Open Access
Asian-Australas J Anim Sci
Vol. 31, No. 8:1073-1077 August 2018
https://doi.org/10.5713/ajas.17.0675
pISSN 1011-2367 eISSN 1976-5517

AJAS
Asian-Australasian Journal of Animal Sciences

Microbiological safety of processed meat products formulated with low nitrite concentration — A review

Soomin Lee[1], Heeyoung Lee[1], Sejeong Kim[1], Jeeyeon Lee[1], Jimyeong Ha[1], Yukyung Choi[1], Hyemin Oh[1], Kyoung-Hee Choi[2,*], and Yohan Yoon[1,*]

* **Corresponding Authors:**
Kyoung-Hee Choi
Tel: +82-63-850-6911, **Fax:** +82-63-850-6911,
E-mail: kheechoi@wonkwang.ac.kr
Yohan Yoon
Tel: +82-2-2077-7585, **Fax:** +82-2-710-9479,
E-mail: yyoon@sm.ac.kr

[1] Risk Analysis Research Center, Sookmyung Women's University, Seoul 04310, Korea
[2] Department of Oral Microbiology, College of Dentistry, Wonkwang University, Iksan 54538, Korea

ORCID
Soomin Lee
https://orcid.org/0000-0003-1811-7365
Heeyoung Lee
https://orcid.org/0000-0001-6115-9179
Sejeong Kim
https://orcid.org/0000-0001-9741-8056
Jeeyeon Lee
https://orcid.org/0000-0002-5885-6835
Jimyeong Ha
https://orcid.org/0000-0001-7973-7926
Yukyung Choi
https://orcid.org/0000-0002-9970-0132
Hyemin Oh
https://orcid.org/0000-0002-8073-7242
Kyoung-Hee Choi
https://orcid.org/0000-0001-7778-2053
Yohan Yoon
https://orcid.org/0000-0002-4561-6218

Submitted Sept 11, 2017; Revised Oct 27, 2017; Accepted Mar 5, 2018

Abstract: Nitrite plays a major role in inhibiting the growth of foodborne pathogens, including *Clostridium botulinum* (*C. botulinum*) that causes botulism, a life-threatening disease. Nitrite serves as a color-fixing agent in processed meat products. However, *N*-nitroso compounds can be produced from nitrite, which are considered as carcinogens. Thus, consumers desire processed meat products that contain lower concentrations (below conventional concentrations of products) of nitrite or no nitrite at all, although the portion of nitrite intake by processed meat consumption in total nitrite intake is very low. However, lower nitrite levels might expose consumers to risk of botulism poisoning due to *C. botulinum* or illness caused by other foodborne pathogens. Hence, lower nitrite concentrations in combination with other factors such as low pH, high sodium chloride level, and others have been recommended to decrease the risk of food poisoning. In addition, natural compounds that can inhibit bacterial growth and function as color-fixing agents have been developed to replace nitrite in processed meat products. However, their antibotulinal effects have not been fully clarified. Therefore, to have processed meat products with lower nitrite concentrations, low pH, high sodium chloride concentration, and others should also be applied together. Before using natural compounds as replacement of nitrite, their antibotulinal activities should be examined.

Keywords: Processed Meat; Nitrite; Microbial Safety; Substitute

INTRODUCTION

Nitrite has been used in processed meat products to enhance color, flavor, storage, and antioxidant activity. It is also used to control foodborne pathogens and lipid rancidification [1,2]. Under anaerobic environmental conditions, nitrite can control *Clostridium botulinum* (*C. botulinum*) germination and the growth of various foodborne pathogens such as *Listeria monocytogenes*, *Clostridium perfringens*, *Achromobacter*, *Aerobacter*, *Escherichia*, *Flavobacterium*, and *Micrococcus* spp. [3]. However, nitrite can be converted into *N*-nitroso compounds known as carcinogens by responding to amines generated from products under acidic conditions, and such conversion can be accelerated by high cooking temperature [3-4]. Therefore, safety issues about nitrite have been raised [2]. Meat products with lower nitrite (below conventional concentration of products) concentrations have been produced [5]. However, Doyle and Glass [6], and Gwak et al [7] have suggested that there are problems related to microbial safety of processed meat products with no or low nitrite concentrations. Therefore, the objective of this short communication was to review food safety of processed meat products formulated with no or low nitrite concentrations.

