risks of cured meat products, either by allowing the reduction and/or even elimination of nitrite/nitrate salts.

NITRITE/NITRATE EFFECTS

Although nitrate and nitrite salts are used as curing salts, the active curing agent is nitrite, which requires the prior conversion of nitrate to nitrite by nitrate-reducing bacteria (species of *Achromobacter, Micrococcus, Lactobacillus*, or *Staphylococcus*) that are naturally present in meat (*3, 28*). Thus, nitrate salts are generally used as reserve agents for the generation of nitrite in products that require a long processing period.

Biochemically, most of the favourable actions of curing salts directly or indirectly result from the $NO^{\bullet}$ formed from the decomposition of nitrite in solution. Likewise, the formation of undesirable compounds, such as N-nitrosamines, originates from the reactivity of intermediate components of these reactions (Fig. 1).

<Fig.1 should appear here>

Curing in meat products

Soon after nitrite is added to the meat batter, the haem iron present in the deoxymyoglobin (DMb; $MbFe^{+2}$) or oxymyoglobin (OMb; $MbFe^{+2}O_2$) is oxidized, as evidenced by the characteristic brown colour of metmyoglobin (MMb; $MbFe^{+3}$). According to the chemical equilibrium of nitrosylated species, nitrite in an acidic medium (pH 5.8 to 6.2) produces nitrous acid (HNO_2, pKa=3.42 at 25 °C), which is in equilibrium with its anhydrous dinitrogen trioxide (N_2O_3). Under reducing conditions and in the absence of light and oxygen, N_2O_3 decomposes into nitrogen dioxide (NO_2^-) and $NO^{\bullet}$. Then, $NO^{\bullet}$ reacts with DMb to form nitrosylmyoglobin (NOMb; $MbFe^{+2}NO$) and/or with MMb to form nitrosylmetmyoglobin ($MbFe^{+3}NO$), a transient pigment that is converted to NOMb (*1,29*). Thus, the binding of $NO^{\bullet}$ to myoglobin generates the pinkish-red colour pigment NOMb in the raw cured products or the pinkish colour nitrosyl-haemochrome (NOhemo) in the cooked cured products due to the denaturation of the pigment globin protein (Fig. 1a). The generation of these pigments is responsible for developing the characteristic colours of these products (*30*).

In addition, $NO^{\bullet}$ stabilizes the haem iron of the meat pigments in cured products, limiting the pro-oxidant activity of iron. The presence of transition metals, especially iron (both haem and nonheme), is among the main factors that determines the oxidative stability of meat because they can catalyse different processes and stages of lipid oxidation (*31*). Lipid autoxidation is a complex process that involves the formation (initiation stage) and propagation of lipid free radicals, beginning with the oxidation of unsaturated lipids by reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2), superoxide anion ($O_2^{\bullet}$) and hydroxyl radicals ($OH^{\bullet}$); then, lipid peroxyl radicals ($L^{\bullet}$, $LOO^{\bullet}$) and hydroperoxides (LOOH) are formed that are further degraded into secondary compounds (*2,29*). The

main secondary compounds released include alcohols, ketones, alkanes, aldehydes, ethers, and hydrocarbons, which are responsible for the deterioration of sensory aspects, such as odours and flavours associated with lipid oxidation (*31*). $NO^{\bullet}$ chelates free metals and sequesters oxygen and other ROS, inhibiting the initiation of lipid oxidation (Fig. 1b). Moreover, $NO^{\bullet}$ shuts down lipid autoxidation by generating nonradical compounds and reacting with lipid peroxyl radicals (*2*). Therefore, the binding of $NO^{\bullet}$ to the haem iron of myoglobin prevents its oxidization and destroys the radical chain reactions of lipid oxidations, maintaining the characteristic flavour of cured meat products.

Antimicrobial action

Meat contains nutrients that, under ideal conditions, promote the growth of desirable and undesirable microorganisms. Desirable microorganisms are generally involved in the formation of sensory characteristics and stability of products, as occurs in the fermentation process. Undesirable microbes accelerate the deterioration of products and may cause disease in consumers. In general, the profile of the microbiota present in the meat product is influenced by the initial bacterial count in the raw material, the additional microbial load from the added ingredients, the sanitary conditions during handling, the processing techniques applied, the postprocessing handling method used, the type of packaging, and the distribution and storage conditions (*32*).

Curing salts can extend the shelf life of meat and meat products by inhibiting the outgrowth of pathogenic and spoilage bacteria. The predominant bacteria associated with the spoilage of refrigerated meat products are *Brochothrix thermosphacta*, *Carnobacterium* ssp, *Lactobacillus* ssp, *Leuconostoc* ssp, and *Weissella* ssp, which cause defects such as sour off-flavours, discolouration, gas production, slime production and decreased pH. Nitrite has an inhibitory effect on the growth of several spoilage bacteria, such as *Enterobacteriaceae* and *B. thermosphact*; however, the presence of nitrite combined with microaerophilic conditions favours the growth of psychotropic lactic acid bacteria (*33*). Nitrite is also recognized for its bacteriostatic and bactericidal effects against pathogenic bacteria, such as *Escherichia coli*, *Salmonella* spp., *Listeria monocytogenes*, *Staphylococcus aureus* and *Clostridium* spp. (*2,3*).

The antimicrobial action of nitrite is also attributed to reactions associated with the decomposition of nitrite to $NO^{\bullet}$ (Fig. 1c). More precisely, this activity is directly related to the level of oxidative stress caused by peroxynitrite ($ONOO^{-}$), which is formed by the reaction of nitrite with H_2O_2 and $NO^{\bullet}$ with $O_2^{\bullet}$ (*3*). Peroxynitrite is in equilibrium with peroxynitrous acid (ONOOH) under physiological conditions, and both compounds are powerful oxidants and nitrating agents. They lead to the oxidation and nitration of proteins, DNA, and lipids through direct or indirect oxidative reactions

radical-mediated mechanisms (*2,3*). Therefore, adding nitrite improves the microbial safety of meat products.

Although different bacteria are influenced by nitrite/nitrate, curing salts are added to specifically control *C. botulinum*, a strictly anaerobic gram-positive rod-shaped (bacillus) bacterium that causes food poisoning (botulism) by a potent and lethal neurotoxin. Botulinic neurotoxins inhibit the release of acetylcholine at synapses and the electrical stimuli responsible for muscle contraction; these process cause paralysis and suffocation, which can lead to death. It is estimated that intoxication can occur with the consumption of foods containing 30-50 ng of toxins (*34*). *C. botulinum* strains are classified as Group I, II, III, or IV according to the most prevalent way in which the bacterium obtains energy (proteolytic or saccharolytic mechanism) and the threshold and optimal temperature and pH for growth. With respect to public health, the most dangerous spore-formers in low-acid chilled foods (such as meat) are from Group II, the psychrotrophic nonproteolytic strains, which can germinate and grow at temperatures as low as 3 °C (*35*). Although lower storage temperatures (less than 2 °C) tend to reduce or impede the production of toxins, maintaining this temperature throughout the distribution and storage chain to the point of sale is very challenging, especially in developing countries. Therefore, nonproteolytic *Clostridium* can only be controlled through the presence of residual nitrite, which prevents the germination of spores and, consequently, inhibits the production of toxins.

Nitrites show antibotulinal activity in a heated system by preventing vegetative cell growth from spores and inhibiting the cell division of any vegetative cell. In addition to the action of the peroxynitrite formed, the main inhibition of *C. botulinum* by nitrite may involve a reaction between $NO^{\bullet}$ and important iron–sulphur enzymes (*e.g.* ferredoxin and pyruvate-ferredoxin oxidoreductase) necessary for energy production in clostridial vegetative cells (*36,37*). However, despite the high lethality of *C. botulinum*, the survival or growth of this microorganism in the reformulated product is not considered in many studies on the replacement and/or reduction of nitrite in meat products.

N-Nitrosamine formation

N-Nitrosamines are a class of compounds that contain a nitrous group (-N-N=O) attached to a secondary amine (R_2-), which can present different radicals (generally hydrocarbons), allowing a wide variety of compounds. Once present in the human body, these compounds undergo enzymatic metabolization and produce the diazonium radical, which is a very reactive electrophile that can react with cellular macromolecules, such as nitrogenous bases (nucleophilic species) present in DNA (*12*).

N-Nitrosamines are divided into volatile N-nitrosamines and non-volatile N-nitrosamines. The former are typically potent carcinogenic nitrosamines present in processed meat products, including

N-nitrosodimethylamine (NDMA), N-nitrosodiethylamine (NDEA), N-nitrosopiperidine (NPIP), N-nitrosopyrrolidine (NPYR), N-nitrosomorpholine (NMOR), N-nitrosomethylethylamine (NMEA), N-nitroso-di-n-propylamine (NDPA) and N-nitrosodibutylamine (NDBA). Among the volatile compounds for which N-nitrosamines have been studied, only 18 % are considered noncarcinogenic, while the nonvolatile N-nitrosamines are weakly carcinogenic or assumed to be noncarcinogenic (*38*). The lack of carcinogenic potential by some N-nitrosamines is attributed to their chemical structure. Species with high molecular mass radicals, as well as extensive carbon chains, are less susceptible to metabolization and consequently the release of diazonium radicals due to steric hindrance. Therefore, N-nitrosamines of lower molecular mass, such as NDMA and NDEA, have the highest mutagenic potential (*12*).

