



Human safety controversies surrounding nitrate and nitrite in the diet

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ABSTRACT

Nitrate and nitrite are part of the human diet as nutrients in many vegetables and part of food preservation systems. In the 1950s and 1960s the potential for formation of nitrosamines in food was discovered and it ignited a debate about the safety of ingested nitrite which ultimately focused on cured meats. Nitrate impurities in salt used in the drying of meat in ancient times resulted in improved protection from spoilage during storage. This evolved into their deliberate modern use as curing ingredient responsible for 'fixing' the characteristic color associated with cured meats, creating a unique flavor profile, controlling the oxidation of lipids, and serving as an effective antimicrobial. Several critical reports and comprehensive reviews reporting weak associations and equivocal evidence of nitrite human health safety have fostered concerns and debate among scientists, regulators, press, consumer groups, and consumers. Despite periodic controversy regarding human health concerns from nitrite consumption, a building base of scientific evidence about nitrate, nitrite, heme chemistry, and the overall metabolism of nitrogen oxides in humans has and continues to affirm the general safety of nitrate/nitrite in human health. As nitrite based therapeutics emerge, it is important to consider the past controversies and also understand the beneficial role in the human diet.

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Introduction

Although naturally present in a variety of foods, most discussion of nitrate and nitrite centers on cured meats because of the deliberate addition as a curing ingredient. Therefore, this discussion will focus on meat curing and the regulations that have evolved in meat products. They represent a template for many safety questions that can arise as nitrite based therapeutics are developed.

Nitrate serves as a precursor to nitrite and in cured meats; nitrite fixes color, contributes to the cured meat flavor, helps in the inhibition of the growth of microorganisms, specifically *Clostridium botulinum*, and effectively controls rancidity by inhibiting lipid oxidation [1]. The meat and poultry industry has greatly benefited from the use of sodium nitrite by allowing for the production of products with unique colors, textures, and flavors; improved food safety; and an extended shelf-life with excellent storage stability [2,3]. The use of sodium nitrite for curing, however, has not been without controversy. Due to a strong public debate in the 1970s concerning the potential to yield carcinogenic nitrosamines, the use of nitrite for curing was nearly banned [4,5]. As a result, several steps were taken by both industry and government to significantly reduce the risk of nitrosamine formation and alleviate potential human health concerns. Since that time, health concerns involving

risks related to cancer, believed to be directly related to the consumption of nitrite cured meat and poultry products, have periodically resurfaced. Why the debate has not encompassed all ingested sources of nitrite is unknown and perhaps a socio-political question.

Research conducted since the mid-1980s has suggested that nitrite is a significant molecule important for human health. New scientific discoveries are now providing a better understanding of the profound and important roles nitrite plays in human physiology. Dietary nitrate from vegetable consumption, for example, has been shown to serve as a significant source for the endogenous production of nitrite and nitric oxide in the human body [6]. As a product of enzymatic synthesis in humans, nitric oxide controls blood pressure, immune response, wound repair, and neurological functions [7]. Recent research has clearly shown that nitric oxide can be produced directly from nitrite and is involved in controlling blood flow in cardiac muscle and potentially other tissues [8,9]. Further, the normal production of nitric oxide and nitrite may prevent various types of cardiovascular disease including hypertension, atherosclerosis, and stroke [7,8].

Dietary sources of nitrate and nitrite

The World Health Organization estimates the mean daily dietary intake of nitrate is from 43 to 141 mg [10]. Exogenous sources for human intake of nitrate are primarily derived from plant derived foods and drinking water with approximately 80% of total

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nitrate intake being attributed to food and another 14% to water [10,11]. Vegetables actually constitute a large component of the dietary intake of nitrate. The National Academy of Sciences [12] reported that 87% of dietary nitrate intake associated with food is derived from vegetables. Further, research by Cassens [5] reported similar findings indicating vegetables are responsible for 85% of dietary nitrate intake. In Great Britain, Knight, Forman, Aldabbagh, and Doll [13] estimated that vegetables contribute over 90% of dietary intake of nitrate. Spinach, beets, radishes, celery, lettuce, cabbage, and collard greens are a few examples of various vegetables found to contain high concentrations of naturally occurring nitrates.