INTAKE OF NITRITE THROUGH CONSUMPTION OF PROCESSED MEAT

The U.S. limits the ingoing level of sodium (or potassium) nitrite for curing. The maximum nitrite levels for comminuted, massaged or pumped, immersion cured, and dry cured meat and poultry products are 156, 200, 200, and 625 ppm, respectively [8]. Maximum sodium nitrite levels for immersion-cured bacon, pumped and/or massaged bacon, and dry cured bacon are set at 120 (or 148 ppm potassium nitrite), 120 (or 148 ppm potassium nitrite), and 200 (or 246 ppm potassium nitrite) ppm, respectively [8]. EU and European countries have also limited the ingoing level of sodium (or potassium) nitrite for processed meat products. The maximum ingoing level of nitrite for cured and canned meat products is 150 ppm in EU [9]. Codex Committee on Food Additives (CCFA) also has regulation for nitrite levels in heat-treated processed meat and processed comminuted meat. For cured ham and cooked cured pork shoulder, the maximum residual nitrite level is 80 ppm. Also, residual nitrite levels in luncheon meat, cooked cured chopped meat are limited to 80 ppm except corned beef whose maximum residual nitrite level is 30 ppm [10]. Korea and Japan mandate the residual nitrite concentration be <70 ppm for processed foods (such as meat products, meat extract processed products, edible beef tallow, and edible pork), up to 50 ppm for fish sausages, and 5 ppm for salted pollack roe and salmon roe [11,12]. However, these concentrations are not observed in processed meat products in markets because nitrite levels in products will decrease continuously due to storage temperature, acidity, heating temperature, storage time, and the presence of other food additives [3]. In the US, levels of residual nitrite and nitrate in cured meat products are 0.64 to 7.31 and 14.81 to 78.81 ppm, respectively [13]. In South Korea, average residual nitrite levels for ham, sausage, and bacon are 16.6, 4.6 ppm, and 15.8 ppm, respectively [14], which are much lower than concentrations listed in regulations. Archer [15] has suggested that ingested nitrites and nitrates are mostly from vegetables and saliva rather than from cured meat. Chung et al [16] have reported that lettuce and spinach have nitrate concentrations of 2,430 and 4,259 ppm, respectively. These are considerably higher than those in meat products reported by Keeton et al [13]. White [17] has found that 81.2% of nitrate intake by Americans is from vegetables, which is higher than nitrate intake from cured meat products (14.7%). Hord et al [18] have also estimated that around 80% of dietary nitrates come from vegetables. Consumed nitrate is absorbed to the plasma through the proximal small intestine [19], and about 65% to 70% of nitrate in plasma is excreted by passive urinary secretion. There are two active secretion systems, colonic and salivary, and the latter is more important due to 25% of entered nitrate is recycled via salivary secretion [19]. One fifth of the recycled nitrate (ca. 5% of the total entered nitrate) is converted to nitrite by microorganisms in oral cavity [19,20]. The nitrite from salivary source account for about 93% of the total ingested nitrite even though it seems like small amount [21]. These results indicate that the portion of nitrite intake by consuming processed meat might be low among total nitrite intake.

MICROBIAL INHIBITION BY LOW NITRITE CONCENTRATIONS

Nitrite in processed meats can inhibit the growth of foodborne pathogens and food spoilage bacteria through various mechanisms, including oxygen uptake and oxidative phosphorylation interruption, formation of nitrous acid and NOs, and interruption of critical enzymes in bacterial metabolism such as aldolase [22]. Nitrite has been used to prevent and control *C. botulinum* growth and other pathogens in processed meat products (Table 1). When nitrite concentration is increased, inhibition for the growth and toxin production of *C. botulinum* is also increased [23]. Robinson et al [24] have examined the effect of nitrite on *C. botulinum* toxin production and reported that the addition of 100 ppm nitrite to pasteurized and cured meats can result in 59% probability of toxin production by *C. botulinum*, which was much higher than the 1% probability of toxin production by *C. botulinum* in the presence of 300 ppm nitrite during storage at 20°C. After 84-day of storage at 27°C, *C. botulinum* toxin has been detected in 7 of 10 bacon samples containing 60 ppm nitrite. However, *C. botulinum* toxin was detected in only 1 of 10 bacon samples containing 340 ppm nitrite [25]. Cui et al [11] have suggested that a significant reduction in nitrite concentrations might lead to increased risk of food poisoning caused by *C. botulinum*. A

