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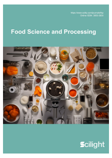

*Review*

# Challenges and Prospects for Nitrite Substitution in Processed Meats

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**Abstract:** Curing salts, nitrite and nitrate salts, are multifunctional additives in meat matrices, responsible for generating and maintaining desirable sensory attributes throughout storage, as well as ensuring microbiological safety, particularly against *Clostridium botulinum*, the causative agent of a highly lethal intoxication in humans. However, the undesirable formation of N-nitrosamines, potentially carcinogenic compounds, is also associated with nitrite addition. Consequently, regulatory reevaluations and scientific investigations have sought alternatives to these curing salts that balance food safety and sensory acceptance while mitigating health risks linked to nitrite consumption. This review presents and updates the principal strategies explored for the partial or complete replacement of nitrite in meat products, covering well-established approaches such as the use of colorants, biopreservation techniques, and the incorporation of natural nitrate sources, as well as an unprecedented perspective by examining, for the first time, the potential of S-nitrosothiols as viable substitutes.

**Keywords:** meat curing; S-nitrosothiol; colorants; biopreservation

## 1. Introduction

Most meat products, particularly ready-to-eat (RTE) items, are mandatorily cured, meaning that curing salts, comprising nitrite salts and their precursors (nitrate salts), are added. Nitrate is used only in "slow-processed" products, such as dry-cured ham and salami, because it functions as a nitrite reservoir, maintaining optimal nitrite levels throughout processing. Curing salts are considered multifunctional additives, as they perform several roles within the meat matrix, including microbial control, development of desirable sensory attributes (color, flavor, and aroma), and oxidative stability during storage, thereby ensuring safe products with attractive characteristics throughout their shelf life [1]. Biochemically, curing consists of a series of reactions and chemical equilibria that culminate in the conversion of nitrite into nitric oxide (NO), a species with high affinity for metal ions such as iron, which is central to all desirable curing processes [2]. The Fe–NO bond in the myoglobin pigment produces nitrosomyoglobin (a pinkish-red pigment) and stabilizes iron in its reduced state (Fe$^{2+}$), thereby limiting oxidative reactions and extending product shelf life [3]. The Fe–NO interaction with microbial enzymes can inhibit the metabolism of major pathogenic microorganisms and prevent foodborne diseases, such as human botulism, caused by *Clostridium botulinum*, a highly resistant spore-forming bacterium that produces a lethal neurotoxin [1].

A paradox exists regarding the relationship between curing salts and consumer health. On one hand, nitrite prevents botulinum intoxication; on the other, it can form undesirable species that act as precursors of N-nitrosamines; compounds of major long-term public health concern due to their mutagenic and genotoxic potential and strong oncogenic effect in humans [4]. For these reasons, the International Agency for Research on Cancer (IARC) concluded that ingested nitrates or nitrites are probable human carcinogens under conditions that favor endogenous nitrosation, and thus cured meat products were classified as Group 1 carcinogens [5]. Although added nitrite degrades during processing and storage, its residual presence remains concerning due to its potential to form N-nitrosamines. In meat products, N-nitrosamines may be endogenous, formed during processing, particularly under high-temperature conditions, or exogenous, formed during digestion, since N-nitrosation reactions are catalyzed under acidic conditions [3,6,7].

Growing concerns have driven regulatory reassessments in several countries to reduce residual levels of these compounds in foods. In addition, the minimum amount required to ensure both desirable sensory characteristics, such as cured color, and microbiological safety varies across products, making the balance between efficacy and safety a persistent challenge for industry. In Brazil, the maximum allowable residual levels are 300 mg/kg for sodium or potassium nitrite (International Numbering System—INS 249 and 250, respectively) and 150 mg/kg for sodium or potassium nitrate (INS 251 and 252, respectively) [8]. In the European Union, Regulation (EU) 2023/2108 reduced nitrite limits to 80 mg/kg for non-heat-treated cured meats and 50 mg/kg for heat-treated products, with residual limits of 25–45 mg/kg throughout shelf life [9]. The Codex Committee on Food Additives (CCFA/FAO/WHO) establishes a maximum limit of 80 mg/kg for cured meat products [10].

Additionally, there is a growing consumer trend towards clean-label meat products, i.e., those formulated with reduced or no added chemical additives. It is worth noting that nitrite and nitrate naturally occur in human saliva and in foods such as vegetables and potable water [11]. Likewise, N-nitrosamines have been reported in water, plant- and animal-based foods and beverages, cigarettes, pesticides, and cosmetics [12].

Given the evident need to partially or completely remove curing salts from meat formulations, various substitution strategies and technological approaches have been investigated. This review presents a systematic and up-to-date analysis of the different categories of strategies for nitrite replacement, based on recent studies, critically highlighting the advantages and limitations of each approach (Figure 1). These strategies include the use of colorant agents to achieve a cured-like appearance, biopreservation techniques (such as microbiota control and the application of bacteriocins, organic acids, essential oils, and plant extracts), as well as combined technological approaches and the incorporation of natural nitrate sources. As a novel contribution, this review explores the emerging use of S-nitrosothiols as a nitrite substitute. In addition, the review discusses the industrial feasibility and safety of these substitutes, while also addressing key knowledge gaps and future research and development needs.