Several factors affect the accumulation of nitrate in vegetables and can allow for a wide range of nitrate concentration. Leafy vegetables such as lettuce and spinach tend to have higher levels of nitrate than seeds or tubers [6,14]. Application of fertilizers generally results in a greater uptake of nitrogen in vegetables, resulting in higher nitrate content [9,15]. Nitrate uptake, nitrate reductase activity, growth rate and growth conditions (e.g., soil temperature, intensity of light, level of rainfall, etc.) all significantly affect the ultimate nitrate content of vegetables [6]. Further, processing methods such as heat treatments and storage conditions can cause the loss of nitrate. For example, increased storage temperatures have been found to decrease the nitrate content of vegetables through increased bacterial facilitated reduction of nitrate to nitrite [16].

Humans generally consume 1.2–3.0 mg of nitrite per day [10]. Surprisingly, saliva accounts for approximately 93.0% of the total daily ingestion of nitrite while foods account for a very small portion of the overall daily nitrite intake. This is due to the chemical reduction of salivary nitrate to nitrite by commensal bacteria in the oral cavity. Cured meats have been reported to comprise 4.8% of daily nitrite intake and vegetables accounting for just 2.2% [11]. Before modern meat curing processes were adjusted, the proportion of ingested nitrite ascribed to cured meats was higher as the National Academy of Sciences [12] reported that 39% was contributed by cured meats, 34% by bakery goods and cereals, and 16% by vegetables. Table 1 displays the nitrate and nitrite content of some common foods purchased from supermarkets across the United States reported by Buege et al. [17].

In 2009, an additional nationwide survey of cured meats and vegetables was conducted to assess nitrate and nitrite content (Table 2). Reviewing the residual nitrate and nitrite content of commercial cured meat products [18], it was concluded that consistently lower levels of residual nitrate and nitrite than those from a survey reported by the National Academy of Sciences in 1981 existed. Keeton et al. [18] reported residual nitrite levels of 7 ppm in cooked sausages (hot dogs), 7 ppm in bacon, and 7 ppm in hams which have fallen from 10–31 ppm, 12–42 ppm, and 16–37 ppm,

respectively when compared to data reported in the NAS [12] study. These levels and findings are consistent with those from an earlier nationwide survey conducted by Buege et al. [17]. Results from both studies were in agreement showing that an approximately 80% reduction in nitrite levels existed in products investigated in these two surveys compared to those from a similar survey conducted in 1975 [12]. Thus, consumption of a typical 45–50 g American hot dog would result in ingestion of only 0.4 mg of nitrite and 1.6 mg of nitrate. Based on this data, it can be concluded that cured meats provide minimal contributions to the human intake amounts of nitrate and nitrite.

The history and use of nitrate and nitrite in foods

Meat and poultry curing is one of the oldest forms of food preservation that has evolved over the centuries [19]. Before the discovery of refrigeration, high moisture foods were preserved by methods effective for controlling spoilage well past harvesting to extend food supplies for a significant period of time. Drying to decrease water activity, smoking, salting, marinating, or pickling foods were also commonly used methods of preservation [2]. Modern day curing technologies can still be directly related to early curing procedures.

The exact historical origin of meat curing is unknown but is believed to have been discovered by accident [1,20,21]. The history of meat processing refers to several accounts of the contamination of salt with saltpeter (potassium nitrate) resulting in a stable red color in the meat [4]. It is unclear whether the saltpeter-cured characteristics were deemed desirable before the 10th century, but during and after the 10th century, the Romans were intentionally adding saltpeter to meat to obtain the desired red color and distinctive flavor. In the 19th century it was discovered that salt (sodium chloride) itself did not produce a “cured color” which led to several investigations to better understand the curing process [20].

In the early 1900s, unsatisfactory and irregular curing was commonplace and was likely associated with the use of both nitrite and nitrate together. Recognizing the potential of “unfit” or unwholesome foods being produced if excessively high levels of nitrate and/or nitrite were used, the USDA Bureau of Animal Industry sponsored a series of experiments to better understand the formulation level/safety relationship in the 1920s [22]. From these experiments, the following conclusions were made:

- (1) “From one-fourth to 1 oz. of sodium nitrite is sufficient to fix the color in 100 lb, (equivalent to 154–617 ppm (mg/kg)) the exact quantity depending on the meat to be cured and process to be employed;

Table 1
The nitrate and nitrite content of fresh foods collected from stores in four U.S. cities in Spring 2000.^a