Table 1. Various nitrite levels to control microbial growth

Object	Target bacteria or toxin	Nitrite levels for inhibition of microbial growth (ppm)	Reference
Pasteurized and cured meats	*C. botulinum* toxin	100, 300	[25]
Bacon	*C. botulinum* toxin	60, 340	[26]
Meat products	Spore-forming pathogens (e.g. *C. perfringens* and *B. cereus*)	100-200	[32]
Broth	*C. perfringens*	50, 100	[33]
Pork sausage	*E. faecalis*	200	[34]

lowered concentration of nitrite in meat products can result in overgrowth of food-spoilage bacteria (*Lactobacillus* spp., *Enterococcus* spp., and *Pseudomonas* spp.) and foodborne pathogens (*L. monocytogenes*, *Salmonella*, and *Staphylococcus aureus*) in processed meat products [5,26-30].

Nitrite is also effective against other spore-forming pathogens such as *C. perfringens* and *Bacillus cereus* because 100 to 200 ppm nitrite can prevent the outgrowth of germinated spores [31]. Myers et al [32] have investigated the effect of lower nitrite concentrations (0, 50, and 100 ppm) on *C. perfringens* outgrowth in broth system during thermal process and cooling cycle used for processed meat products. It has been found that both nitrite concentration and temperature have significant effects on *C. perfringens* populations and spore survival, suggesting that lower nitrite concentrations are associated with a risk of *C. perfringens* outgrowth [32]. Sameshima et al [33] have reported that the growth of *Enterococcus* faecalis at 10°C is twice slower in pork sausage containing 200 ppm nitrite under vacuum packaging compared to that in sausage containing no nitrite. These results suggest that lower nitrite concentrations will allow more toxin production of *C. botulinum* and more growth of foodborne pathogens in processed meat products. Therefore, the overgrowth of pathogens in meat products formulated with lower nitrite concentrations should be controlled with other combinations.

COMBINATIONS TO IMPROVE MICROBIOLOGICAL INHIBITION

Gunvig et al [34] have developed a model to predict the interface between growth and no growth of *C. botulinum* in meat products (bologna type sausage, chicken cold cuts, and pork cold cuts), containing various levels of sodium nitrite (0 to 150 ppm), sodium lactate (0 to 3%), and sodium chloride (1.2% to 2.4%) with different pH levels (5.4 to 6.4) at 4°C to 12°C. They found that *C. botulinum* did not grow in the presence of 72 to 150 ppm nitrite irrespective of pH, levels of sodium chloride, levels of sodium lactate, or temperature. However, in the presence of 60 ppm nitrite, growth of *C. botulinum* was increased at pH>6, higher storage temperatures, and lower concentrations of sodium chloride and sodium lactate. Sixty ppm nitrite was not sufficient to inhibit *C. botulinum* [34]. Thus, the use of other antimicrobial systems is recommended for products with such low levels of nitrite. Johnson [35] has reported that the minimum pH value for *C. botulinum* growth was 4.6 for Group I and 5.0 for Group II. The minimum sodium chloride concentration for inhibiting *C. botulinum* growth was 10% for Group I and 5% for Group II. Recently, Doyle and Glass [6] have assessed the effect of a combination of sodium chloride and sodium nitrite on *L. monocytogenes* growth, using ComBASE (www.combase.cc/) and found that the antilisterial effect of 100 ppm sodium nitrite plus 0.5% to 5.5% sodium chloride is increased with increasing sodium chloride concentration. Harrison et al [36] have reported that ground beef jerky with a nitrite and salt cure mix can decrease *E. coli* O157:H7 cell counts compared to meat without such cure mix during drying. However, *S. aureus* is resistant to 8% sodium chloride and up to 100 ppm nitrite [37]. In the presence of 100 ppm nitrite at pH 6.0, a 4-log increment of *L. monocytogenes* cell counts in the presence of 0.5% sodium chloride has been observed after storing at 37°C for 8 h or 5°C for 287 h. However, in the presence of 4.5% sodium chloride, storing at 37°C for 11 h or 5°C for 479 h is required to induce a 4-log increase of *L. monocytogenes* [38]. McClure et al [39] have reported that *L. monocytogenes* does not grow within 21 days at 20°C and pH 5.3 with addition of 50 ppm nitrite. Indeed, at pH 6.0 and 10°C, the inhibitory effect of nitrite is minimal after adding 400 ppm $NaNO_2$. Therefore, microbiological safety of processed meat products with lower nitrite levels can be improved by a combination of pH and sodium chloride concentration. Gwak et al [7] and Tompkin [40] have also suggested that increased sodium chloride levels can control the growth of harmful bacteria in processed meat products with lower nitrite concentrations because the antimicrobial effect of nitrite is influenced by sodium chloride concentration, pH, reductants, iron content, and so on.