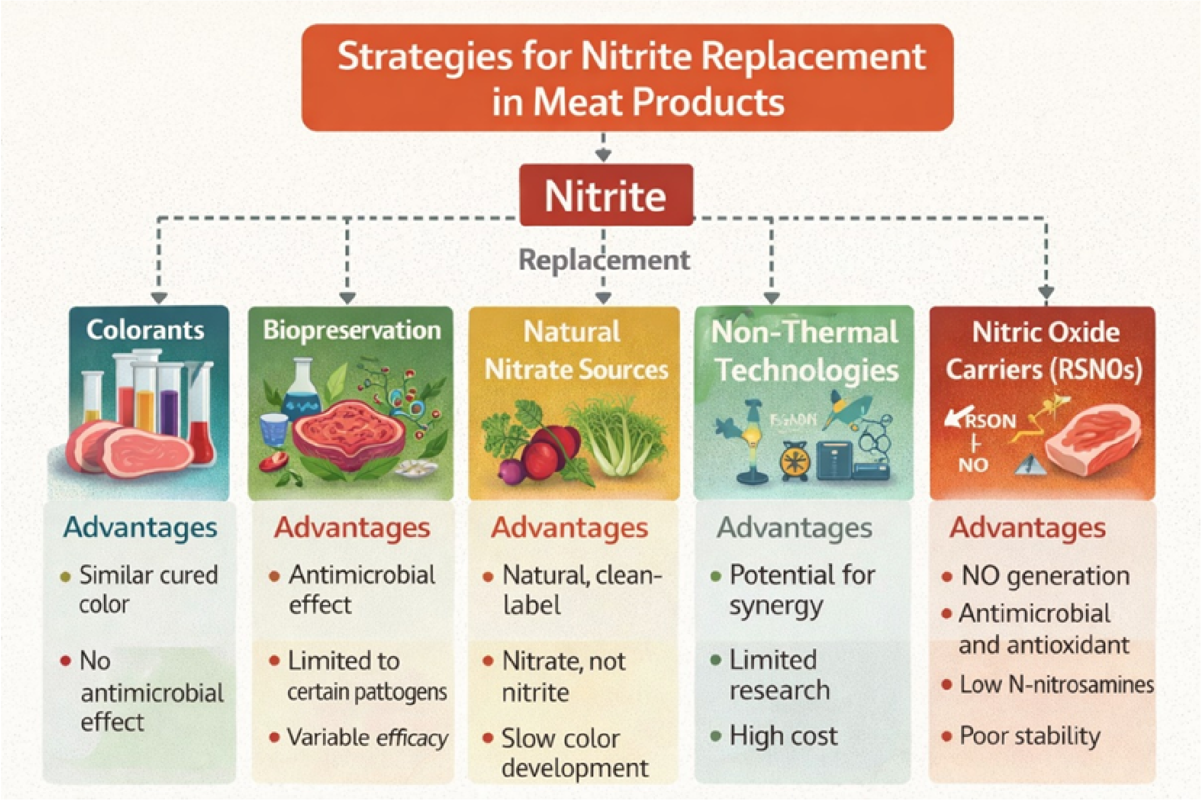

**Figure 1.** Comparative overview of nitrite replacement strategies in meat products highlighted in this review.

## 2. Nitrite Replacement

### *2.1. Use of Food Colorants*

Although sodium nitrite produces the desirable cured colour in meat products, its use is frequently complemented by the addition of food colorants, particularly in heat-treated products. This practice is regulated and widely adopted by industry to ensure colour uniformity and standardization, and to assist in preserving colour during storage. Consequently, certain colorants are permitted by food safety authorities in many countries.

Colorants may be classified as natural or synthetic according to their origin or method of production. Natural colorants are obtained from plants, microorganisms, or insects, such as carmine from cochineal insects, annatto (*Bixa orellana* L.) derived pigments, and pigments produced by inoculation of the fungus *Monascus ruber*, which also exhibits antioxidant activity [13]. Synthetic colorants are laboratory-synthesized compounds, for example, Allura Red (INS 129). According to Castro et al. [14], synthetic and natural colorants present distinct advantages and limitations. In general, synthetic colorants tend to show high stability to light, oxygen, heat, and pH; uniformity; high colouring power; low microbial contamination risk; and relatively low production cost. However, the use of synthetic additives has been declining due to potential health concerns and changing consumer preferences toward products with reduced or no synthetic additives.

Natural colorants are attractive because, beyond imparting colour, they may provide functional and preservative effects. Additionally, this approach can be more sustainable when raw material is derived from edible by-products or otherwise unsaleable vegetable material [15]. These authors evaluated labels of various Polish meat products with respect to colorant usage. The most common commercial colorants included carmine, cochineal extract, paprika extract, betanin, caramel, and bixina, with a predominance in sausage products.

Overall, the use of colorants in meat products fits within strategies for partial nitrite replacement, or total replacement when combined with other techniques.

### *2.2. Biopreservation Techniques*

The application of biopreservative agents in meat and meat products aims primarily at ensuring food safety against spoilage organisms and pathogens, either as a substitute for an existing preservation technique or as a component of hurdle technology [16,17]. Biopreservation may also reduce lipid oxidation and stabilise product colour [16,18–20]. Biopreservation approaches include: (1) application of microbial cultures that act directly by competition for nutrients and space or indirectly through production of inhibitory compounds (primary or secondary metabolites) such as bacteriocins, organic acids, hydrogen peroxide and enzymes; (2) direct application of metabolites (primary or secondary); and (3) use of plant-derived metabolites such as extracts or essential oils.

#### 2.2.1. Bacteriocins

Bacteriocins are metabolites composed of peptides or proteins whose amino-acid composition confers specific biochemical properties, antimicrobial spectra, and modes of action [16,18]. Examples include nisin, pediocin, lacticin, lactococcin, leucocin, plantaricin, enterocin, and carnobacteriocins. Generally, bacteriocins are colourless, odourless, and tasteless. They are typically sensitive to proteolytic enzymes, and their activity depends on environmental parameters such as pH, temperature, and substrate characteristics [21].

Main application strategies for bacteriocins in meat systems comprise: (1) direct inoculation of bacteriocin-producing strains as starter or protective cultures; (2) direct addition of purified bacteriocin as a food additive; (3) incorporation of purified bacteriocin in packaging materials; and (4) encapsulation of bacteriocins [21]. Cultures may be applied fresh or lyophilized, either incorporated into the product mass or surface-sprayed onto fresh or finished products [16].