In meat, N-nitrosamines are formed during processing through reactions between secondary amines and nitrosating agents, such as N_2O_3, which are derived from the curing reactions of nitrite to $NO^{\bullet}$ (Fig. 1d) (*12,38*). In the food processing industry, ascorbate or iso-ascorbate (or erythorbate) salts are commonly used to accelerate curing reactions and, consequently, reduce processing time and the availability of nitrosating agents to form N-nitrosamines (*3*). However, according to Majou and Christieans (*3*), ascorbate can also participate in the reduction cycle of metmyoglobin reductase (MMR), generating $NO^{\bullet}$ from nitrite, which binds to DMb and maintains the production of $O_2^{\bullet}$ and H_2O_2; these increase peroxynitrite production and promoting antimicrobial activity.

The N-nitrosamines found in cured meat products are called preformed N-nitrosamines, and their inhibition during processing remains a major challenge for the safety of meat products. The levels of N-nitrosamines in meat products vary widely, from undetectable (<1 μg/kg) to several thousand parts per billion (μg/kg) orders of magnitude, depending on the type of N-nitrosamines (*14,38*). In general, exposure of the product to high temperatures during processing or preparation for consumption tends to increase the content of pre-formed nitrosamines. This occurs because elevated temperatures catalyse the formation of essential substrates for the N-nitrosation reaction, such as nitrogen oxides and secondary amines (*12*). However, in addition to the N-nitrosamines found in different types of food products (*14*), they can also be formed endogenously during food digestion under acidic conditions (pH close to 3.0) in the human stomach. It is estimated that endogenous formation contributes 45–75 % of the total human exposure to NOC (*39*). According to Hinuma *et al.* (*40*), the stomach is an important place for nitrosation because the precursors of N-nitrosamines, such as secondary amines and nitrite, enter the stomach in foods with saliva, and the acidity of gastric juice may be appropriate for nitrosation. Importantly, nitrite is also formed in the human body from the reduction of nitrate by bacteria in the oral cavity; therefore, humans are primarily exposed to nitrite from the consumption of vegetables and water, which are the main sources of nitrate intake (*4,40*).

NON-THERMAL TECHNOLOGIES

Antimicrobial technologies in food processing can be classified as thermal or nonthermal. During thermal processing, food is preserved by exposure to a very high temperature, which decreases microbial growth or contamination from food but also leads to some deleterious effects on food products, such as a loss of heat-sensitive nutritional components, textural changes in food, changes in rheological characteristics and changes in sensory attributes (*27*). In nonthermal processing, food is processed at near room temperature and is not damaged due to heat-sensitive nutritious materials; these processes results in a reduction in the microbial load in food with an increase in shelf life, achieving good sensory and textural characteristics (*41*). Various researchers have proposed the use of nonthermal techniques to reformulate meat products by reducing or replacing curing salts to enhance the quality of products and meet consumer expectations.

Irradiation

Food irradiation involves exposing food to a controlled amount of ionizing radiation (energy). With irradiation, little or no heating is applied to the food matrix, nothing is added to the food, and less energy and water are needed for processing. Additionally, irradiation can be applied to either packaged or bulk food in large or small quantities. The amount of energy absorbed, which is expressed in grey (Gy) – equivalent to one unit of joules per kilogram of food (J/kg), can be disrupted by several factors, including physicochemical properties (temperature, physical state, and density) of the food to be irradiated, the dose applied, the exposure time and the distance of the food to the radiation source (*42,43*).

Ionizing radiation can be classified into high-speed electrons, alpha (α; composed of two protons and two neutrons) and beta (β; composed only of electrons), and elementary particles of electromagnetic waves, X-rays and gamma rays (γ), which have zero charge, and the latter being the most energetic (*43*). Stronger interactions of radiation with matter are known to occur for high-energy charged particles emitted from electron beams than for photons of gamma rays and X-rays. Therefore, the three types of radiation used for treating foods show different penetration and effects with respect to the microbiological and physicochemical qualities of foods (*44*). Gamma particles can penetrate matter more intensely by emitting continuous rays at a predictable rate and, therefore, are the most important type of radiation in the food processing industry. In general, the radioactive isotopes cobalt-60 (^{60}Co) and caesium-137 (^{137}Cs) are used for the emission of gamma rays (*41,43*). However, the future use of these isotopes is uncertain due to the rising prices of cobalt-60, increased public concerns about the safety of radioactive materials and issues related to their safe disposal (*44*). This

safety concern has provided an opportunity for the application of electron beams in food products; electron beam accelerators are used to generate high-energy electrons (of 10 mega-electronvolts – MeV) that can penetrate up to 39 mm deep in food with high moisture content (*41*). As the energy of ionizing radiation is high but lower than the threshold of nuclear reactions, food does not become radioactive (*43*). Therefore, the use of ionizing radiation in food processing has been approved and regulated in as many as 60 countries.

Irradiation can increase the shelf life of foods and increase their safety. The process promotes chemical, physical, and biological changes in the structure of microorganisms, preventing their multiplication; direct irradiation results in the unfolding of DNA and damage to nucleic acids, and the ionization of water molecules results in oxidative damage to microbial cells (*41,42*). According to Ehlermann (*45*), the effects of irradiation applications in food processing to control the growth of microorganisms can be classified as radappertization, radicidation, or radurization, which are differentiated by the dose of radiation applied. Radappertization involves the use of extremely high doses of radiation (greater than 10 kGy), ranging between 30 and 50 kGy. These doses can kill all microorganisms in foodstuffs up to the level of spores and are typically used in the sterilization process. During radicidation, moderate doses of radiation (between 1 and 10 kGy) are used to reduce spoilage and microbial pathogens, resulting in food pasteurization. Low doses (less than 1 kGy) are used in the radurization process to inhibit respiration, control microbial growth in fresh meats, slow the ripening of vegetables, and prevent infestations of insects and parasites in grains and fruits.

Irradiation alters food components in meat, particularly lipids and proteins, but can preserve the original quality of the meat (*46*). Fats begin to oxidize when exposed to radiation, leading to rancid off-flavours, oxidation of the meat pigment, and unfavourable colour changes (*47,48*). Nevertheless, food irradiation at doses up to 10 kGy does not cause any unique nutritional issues (*46*).

The recent results obtained using irradiation in cured meat processing related to microbial control and/or N-nitrosamine formation are presented in Table 1. When doses lower than 6.0 kGy were applied, a significant, dose-dependent reduction in vegetative cells was reported for spoilage microorganisms and pathogens (*49,50*). In cooked ham, Silva *et al.* (*51*) reported that the number of *C. sporogenes* spores recovered decreased with increasing doses of radiation (up to 6.0 kGy), but the radiation sensitivity (D-values) decreased from 1.98 kGy in uncured samples to 1.81 and 1.57 kGy in cured samples. According to these authors, the microbiological safety was guaranteed with 50 mg/kg nitrite and irradiation at a dose of 1.5 kGy. Nevertheless, Dutra *et al.* (*5*) reported that gamma irradiation greater than 10 kGy could inactivate *C. botulinum* spores, regardless of the addition of nitrite salts. However, the resistance of spores to irradiation depends upon multiple factors, such as the type of spores (proteolytic or nonproteolytic strains), the temperature of the product and

environment, the medium of treatment, and the initial dose of microorganisms; compared to nonproteolytic strains, proteolytic *C. botulinum* spores are more resistant to irradiation (*56*).

<Table 1 should appear here>

The radiolysis (dissociation of molecules) caused by gamma radiation also helps reduce the amount of residual nitrite and N-nitrosamines in meat products. Furthermore, products that handle irradiated nitrites cannot function as nitrosating agents; therefore, the formation of N-nitrosamines can be inhibited (*53-55,57-58*). Although positive bactericidal effects and N-nitrosamine degradation have been verified, the use of radiation may be limited due to changes in the sensory characteristics of the product. Considering that radiolysis is not selective, the lysis of other substances, such as water, can also occur; this process releases hydroxyl radicals ($OH^{\bullet}$), which can increase the extent of lipid oxidation in food products. Lipid oxidation induced by radiolysis produces compounds, such as sulfurates, can negatively modify aroma and taste, impairing the sensory acceptance of meat products (*50,59-61*). Although irradiation intensifies lipid oxidation, Dutra *et al.* (*60*) observed that adding nitrite to emulsified sausages had a greater effect than irradiation on the quality parameters evaluated; even at low levels (~75 ppm), the use of irradiation decreased the deleterious effects of irradiation at doses as high as 20 kGy.