Food product	Nitrate		Nitrite	
	Mean concentration (ppm)	Range (ppm)	Mean concentration (ppm)	Range (ppm)
Beets	2756	1680–3590	10.0	2.1–29.8
Spinach	2333	535–3660	7.0	0.0–12.9
Radishes	1680	764–2500	0.1	0.0–1.0
Celery	1544	316–3320	1.6	0.0–5.2
Iceberg lettuce	786	347–1080	0.2	0.0–1.7
Cabbage	573	193–976	2.4	0.0–12.6
Green beans	386	165–611	0.5	0.0–2.5
Strawberries	173	105–293	2.0	0.0–7.1
Bananas	137	88–214	2.1	0.0–9.5
Mushrooms	590	19–85	8.0	0.0–38.0
Green peppers	33	8–55	0.4	0.0–3.0

^a Amounts are reported in ppm (mg/kg) Table is reproduced from Buege, Weiss, and Elliffson [17].

Table 2The nitrate and nitrite content of fresh foods collected from stores in five U.S. cities in 2009.^a

Food product	Nitrate		Nitrite	
	Mean concentration (ppm)	Range (ppm)	Mean concentration (ppm)	Range (ppm)
Broccoli	394	29–1140	0.6	0.01–9.5
Cabbage	418	37–1831	0.1	0.01–0.4
Celery	1496	20–4269	0.1	0.02–0.5
Lettuce	850	79–2171	0.6	0.01–9.7
Spinach	2797	65–8000	8.0	0–137.2
Cured, dried, uncooked sausage	113	0.1–2289	0.8	0.03–9.7
Cured, cooked sausage	32	0.8–541	7.6	0.1–29.3
Fermented, cooked sausage	46	1.8–320	0.8	0–26.7
Whole-muscle, brine cured, uncooked	14	3.5–32	6.8	0.2–36.5
Whole-muscle, brine cured, cooked	16	0.2–108	7.5	0.03–27.6
Whole-muscle, dry cured, cooked	106	0.4–1366	1.5	0.02–16.2

^a Amounts are reported in ppm (mg/kg) Table is reproduced from Keeton et al. [18].

- (2) “Meats cured with sodium nitrite need to contain no more nitrite than meats cured with nitrate, and are free from the unconverted nitrate regularly present in nitrate-cured meats;”
- (3) “A shortening of the customary curing period may be obtained by the use of nitrite.”

Early US regulatory discussion on the use of nitrate from saltpeter can be found in the regulation 18 Bureau of Animal Industry Order 218 [22]. With these conclusions, the first USDA regulations for nitrate and nitrite use in meat products were established in 1925 permitting no more than 200 ppm (mg/kg) added levels of nitrate, nitrite, or combinations thereof based on the meat weight. Further research and findings regarding a better understanding of the curing capacity of nitrate, the early concern about nitrosamine formation, and the impact of reductants and acidulants, or so called “cure accelerators” resulted in a re-clarification of curing regulations in 1970 that still exist today [4]. Table 3 illustrates the maximum allowable limits for various curing agents and curing methods for cured meat and poultry products [23].

Additional regulatory limits exist for curing ingredients used in bacon which were established in 1978. Nitrate is not permitted in bacon so that actual concentrations of nitrite can be controlled more precisely. Further, bacon is required to also have either 550 ppm added sodium erythorbate or sodium ascorbate, regardless of curing method, to inhibit the potential for nitrosamine formation during frying [24].

The change from nitrate to nitrite use by the meat industry was not a fast one [22]. In 1930, over 54% of packers were using nitrate compared to only 17% using nitrite. A survey of nitrate and nitrite levels in 1936 reported samples obtained at retail outlets contained an average of 3–86 ppm nitrite but also had 160–3900 ppm nitrate. In 1937, a similar survey reported an average of 16–102 ppm nitrite and 210–3000 ppm nitrate. From the period of 1970–1980, a marked decline in the amount of nitrate being used by the meat industry was found and likely due to the following:

- 1) A better understanding of meat curing and meat curing chemistry;
- 2) The wider use of cure accelerators such as sodium or potassium ascorbate and erythorbate, or their acid form;
- 3) Regulatory changes for curing ingredients and processing that no longer allowed the use of nitrate in bacon and;
- 4) A growing consumer concern about the potential negative impacts of consuming nitrite/nitrate containing meat products underlined by the discovery of possible nitrosamine formation.

