Original Article
Toxicology International, *Sept-Dec 2016 / Vol-23 / Issue-3, 236-239*
ISSN (Print) : 0971-6580
ISSN (Online) : 0976-5131
DOI: 10.22506/ti/2016/v23/i3/146716

Study of nitrite in hamburgers supplied in Tehran, Iran

Nabi Shariatifar[1], Mohammad Rezaei[1,2], Rezvan Rahimnia[3]

[1]Department of Environmental Health Engineering, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran,
[2]Department of Biology, Payam-e Noor University, Tehran, Iran,
[3]Department of Environment, Payam-e Noor University, Tehran, Iran
* Rahimniarezvan@yahoo.com

Abstract

One of the food additives that adds color, taste, and protection against micro-organisms is nitrite; however, when nitrite content exceeds a specific level, it acts as a toxic element and may cause cancer. Using chemical preservatives in natural and organic foods is not legal, so that traditional curing agents are used for cured meat. Therefore, using nitrite on hamburgers is not legal. Purpose of the present study is to measure nitrite content of hamburger products in Tehran's market in 2015. To this end, 120 samples were collected randomly and analyzed following Official AOAC Method 973. The results showed that residual nitrite in the sample was at the range 28-100 mg/kg ($p<0.001$). Nitrite was detected in 83.3% of the samples; which indicated an undesirable condition and failure to observe sodium nitrite standards at hamburger factories.

Keywords: Sodium nitrite; hamburgers; food safety; spectrophotometric

Received: 27-05-2016 **Revised:** 26-07-2016 **Accepted:** 05-08-2016

1. Introduction

Because micro-organisms can easily thrive on the rich supply of nutrient of meat and its side-products with high moisture content, meat and the similar products can easily lose their quality. Microorganisms are the main reason that cut shelf life of meat products (1). Nitrite induces bacteriostatic effects on the specific species of bacteria that are responsible for microbial spoilage of meat. It also affects different types of microorganisms differently so that different inhibition mechanisms are need to control different microorganisms. Along with improving safety of food, sodium nitrate improves the shelf life of meat products (2). Achieving microbiological safety by adding nitrite to cured meat, probably, is the most critical function of the curing process(3, 4).

In addition to its controlling effect on many micro-organisms; nitrite's inhibition effect on Clostridium botulinum is of greatest importance. The microorganism generates a heat-resistant spores, which enables it to survive the normal cooking temperature. Nitrite/nitrate inhibits spore germination so that production of deadly toxin by the vegetative cells is avoided(5).

One of the most toxic substance is produced by C. Botulinum and upon digestion, it can cause botulism. The disease is the top lethal food-borne disease that cause 20-50% mortality rate. It is notable that antimicrobial activity of nitrate and nitrite is a function of other factors such as PH and salt content(6, 7).

Salt is a growth inhibition factor of micro-organism through attenuating the water content available to the microorganism. It also functions synergistically with nitrite to inhibit growth of microorganism (8). There are hard evidences that solutions 5% sodium chloride effectively inhibits growth of C. Botulinum under optimal growth condition. Still, excessive salt content makes the products unpalatable and thus undesirable to the consumers. Therefore, nitrite is needed along with salt to inhibit growth of C.botuinum. Additionally, pH of the meat product is another key factor in controlling C.botulinum,

* *Author for correspondence*

and other pathogens and spoilage microorganism (9, 10). This is can be attributed to un-dissociated nitrous acid that yields nitric oxide; which needs acidic pH. With one unit decrease in pH, the antimicrobial activity increases about two units. Thus, the lower the pH, the stronger the antimicrobial activity of nitrite(10).

At any rate, stability of nitrite reduces with reduction of pH; thus, it is vital to add salt in cured meat for its synergistic effect with nitrite(11).

The purpose of the present study is to examine the main categories of hamburger products with respect to their nitrite content. To this end, the samples were collected randomly from Tehran's market as representative of cured meat products consumption by Iranian population.

2. Material and methods

Sampling: Totally, 120 hamburger samples were procured from the local retailers in Tehran in 2015.

Chemicals and reagents: All the reagents utilized for the purpose of this study were of analytical grade purity, while all the chemical substances and the standards were procured from Merck (Darmstradt, Germany).

Sample preparation: The samples were chopped into pieces and homogenization was done mechanically. To determine nitrite level, 5gr of each sample was placed in a 50ml beaker filled with 40ml distilled water and then heated up to 80°C; meanwhile, the solution was mixed thoroughly using a glass rod before being transferred to a 500ml volumetric flask. The beaker and rod were washed with enough distilled water until volume of the solution reached to 300ml. Then, the flask was put into a stream bath for 2hr under occasional shaking. Then the solution was cooled down to ambient temperature and the solution volume was increased to 500ml by adding distilled water while being remixed and filtered. Afterward, 2.5ml sulphanilamide reagent (5-50 µg $NaNO_2$ per 50ml) was added, mixed, and diluted. Color of the solution was given 15min to develop. Then, a portion of the solution was transferred to the photometer cell and the absorption features were determined at 540nm against a blank of distilled water with 2.5 ml sulfanilamide reagent and 2.5ml NED reagent. Once again, distilled water was used as negative control and sodium nitrite solution was used as a positive control(11, 12).