Therefore, the use of irradiation has great potential as a tool for reformulating meat products aimed at reducing nitrite levels, contributing to the reduction of residual nitrite and N-nitrosamines while ensuring microbiological safety. However, the level of nitrite reduction and irradiation dosage applied must be evaluated for each specific meat product (emulsified, fermented, smoked, *etc*.), as well as the technical and financial feasibility of this type of application. Moreover, it is crucial to change the current consumer perceptions about irradiation since it restricts the growth of this technology in the food industry sector.

Plasma

Cold plasma is an emerging, economical and environmentally friendly technology with potential applications in the food and bioprocessing industry, including microbial decontamination, enzyme inactivation, shelf-life extension, and physicochemical modification (*62*).

Plasma is the fourth state of matter and is formed by gases subjected to extremely high energies; as a result, molecular agitation overcomes the energy of external electrons. Then, the released electrons form a shapeless, electrically neutral mass composed of electrons and dissociated nuclei (*63*). Basically, plasma treatment can be divided into two types: thermal plasma and cold plasma (nonthermal). Thermal plasma produces a large amount of energy by utilizing high

temperature. Cold plasma is a nonthermal treatment that works in the temperature range of 25–65 °C (*41,64*). Cold plasmas can be induced and sustained through an electric discharge in a gas at atmospheric or low pressures, using the corona, dielectric barrier discharge (DBD), or gliding arc discharge configurations (*65*); cold plasmas can be obtained using air and feed gases, such as helium, nitrogen, and argon, or a mixture of gases, which are subjected to different levels to increase the kinetic energy of electrons. Depending on the discharge gas, the cold plasma process yields different biologically active agents: reactive oxygen species (ROS), including atomic oxygen, ozone (O_3), hydroxyl radical ($OH^{\bullet}$), and hydrogen peroxide (H_2O_2); and reactive nitrogen species (RNS), such as peroxynitrite ($ONOO^{\bullet}$), nitrate (NO_3^-), nitrite (NO_2^-), their corresponding acids, and nitrogen oxides (NO_x) (*63, 64*). The reactive species and their concentration in the plasma vary depending on many factors, including the gas in which plasma is induced, the configuration of the plasma source, the power input to the gas, the duration of treatment, and the humidity levels (*65*).

In food processing, cold plasma is an effective strategy for controlling microbial development. The ROS generated from plasma detrimentally interact with vital cellular biomolecules, such as DNA, proteins and enzymes, in cells and may alter the function of biological membranes via interactions with lipids; this process causes unsaturated fatty acid peroxides to form and amino acids in proteins to oxidize (*56,64,65*). The mechanism of action of cold plasma is different for gram-positive and gram-negative bacteria. Cleavage of specific peptidoglycan bonds and oxidation of lipids present in lipopolysaccharides occur in gram-negative bacteria, while oxidative damage to intracellular components, such as DNA, occurs in gram-positive bacteria (*66*). The effectiveness of the antibacterial action depends on numerous factors, including the type of plasma discharge, operating conditions, choice of gas, relative humidity of the product, state (solid or liquid) of the food, and the microorganisms present (*65*).

Due to the formation of RSN during its application, plasma can generate a "natural" source of nitrite. Therefore, in meat processing, cold plasma has been used to induce the curing process and is applied postprocessing (*67*); in addition, cold plasma can be incorporated into the processing steps directly into the meat batter (*68*) or indirectly when applied to water (*63,69-71*) or some ingredient (*72,73*) of the formulation. When plasma is applied to the water that is incorporated into the formulation, this mixture can be considered an additive. According to Jung *et al.* (*69*), the application of plasma in deionized water can favour its acidification (pH decreases from 7 to 2), and a concentration of 782 mg/L nitrite and 358 mg/L nitrate can be formed after 120 min. When the meat mass is directly exposed to cold plasma, a concentration of 66 mg/kg nitrite is formed after 30 min of application (*68*). In general, the level of nitrite production tends to increase with increasing plasma potency (*70*), but since the application of cold plasma increases the temperature of the meat mass,

the optimal nitrite content without exceeding the recommended temperature (<13 °C) was reported to be 42 mg/kg (*71*). Nevertheless, as previously described, it is important to highlight that replacing synthetic nitrites with “natural” nitrite sources does not provide healthier or safer advantages over synthetic nitrite salts.

Similar to the irradiation process, cold plasma produces free radicals and reactive species via gaseous electrical discharge that act as antibacterial agents but can also induce lipid oxidation, especially during the storage of meat products (*62,72*). Lipid oxidation induced by an oxygen-containing cold plasma process can affect the acceptability and shelf life of foods. The nutritional, sensory, and other quality attributes can change depending on the cold plasma process, time, and exposure conditions. In the case of solid foods, however, the lower penetration depth of plasma and the reactive species (10–50 μm depth) affect only the surface and help retain nutrients inside the product (*64*).

In Table 2, we summarize the results obtained to date from cold plasma treatment of meat, both in terms of microbial control (other than *C. botulinum*) and minimization of N-nitrosamines. Although the application of cold plasma in meat processing has high potential, this decontamination technology is a relatively newer approach in the food industry, and more research is needed to optimize the technology for specific applications in meat processing. Since there are no reports on the effect of cold plasma treatment on *C. botulinum* growth and N-nitrosamine levels, further research is needed to clarify its effectiveness in reducing the risks attributed to the use of nitrite/nitrate in meat products.

<Table 2 should appear here>

High-pressure process

High hydrostatic pressure (HHP) technology is applied in food processing mainly to control microbes through enzyme inactivation (*27,76*). In an HPP system, the high pressure (100–1000 MPa) is uniformly distributed by an incompressible (hydraulic) fluid, usually water, to packaged food in a thermally insulated airtight vessel; this process provides a pasteurization effect through the uniform and instantaneous application of isostatic pressure for approximately 10–20 min. According to the isostatic principle, when pressure is applied to a liquid medium in a closed environment, equal pressure is applied to the food product at any point, regardless of its size and shape; therefore, food products of different volumes can be processed within the same batch. Moreover, HPP reduces the influence of temperature on food components. Although the effect of high pressure induces adiabatic heating in water at 3 °C every 100 MPa, the initial pasteurization temperature is controlled within 5–10 °C so that the water temperature will not exceed 30 °C when the pressure reaches 600 MPa (*77,*

78). The efficiency of HPP application can be affected by several parameters related to the process (such as adiabatic heating, decompression time, pressure level, combination of time and pressure, and packaging material), as well as factors intrinsic to the product (pH, water activity, composition, *etc*.) and extrinsic factors (storage and transport conditions) (*27*).

In addition to increasing the health benefits of food products, HPP is a nonthermal technique that potentially enhances the shelf life of food and maintains its quality and safety. In addition to inactivating pathogens, this technology provides several positive effects, as it better preserves the flavour compounds and provides better nutritional value compared to conventional thermal methods (*41*). HPP acts in the range from 5 to 30 °C, which reduces the occurrence of the Maillard reaction and caramelization and prevents the release of new aromatic compounds; consequently, the use of colouring and flavouring agents for meat products is reduced. On the other hand, HPP can affect the microstructure and negatively affect the texture and water retention capacity of certain meat products (*79*). Regarding food safety, although high pressure can eliminate pathogens, a large variety of microbes with different physiological characteristics occur in food products, which leads to highly diverse pressure tolerance characteristics among these microbes. HHP treatment is more effective against eukaryotes, gram-negative bacteria, protozoa, and parasites than against yeast and mould, which inactivate at much higher pressures (*27,77*).

In recent years, the success of HHP against important pathogens in meat products has been reported in several studies (Table 3). HPP exhibits antibacterial activity by modifying the morphology of the microorganism, which causes damage in the cell membranes, the denaturation of proteins and enzymes involved in important genetic mechanisms and chemical reactions that inhibit the growth of microorganisms. The inactivation of microorganisms through HPP may also be related to the rupture of the cell membrane, the formation of ripples and subsequent swelling and reduction in cell volume (*78*). However, although HPP inactivates bacteria, it only partially inactivates spores. Moreover, *C. botulinum* spores are very resistant to HPP, especially at room temperature; in a minced raw chicken inoculated with five nonproteolytic strains of *C. botulinum*, which were cooked and treated at 600 MPa for 2 min at 20 °C, Linton *et al.* (*80*) observed that the spores survived after treatment and germinated during storage. Factors related to bacteria (type, initial count, *etc*.), food matrix (pH, water activity, *etc*.) and treatment conditions (pressure cycles, temperature, *etc*.) influence the resistance of cells and spores to the applied HPP or even the combination of treatments. Similarly, spore recovery after HPP application depends on the medium, additives and incubation conditions (*56*). Therefore, many researchers have combined HHP with sodium nitrite or other antibacterial agents (bacteriocin, plant extract, essential oil, *etc*.) to reduce the addition of sodium nitrite or produce a synergistic antibacterial effect.

<Table 3 should appear here>

Therefore, the application of HHP on an industrial scale has been recommended, mainly for postprocessing and ready-to-eat (RTE) packaged products. In this sense, the use of HHP as a sterilization treatment helps reduce the need to add nitrite to meat products, meeting the growing consumer demand for natural, preservative-free, and minimally processed products. However, research on this technology is scarce, especially regarding the effects on the survival of *C. botulinum* and the quantification and formation of N-nitrosamines in cured meat products.