Concerns associated with nitrate and nitrite

During the 1950 and 1960s, as studies into the chemical reactions of inorganic and organic compounds were conducted, the outside potential to form carcinogenic *N*-nitrosamines was uncovered. These findings were related to observational studies of health among industrial chemical workers and animal model experiments [25,26]. Coincidentally, hepatic toxicity in sheep fed fish meal diets preserved with nitrite was also observed in Norway [27,28]. In 1970, Lijinsky and Epstein published a critical report in *Nature* [29] entitled “Nitrosamines as Environmental Carcinogens”, which showed that nitrosamines were potent and specific carcinogenic compounds. Further, the authors concluded the most appropriate means to address the problem was to eliminate one or the other of nitrosamine precursors (nitrite and secondary amines). This particular paper brought wide spread public attention to the question of safety of nitrite and was followed by intense survey and study of potential public health risks due to food and environmental exposure to nitrite. Since all cured meats were viewed as containing both precursors, consumption of cured meat was considered a potential public health hazard.

US food laws contain a 1958 statute commonly known as the Delaney amendment requiring FDA, (Food and Drug Administration) to not permit use of ingredients in foods that are known to cause cancer. This has resulted in intense debate concerning numerous ingredients and additives in foods when a report of

Table 3Maximum allowable added levels for curing ingredients in meat and poultry in the United States.^a

Curing agent	Curing method			
	Immersion cured (ppm)	Massaged or pumped (ppm)	Comminuted (ppm)	Dry cured (ppm)
Sodium nitrite	200	200	156	625
Potassium nitrite	200	200	156	625
Sodium nitrate	700	700	1718	2187
Potassium nitrate	700	700	1718	2187

^a Limits are based on total formulation/brine weight for immersion cured, massaged, or pumped and raw meat (green) weight for comminuted or dry cured products [23].

carcinogenicity is published. Notable examples of the inquiry and public policy debate include the artificial sweetener, saccharin, and phenolic antioxidants BHA (butylated hydroxyanisole) and BHT (butylated hydroxytoluene) used to inhibit lipid oxidation as well as the use of nitrite and nitrate in cured meat products. With respect to nitrite, the controversy surrounding its use included hearings by a US House of Representatives subcommittee which were held in 1971 and 1972. These were followed by a series of expert panel meetings held from 1973–1977. Members of the panel included USDA, FDA industry, and academic scientists. This group grappled with the public health significance and realization that nitrosopyrrolidine could be formed during frying of bacon. In a social era of developing consumer activism and appointment of activist as government officials, banning nitrite in foods was seriously discussed.

Potential for nitrosamine formation in cured meats was first identified in 1971 and their formation can take place only under special conditions where secondary amines are present, nitrite is available to react, near neutral pH is found, and product temperatures reach greater than 130 °C, such as during the frying of bacon. Because of this fact and the growing concerns and controversy over nitrite usage, a series of proposed regulations were submitted, reviewed, and acted upon in the interest of avoiding a complete ban on nitrite. Proposed nitrite regulations in 1975, centered on nitrosamine formation in bacon and resulted in the reduction of added nitrite in bacon from 200 to 125 ppm. Additional petitions to this proposed rule resulted in a 1978 published final rule requiring the use of 120 ppm added nitrite (or equivalent potassium nitrite of 148 ppm), 550 ppm added sodium ascorbate or erythorbate, and the banning of nitrate addition during bacon processing. The rule also included the establishment of a nitrosamine monitoring and regulatory control program. Within one year of the newly developed monitoring program nearly all bacon manufactured was in compliance with both the regulations and limits specified in the compliance monitoring program. Today, the regulatory controls, and more stringent plant production practices have essentially eliminated all regulatory nitrosamine concerns in meat and poultry products.

Curiously, in the 1990s, despite developing controversies, a new consumer product containing nitrate was introduced. Toothpaste for sensitive teeth is now common and contains high levels (5% or 50,000 ppm (mg/kg)) of potassium nitrate. While under FDA regulatory purview, this newer source of human exposure has had no public controversy which is an interesting social question considering the debate concerning cured meats and the known salivary reduction of nitrate to nitrite.