Calibration curve: Sodium nitrite was measured through comparing the standard curve; the solution was prepared through adding 10, 20, 30, and 40ml nitrite working standard solution to 50ml volumetric flasks; then 2.5ml of sulphanilamide reagent was added and mixed and the process was followed as noted earlier. The calibration curve was drawn by plotting the absorbance against the concentration. The curve was developed to be a straight line to 1µg/ml $NaNo_2$ in the final solution. Level of nitrate in the samples were reported as mg NO_2/kg hamburger. The limits of detection and quantification (LOD and LOQ) were calculated using standard deviation of the y-intercept of the calibration curve: (13)

$$LOD = 3.3 \times SDy/S \text{ and } LOQ = 10 \times SDy/S$$

Where, SDy denotes SD of the y-intercept and S denotes the slope of the regression line.

To ensure quality, 10mg/kg nitrite standard was used as control. Participation in a proficiency test at a nitrite level of 72.2mg/kg in freeze dried meat led to z-score equal with -1.7. (14).

Recoveries: To examine accuracy of the procedure, experiments of recovery were performed. The samples of hamburger were spiked using 10, 50, and 100mg/kg sodium nitrite. The results confirmed 100% recovery after the extraction procedure. The experimental recovery was computed as the difference between two measurement (sample and spiked samples) as follows:

(Total analyte found – analyte originally present) × 100/analyte spike = percentage recovery)

Immediately after sample preparation, the recovery ranges for nitrite was estimated.

3. Statistical analysis

Data analyses were carried out in SPSS V.17 (IBM Corp., Armonk, NY, USA) and p-value <0.001 was taken as statistically significant.

4. Results and Discussion

A chemical additive to meat products is sodium nitrite, which is obtained from nitrates. The additive reacts with the naturally occurring components of protein known as

amines. The reaction might yield nitrosamines, which are carcinogen(15). The carcinogen components can develop in nitrite or nitrate-treated meat or in the digestive tract. Association between sodium-nitrite and stomach cancer is supported by many studies. In the early 1970s and because of development of carcinogenic nitrosamines, the nitrite was known as a hazardous material (16). Nitrite also acts as an inhibitor of microorganisms' activity while adding pleasant flavor to meat products (17). Table 1 lists the mean concentration level at different ranges of the 120 tested samples; while calibration curve data, LOD, LOQ and recovery values are listed in Table 2. Data analyses supported significant difference between sodium nitrite volume and Iran National Standard in 100 samples ($p<0.001$). In another 20 samples (16.7%) was below LOD. Mean levels in factories A-T are given nitrate and nitrite are not allowed for hamburger, while these salts can be used in cooked meat products as a curing agent. According to Iran National Standard codes, nitrite in meat products should be less than 120mg/kg, while it is completely prohibited in hamburger products.

Our results showed that 83.3m/kg nitrite was in the hamburger products of 20 factories (30mg/kg is maximum allowable level). This means risk of cancer for the consumer. Therefore, nitrite level in the meat products under study is a food safety issues. High levels of dietary intake of nitrite has been confirmed by many other studies as well. Studies in the UK, Canada, Colombia, Chile, Japan, Denmark, and Italy have showed that high dietary intake of nitrite can be a cause of gastrointestinal cancer (18-20). Nitrate level measured in products by some of the meat products factories in Semnan-Iran was below the standard limit ($p<0.05$), so that the products were acceptable from the standards codes viewpoint (21). Rezaei et al. (2013) mentioned that the mean nitrite content in the hamburger samples available in Arak-Iran was below the standard limits only in 15 samples (28.1%) (11). Pereira et al. (2012) indicated that the mean nitrate and nitrite content in the meat samplesmet the standards in Brazil(17). Ali (2007) noted that the mean nitrite content in curing meat samples was as per the NED standard method(22).

Our findings are consistent with Babaee et al. (2012) who reported 89.9mg/kg nitrite content in hamburger products available in Mazadaran in 2009; and with Rezaei et al. (2013) who indicated 100mg/kg nitrite in hamburger products available in Arak-Iran(11, 23).

5. Conclusion

The results indicated necessity of a routine monitoring system to ensure that nitrate and nitrite concentrations in meat products and hamburger meet the standards. The monitory system is also needed to assess the effect of different processing methods on nitrate and nitrite levels and their dietary intake.

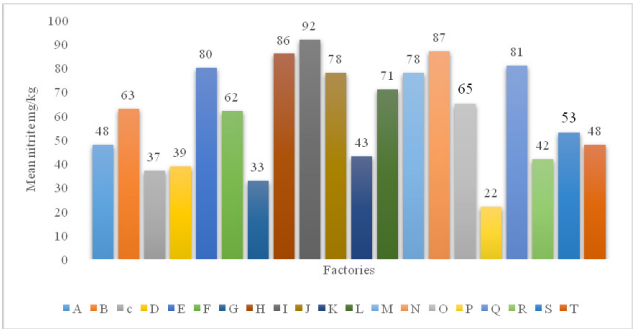


Figure 1. Average nitrite (mg/kg) levels in various factories.