Prospects

Some additional nonthermal technologies—such as pulsed electric field (PEF) and ultrasound—have not been tested as strategies for replacing nitrite in meat products. However, promising results have been obtained in the reduction of other ingredients, such as salt (sodium chloride) and phosphate, with increased diffusion of salting (*90-92*).

PEF processing involves repeated applications of pulses with an electric field strength of 0.1–100 kV/cm to the food between the electrodes; the resulting electric field causes the formation of pores in the cell membranes of microbes (*93*). Although the application of PEF in solid foods is less studied than its application in liquid foods (*41*)—in which researchers aim to sterilize and inactivate enzymes—this technology may reduce the amount of sodium nitrite and the curing time needed by disrupting cellular tissues and affecting the mass transfer processes (*90*).

Ultrasonication is used at different frequencies, including low-frequency (20 kHz–100 kHz), medium-frequency (100 kHz−1 MHz), and high-frequency (1 MHz–100 MHz) ultrasonication; low-frequency ultrasonication produces large shear forces in the medium, whereas high-frequency ultrasonication produces less shear force in the medium (*41*). The propagation of mechanical energy by ultrasonic waves results in a captivation effect, which is characterized by compression (negative pressure) and decompression (positive pressure) zones. The resulting gas bubbles can implode when unstable, promoting an increase in temperature and pressure that exerts mechanical, chemical, and biochemical effects (*94*). Due to the effects caused by cavitation and other acoustic phenomena, ultrasound can affect the technological properties of proteins and meat products; thus, this technology has already been applied to reduce sodium chloride and additives, such as phosphate (*91,92*).

CONCLUSIONS

In addition to ensuring the sensory quality and food safety of meat products, the use of curing salts can promote the formation of N-nitrosamines. Completely replacing curing salts is challenging due to the versatility and effectiveness of these additives in developing the sensory characteristics of

food products and controlling microbial growth. Thus, a validated alternative to curing salts that can be applied on an industrial scale is lacking in the food processing industry. Most alternative technologies have not adequately reduced the use of N-nitrosamines in meat products. Combining technologies may be an effective alternative to curing salts, limiting the generation of N-nitrosamines without impairing the sensory characteristics of the product.

However, there are gaps in the relevant research on antimicrobial effects, particularly the effects of these alternative techniques on highly toxic *C. botulinum*. The difficulty in performing tests on highly resistant spores of *C. botulinum* spores is among the main challenges in this field of food safety research. *C. botulinum* spores are generally resistant to HPP and irradiation, but promising results in terms of inactivation have been achieved when one of these techniques were combined with heat treatment. Likewise, nonthermal technologies can be used to exert synergistic effects on the inactivation of *C. botulinum*.

Although nonthermal technologies generally require higher initial investments, maintenance and high energy inputs compared to conventional thermal processes, these methods achieve high operational efficiency. Future research related to nonthermal technologies should focus primarily on increasing the cost-effectiveness and viability of these technologies to broaden their production and marketing scope in the food processing sector. Through this research, nutritious and safe food can be generated without eliminating the sensory characteristics that make these foods palatable to consumers.

ACKNOWLEDGEMENTS

The authors would like to thank the Higher Education Personnel Improvement Coordination (CAPES/Brazil) for the doctorate scholarships granted to the first author, and the National Council for Scientific and Technological Development (CNPq/Brazil) for the Research Productivity Fellows (PQ) granted to the last two authors.

FUNDING

This work was funding by the National Council for Scientific and Technological Development (CNPq/Brazil; Award Number: 430206/2016-0) and Minas Gerais State Research Support Foundation (FAPEMIG/Brazil; Award Number: CVZ APQ 02904-17).

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

SUPPLEMENTARY MATERIALS

There are no supplementary files.

AUTHORS' CONTRIBUTION

B.F. Andrade participated in the design of the work, data collection, data analysis and interpretation, and drafting the original article. L.R. Carmo and M.S. Tanaka participated in the data analysis and interpretation and drafting of the article. R.A. Torres Filho and A.L.S. Ramos participated in the manuscript critical revision. E.M. Ramos participated in the design of the work, and critical revision and editing of the manuscript.

ORCID ID

B.F. Andrade https://orcid.org/0000-0001-5221-034X
L.R. Carmo https://orcid.org/0000-0001-6207-5217
M.S. Tanaka https://orcid.org/0000-0002-8817-7104
R.A. Torres Filho https://orcid.org/0000-0003-1760-0419
A.L.S. Ramos https://orcid.org/0000-0001-5510-5131
E.M. Ramos https://orcid.org/0000-0002-8240-8151

REFERENCES

1. Honikel KO. The use and control of nitrate and nitrite for the processing of meat products. Meat Sci. 2008;78(1–2):68-76. https://doi.org/10.1016/j.meatsci.2007.05.030

2. Jo K, Lee S, Yong HI, Choi Y-S, Jung S. Nitrite sources for cured meat products. LWT - Food Sci Tech. 2020;129:109583. https://doi.org/10.1016/j.lwt.2020.109583

3. Majou D, Christieans S. Mechanisms of the bactericidal effects of nitrate and nitrite in cured meats. Meat Sci. 2018;145:273-84. https://doi.org/10.1016/j.meatsci.2018.06.013

4. Shakil MH, Trisha AT, Rahman M, Talukdar S, Kobun R, Huda N, *et al*. Nitrites in cured meats, health risk issues, alternatives to nitrites: a review. Foods (Basel, Switzerland). 2022;11(21):3355. https://doi.org/10.3390/foods11213355

5. Dutra MP, Aleixo GC, Ramos ALS, Silva MHL, Pereira MT, Piccoli RH, *et al*. Use of gamma radiation on control of *Clostridium botulinum* in mortadella formulated with different nitrite levels. Radiat Phys Chem. 2016;119:125-9. https://doi.org/10.1016/j.radphyschem.2015.10.008

6. Sindelar J, Milkowski A. Sodium nitrite in processed meat and poultry meats: a review of curing and examining the risk and benefit of its use. White Paper, Series. 2011;3:1-14.

7. ANVISA RDC no. 52, May 14 2019, Provides for food additives authorized for use in meat and meat products (in Portuguese), 272. Sect. 1 (2019).

8. EU. Commission Regulation (EU) 2023/2108 of 6 October 2023 amending Annex II to Regulation (EC) No 1333/2008 of the European Parliament and of the Council and the Annex to Commission Regulation (EU) No 231/2012 as regards food additives nitrites (E 249-250) and nitrates (E 251-252). Document 32023R2108; OJ L, 2023/2108, 9.10.2023. Avaliable from: https://eur-lex.europa.eu/eli/reg/2023/2108/oj

9. CCFA. Codex general standard for food additives (GSFA): CODEX STAN 192-1995. Rome: Codex Committee on Food Additives; Food and Agriculture Organization of the United Nations; World Health Organization, 2021.

10. Milkowski AL. Sources of Exposure to Nitrogen Oxides. In: Bryan NS, Loscalzo J, editors. Nitrite and Nitrate in Human Health and Disease. Cham: Springer International Publishing; 2017. p. 69-82.

11. IARC. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Red Meat and Processed Meat. Lyon, France: International Agency for Research on Cancer; 2018.

12. López-Rodríguez R, McManus JA, Murphy NS, Ott MA, Burns MJ. Pathways for N-Nitroso Compound Formation: Secondary Amines and Beyond. Org Process Res Dev. 2020;24(9):1558-85. https://doi.org/10.1021/acs.oprd.0c00323

13. Bogovski P, Bogovski S. Special report animal species in which n-nitroso compounds induce cancer. Int J Cancer. 1981;27(4):471-4. https://doi.org/10.1002/ijc.2910270408

14. Park J-e, Seo J-e, Lee J-y, Kwon H. Distribution of Seven N-Nitrosamines in Food. Toxicol Res. 2015;31(3):279-88. https://doi.org/10.5487/TR.2015.31.3.279

15. Luzardo-Ocampo I, Ramírez-Jiménez AK, Yañez J, Mojica L, Luna-Vital DA. Technological Applications of Natural Colorants in Food Systems: A Review. Foods (Basel, Switzerland). 2021;10(3):634. https://doi.org/10.3390/foods10030634

16. Shahidi F, Pegg RB. Nitrite-free meat curing systems: Update and review. Food Chem. 1992;43(3):185-91. https://doi.org/10.1016/0308-8146(92)90171-W

17. Barcenilla C, Ducic M, López M, Prieto M, Álvarez-Ordóñez A. Application of lactic acid bacteria

for the biopreservation of meat products: A systematic review. Meat Sci. 2022;183:108661. https://doi.org/10.1016/j.meatsci.2021.108661

18. Campolina GA, Cardoso MG, Caetano ARS, Nelson DL, Ramos EM. Essential Oil and Plant Extracts as Preservatives and Natural Antioxidants Applied to Meat and Meat Products: A Review. Food Tech Biotech. 2023;61(2):212-25. https://doi.org/10.17113/ftb.61.02.23.7883