Nisin and pediocin are bacteriocins that have been extensively investigated for application in meat products, and comparative studies conducted within the same food matrix demonstrate relevant quantitative differences in antibacterial efficacy and persistence of activity during storage. In ground pork subjected to decontamination treatments, Murray and Richard [22] directly compared nisin A and pediocin AcH and reported that nisin application resulted in an immediate reduction of approximately 2.0–2.5 log CFU/g of *Listeria monocytogenes*, whereas pediocin produced a more limited initial reduction of about 1.0–1.5 log CFU/g under the same experimental conditions. During refrigerated storage, samples treated with pediocin exhibited faster microbial recovery, while nisin maintained residual reductions greater than 1.0 log CFU/g for a longer period, indicating greater robustness of its antimicrobial effect in the evaluated meat matrix.

In processed RTE meat products, including sausages and sliced cooked meats, review studies and technological applications indicate that nisin tends to exert a broader inhibitory effect on Gram-positive microbiota, whereas class IIa pediocins act in a more targeted manner against *L. monocytogenes* and are therefore frequently applied as surface treatments to control post-processing contamination [23–25].

Kalschne et al. [26] reported that direct addition of nisin in sliced, vacuum-packaged ham reduced counts by 2–3 log cycles during refrigerated storage (60 days) compared with nisin-free controls. Kumar et al. [27] concluded that a combination of pediocin produced by *Pediococcus pentosaceus* and *Murraya koenigii* in a refrigerated emulsion meat system provided antilisterial and antioxidant activity comparable to conventional nitrite formulations, without altering colour parameters or emulsion stability. Nieto-Lozano et al. [28] indicated that application of pediocin or the producing organism (*Pediococcus acidilactici*) may effectively inhibit *L. monocytogenes* and *Clostridium perfringens* in fermented sausages. Principal modes of action of bacteriocins include inhibition of cell-wall synthesis, permeabilization of the target cell membrane, and inhibition of enzymatic activity [16,18]. Although promising, bacteriocin use as additives may be constrained by high cost and limited efficacy against certain pathogens in finished products [16].

### 2.2.2. Enzymes

Enzymes are proteins that catalyse chemical reaction rates; their activity is influenced by substrate concentration, ionic strength, temperature, and pH—factors that limit their application. Strategies under discussion include inoculation with enzyme-producing microorganisms or direct addition of enzymes.

Certain *Staphylococcus* species (e.g., *S. epidermidis, S. warneri, S. lentus*) isolated from Parma ham have been implicated in generating characteristic product colour via removal of iron from heme and incorporation of zinc, yielding zinc protoporphyrin IX [20,29]. The biosynthesis mechanism remains incompletely elucidated, but one hypothesis is that some bacteria possess ferrochelatase (FECH) activity, favouring iron removal from porphyrin rings and formation of the red pigment [30].

Zhu et al. [31] reported that inoculation with *Lactobacillus plantarum* (7 log CFU/g) can replace up to 50% of sodium nitrite due to the strain's high nitrate- and nitrite-reductase activity, resulting in substantial NO release and low residual nitrate/nitrite levels. Under these conditions, antioxidant activity, like conventional nitrite concentrations, was observed, along with reduced formation of biogenic amines such as tyramine.

Lysozyme, a hen-egg white enzyme with antibacterial potential, is not yet approved for use in the meat industry but has been investigated. Nattress et al. [32] evaluated nisin–lysozyme combinations against meat spoilers (*Brochothrix thermosphacta* and *Carnobacterium* spp.) and suggested synergistic effects that may extend shelf life. Li et al. [33] reported that lysozyme combined with Chinese liquor inhibited growth and enterotoxin production by *S. aureus* in fermented products. Kroll et al. [34] describe lysozyme's bactericidal action via hydrolysis of the β-1,4 glycosidic bonds between N-acetylmuramic acid and N-acetylglucosamine in peptidoglycan.

### 2.2.3. Organic Acids

Organic acids constitute a class of compounds commonly present in plants (e.g., citric, ascorbic, benzoic acids), animal tissues (e.g., lactic, formic, propionic acids) or as microbial metabolites (e.g., lactic acid produced by lactic acid bacteria). These acids may occur naturally in foods or be added to extend shelf life and control pathogens [35]. Use of organic acids and derivatives typically aims at partial nitrite reduction, or full replacement when combined with other strategies, because high concentrations may negatively affect sensory attributes.

Organic acids can be applied by spraying, immersion, injection, incorporation into active packaging films, or by nanoencapsulation. Their antibacterial mechanisms depend on the target microorganism and extend beyond a simple reduction in water activity. In their undissociated form, organic acids readily permeate the bacterial cell membrane and dissociate in the cytoplasm, leading to intracellular acidification and dissipation of the proton motive force. This process compromises cellular energy metabolism by inhibiting ATP synthesis, as cells must expend energy to extrude excess protons and maintain pH homeostasis [36,37]. In parallel, organic acids interfere with the activity of key metabolic enzymes, including dehydrogenases, decarboxylases, and enzymes of the tricarboxylic acid cycle, thereby disrupting carbon flux and redox balance. Inhibition of enzymes such as pyruvate dehydrogenase and fumarase has been associated with reduced NADH generation and impaired central metabolism [38]. Additionally, organic acids disrupt membrane-associated transport systems, reduce amino acid uptake, and alter membrane integrity, which indirectly affects DNA replication and protein synthesis [39]. Collectively, these effects result in metabolic exhaustion, growth inhibition, and eventual bacterial cell death, particularly in anaerobic and facultative anaerobic bacteria commonly associated with meat products.

Lactic acid (2-hydroxypropionic acid; pKa = 3.85) and derivatives can be incorporated into meat formulations as antimicrobials, to enhance perceived saltiness, stabilise colour and act as antioxidants [35]. Redondo-Solano et al. [40] examined ham formulations varying nitrite (0 or 100 ppm) and salt (1 or 2%) combined with potassium lactate and sodium diacetate mixtures (0, 1.5, or 2.5%) on *C. perfringens* spore germination and growth. Although salt reduction increased germination risk, nitrite and/or organic acid salts exhibited a synergistic inhibitory effect.