MICROBIOLOGICAL SAFETY OF NATURAL SUBSTITUTES

Although some consumers prefer no or low nitrite in processed meat products [7], removing nitrite from meat products could be problematic because of its role in microbiological safety. Thus, the food industry has tried to replace synthesized nitrite with vegetables with high nitrite concentrations because consumers are more comfortable with natural sources. Hence, numerous studies have been conducted on natural sources of nitrite. Horsch [41] has compared the antilisterial effect of nitrite with celery juice which contains a high concentration of nitrite and found that the antilisterial effect of sodium nitrite is higher than that of celery juice at the same nitrite concentration. However, King et al [42] have suggested that equivalent nitrite concentrations from purified nitrite and celery juice powder have equivalent effectiveness on the growth of *C. perfringens*. Although theses natural substances possess antibacterial and color-formation effects, and they could be regarded as nitrite substitutes, their fundamental ability to inhibit germination of *C. botulinum* spores should also be examined prior to their industrial use. Cui et al [11] have recently examined antibotulinal activities of 90 fresh herbs and spices, and found that nutmeg (0.05%), sage (0.02%), and clove (0.05) extracts possess antibotulinal activities in a model meat product without compromising its organoleptic properties.

CONCLUSION

The portion of nitrite intake through consuming processed meat in total nitrite intake is relatively low. However, consumers still want to have low nitrite concentration in processed meat products. Low or no nitrite concentration in processed meat products may increase the risk of illness caused by foodborne pathogens. Thus, other factors such as low pH, addition of sodium chloride, antimicrobials, and/or natural sources should be combined with nitrite to control foodborne pathogens, particularly for *C. botulinum* and *L. monocytogenes*. In addition, although some natural substances possess antimicrobial and color-formation effects, their antibotulinal activity must be examined before using them in processed meat products.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

ACKNOWLEDGMENTS

This work was supported by the Wonkwang University in 2017.