19. Kurćubić VS, Mašković PZ, Vujić JM, Vranić DV, Vesković-Moračanin SM, Okanović ĐG, et al. Antioxidant and antimicrobial activity of Kitaibelia vitifolia extract as alternative to the added nitrite in fermented dry sausage. Meat Sci. 2014;97(4):459-67. https://doi.org/10.1016/j.meatsci.2014.03.012

20. Guimarães AS, Guimarães JS, Rodrigues LM, Fontes PR, Ramos AdLS, Ramos EM. Assessment of Japanese radish derivatives as nitrite substitute on the physicochemical properties, sensorial profile, and consumer acceptability of restructured cooked hams. Meat Sci. 2022;192:108897. https://doi.org/10.1016/j.meatsci.2022.108897

21. Sindelar JJ, Cordray JC, Sebranek JG, Love JA, Ahn DU. Effects of varying levels of vegetable juice powder and incubation time on color, residual nitrate and nitrite, pigment, ph, and trained sensory attributes of ready-to-eat uncured ham. J Food Sci. 2007;72(6):S388-S95. https://doi.org/10.1111/j.1750-3841.2007.00404.x

22. Andrade BF, Guimarães AS, do Carmo LR, Tanaka MS, Fontes PR, Ramos AdLS, Ramos EM. S-nitrosothiols as nitrite alternatives: Effects on residual nitrite, lipid oxidation, volatile profile, and cured color of restructured cooked ham. Meat Sci. 2024;209:109397. https://doi.org/10.1016/j.meatsci.2023.109397

23. Kanner J, Juven BJ. S-Nitrosocysteine as an antioxidant, color-developing, and anticlostridial agent in comminuted Turkey meat. J Food Sci. 1980;45(5):1105-12. https://doi.org/10.1111/j.1365-2621.1980.tb06497.x

24. Shpaizer A, Nussinovich A, Kanner J, Tirosh O. S-Nitroso-N-acetylcysteine generates less carcinogenic N-nitrosamines in meat products than nitrite. J Agric Food Chem. 2018;66(43):11459-67. https://doi.org/10.1021/acs.jafc.8b04549

25. Kanner J, Shpaizer A, Nelgas L, Tirosh O. S-Nitroso-N-acetylcysteine (NAC-SNO) as an antioxidant in cured meat and stomach medium. J Agric Food Chem. 2019;67(39):10930-6. https://doi.org/10.1021/acs.jafc.9b03741

26. Shpaizer A, Kanner J, Tirosh O. S-Nitroso-N-acetylcysteine (NAC–SNO) vs. nitrite as an anti-clostridial additive for meat products. Food Funct. 2021;12(5):2012-9.

https://doi.org/10.1039/D0FO02839H

27. Allai FM, Azad ZRAA, Mir NA, Gul K. Recent advances in non-thermal processing technologies for enhancing shelf life and improving food safety. Appl Food Res. 2023;3(1):100258. https://doi.org/10.1016/j.afres.2022.100258

28. Sebranek JG, Bacus JN. Cured meat products without direct addition of nitrate or nitrite: what are the issues? Meat Sci. 2007;77(1):136-47. https://doi.org/10.1016/j.meatsci.2007.03.025

29. Møller JKS, Skibsted LH. Nitric Oxide and Myoglobins. Chem Rev. 2002;102(4):1167-78. https://doi.org/10.1021/cr000078y

30. Ramos EM, Gomide LAM. Meat quality assessment: Fundamentals and methodologies (in portuguese). 2nd ed. Viçosa, MG, Brazil: Editora UFV; 2017.

31. Domínguez R, Pateiro M, Gagaoua M, Barba FJ, Zhang W, Lorenzo JM. A comprehensive review on lipid oxidation in meat and meat products. Antioxidants (Basel). 2019;8(10). https://doi.org/10.3390/antiox8100429

32. Jay JM, Loessner MJ, Golden DA. Modern food microbiology. 7th ed ed. New York: Springer New York; 2005.

33. Borch E, Kant-Muermans M-L, Blixt Y. Bacterial spoilage of meat and cured meat products. Int J Food Microbiol. 1996;33(1):103-20. https://doi.org/10.1016/0168-1605(96)01135-X

34. Hill KK, Xie G, Foley BT, Smith TJ, Munk AC, Bruce D, *et al.* Recombination and insertion events involving the botulinum neurotoxin complex genes in *Clostridium botulinum* types A, B, E and F and *Clostridium butyricum* type E strains. BMC Biol. 2009;7:66. https://doi.org/10.1186/1741-7007-7-66

35. Peck MW. *Clostridium botulinum* and the safety of minimally heated, chilled foods: an emerging issue? J Appl Microbiol. 2006;101(3):556-70. https://doi.org/10.1111/j.1365-2672.2006.02987.x

36. Cammack R, Joannou CL, Cui XY, Torres Martinez C, Maraj SR, Hughes MN. Nitrite and nitrosyl compounds in food preservation. Biochimica et Biophysica Acta - Bioenergetics. 1999;1411(2-3):475-88. https://doi.org/10.1016/S0005-2728(99)00033-X

37. Carpenter CE, Reddy DS, Cornforth DP. Inactivation of clostridial ferredoxin and pyruvate-ferredoxin oxidoreductase by sodium nitrite. Appl Environ Microbiol. 1987;53(3):549-52. https://doi.org/10.1128/aem.53.3.549-552.1987

38. Xie Y, Geng Y, Yao J, Ji J, Chen F, Xiao J, et al. N-nitrosamines in processed meats:

Exposure, formation and mitigation strategies. J Agric Food Res. 2023;13:100645. https://doi.org/10.1016/j.jafr.2023.100645

39. Tricker AR. N-nitroso compounds and man: sources of exposure, endogenous formation and occurrence in body fluids. Eur J Cancer Prev. 1997;6(3).

40. Hinuma K, Matsuda J, Tanida N, Hori S, Tamura K, Ohno T, *et al.* N-nitrosamines in the stomach with special reference to in vitro formation, and kinetics after intragastric or intravenous administration in rats. Gastroenterol Jpn. 1990;25(4):417-24. https://doi.org/10.1007/bf02779329

41. Jadhav HB, Annapure US, Deshmukh RR. Non-thermal Technologies for Food Processing. Front Nut. 2021;8. https://doi.org/10.3389/fnut.2021.657090

42. Ravindran R, Jaiswal AK. Wholesomeness and safety aspects of irradiated foods. Food Chem. 2019;285:363-8. https://doi.org/10.1016/j.foodchem.2019.02.002

43. Indiarto R, Irawan AN, Subroto E. Meat Irradiation: A Comprehensive Review of Its Impact on Food Quality and Safety. Foods (Basel, Switzerland). 2023;12(9):1845. https://doi.org/10.3390/foods12091845

44. Rather SA, Hussain PR, Suradkar PP, Ayob O, Sanyal B, Tillu A, *et al.* Comparison of gamma and electron beam irradiation for using phyto-sanitary treatment and improving physico-chemical quality of dried apricot and quince. J Radiat Res Appl Sci. 2019;12(1):245-59. https://doi.org/10.1080/16878507.2019.1650223

45. Ehlermann DAE. Particular applications of food irradiation: Meat, fish and others. Radiat Phys Chem. 2016;129:53-7. https://doi.org/10.1016/j.radphyschem.2016.07.027

46. Khalid W, Maggiolino A, Kour J, Arshad MS, Aslam N, Afzal MF, *et al.* Dynamic alterations in protein, sensory, chemical, and oxidative properties occurring in meat during thermal and non-thermal processing techniques: A comprehensive review. Front Nutr. 2023;9. https://doi.org/10.3389/fnut.2022.1057457

47. Brewer S. Irradiation effects on meat color - a review. Meat Sci. 2004;68(1):1-17. https://doi.org/10.1016/j.meatsci.2004.02.007

48. Rodrigues LM, Sales LA, Fontes PR, Torres Filho RA, Andrade MPD, Ramos AdLS, Ramos EM. Combined effects of gamma irradiation and aging on tenderness and quality of beef from Nellore cattle. Food Chem. 2020;313:126137. https://doi.org/10.1016/j.foodchem.2019.126137

49. Silva DRG, Moura APR, Haddad GBS, Souza PM, Ramos AdLS, Hopkins DL, Ramos EM. Reducing the safety risk of low nitrite restructured sliced cooked ham by gamma radiation. J Food Process Preserv. 2021;45(1):e15071. https://doi.org/10.1111/jfpp.15071

50. Rodrigues I, Baldini A, Pires M, Carvalho Barros J, Fregonesi R, Gonçalves de Lima C, *et al.* Gamma ray irradiation: A new strategy to increase the shelf life of salt-reduced hot dog wieners. LWT- Food Sci Tech. 2021;135:110265. https://doi.org/10.1016/j.lwt.2020.110265