Sorbic acid (2,4-hexadienoic acid; pKa = 4.76) and salts, obtained from rowan berries (*Sorbus aucuparia*) or synthetically produced, are authorised as antifungal and antimicrobial preservatives in many jurisdictions [41]. Khanipour et al. [42] demonstrated that combined NaCl and sorbate effectively reduce *C. sporogenes* in high-moisture, mildly acidic culture conditions. However, sorbate has been associated with cytotoxic, genotoxic, and allergenic effects [43].

The combined use of bacteriocins, enzymes, and organic acids as nitrite alternatives offers microbial safety benefits while reducing nitrite-associated health risks and meeting consumer demand for more natural products. Nonetheless, further research is required to evaluate efficacy, safety, and commercial feasibility across diverse product types and processing conditions. Once microbiological preservation is demonstrated, complementary strategies are typically needed to achieve full substitution of sodium nitrite.

#### 2.2.4. Essential Oils and Plant Extracts

Essential oils and extracts, obtained from plants, vegetables, and fruits, are complex mixtures of terpenic hydrocarbons, monoterpene and sesquiterpene alcohols, aldehydes, ketones, polyphenols, esters, and may also contain fatty acids, oxides and sulfur-derived compounds. Constituents may be present in major or trace amounts and often display volatility and odour. These compounds typically exhibit antimicrobial activity against spoilage organisms and pathogens, and antioxidant properties [44,45]. Essential oils are commonly obtained by mechanical/thermal processes (e.g., distillation) together with hydrolates, whereas extracts are produced by solvent extraction of the raw material [46]. Oils and extracts may be applied directly to products, incorporated into packaging, embedded in edible coatings (pectin, chitosan, alginate, gelatin), or microencapsulated.

Natural antimicrobial compounds include eugenol (clove), thymol (thyme/oregano), carvacrol (oregano), vanillin (vanilla), allicin (garlic), cinnamaldehyde (cinnamon) and allyl isothiocyanate (mustard) [47]. Falleh et al. [48] report that oil constituents may act bacteriostatically (inhibiting growth) or bactericidally (killing cells). For hydrophobic compounds, the accepted mode of action is membrane disruption, causing structural loss, permeability alteration, leakage of cellular constituents, and impaired membrane functions [45,49].

Reformulating of cooked pork sausages with coriander essential oil (CEO) and different levels of sodium nitrite, Šojić et al. [50] reported that reduced concentration of sodium nitrite (60 mg/kg) in combination with 0.12 μL/g of CEO resulted in satisfying redness (a*) and improved oxidative (measured by TBARS test) and microbial stability (total plate count) during refrigerated storage. Šojić et al. [51] also found that reformulation of cooked pork sausages with nitrite (50 mg/kg), tomato pomace extract (0.075 μL/g), and organic peppermint essential oil (0.075 μL/g) reduced oxidation and residual nitrite levels. The extract intensified red colour index while the oil reduced total plate counts by approximately 38% relative to the control (nitrite; 100 mg/kg). Kramer et al. [52] demonstrated that 0.4 g/kg of hop extract (rich in β-acids) in mortadella reduced *L. monocytogenes* by 3 log cycles during 28-day storage at 7 °C relative to formulations without hop; doubling the extract to 0.8 g/kg enhanced effects. Kim and Chin [53] reported that addition of paprika oleoresin solution (0.1%) permitted a 75% reduction in nitrite content in mortadella formulations without altering physicochemical properties, texture, microbial counts, or lipid oxidation; the oleoresin's effect is attributed to antioxidants such as vitamins C and E, tocopherols and carotenoids.

Despite positive antimicrobial effects, essential oils and extracts have limitations. Production of bioactive compounds can be costlier and less effective than synthetic additives; extracts may carry mycotoxins produced by filamentous fungi and can adversely affect sensory attributes [50]. Essential oils and extracts are often unstable, degrading under light, heat, humidity and in the presence of metals, hindering preservation of functional properties during storage. Their use may markedly change product aroma and provoke consumer rejection [45]. Ferraz et al. [46] also note potential environmental impacts from large-scale production, since certain components can be toxic to organisms across trophic levels.

### *2.3. Use of Natural Nitrate Sources*

Plant extracts may be incorporated into meat formulations to supply nitrite via endogenous conversion of nitrate under specific conditions. Most vegetables contain significant natural nitrate concentrations. According to Flores and Toldrá [54], addition of plant extracts during processing can be implemented in two principal ways: (1) direct addition of extract to brine or other ingredients followed by inoculation with starter cultures capable of reducing nitrate to nitrite during manufacture; or (2) addition of "cultivated" or "pre-fermented" extracts, in which the plant extract is fermented by bacteria with nitrate-reductase activity prior to use. Guimarães et al. [55] state that extracts may be applied as powder (from drying fresh vegetables), aqueous solutions, or as residues from aqueous extraction; such extracts may also contain phenolic compounds that act synergistically with nitrite as antioxidants.

Santamaria [56] proposed a classification for nitrate levels in vegetables: very low (<200 mg/kg), low (200–500 mg/kg), medium (500–1000 mg/kg), high (1000–2500 mg/kg), and very high (>2500 mg/kg). Vegetables expected to present very high nitrate concentrations include radish and arugula (Brassicaceae); beetroot, chard and spinach (Chenopodiaceae); lettuce (Asteraceae); and celery (Apiaceae).