In 1979, a study conducted by Dr. Paul Newberne from the Massachusetts Institute of Technology (MIT), investigating cancer of the lymphatic system in rats, became the centerpiece of heated debates including the USDA, FDA (Food and Drug Administration), media, the meat industry, scientists, and many others [30]. Because of the important public health implications, the study data was reevaluated by an Interagency Working Group convened by the U.S. Food and Drug Administration (FDA). The interagency working group responded to the FDA in 1980 “that no demonstration could be found that the increased incidences of these tumors were induced by the ingestion of sodium nitrite” [31]. During this period, a special National Academy of Sciences (NAS) committee was also created to comprehensively review the available literature and risk benefit information on nitrate and nitrite. This extensive review resulted in two reports by NAS entitled “The Health Effects of Nitrate, Nitrite, and N-Nitroso Compounds” and “Alternatives to the Current Use of Nitrite in Foods” issued in 1981 and 1982, respectively [12,32]. These two exhaustive reports, addressed the concerns of nitrite usage among regulatory authorities. After the release of these reports and the changed USDA regulations, the public and

regulatory controversy around nitrite subsided. An excellent review of the scientific and social political discussions during this period was written by Cassens [4].

The 1982 National Academy of Sciences report also called for a more thorough evaluation of nitrite in cancer bioassays and thus it was nominated by FDA for study in the National Toxicology Program (NTP). This study was completed in the 1990s and the results were peer reviewed by a panel at a meeting held on May 18, 2000. The review panel concluded that there was no evidence that nitrite induced carcinogenicity in any major tissues of male and female rats and male mice. There was only equivocal evidence for carcinogenicity in the forestomach of female mice [33]. This “gold standard” of cancer bioassays was, at the time, the definitive statement of safety for nitrite as an ingredient.

Separately, in 1998, the state of California proposed classification of nitrite as a developmental and reproductive toxicant (DART) under their “Proposition 65” law (Safe Drinking Water and Toxic Enforcement Act). In the process, the California Office of Environmental Health Hazard Assessment (OEHHA) staff produced a Hazard Identification Document to support the proposal [34]. The consequence of listing nitrite as DART would have resulted in a requirement to place warning labels on all cured meat products. However, at a public hearing on June 2, 2000, the state’s independent technical review committee voted seven to one against this listing. The NTP and Proposition 65 reviews of nitrite safety in the context of nitrite benefits have been summarized by Archer [11].

Beyond toxicological studies, diet, lifestyle, and disease relationships have long been studied by epidemiologists and methods for conducting these investigations have become increasingly more sophisticated. This has been particularly true in the past several decades with the advent of large public health databases that have been used retrospectively and prospectively to examine statistical relationships between many factors and human health. Human nutritional epidemiology is now considered an important component in making dietary recommendations. Epidemiological investigations into nitrite, nitrate and nitrosamine exposure and a variety of health outcomes have been widely published and have been used in deliberations by governmental bodies. Single studies have often been widely communicated in the nonscientific media. As information involving sensitive topics surrounding human health surfaces, media normally and commonly formulate opinionated pieces, often with incomplete explanations of study limitations, uncertainty, or conflicting evidence.

In the 1990s, for example, a series of epidemiological studies reported consumption of cured meats was related to brain cancer and childhood leukemia [35–38]. One such example of this was a Washington Post news piece by Maugh [39] who reported that “Children who eat more than 12 hot dogs per month have nine times the normal risk of developing leukemia” and was based on the epidemiological study “Processed meats and risk of childhood leukemia” written by Peters et al. [37]. In general, where positive epidemiological associations have been found, they were weak and not clearly supported by other independent scientific evidence, thus not warranting any cause for public alarm [40–42].

In 2006, another review of the carcinogenicity of nitrite and nitrate was conducted by a working group composed of epidemiologists, toxicologists, and cancer researchers and convened by the International Agency for Research on Cancer (IARC); a part of the United Nations sponsored World Health Organization headquartered in Lyon, France [40,43,44]. The results of this review were published in a summary form shortly after the meeting [45] and in a full monograph in 2010 [46]. IARC, like NTP, follows a very structured “decision tree” process in making conclusions about substances under review as potential human carcinogens. Their final conclusion was as follows:

“Ingested nitrate or nitrite under conditions that result in endogenous nitrosation is *probably carcinogenic to humans* (Group 2A) [47].”