51. Silva DRG, Haddad GBS, de Moura AP, de Souza PM, Ramos AdLS, Hopkins DL, Ramos EM. Safe cured meat using gamma radiation: Effects on spores of *Clostridium sporogenes* and technological and sensorial characteristics of low nitrite cooked ham. LWT- Food Sci Tech. 2021;137:110392. https://doi.org/10.1016/j.lwt.2020.110392

52. Wei F, Xu X, Zhou G, Zhao G, Li C, Zhang Y, *et al.* Irradiated Chinese Rugao ham: Changes in volatile N-nitrosamine, biogenic amine and residual nitrite during ripening and post-ripening. Meat Sci. 2009;81(3):451-5. https://doi.org/10.1016/j.meatsci.2008.09.005

53. Ahn HJ, Kim JH, Jo C, Lee JW, Yook HS, Byun MW. Effects of gamma irradiation on residual nitrite, residual ascorbate, color, and N-nitrosamines of cooked sausage during storage. Food Control. 2004;15(3):197-203. https://doi.org/10.1016/s0956-7135(03)00047-1

54. Ahn HJ, Kim JH, Jo C, Lee CH, Byun MW. Reduction of carcinogenic N-nitrosamines and residual nitrite in model system sausage by irradiation. J Food Sci. 2002;67(4):1370-3. https://doi.org/10.1111/j.1365-2621.2002.tb10291.x

55. Ahn HJ, Jo CR, Kim JH, Chung YJ, Lee CH, Byun MW. Monitoring of nitrite and N-Nitrosamine levels in irradiated pork sausage. J Food Prot. 2002;65(9):1493-7. https://doi.org/10.4315/0362-028x-65.9.1493

56. Munir MT, Mtimet N, Guillier L, Meurens F, Fravalo P, Federighi M, *et al.* Physical Treatments to Control *Clostridium botulinum* Hazards in Food. Foods (Basel, Switzerland). 2023;12(8):1580. https://doi.org/10.3390/foods12081580

57. Ahn HJ, Kim JH, Jo C, Yook HS, Byun MW. Radiolytic characteristics of nitrite by gamma irradiation. Food Chem. 2003;82(3):465-8. https://doi.org/10.1016/s0308-8146(02)00591-5

58. Ahn HJ, Yook HS, Rhee MS, Lee CH, Cho YJ, Byun MW. Application of Gamma Irradiation on

Breakdown of Hazardous Volatile N-Nitrosamines. J Food Sci. 2002;67(2):596-9. https://doi.org/10.1111/j.1365-2621.2002.tb10644.x

59. Ahn DU, Lee EJ, Feng X, Zhang W, Lee JH, Jo C, et al. Mechanisms of volatile production from non-sulfur amino acids by irradiation. Radiat Phys Chem. 2016;119:64-73. https://doi.org/10.1016/j.radphyschem.2015.09.008

60. Dutra MP, Cardoso GP, Fontes PR, Silva DRG, Pereira MT, Ramos AdLS, Ramos EM. Combined effects of gamma radiation doses and sodium nitrite content on the lipid oxidation and color of mortadella. Food Chem. 2017;237(1):232-9. https://doi.org/10.1016/j.foodchem.2017.05.108

61. Feng X, Moon SH, Lee HY, Ahn DU. Effect of irradiation on the parameters that influence quality characteristics of raw turkey breast meat. Radiat Phys Chem. 2017;130:40-6. https://doi.org/10.1016/j.radphyschem.2016.07.015

62. Gavahian M, Chu Y-H, Mousavi Khaneghah A, Barba FJ, Misra NN. A critical analysis of the cold plasma induced lipid oxidation in foods. Trends Food Sci Technol. 2018;77:32-41. https://doi.org/10.1016/j.tifs.2018.04.009

63. Inguglia ES, Oliveira M, Burgess CM, Kerry JP, Tiwari BK. Plasma-activated water as an alternative nitrite source for the curing of beef jerky: Influence on quality and inactivation of *Listeria innocua*. Innov Food Sci Emerg Technol. 2020;59:102276. https://doi.org/10.1016/j.ifset.2019.102276

64. Mandal R, Singh A, Pratap Singh A. Recent developments in cold plasma decontamination technology in the food industry. 2018;80:93-103. https://doi.org/10.1016/j.tifs.2018.07.014

65. Misra NN, Jo C. Applications of cold plasma technology for microbiological safety in meat industry. Trends Food Sci Technol. 2017;64:74-86. https://doi.org/10.1016/j.tifs.2017.04.005

66. Lee J, Lee CW, Yong HI, Lee HJ, Jo C, Jung S. Use of atmospheric pressure cold plasma for meat industry. Food Sci Anim Resour. 2017;37(4):477-85. https://doi.org/10.5851/kosfa.2017.37.4.477

67. Souza VR, Illera AE, Keener KM. High voltage atmospheric cold plasma technology as a food safety intervention for decontamination of cutting tools during ready-to-eat poultry meat slicing. Innov Food Sci Emerg Technol. 2022;80:103065. https://doi.org/10.1016/j.ifset.2022.103065

68. Jung S, Lee J, Lim Y, Choe W, Yong HI, Jo C. Direct infusion of nitrite into meat batter by atmospheric pressure plasma treatment. Innov Food Sci Emerg Technol. 2017;39:113-8.

https://doi.org/10.1016/j.ifset.2016.11.010

69. Jung S, Kim HJ, Park S, Yong HI, Choe JH, Jeon HJ, *et al.* Color developing capacity of plasma-treated water as a source of nitrite for meat curing. Korean J Food Sci Anim Resour. 2015;35(5):703-6.
https://doi.org/10.5851/kosfa.2015.35.5.703

70. Luo J, Yan W, Nasiru MM, Zhuang H, Zhou G, Zhang J. Evaluation of physicochemical properties and volatile compounds of Chinese dried pork loin curing with plasma-treated water brine. Sci Rep. 2019;9(1):13793.
https://doi.org/10.1038/s41598-019-50351-5

71. Lee J, Jo K, Lim Y, Jeon HJ, Choe JH, Jo C, *et al.* The use of atmospheric pressure plasma as a curing process for canned ground ham. Food Chem. 2018;240:430-6.
https://doi.org/10.1016/j.foodchem.2017.07.148

72. Marcinkowska-Lesiak M, Wojtasik-Kalinowska I, Onopiuk A, Stelmasiak A, Wierzbicka A, Poltorak A. Application of atmospheric pressure cold plasma activated plant protein preparations solutions as an alternative curing method for pork sausages. Meat Sci. 2022;187:108751.
https://doi.org/10.1016/j.meatsci.2022.108751

73. Kim JW, Lee HJ, Shin D-J, Baek KH, Yong H-I, Jung S, *et al.* Enrichment of nitrite in onion powder using atmospheric pressure plasma and egg whites for meat curing. LWT- Food Sci Tech. 2021;135:110050.
https://doi.org/10.1016/j.lwt.2020.110050

74. Yong HI, Lee SH, Kim SY, Park S, Park J, Choe W, *et al.* Color development, physiochemical properties, and microbiological safety of pork jerky processed with atmospheric pressure plasma. Innov Food Sci Emerg Technol. 2019;53:78-84.
https://doi.org/10.1016/j.ifset.2017.09.005

75. Gök V, Aktop S, Özkan M, Tomar O. The effects of atmospheric cold plasma on inactivation of *Listeria monocytogenes* and *Staphylococcus aureus* and some quality characteristics of pastırma—A dry-cured beef product. Innov Food Sci Emerg Technol. 2019;56:102188.
https://doi.org/10.1016/j.ifset.2019.102188

76. Oliveira TLCd, Ramos ALS, Ramos EM, Piccoli RH, Cristianini M. Natural antimicrobials as additional hurdles to preservation of foods by high pressure processing. Trends Food Sci Technol. 2015;45(1):60-85.
https://doi.org/10.1016/j.tifs.2015.05.007

77. Huang H-W, Hsu C-P, Wang C-Y. Healthy expectations of high hydrostatic pressure treatment in food processing industry. J Food Drug Anal. 2020;28(1):1-13.

https://doi.org/10.1016/j.jfda.2019.10.002

78. Aganovic K, Hertel C, Vogel RF, Johne R, Schlüter O, Schwarzenbolz U, *et al.* Aspects of high hydrostatic pressure food processing: Perspectives on technology and food safety. Compr Rev Food Sci Food Saf. 2021;20(4):3225-66.
https://doi.org/10.1111/1541-4337.12763

79. Roobab U, Afzal R, Ranjha MMAN, Zeng X-A, Ahmed Z, Aadil RM. High pressure-based hurdle interventions for raw and processed meat: a clean-label prospective. Int J Food Sci Tech. 2022;57(2):816-26.
https://doi.org/10.1111/ijfs.15499

80. Linton M, Connolly M, Houston L, Patterson MF. The control of *Clostridium botulinum* during extended storage of pressure-treated, cooked chicken. Food Control. 2014;37:104-8.
https://doi.org/10.1016/j.foodcont.2013.09.042