In fermented sausages, the application of natural nitrate sources based on beetroot and radish has been investigated with different approaches, reflecting differences in their technological and functional effects. Regarding beetroot, Sucu and Turp [57] evaluated the use of beetroot powder as a nitrate substitute in fermented beef sausage (sucuk type) and observed that the incorporation of this ingredient resulted in a significant improvement in color stability, as evidenced by increased redness (a*) values. In addition, the authors reported a reduction in residual nitrate content in the final products, without negative effects on sensory properties, as no significant differences were observed compared with control samples, indicating consumer acceptability.

In contrast, studies investigating the use of radish in fermented sausages highlight its potential as a natural nitrite source due to its high nitrate/nitrite content, as well as the presence of bioactive compounds with antioxidant properties. Previous studies indicate that radishes contain relevant amounts of phenolic compounds and vitamin C, which are associated with reduced lipid oxidation during the processing and ripening of fermented meat products [58,59]. In addition, the nutritional profile of radish, characterized by dietary fiber, minerals, and bioactive compounds such as glucosinolates and isothiocyanates, suggests complementary functional effects that may positively influence product stability and nutritional characteristics [60].

Ozaki et al. [61] evaluated the addition of 0.5% and 1% (*w*/*w*) powdered radish and beetroot extracts together with a *S. carnosus* starter culture in fermented salamis. Radish extract contained higher nitrate (16,263 mg/kg) than beetroot extract (14,037 mg/kg). Generally, the addition of vegetable extracts reduced moisture and water activity (aw) and increased weight loss in salamis. Extracted formulations showed greater pH reduction versus control, providing an important barrier to unwanted microbial growth. Beetroot extract produced darker salami colour due to high betacyanin content. The optimal extract concentration for desirable pH, colour, nitrate/nitrite content and lactic acid bacteria development was 1%.

Riel et al. [62] investigated the replacement of sodium nitrite in mortadella by powdered parsley extract at 1.07, 2.14, and 4.29 g/kg. The highest extract concentration yielded lower *L. monocytogenes* counts and reduced residual nitrite relative to nitrite controls. Extracts did not affect texture, colour, water activity, or sensory attributes (appearance, flavour, and texture). Guimarães et al. [55] reported that radish derivatives (powder, aqueous solution and extraction residues) exhibit high nitrate content and appreciable levels of antioxidative compounds (ascorbic acid and phenolics). The application of these radish derivatives on restructured hams led to a difference only in terms of the residual nitrite content of the samples elaborated with sodium nitrite (150 mg/kg), and they presented a colour hue similar to that of the products with lower nitrite content (40 mg/kg) that was stable throughout the storage time [63].

Because nitrate/nitrite are supplied indirectly from vegetable sources, products may be positioned as “organic” or “natural”, which increases consumer appeal [64]. However, the use of plant extracts does not prevent N-nitrosamine formation, since residual nitrite under nitrosating conditions can still generate these undesirable compounds. Therefore, claims such as “uncured” or “no nitrate/nitrite added” can be misleading [2,54]. Other disadvantages include potential pesticide residues and certain mycotoxins, as well as sensory alterations limiting applicability [54,65]. Moreover, vegetable nitrate concentrations are highly variable, depending on plant nitrate uptake and accumulation capacity, plant part used (petiole, leaf, root, stem, inflorescence, tuber, bulb), and factors influencing plant development such as nitrate-reductase activity, growth rate, environmental conditions (soil temperature, light intensity, precipitation), fertilizer application, agricultural practices, genetics and processing methods [56,66].

### *2.4. Nitric Oxide Carriers*

Chemical carriers are donor or transport species for active substances that may be produced or added directly to a system or incorporated into encapsulation matrices (polymers or nanomaterials) to regulate release at specific sites and conditions, preventing premature decomposition of the active species [67,68]. NO carriers represent a relatively stable form of NO occurring naturally in humans and are also found in animals and plants [68]. NO carriers have been synthesised for biomedical applications, agricultural uses to improve plant defence and growth, and more recently as potential alternatives to direct nitrite addition in meat products.

*In vivo*, NO carriers act as signalling molecules in physiological and pathophysiological processes [69]. NO produced in skeletal muscle, for example, functions as a secondary messenger in numerous physiological processes via the cyclic guanosine monophosphate (cGMP) pathway and cGMP-independent mechanisms such as S-protein nitrosylation [70]. Processes relevant to the conversion of muscle to meat, such as rigor mortis, are influenced by NO and S-protein nitrosylation. By modulating glycolytic metabolism, $Ca^{2+}$ release, apoptosis, and proteolysis, NO release may correlate with fresh meat quality [70,71]. Thiol-containing amino acids or peptides (–SH), also termed mercaptans, have high potential as NO carriers *in vivo*; examples include L-cysteine (L-Cys), glutathione (GSH) and N-acetylcysteine (NAC) [72].

Thiols serve as carriers by incorporating NO via an S–N bond (RS–NO), forming S-nitrosothiols (RSNOs). S-nitrosylated species of L-Cys, NAC, and GSH correspond to S-nitroso-L-cysteine (Cys-SNO), S-nitroso-N-acetylcysteine (NAC-SNO), and S-nitroso-glutathione (GSNO) [73]. In biomedicine, other NO donor classes include organic nitrates, N-diazeniumdiolates, furoxans, metal nitrosyl complexes, and nitrobenzenes [68]. RSNOs are widely studied due to favourable efficacy and low toxicity. Pharmacologically, GSNO is stored as a powder at −20 °C, protected from light and under an inert atmosphere [69]. Exogenous administration of RSNOs (Cys-SNO, GSNO, NAC-SNO) is typically intravenous because of their low stability. Therefore, to address instability and poor oral bioavailability, RSNOs have been conjugated to hydrophilic (alginate, chitosan) or hydrophobic polymer matrices, or structurally modified to alter molecular weight, hydrophilicity, and charge properties [69,74]. Tsikas et al. [74] proposed synthesising S-nitroso-N-ethyl ester of N-acetyl-L-cysteine (NACET-SNO), wherein ethyl esterification of NAC yields NACET prior to nitrosation; esterification reduces negative charge at physiological pH and improves membrane permeability, and NACET exhibits greater antioxidant potential and solubility in aqueous and organic solvents than NAC. According to Pelegrino et al. [75], chemical modification of chitosan by S-nitrosation yields S-nitroso-chitosan, also reported for medicinal applications.