When carefully examined, this conclusion appears to be very narrow in scope. Under certain conditions, ingested amines and amides can be nitrosated to form carcinogenic nitrosamines. However, nitrosation of thiols as part of physiological control mechanisms is an evolving area of research. Additionally, most ingested nitrite is formed in saliva, so swallowing saliva in combination with virtually any food could be considered to result in potential formation nitrosated compounds. Thus, questions about the appropriateness of the IARC conclusion exist [48].

The relationship between diet and cancer continues to be studied by researchers and headlined in the media. Several reports have asserted that red meat and/or processed meat consumption have been associated with higher rates of certain types of cancer. A 2007 report from the World Cancer Research Fund (WCRF), a confederation of cancer research and treatment advocacy groups including the American Institute for Cancer Research (AICR), represented a major global initiative to link diet to cancer [49]. This WCRF report – Food, Nutrition, Physical Activity and the Prevention of Cancer: a Global Perspective – was released in November 2007. The report included 10 broad recommendations for cancer prevention regarding diet, lifestyle, and exercise. Specifically, the report made a recommendation to limit red meat consumption to no more than 18 oz. (cooked) per week and eliminate processed meat consumption entirely. A subsequent February 2009 Policy Report was issued to seek international public policy changes to promote their opinions on how dietary changes could reduce cancer incidence [49]. The conclusions and recommendations made by WCRF were largely based on epidemiological associations of weak magnitude and have been challenged by researchers both associated and independent of the meat and poultry industry [50–58]. The intense debate will continue.

The purpose and function of using nitrate and nitrite in the meat industry

Nitrite is considered an essential curing ingredient responsible for ‘fixing’ the characteristic color associated with cured meats, creating a unique flavor profile that is distinguishable from products not containing nitrite, providing control of the oxidation of lipids, and serving as an effective antimicrobial by itself or synergistically with other ingredients [21,59,60]. Nitrate, also considered a curing ingredient, is only effective if first reduced to nitrite.

Quality impact from curing

The fixation of a desirable red color, shaded pink, is the most obvious effect from nitrite addition and is often considered an extremely important attribute for consumer acceptance [61]. Much of the nitrite added during the product manufacturing is either depleted through a series of nitrogen oxide reactions or during product manufacture and storage. Between 10 and 20% of the originally added nitrite is typically present after the manufacturing process [62,63]. These levels of nitrite, referred to as residual nitrite, slowly decline over the storage life of cured meat products until they are often non-detectable [17,18,64–66]. To maintain a cured meat color throughout extended shelf-life, it is generally accepted that a small amount (10–15 ppm) of residual nitrite is needed to serve as a reservoir for the re-generation of cured meat pigment lost from oxidation and light-induced iron–nitric oxide dissociation [67].

Nitrite chemistry and the associated reactions likely play a role in imparting the unique flavor resulting from the addition of nitrite

[68]; however, the specific compounds involved are still not yet known. A proposed reason for cured flavor differences between products containing nitrite and those without is due to the nitrite-related suppression of oxidation products; thus controlling lipid oxidation related flavor compound development [69]. However, sensory research suggests that cured flavor is not solely a result of retarding lipid oxidation but instead a combination of a complex cured aroma and flavor in cooperation with a lack of rancid flavors.

One of the most noteworthy properties of nitrite is its ability to effectively delay the development of oxidative rancidity which is as leading reason for the deterioration of product quality. Like many compounds that can be either oxidized or reduced, at high concentrations along with appropriate cofactors, nitrite can become a pro-oxidant. However, at levels used in cured meats it serves as an antioxidant. The antioxidant effect of nitrite is likely due to the same mechanisms responsible for cured color development involving reactions with heme proteins and metal ions, chelating of free radicals by nitric oxide, and the formation of nitroso- and nitrosyl compounds having antioxidant properties [70]. Because nitrite is highly effective as an antioxidant, synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are not used in a majority of cured meats.

Microbiological safety impact from curing

Nitrite has been shown to have varying degrees of effectiveness in either preventing or controlling the growth of certain bacteria. A recent risk–benefit review of nitrite included a discussion of the antibacterial benefits of nitrite in cured meat products [48]. The inhibitory mechanism which results in the effects nitrite has on some bacteria likely differs among bacterial species and is not considered effective for controlling gram-negative enteric pathogens such as *Salmonella* and *Escherichia coli* [71]. Bauermann [72] concluded in a study comparing the coliform levels in poultry products with and without nitrite that sodium nitrite does provide improved bacterial shelf-life. Buchanan and Solberg [73] found a bacteriostatic action of nitrite on *Staphylococcus aureus* and suggested their results provided evidence that *S. aureus* may be effectively controlled with 200 ppm of nitrite. However, other researchers have reported conflicting results as Bayne and Michener [74] reported no effect on the control of *Staphylococcus*, *Salmonella*, or spoilage bacteria present in frankfurters whether or not nitrite was included.