REFERENCES

1. Horsch AM, Sebranek JG, Dickson JS, et al. The effect of pH and nitrite concentration on the antimicrobial impact of celery juice concentrate compared with conventional sodium nitrite on *Listeria monocytogenes*. Meat Sci 2014;96:400-7.
2. Sebranek JG, Bacus JN. Cured meat products without direct addition of nitrate or nitrite: what are the issues? Meat Sci 2007;77:136-47.
3. Krause BL, Sebranek JG, Rust RE, Mendonca A. Incubation of curing brines for the production of ready-to-eat, uncured, no-nitrite-or-nitrate-added, ground, cooked and sliced ham. Meat Sci 2011;89:507-13.
4. Knechtges P. Food safety: theory and practice. Chapter5. Food safety: engineering controls and technology. Burlington, MA, USA: Jones & Bartlett Learning; 2012.
5. Ha J, Gwak E, Oh M-H, et al. Kinetic behavior of *Salmonella* on low $NaNO_2$ sausages during aerobic and vacuum storage. Korean J Food Sci An 2016;36:262-6.
6. Doyle ME, Glass KA. Sodium reduction and its effect on food safety, food quality, and human health. Compr Rev Food Sci Food Saf 2010;9:44-56.
7. Gwak E, Oh M-H, Park B-Y, et al. Probabilistic models to predict *Listeria monocytogenes* growth at low concentrations of $NaNO_2$ and NaCl in frankfurters. Korean J Food Sci An 2015;35:815-23.
8. USDA (U.S. Department of Agriculture). Processing inspector's calculations handbook. Food Safety and Inspection Service. Washington, DC, USA: USDA; 1995.
9. EUR Lex [Internet]. EU Commission Regulation (EU) No 1129/2011 of 11 November 2011 amending Annex II to Regulation (EC) No 1333/2008 of the European Parliament and of the Council by establishing a Union list of food additives Text with EEA relevance [cited 2017 May 26]. Available from: http://eur-lex.europa.eu/eli/reg/2011/1129/oj
10. CCFA (Codex Committee on Food Additives). General standard for food additives, CODEX STAN 192-1995. Rome, Italy: Pectin; Food and Agriculture Organization of the United Nations; 2015.
11. Cui H, Gabriel AA, Nakano H. Antimicrobial efficacies of plant extracts and sodium nitrite against *Clostridium botulinum*. Food Control 2010;21:1030-6.
12. MFDS (Ministry of Food and Drug Safety). Korea food additives code [internet]. Cheongju, Korea: Ministry of Food and Drug Safety; 2013 [cited 2018 May 30]. Available from: http://www.mfds.go.kr/index.do?x=0&searchkey=title:contents&mid=1013&searchword=식품첨가물&y=0&division=&pageNo=1&seq=11290&sitecode=2017-12-11&cmd=v
13. Keeton JT, Osburn WN, Hardin MD, Bryan NS. A national survey of the nitrite/nitrate concentrations in cured meat products and non-meat foods available at retail-NPB #08-124. National Pork Board Research Report; 2009.
14. KFSRI (Korea Food Safety Research Institute) [Internet]. Wanju, Korea: Nitrite and food safety [cited 2015 June 5]. Available at: http://www.kfsri.or.kr/board/board.asp?bid=bodo
15. Archer DL. Evidence that ingested nitrate and nitrite are beneficial to health. J Food Prot 2002;65:872-5.
16. Chung SY, Kim JS, Kim M, et al. Survey of nitrate and nitrite contents of vegetables grown in Korea. Food Addit Contam 2003;20:621-8.
17. White JW. Relative significance of dietary sources of nitrate and nitrite. J Agric Food Chem 1975;23:886-91.
18. Hord NG, Tang Y, Bryan NS. Food sources of nitrates and nitrites: the physiologic context for potential health benefits. Am J Clin Nutr 2009;90:1-10.
19. Walker R. The metabolism of dietary nitrites and nitrates. In: Wilson WS, Ball AH, Hinton RH, editors. Managing risks of nitrates to humans and the environment. Cambridge, UK: The Royal Society of Chemistry; 1999. pp. 250-8.
20. L'hirondel J-L. Are dietary nitrates a threat to human health? In: Morris J, Bates R, editors. Fearing food: risk, health and environment. Oxford, UK: Butterworth and Heinemann; 1999. pp. 38-47.
21. Cassens RG. Residual nitrite in cured meat. Food Technol 1997;51:53-5.
22. Weiss J, Monika G, Schuh V, Salminen H. Advances in ingredient and processing systems for meat and meat products. Meat Sci 2010;86:196-213.
23. Huhtanen CN, Wasserman AE. Effect of added iron on the

formation of clostridial inhibitors. Appl Microbiol 1975;30: 768-70.