In meat systems, Emi-Miwa et al. [76] reported that RSNOs, nitrosated amino acids (nitrosoproline, nitrotyrosine), nitrosomyoglobin, and gaseous nitrogen oxides (N_xO_x) may form naturally in conventional formulations containing sodium nitrite, although RSNO levels were low compared with gaseous derivatives such as NO and nitrous oxide ($N_2O$). Recently, RSNOs have been proposed as curing agents and potential full replacements for sodium nitrite in meat products. This approach is based on the same functional endpoints as nitrite (Figure 2), antioxidant and antimicrobial activity, and development of cured colour [77–79], but offers the advantage of reducing preformed or digestion-mediated N-nitrosamine formation, since S-nitrosylation is kinetically favoured over N-nitrosylation [7,80].

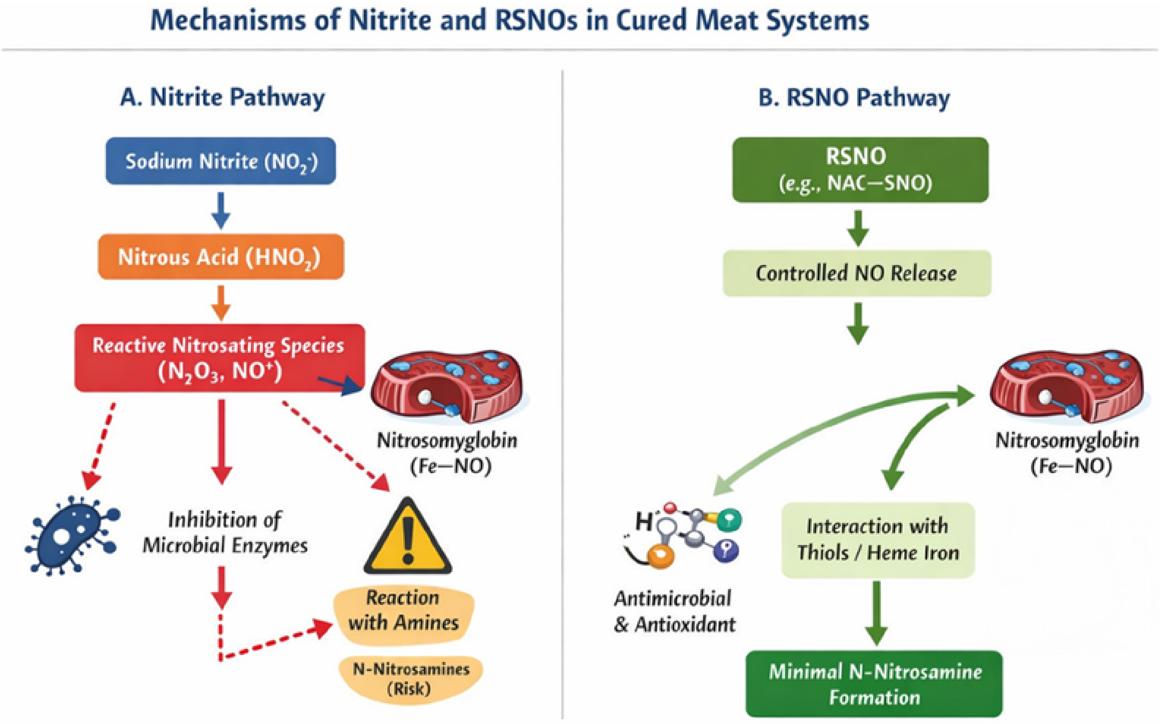

**Figure 2.** Schematic comparison of meat curing mechanisms using sodium nitrite and S-nitrosothiol (RSNO). Nitrite promotes cured color and antimicrobial effects but may generate N-nitrosamines, whereas RSNOs provide controlled nitric oxide (NO) release, enabling cured color development with minimal N-nitrosamine formation.

In turkey meat processing simulations, Kanner and Juven [81] observed that equivalent addition of Cys-SNO and sodium nitrite effectively produced a cured-like colour, reduced lipid oxidation, and decreased *C. botulinum* spore counts. Shpaizer et al. [7] reported that RSNO addition provided greater colour stability and a larger reduction in nitrosamine formation compared to nitrite salts; NAC-SNO was the most effective (≈99% reduction of N-NAs) during processing, storage, and digestion, possibly due to a longer half-life ($t_{1/2}$ = 107 h) relative to Cys-SNO ($t_{1/2}$ = 2.8 h) at 37 °C. Kanner et al. [77] demonstrated that at pH 3.0 (simulating gastric juice), only NAC-SNO exhibited antioxidant activity. Shpaizer et al. [78] reported comparable effects of nitrite and NAC-SNO on the growth of *C. sporogenes* in meat broth.

Technological properties of restructured cooked ham were assessed by Andrade et al. [79] at various nitrite-equivalent levels (25–300 mg NEq/kg) using two RSNOs—S-nitroso-N-acetylcysteine (NAC-SNO) and the ethyl ester derivative (NACET-SNO). Results indicated that 100–200 mg NEq/kg of RSNOs sufficed to achieve comparable antioxidant activity, instrumental colour, and volatile profiles. However, higher concentrations were necessary to ensure a more stable cured colour over 2 h at 4 °C and to achieve a sensory perception equivalent to nitrite-containing products. The authors attributed reduced colour stability to significantly lower residual nitrite levels generated by RSNOs compared with conventional nitrite, which may compromise product stability during extended storage. Andrade et al. [82] found that despite slightly higher lipid oxidation in sliced products treated with NAC-SNO, substitution yielded a sensory profile characteristic of cooked cured products with higher overall acceptability and increased intensity of characteristic flavour. Thus, the addition of 150 mg NEq/kg NAC-SNO was equivalent to the same nitrite concentration commonly used in cooked cured hams and is suggested as a viable nitrite substitute for achieving desirable stability and sensory properties in restructured cooked hams.