Even though the specific inhibitory mechanisms of nitrite are not well known, its effectiveness as an antimicrobial is dependent on several factors including residual nitrite level, pH, salt concentration, reductants present, iron content, and others [71]. As an example, nitrite inhibits bacteria more effectively at low pH [75,76] suggesting the antimicrobial action of nitrite is associated with the generation of nitric oxide or nitrous acid. However, the tolerance of nitric oxide by bacteria varies from acting as a metabolite for some to being toxic for others [77]. The presence of nitrous acid has also been suggested to contribute to the antibacterial impact of nitrite. Therefore, it is likely that nitrite reactions related to the development of cured meat color are also important for the antimicrobial properties attributed to nitrite.

Before 2000, *C. botulinum* was the most widely recognized pathogen associated with an impact from nitrite addition. This antibotulinal effect on *C. botulinum* in thermally processed meat product systems takes place at two different stages in the life cycle of the microorganism. The first *C. botulinum* controlling effect of nitrite is the inhibition of vegetative cells emerging from surviving spores. The second controlling effect is preventing cell division in any vegetative cells [20].

The main portion of nitrite added to cured meats is for *C. botulinum* control whereas only a small portion (roughly 25 ppm or less) is needed for color development [78]. As nitrite levels increase, inhibition of *C. botulinum* growth and toxin production also increases [79]. The level of added nitrite is believed to have more impact than the residual level during storage in providing inhibitory control of *C. botulinum* which suggests the formation of anti-bacterial compounds as a result of nitrite-related reactions may be significant [80].

Over the last 20 years, a greater appreciation for the contribution nitrite has in protection from other food-borne pathogens has developed. Models developed to predict bacterial growth of pathogens such as *Listeria monocytogenes*, show improved effectiveness of antimicrobials like sodium lactate and sodium diacetate in the presence of nitrite [81–88]. Other estimations predict that growth rates of pathogens such as *L. monocytogenes*, *E. coli* O157:H7, *S. aureus* and *Bacillus cereus* are reduced in the presence of nitrite at levels used in cured meat and poultry products [48].

Alternative curing methods

The substantiating importance of meat and poultry curing can be demonstrated by the widespread interest in “natural curing” of natural or organic meat and poultry products. Although natural and organic labeling standards ban the addition of any synthetic chemical, including nitrite and nitrate, the importance of curing for safety of preserved meat products coupled with the quality demands from consumers has resulted in a novel approach of curing called “indirect” or “natural curing”. These products are characterized by use of vegetable sources of nitrate such as celery powder, high in naturally accumulating nitrate, coupled with addition of nitrate reducing bacteria to facilitate *in situ* generation of nitrite during the manufacturing process. The nitrosyl heme pigment generated by this technique is identical to that resulting from direct nitrite addition. [89–93].

Summary

Humans have been consuming nitrate and nitrite since the beginning of time in a variety of foods including vegetables and cured meats. Since the controversies about the safety of nitrite that started in the mid-20th century, much has been learned about nitrite and heme chemistry and the overall metabolism of nitrogen oxides in humans. The ongoing research focused on the metabolism of nitric oxide, nitrite, and nitrate appears to reaffirm the general benefits of nitrate/nitrite in human health. Yet disagreement about health impacts of dietary nitrite and nitrate, particularly in cured meats still exist despite changes in meat curing practices that minimize potential for nitrosamine formation. The challenge to scientists is twofold. First, is to continually broaden their understanding of curing in the context of human physiology and metabolism of nitrite. The second is to effectively educate a broad community of public health scientists, nutritionists, and the general public about the fundamental role of nitrite in biology in order to address concerns about adverse health effects from dietary exposure. It is important to consider the historical debates surrounding nitrite and nitrate in our diet in order to effectively and safely develop therapeutics or nutritional strategies to replete NO homeostasis in patients.

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