Table 1. Mean levels and ranges (mg/kg) for nitrite in the hamburger samples

	20-40	40-60	60-80	80-100	<LOD	>LOD
Sample	30	10	32	28	20	100
Range	28- 39	43-60	69-76	82-100	0	28-100
Mean±SD	32.3	59.8	71.7	91.7	0	61.8±3.2

Table 2. Characteristic of the analytical method for nitrite

Calibration curve	R^2	Linearity (mg/kg)	LOD (mg/kg)	LOQ (mg/kg)	Recovery (%)
y = 0.1023x + 0.0421	0.9979	2.5-15	0.5	1.5	81-95

6. Acknowledgments

The authors wish to thank the Institute for Environmental Research (IER), Tehran University of Medical Sciences, Tehran, Iran.

7. Conflict of Interest

None Declared

8. References

1. Garriga M, Grebol N, Aymerich M, Monfort J, Hugas M. Microbial inactivation after high-pressure processing at 600 MPa in commercial meat products over its shelf life. Innovative Food Science & Emerging Technologies. 2004;5(4):451-7.
2. Tarr H. The action of nitrites on bacteria. Journal of the Fisheries Board of Canada. 1941;5(3):265-75.
3. Sebranek JG, Bacus JN. Cured meat products without direct addition of nitrate or nitrite: what are the issues? Meat science. 2007;77(1):136-47.
4. Honikel K-O. The use and control of nitrate and nitrite for the processing of meat products. Meat science. 2008;78(1):68-76.
5. Sugiyama H. Studies on factors affecting the heat resistance of spores of Clostridium botulinum. Journal of bacteriology. 1951;62(1):81.
6. Chin J, Association APH. Control of communicable diseases manual: American Public Health Association Washington, DC; 2000.
7. Sobel J, Tucker N, Sulka A, McLaughlin J, Maslanka S. Foodborne botulism in the United States, 1990-2000. Emerging infectious diseases. 2004;10:1606-11.
8. Barak A. Method of treating liquids to inhibit growth of living organisms. Google Patents; 2000.
9. Ghaly AE, Dave D, Budge S, Brooks M. Fish spoilage mechanisms and preservation techniques: review. American Journal of Applied Sciences. 2010;7(7):859.
10. Buchanan RL, Stahl HG, Whiting RC. Effects and interactions of temperature, pH, atmosphere, sodium chloride, and sodium nitrite on the growth of Listeria monocytogenes. Journal of Food Protection®. 1989;52(12):844-51.
11. Rezaei M, Shariatifar N, Jahed Khaniki G, Javadzadeh M. Nitrite in hamburgers in Arak, Iran. Food Additives & Contaminants: Part B. 2013;6(4):285-8.
12. Rezaei M, Fani A, Moini AL, Mirzajani P, Malekirad AA, Rafiei M. Determining nitrate and nitrite content in beverages, fruits, vegetables, and stews marketed in Arak, Iran. International Scholarly Research Notices. 2014;2014.
13. ICH. Guidance for industry - Q2B validation of analytical procedures: methodology. European Medicines Agency Geneva: ICH. 1995.
14. FAPAS. Food Analysis Performance Assessment Scheme. Proficiency Testing Report series 151, round 19, March 2002. The Food and Environment Research Agency, Sand Hutton, York YO41 1LZ. 2002.
15. Pierson MD, Smoot LA, Robach MC. Nitrite, nitrite alternatives, and the control of Clostridium botulinum in cured meats. Critical Reviews in Food Science & Nutrition. 1983;17(2):141-87.
16. Pegg RB, Shahidi F. 12 Processing of Nitrite-Free Cured Meats. Advanced Technologies For Meat Processing. 2006:309.
17. Pereira EA, Petruci JF, Cardoso AA. Determination of nitrite and nitrate in Brazilian meats using high shear homogenization. Food Analytical Methods. 2012;5(4):637-42.
18. Hsu J, Arcot J, Lee NA. Nitrate and nitrite quantification from cured meat and vegetables and their estimated dietary intake in Australians. Food Chemistry. 2009;115(1):334-9.
19. Tannenbaum S, Weisman M, Fett D. The effect of nitrate intake on nitrite formation in human saliva. Food and cosmetics toxicology. 1976;14(6):549-52.
20. Knekt P, Järvinen R, Dich J, Hakulinen T. Risk of colorectal and other gastro-intestinal cancers after exposure to nitrate, nitrite and N-nitroso compounds: a follow-up study. International journal of cancer. 1999;80(6):852-6.
21. Nasehinia H, Mahdinia S, Ghorbani R, Nouri SM. Nitrite concentration in distributed sausage in semnan province. 2008.
22. Ali Hak. Batch and flow-injection spectrophotometric determination of nitrite in curing meat andnitrate in wastewater samples: Salahaddin University-Erbil; 2007.
23. Babaee Z, Bagheri GA, Salehifar E, Javadian B, Karimzadeh L. Determination of nitrate and nitrite residue in meat product produced in some city of Mazandaran in 2009. J Mazand Univ Med Sci. 2012;22(1):228-33.