Although RSNOs show high potential for nitrite replacement, important knowledge gaps remain regarding application across the wide range of product types (poultry, pork, beef, goat, etc.), formulations and processing methods (cooking, smoking, drying, fermentation), and processing times that must be evaluated to validate use. The principal limitation for the RSNO application in foods is their high instability. RSNOs used in meat model systems are synthesised in acidic solutions (≈pH 2.0) and applied immediately after preparation [77]. S-nitrosothiol formation is a relatively simple S-nitrosation reaction between a sulfhydryl group (–SH) and nitrosating species such as nitrite. At the molecular level, nitrite is protonated under acidic or processing conditions to form nitrous acid ($HNO_2$), which decomposes into reactive nitrosating species, particularly dinitrogen trioxide ($N_2O_3$). These electrophilic species selectively react with nucleophilic thiolate anions ($RS^-$) of cysteine residues or low-molecular-weight thiols, yielding the S–N bond characteristic of RSNO [7,83]. Once formed, RSNOs function as relatively stable NO carriers and can undergo homolytic cleavage to release NO, metal-catalyzed decomposition, or S→N transnitrosation, transferring the nitrosyl group to secondary amines. These pathways are strongly modulated by pH, oxygen tension, temperature, and transition metals such as iron and copper [7,77]. Importantly, compared to free nitrite, RSNO-mediated nitrosylation directs nitric oxide preferentially toward thiols and heme iron rather than indiscriminate amine nitrosation, thereby supporting cured meat color development, antimicrobial activity, and antioxidant effects while substantially limiting the formation of carcinogenic N-nitrosamines, particularly in the case of more stable RSNOs such as S-nitroso-N-acetylcysteine [77].

Stability of RSNOs depends on intrinsic factors (RSNO structure, primary vs. tertiary; spatial conformation—syn/anti) and extrinsic factors (RSNO concentration, medium pH, temperature, light, presence of metal ions such as copper) [73,84]. Therefore, industrial deployment faces challenges in RSNO synthesis, stability, and application form, and process development is required to optimise production and handling of this novel additive. For large-scale commercialisation, desirable additive characteristics include ready-to-use formulation at high concentration, an appropriate physical state (solid or liquid), minimal refrigeration requirements, and extended shelf life. In this context, Andrade et al. [85] studied the stabilization of NAC-SNO solutions at different concentrations (200, 300, and 400 mM), followed by salt impregnation and lyophilization, to develop NAC-SNO powder for industrial application. The stability of the powders increased with concentration, with half-life ($t_{1/2}$ values) of up to 100 days at the highest concentration under refrigeration temperature (2 °C). According to these authors, NAC-SNO powder, stored refrigerated or frozen, is an interesting optimization strategy for industrial use, as it presents good yield (approximately 79%), stability, and practicality.

## 3. Key Findings, Research Gaps, and Future Perspectives

This review synthesizes current scientific evidence on strategies aimed at reducing or replacing nitrite in processed meat products (summarized in Table 1), highlighting both the progress achieved and the limitations that remain. Overall, the literature consistently demonstrates that, to date, no alternative is simultaneously safe, technologically robust, and industrially feasible that can fully replicate all the multifunctional roles of sodium nitrite, including antimicrobial protection, particularly against *C. botulinum*, cured color development, oxidative stability, and sensory quality, when applied as a stand-alone substitute. Consequently, most of the investigated approaches should be regarded as partial replacements or as components of combined preservation systems rather than complete substitutes.

**Table 1.** Main strategies investigated for nitrite replacement in meat products, their mechanisms, advantages, limitations, and representative references.

| Strategy | Main Mechanism of Action | Advantages | Limitations | Representative References |
|---|---|---|---|---|
| Food colorants | Direct color enhancement via pigments | Stable color; easy application | No antimicrobial activity; limited oxidative protection | [13,14] |
| Bacteriocins | Membrane permeabilization; pore formation; inhibition of cell wall synthesis | Strong anti-Listeria activity; natural origin | Narrow antimicrobial spectrum; cost | [21,22] |
| Organic acids | Intracellular acidification; collapse of proton motive force; inhibition of ATP synthesis and TCA cycle enzymes | Effective microbial control; low cost | Sensory impact at high concentrations | [35,40] |
| Enzymes and starter cultures | Reduction of nitrate to nitrite; pigment formation | Clean-label perception; traditional processing | Process-dependent; variable efficacy | [29–31] |
| Essential oils and plant extracts | Membrane disruption; oxidative stress induction; enzyme inhibition | Antimicrobial and antioxidant properties | Flavor alteration; instability | [44.48,49,50] |
| Natural nitrate sources | Indirect nitrite generation via microbial nitrate reduction | Clean-label appeal | Variable nitrate content; color and flavor alteration; potential nitrosamine formation | [54–56] |
| NO carriers (RSNOs) | Controlled NO release; S-nitrosylation; interaction with thiols and heme iron | Cured color; antibotulinic and antioxidant effects; reduced N-nitrosamine formation | Stability and regulatory challenges | [7,78,79,82] |

Established strategies, such as the use of colorants, biopreservation techniques, organic acids, essential oils, and plant-derived nitrate sources, have shown variable effectiveness depending on product type, formulation, processing conditions, and storage environment. These approaches tend to perform best when applied within hurdle technology frameworks, where individual limitations can be mitigated through synergistic effects. However, their performance remains highly product-specific, which limits broad generalization across different meat matrices and processing technologies.

The use of natural nitrate sources, although aligned with market demand for products labelled as “natural,” may mislead consumers regarding product safety. This is because the risks associated with N-nitrosamine formation are not eliminated but rather shifted to an indirect source. Moreover, this strategy introduces substantial compositional variability, compromising process predictability and industrial control, and may undesirably affect product stability and sensory attributes.