81. Higuero N, Ramírez MR, Vidal-Aragón MdC, Cava R. Influence of high-pressure processing and varying concentrations of curing salts on the color, heme pigments and oxidation of lipids and proteins of Iberian dry-cured loins during refrigerated storage. LWT- Food Sci Tech. 2022;160:113251.
https://doi.org/10.1016/j.lwt.2022.113251

82. Lopez CM, Dallolio G, Bonilauri P, Rebecchi A. Strategies for Nitrite Replacement in Fermented Sausages and Effect of High Pressure Processing against *Salmonella* spp. and *Listeria innocua*. Foods (Basel, Switzerland). 2021;10(11):2617.
https://doi.org/10.3390/foods10112617

83. Balamurugan S, Inmanee P, Souza J, Strange P, Pirak T, Barbut S. Effects of High Pressure Processing and Hot Water Pasteurization of Cooked Sausages on Inactivation of Inoculated *Listeria monocytogenes*, Natural Populations of Lactic Acid Bacteria, Pseudomonas spp., and Coliforms and Their Recovery during Storage at 4 and 10°C. J Food Prot. 2018;81(8):1245-51.
https://doi.org/10.4315/0362-028x.Jfp-18-024

84. Lavieri NA, Sebranek JG, Cordray JC, Dickson JS, Horsch AM, Jung S, *et al.* Effects of Different Nitrite Concentrations from a Vegetable Source with and without High Hydrostatic Pressure on the Recovery of *Listeria monocytogenes* on Ready-to-Eat Restructured Ham. J Food Prot. 2014;77(5):781-7.
https://doi.org/10.4315/0362-028X.JFP-13-401

85. Myers K, Cannon J, Montoya D, Dickson J, Lonergan S, Sebranek J. Effects of high hydrostatic pressure and varying concentrations of sodium nitrite from traditional and vegetable-based sources on the growth of *Listeria monocytogenes* on ready-to-eat (RTE) sliced ham. J Food Prot.

2013;94(1):69-76.
https://doi.org/10.1016/j.meatsci.2012.12.019

86. Myers K, Montoya D, Cannon J, Dickson J, Sebranek J. The effect of high hydrostatic pressure, sodium nitrite and salt concentration on the growth of *Listeria monocytogenes* on RTE ham and turkey. Meat Sci. 2013;93(2):263-8.
https://doi.org/10.1016/j.meatsci.2012.09.007

87. Cava R, Higuero N, Ladero L. High-pressure processing and storage temperature on *Listeria monocytogenes*, microbial counts and oxidative changes of two traditional dry-cured meat products. Meat Sci. 2021;171:108273.
https://doi.org/10.1016/j.meatsci.2020.108273

88. Pietrzak D, Fonberg-Broczek M, Mucka A, Windyga B. Effects of high pressure treatment on the quality of cooked pork ham prepared with different levels of curing ingredients. High Pressure Res. 2007;27(1):27-31.
https://doi.org/10.1080/08957950601091087

89. Hayman MM, Baxter I, O'Riordan PJ, Stewart CM. Effects of high-pressure processing on the safety, quality, and shelf life of ready-to-eat meats. J Food Prot. 2004;67(8):1709-18.
https://doi.org/10.4315/0362-028x-67.8.1709

90. Stoica M, Antohi VM, Alexe P, Ivan AS, Stanciu S, Stoica D, *et al.* New strategies for the total/partial replacement of conventional sodium nitrite in meat products: a review. Food Bioproc Tech. 2022;15(3):514-38.
https://doi.org/10.1007/s11947-021-02744-6

91. Pinton MB, Correa LP, Facchi MMX, Heck RT, Leães YSV, Cichoski AJ, *et al.* Ultrasound: A new approach to reduce phosphate content of meat emulsions. Meat Sci. 2019;152:88-95.
https://doi.org/10.1016/j.meatsci.2019.02.010

92. Barretto TL, Bellucci ERB, Barbosa RD, Pollonio MAR, Romero JT, da Silva Barretto AC. Impact of ultrasound and potassium chloride on the physicochemical and sensory properties in low sodium restructured cooked ham. Meat Sci. 2020;165:108130.
https://doi.org/10.1016/j.meatsci.2020.108130

93. Bhat ZF, Morton JD, Mason SL, Bekhit AE-DA. Current and future prospects for the use of pulsed electric field in the meat industry. Crit Rev Food Sci Nutr. 2019;59(10):1660-74.
https://doi.org/10.1080/10408398.2018.1425825

94. Alarcon-Rojo AD, Janacua H, Rodriguez JC, Paniwnyk L, Mason TJ. Power ultrasound in meat processing. Meat Sci. 2015;107:86-93.
https://doi.org/10.1016/j.meatsci.2015.04.015

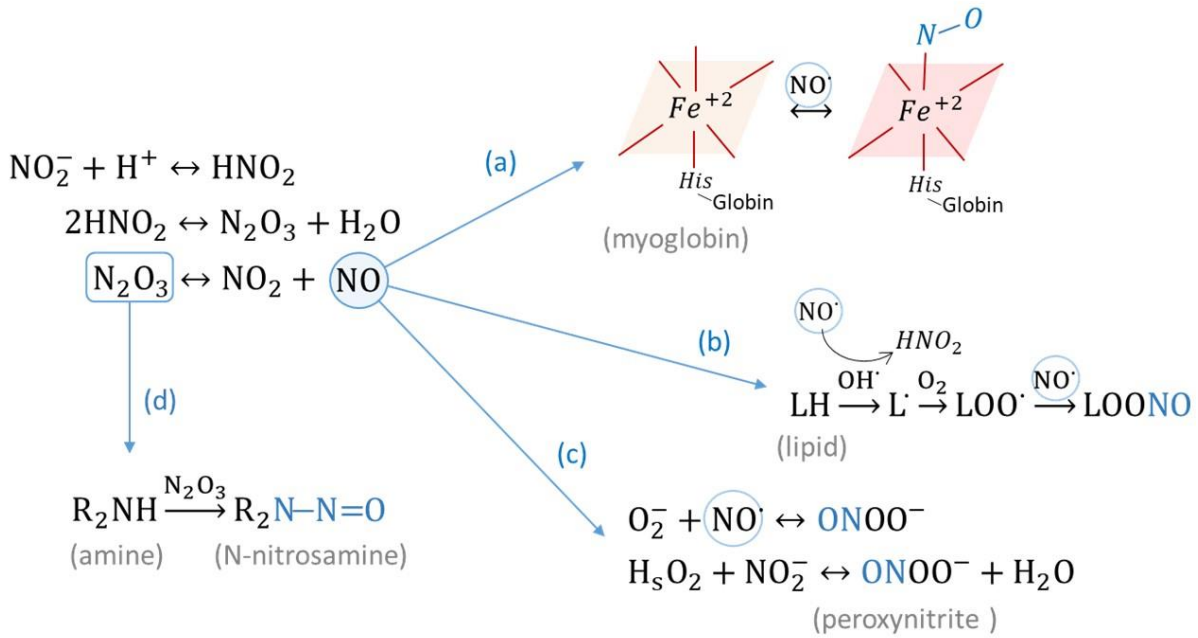

Fig. 1. The mechanisms of nitrite actions in meat curing: (a) development of cured color, (b) antioxidant effect; (c) antimicrobial effect; and (d) N-nitrosamine formation

Table 1. Recent research with gamma irradiation application for microbial control and reduction of N-nitrosamines in meat products.

Reference	Product	Assessment	Main conclusions
Dutra *et al.* (*5*)	Mortadella	Effect of different gamma irradiation doses (0, 10 and 20 kGy) associated with different levels of nitrite (0, 150 and 300 mg/kg) in products inoculated with spores of *Clostridium botulinum* (10^7 spores/g).	Gamma irradiation of 10 kGy is capable of inactivating *C. botulinum*, regardless of the addition of nitrite salts.
Silva *et al.* (*49*)	Cooked and sliced ham	Effect of different gamma irradiation doses (0.5, 1.0, 1.5 and 2.0 kGy) on *Listeria monocytogenes* (10^6 CFU/g) survival and development in products formulated with different levels of nitrite (0, 50 and 150 mg/kg).	There was a synergistic effect between the use of nitrite and irradiation against *Listeria*, being estimated to eliminate *Listeria* irradiation with 1.5 kGy for 150 mg/kg of nitrite and irradiation with 1.8 kGy for 50 mg/kg of nitrite.
Rodrigues *et al.* (*50*)	Sausage	Effect of different gamma irradiation doses (1.5, 3.0 and 4.5 kGy) associated with reduced sodium content (2 and 1.25 %) on microbiological safety and shelf life of cured (150 mg/kg nitrite) cooked sausages.	The reduction in microbial load was dose dependent; the minimum dose (1.5 kGy) provided reductions of up to 6 log cycles in lactic acid bacteria.
Silva *et al.* (*51*)	Cooked ham	Effect of different gamma irradiation doses (0, 1.5, 3.0, 4.5 and 6.0 kGy) associated with different levels of sodium nitrite (0, 50 and 150 mg/kg) on *Clostridium sporogenes* (10^7spores/g).	The guarantee of microbiological safety was obtained with 50 mg/kg of nitrite and irradiation with a dose of 1.5 kGy.
Wei *et al.* (*52*)	Chinese Rugao ham	Effect of gamma irradiation application (5 kGy, rate 10-15 kGy/h) on the profile of biogenic amines, N-nitrosamines and residual nitrite in products added with 150 mg/kg of nitrite.	Irradiation was able to reduce the residual nitrite content. The levels of some biogenic amines (tyramine and putrescine) decreased and others (spermidine, cadaverine, tryptamine and phenylethylamine) increased. Irradiation is capable of degrading volatile N-nitrosamines.