24. Robinson A, Gibson AM, Roberts TA. Factors controlling the growth of *Clostridium botulinum* types A and B in pasteurized, cured meats V. Prediction of toxin production: Non-linear effects of storage temperature and salt concentration. J Food Technol 1982;17:727-44.
25. Christiansen LN, Tompkin RB, Shaparis AB, et al. Effect of sodium nitrite on toxin production by *Clostridium botulinum* in bacon. Appl Microbiol 1974;27:733-7.
26. Jo H, Park B, Oh M, et al. Probabilistic models to predict the growth inhibition time for *Pseudomonas* spp. in processed meats formulated with NaCl and $NaNO_2$. Korean J Food Sci An 2014;34:736-41.
27. Lee S, Lee H, Kim S, et al. Probabilistic models to describe the effect of $NaNO_2$ in combination with NaCl on growth inhibition of *Lactobacillus* in frankfurters. Meat Sci 2015;110:302-9.
28. Lee H, Gwak E, Lee S, et al. Model to predict growth/no growth interfaces of *Enterococcus* as a function of NaCl and $NaNO_2$. J Food Saf 2016;36:537-47.
29. Lee J, Gwak E, Ha J, et al. Mathematical model for predicting the growth probability of *Staphylococcus aureus* in combination of NaCl and $NaNO_2$ under aerobic or evacuated storage conditions. Korean J Food Sci An 2016;36:752-9.
30. Lee J, Gwak E, Lee H, et al. Effect of low $NaNO_2$ and NaCl concentrations on *Listeria monocytogenes* growth in emulsion-type sausage. Asian-Australas J Anim Sci 2017;30:432-8.
31. Redondo-Solano M. Effect of sodium nitrite, sodium erythorbate and organic acid salts on germination and outgrowth of *Clostridium perfringens* spores in ham during abusive cooling [master's thesis]. Lincoln, NE, USA: University of Nebraska; 2011.
32. Myers MI, Sebranek JG, Dickson JS, et al. Implications of decrease nitrite concentrations on *Clostridium perfringens* outgrowth during cooling of ready-to-eat meats. J Food Prot 2016; 79:153-6.
33. Sameshima T, Takeshita K, Miki T, et al. Effect of sodium nitrite and sodium lactate on the growth rate of lactic acid spoilage bacteria isolated from cured meat products. Jpn J Food Microbiol 1998;13:159-64.
34. Gunvig A, Hansen F, Borggard C. A mathematical model for predicting growth/no-growth of psychrotrophic *C. botulinum* in meat products with five variables. Food Control 2013;29: 309-17.
35. Johnson EA. *Clostridium botulinum*. In: Doyle MP, Beuchat LR, Montville TJ, editors. Food microbiology, fundamentals and frontiers. 3rd edition, Washington DC, USA: ASM Press; 2007. p. 401-21.
36. Harrison J, Harrison M, Rose B. Survival of *E. coli* O157:H7 in ground beef jerky assessed on two plating media. J Food Prot 1998;61:11-3.
37. Whiting RC, Sackitey S, Calderone S, Morley K, Philips JG. Model for the survival for *Staphylococcus aureus* in nongrowth environments. Int J Food Microbiol 1996;31:231-43.
38. Buchanan RL, Stahl HH, Whiting RC. Effects and interactions of temperature, pH, atmosphere, sodium chloride and sodium nitrite on the growth of *Listeria monocytogenes*. J Food Prot 1989;52:844-51.
39. McClure PK, Kelly TM, Roberts TA. The effects of temperature, pH, sodium chloride and sodium nitrite on the growth of *Listeria monocytogenes*. Int J Food Microbiol 1991;14:77-82.
40. Tompkin RB. Nitrite. In: Davidson PM, Sofos JN, Branen A, editors. Antimicrobials in food. 3rd edition. Boca Raton, FL, USA: CRC Press; 2005. p. 169-236.
41. Horsch, A. The effect of pH and nitrite concentration on the antimicrobial impact of celery juice compared with sodium nitrite on *Listeria monocytogenes* [master's thesis]. Ames, IA, USA: Iowa State University; 2013.
42. King AM, Glass KA, Milkowski AL, Sinderlar JJ. Comparison of effect of curing ingredients derived from purified and natural sources on inhibition of *Clostridium perfringens* outgrowth during cooling of deli-style turkey breast. J Food Prot 2015; 78:1527-35.