In formulations where nitrite reduction is compensated using colorants or biopreservatives, a lower residual nitrite content at the end of processing is expected, as myoglobin continues to act as the primary nitric oxide acceptor regardless of the presence of the substitute [3]. Although lower residual nitrite levels are associated with reduced nitrosamine formation, they may be insufficient to ensure color stability and, more critically, to provide adequate microbial protection during storage.

In parallel with direct substitution strategies, non-thermal technologies, including high-pressure processing (HPP), pulsed electric fields (PEF), pulsed light, cold plasma, and irradiation, have been investigated as means to reduce nitrite usage in meat products. These technologies offer the advantage of reducing microbial load with less impact on sensory and nutritional characteristics compared with conventional thermal treatments [2]. However, unlike nitrite, which provides persistent and multifunctional antimicrobial action, these approaches do not effectively inhibit the growth of surviving microorganisms or post-processing recontamination, particularly in RTE meat products. Their efficacy is strongly dependent on the food matrix and storage conditions. Accordingly, such technologies are more appropriately considered complementary tools within multi-hurdle systems and are often combined with nitrite substitutes to enhance antimicrobial efficacy and reduce N-nitrosamine formation. Despite their synergistic potential, these combinations are frequently industrially unfeasible due to increased process complexity and additional processing steps, which likely explains their limited coverage in the literature.

Among emerging alternatives, S-nitrosothiols (RSNOs) stand out as a particularly promising and mechanistically innovative approach. By acting as direct NO donors, RSNOs enable cured color formation, inhibit lipid oxidation, and exert antimicrobial effects comparable to those of conventional nitrite, while consistently reducing N-nitrosamine formation during processing, storage, and simulated digestion. At nitrite-equivalent concentrations, RSNOs have been shown to produce technological and sensory characteristics like those of nitrite-cured products [77,79,82]. Although Shpaizer et al. [78] demonstrate that NAC-SNO, under the same conditions and concentrations, acts as an anti-clostridial compound like sodium nitrite in meat products, important challenges remain, including chemical instability, sensitivity to light and temperature, interactions with metal ions, and more studies involving toxigenic *C. botulinum* strains relevant to humans.

Despite extensive research efforts, significant gaps still hinder the large-scale implementation of nitrite alternatives. Most studies have been conducted under laboratory or pilot-scale conditions, with limited validation under realistic industrial processing, distribution, and retail scenarios. In addition, systematic evaluations across different meat species, product categories, and processing methods remain scarce. Comprehensive toxicological assessments, economic feasibility analyses, and consumer acceptance studies are also limited.

Future research should therefore prioritize integrative, application-oriented approaches rather than seeking a universal one-to-one replacement for nitrite. Progress in this field will depend on coordinated efforts among researchers, regulatory authorities, and the meat industry to develop solutions that ensure microbial safety, technological and sensory quality, regulatory compliance, and consumer acceptance.

## 4. Industry Challenges

Reformulating meat products to remove additives presents a major challenge because reformulated products must match conventional counterparts in sensory quality (appearance, texture, and flavour), microbiological safety, and stability over shelf life. It is essential to consider specific product and process characteristics, production cost (which affects retail price), and the logistical and climatic context of each country. In Brazil, for instance, tropical climate and large territorial extent can affect cold-chain conditions during distribution and at points of sale, reducing product shelf life.

Adoption of alternatives may markedly increase costs, reducing product competitiveness, since natural ingredients and additional processes to ensure equivalent microbiological safety raise quality-control expenses. For example, some alternatives, such as plant extracts rich in nitrates, require chemical conversions that may be less controllable and necessitate process adjustments, increasing operational costs and complicating production standardisation.

A further critical aspect is the need for a strict cold chain for storage and transport of these substitutes, because many compounds, especially natural ones, are more susceptible to microbial spoilage and loss of sensory and functional properties under inadequate temperatures. Maintaining specific temperatures across the logistic chain imposes additional infrastructure and transport costs, which can substantially increase the final product price. This situation particularly affects competitiveness when compared to curing salts, which do not demand the same logistical controls.

One major obstacle to implementing new technologies and substances for nitrite reduction is the requirement to demonstrate their stability, behaviour during production processes, and effects on food matrices. Thus, understanding how substitutes behave during storage, transport, and processing without compromising product quality and safety is imperative. These challenges necessitate further research and development and a deeper understanding of substitute behaviour in different physical states and interactions with other ingredients to ensure final food products meet flavour, safety, and shelf-life requirements without jeopardising consumer health.

Moreover, the cold chain during product shelf life is crucial for microbiological safety and quality of most meat products. Proposed replacement techniques are generally combined with refrigeration to ensure efficacy and extend shelf life, thereby increasing reliance on rigorous temperature control, advanced monitoring technologies and robust refrigeration infrastructure—factors that raise operating costs.

Addressing these challenges requires collaboration among scientists, regulatory authorities and the food industry to develop effective, safe alternatives aligned with regional and global regulations, while delivering organoleptic and sensory characteristics acceptable to consumers. Availability and cost of proposed ingredients and processes must be considered, since these factors may prevent industrial adoption regardless of technological potential.

It should be emphasised that, despite extensive research, no commercial substitute to date matches sodium nitrite in providing identical functional properties while ensuring safety against N-nitrosamine formation.

## Author Contributions

B.F.A.: conceptualization, methodology, data curation, writing—original draft preparation; L.R.d.C.: writing—original draft preparation. R.d.A.T.F., P.R.F. and A.d.L.S.R.: writing—reviewing and editing. E.M.R: conceptualization, methodology, writing—reviewing and editing. All authors have read and agreed to the published version of the manuscript.

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The authors declare no conflict of interest.

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No AI tools were utilized for this paper.

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