Ahn *et al.* (*53*)	Smoked sausage	Effect of different gamma irradiation doses (0, 5, 10 and 20 kGy) on products added with 150 mg/kg of nitrite.	The application of 10 and 20 kGy reduced the formation of N-nitrosodimethylamine (NDMA) in the vacuum-packed product or under aerobic conditions. N-nitrosopyrrolidine (NPYR) was not detected in irradiated samples, regardless of concentration.
Ahn *et al.* (*54*)	Sausage	Effect of applying different irradiation doses (0, 5,10, 20 and 30 kGy) on products added with 156 mg/kg of nitrite, stored in aerobic and anaerobic packaging.	Irradiation to control nitrosamines was more effective in products stored in aerobic packaging, with a reduction effect starting at 10 kGy. In anaerobic packaging, a minimum dose of 20 kGy was required.
Ahn *et al.* (*55*)	Sausage	Effect of irradiation (0, 5, 10 and 20 kGy) and different nitrite levels (75 and 150 mg/kg) on N-nitrosamine content during storage (4 weeks / 4 °C).	The effect of irradiation on N-nitrosamines was observed with storage. A dose equal to or greater than 10 kGy was necessary to control N-nitrosodimethylamine (NDMA) and N-nitrosopyrrolidine (NPYR).

CFU=colony forming units

Table 2. Recent research with cold plasma application for microbial control and reduction of N-nitrosamines in meat products.

Reference	Product	Assessment	Main conclusions
Inguglia *et al.* (*63*)	*Jerky beef*	Effect of applying cold plasma (20 kHz; supplied with air or nitrogen) in brine water with different nitrite contents (0, 50 and 150 mg/kg) in products inoculated with *Listeria innocua*.	Only plasma-treated samples had residual nitrite concentrations. A reduction of 0.5 log CFU/mL of *Listeria* was verified in the samples treated with plasma, regardless of the nitrite content.
Marcinkowska-Lesiak *et al.* (*72*)	Sausage	Effect of plasma application in plant solutions (soybean and lentil) as a substitute for nitrite (75 mg/kg) on microbiological and technological characteristics.	There was no difference in residual nitrite content (50 mg/kg) and in the development of aerobic bacteria, regardless of treatment.
Kim *et al.* (*73*)	Sausage	The effect of adding onion powder treated with plasma (550 W and 25 kHz) and without sodium nitrite on carcinogenic potential was evaluated using the Ames test.	Onion powder treated with plasma did not show an increase in the number of His+ revertant colonies, suggesting safety for application.
Yong *et al.* (*74*)	*Jerky beef*	Effect of applying cold plasma (4 kHz; air) for 0, 20, 40 and 60 min after marinating pork meat in brine (with and without nitrite).	The application of cold plasma for 40 minutes was sufficient to reach the same nitrite concentration as in the conventional formulation (150 mg/kg). Greater antibacterial action against *Staphylococcus spp*. and *Bacillus spp.* was verified in samples treated with cold plasma.
Gök *et al.* (*75*)	Pastrami	Effect of application time (0, 180 and 300 s) and composition of oxygen, argon and two oxygen/argon mixtures (25:75 and 50:50) of cold plasma on the control of *Staphylococcus aureus* (10^6 CFU/g) and *Listeria monocytogene* (10^6 CFU/g).	There was a reduction in the counts of *S. aureus* (0.85 log CFU/cm^2) and *L. monocytogenes* (0.83 log CFU/cm^2), regardless of the time of application. Plasma from oxygen gas was more effective for the reduction of *L. monocytogenes*.

CFU=colony forming units

Table 3. Recent research with high hydrostatic pressure (HHP) application for microbial control and reduction of N-nitrosamines in meat products.

Reference	Product	Assessment	Main conclusions
Linton *et al.* (*80*)	Cooked chicken	Effect of HHP applied at 600 MPa for 2 min at 20 °C in uncured minced cooked chicken made without nitrite and inoculated with five nonproteolytic strains of *C. botulinum* (10^4 spores/g). They investigated oxygen-permeable packaging, and the addition of 2 % sodium lactate and *Weissella viridescens* (10^8 CFU/g) to control *C. botulinum*.	The HPP treatment does not inactivate bacterial endospores and the conditions in cooked chicken could support the growth of *C. botulinum*. *W. viridescens* was not suitable for use as a protective culture to control the growth of *C. botulinum, but g*ermination and growth was controlled whit 2 % addition of sodium lactate and when oxygen-permeable packaging was used.
Higuero *et al.* (*81*)	Dry-cured sirloin (sliced)	Effect of HHP treatment at 600 MPa for 7 min on technological and physicochemical characteristics of products made with reduced amounts of sodium nitrite and nitrate (150, 75, 37.5 and 0 mg/kg), sliced and stored under refrigeration for 240 days.	The HPP treatment applied (600 MPa, 7 min) minimally affected the color and oxidation of lipids and proteins, regardless of the added sodium nitrite/nitrate content. The reduction of nitrate/nitrite in up to 75 % proved to be feasible without affecting the instrumental color, lipid and protein oxidation.
Lopez *et al.* (*82*)	Fermented sausage	Effect of HHP applied at 593 MPa for 290s in fermented sausages made without nitrite and inoculated with *Listeria innocua* (10^6 CFU/g) and *Salmonella enterica* (10^6 CFU/g) strains.	Sausages that were treated with HHP reduced by up to 4.74 and 3.83 log CFU/g for *L. innocua* and *Salmonella spp.,* respectively.
Balamurugan *et al.* (*83*)	Cooked Sausages	Effects of HHP (600 MPa for 3 min) and hot water (HW; 75°C for 15 min) pasteurization on the inactivation of inoculated *Listeria monocytogenes* (10^8 CFU/g) in vacuum-packaged cooked sausages and their recovery during storage at 4 and 10°C for 35 days.	The combined effect of HPP and HW resulted in a > 6 log reduction in the number of *L. monocytogenes* inoculated during storage, regardless the temperature.

Lavieri *et al.* (*84*)	Restructured ham (sliced)	To investigate the effect of 0.50 and 100 mg/Kg of vegetable source nitrite and the use of HHP at 400 and 600 MPa for 4 min on the *Listeria monocytogenes* count inoculated (10^5 CFU/g) into slices of restructured ham.	Treatment of 600 MPa HHP for 4 min led to populations of *L. monocytogenes* below the limit of detection, independent of the added nitrite concentration.
Myers *et al.* (*85*)	Cooked ham (sliced)	Effect of added nitrite source (chemical or vegetable powder) and HHP (600 or 400 MPa for 3 min) on *Listeria monocytogenes* (10^6 CFU/g) growth and physicochemical and technological characteristics.	The application of HHP at 600 MPa, regardless of the nitrite source used, reduced the number of *L. monocytogenes* by more than 3 log CFU/g, whereas the use of 400 MPa had a minimal effect. Furthermore, the use of HHP did not change the concentration of residual nitrite in the formulations.
Myers *et al.* (*86*)	Cooked ham and turkey (sliced)	Effect of HHP treatment at 600 MPa for 3 min in products made with different meat species and varying sodium nitrite concentration (0 and 200 mg/kg), both inoculated (10^5 CFU/g) with *Listeria monocytogenes*, over 28 days of refrigerated storage.	The use of HHP reduced *L. monocytogenes* counts by more than 3 log CFU/g after 182 days in storage, while cured products achieved growth.
Cava *et al.* (*87*)	Salchichón and Dry-cured loin	The effect of applying HPP (600 MPa, 8 min) on the control of *L. monocytogenes* was evaluated in products made with 150 mg/kg of nitrite, sliced and vacuum-packed.	Average reductions of 2.30 log CFU/g in the *L. monocytogenes* population were observed immediately after high-pressure treatment.
Pietrzak *et al.* (*88*)	Cooked ham	Effects of HHP (600 MPa, 10 min, 20 °C) on the control of *Salmonella*, coliforms and *Staphylococcus aureus* in nitrite-reduced ham (50 and 80 mg/kg).	The reduction of sodium nitrite did not affect the microbiological quality when associated with high pressure after 8 weeks of storage.
Hayman *et al.* (*89*)	Pastami, Strassburg,	Effect of HHP (600 MPa, 20°C, for 180 s) on shelf-life extension and safety against *L. monocytogenes*.	High pressure was efficient in controlling aerobic and anaerobic bacteria, generating counts below

	Sausage and Cajun meat	detectable limits and lower than untreated ones after 95 days of storage at 4 °C.

CFU=colony forming units