

## Nitrite Content in Cured Meats Samples at Khartoum State (Sudan)

MANAL MOHAMMED ABD ALSHAFFEA AHMED

National Laboratory for Public Health, Khartoum (Sudan)

OMER ADAM MOHAMED GIBLA

ESRAA OMER ADAM MOHAMMED

College of Science, Sudan University of Science and Technology

### Abstract

*The aim of this study was to measure nitrite ions ( $NO_2$ ) residue in some processed meats samples produced by different companies. Thirty samples were obtained from the local markets in Khartoum state. The collected samples include Sausages, Martidala, Beef burger, Basterma, Hot dogs and Frankfurters. Nitrite ions were extracted from each sample by hot water and their concentrations were measured by Uv-Vis spectrophotometry. Three samples showed no nitrite content. Thirty samples showed nitrite concentrations below the permissible range which is (130mg/Kg) according to national regulation for food additives (Sudan, 2006). The highest mean values were found in Hot dog samples (4.5mg/kg) and the lowest mean values were observed in Sausages samples (0.66mg/kg). The concentrations were expressed as sodium nitrite ( $NaNO_2$ ).*

**Key words:** nitrite content, cured meats, permissible range, Uv-Vis spectrophotometry, clostridium botulinum, nitrosylation agent

### INTRODUCTION

For centuries ago, meat had been preserved with salt. At certain levels, salt prevents growth of some types of bacteria which causes meat spoilage. Salt prevents bacterial growth,

either because of its direct inhibitory effect, or because of the drying effect it has on meat. Most types of bacteria require substantial amounts of moisture to live and grow (Epley, Addis, and Joseph J, 1992). Cured meats have specific properties including, pink color, characteristic flavor and texture (Borchert and Cassens 1998). Curing is the treatment applied to food or other products, to prevent decay by addition of salt or smoke. The curing process refers to food preservation by drying or removal of water. It is difficult to track the exact origin of the curing practice in human civilization, as it had been present in history for thousands of years (Roberts, 1996). The main purpose of curing was to preserve the food in times, where, refrigeration was not available (Redondo-Solano, 2011).

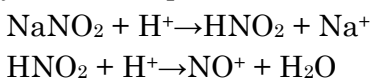
Sodium nitrite and potassium nitrate have a long history of use as curing ingredients, and by the close of the 19th century the scientific basis of the process were becoming well understood. It was realized, for example, that nitrate must be converted to nitrite which help the curing process to proceed. This process has two purposes. One is “cosmetic”, where they give the meat a pinkish-reddish color and prevent it from turning brown. The second is the prevention of clostridium botulinum growth, the microorganism that produces the deadly botulism toxin (Borchert and Cassens, 1998). Sodium nitrite ( $\text{NaNO}_2$ ) is commonly added to meat products which are kept for an extended period in a cold, but not frozen state. This compound is produced from nitrogen oxides or nitrous fumes that are obtained after the catalytic oxidation of ammonia with air in a controlled environment. The nitrogen oxides are then absorbed in sodium carbonate or sodium hydroxide solutions to obtain pure, white to yellowish color (Redondo-Solano, 2011). The chemical reaction is:

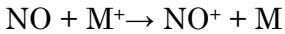
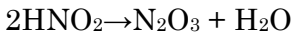


Nitrite, in comparison to nitrate, is a much more reactive compound. Like nitrate, the pKa of nitrous acid (HNO<sub>2</sub>) is relatively low, 3.3. Most of this compound would be found as the nitrite anion (NO<sub>2</sub><sup>-</sup>) in biological or meat curing conditions, but, some of nitrous acid would be found. The nitrite ion must be reduced to act as anitrosating/ nitrosylating agent. Acidification of nitrite provides one of the best methods to form nitric oxide. In the presence of mineral acids and other reducing compounds, nitrite may be non-enzymatically reduced to nitric oxide. However, the anhydrous form of nitrous acid, dinitrogen trioxide, N<sub>2</sub>O<sub>3</sub>, is thought to be one of the main nitrosating compounds (Lundberg et al; 2008). Reduction of nitrite in meat curing systems is essential to provide nitrosation/nitrosylation reactions and is impacted by many factors including pH, temperature, endogenous compounds, and other added ingredients. In vivo, the nitrite produced by bacteria in the oral cavity is readily reduced to nitric oxide in the acid conditions of the stomach and provides a supplement nitric oxide source (Lundberg et al 2008).

Early researchers noted, the color change, of hemoglobin with the addition of nitrite. This reaction is known to form nitrosylmetmyoglobin, which renders hemoglobin unable to transport oxygen. Cyanosis caused by ingesting sodium nitrite or nitrite containing food has been reported throughout medical literature (Sullivan, 2011).

Several studies demonstrated the presence of high amounts of nitrosamines in cured products, when subjected to high temperatures. There are more than 300 different types of nitrosamines and about 97% of them have been shown to be teratogenic in laboratory animals (Redondo-Solano, 2011). The chemical process that leads to the formation of nitrosamines in meat systems is depicted as.





Primary amine  $\text{RNH}_2 + \text{NO}^+ \rightarrow \text{RNH-N} = \text{O} + \text{H}^+ \rightarrow \text{ROH} + \text{N}_2$

Secondary amine  $\text{R}_2\text{NH} + \text{NO}^+ \rightarrow \text{R}_2\text{N-N} = \text{O} + \text{H}^+$

Tertiary amine  $\text{R}_3\text{N} + \text{NO}^+ \rightarrow$  no nitrosamine formation

This process involves the same reactions leading to the formation of nitric oxide and nitrous acid; therefore, the same conditions may lead to the reduction of nitrite which favors nitrosamine formation. The secondary amines form the more stable nitrosamines. Amines are present in very low concentrations in fresh meat products in the form of creatine, creatinine or free amino acids like proline or hydroxyproline (Honikel, 2008).

Nitrite itself can be toxic if consumed in very high quantities. It can interact chemically with hemoglobin and interrupt the normal oxygen transportation in humans (Redondo-Solano, 2011).

Today, the use of sodium nitrite is acknowledged by regulatory authorities around the world, as an important contributor to food safety. In addition, a rapidly expanding body of scientific research is demonstrating that, the advantages of nitrite extend well beyond food safety to include a diverse and critical array of potent benefits for human health.

Nitrite concentrations decline in meat mixtures during processing. Many factors influence the rate of nitrite reduction in meat. Gary Anthony Sullivan (2011) and Greenwood (1940) proposed six factors that may influence nitrite loss, which are Time and temperature employed during processing, Amount of protein, fat, and carbohydrate, Concentration of salt, Concentration of nitrate and nitrite, Number of microorganisms, and Acidity.

Many countries have developed specific, regulations to control the amount of nitrate and nitrite present in meat products. Most of the regulations specify the amount of nitrite that must be added to the product as a part of the manufacturing process (Roberts, 1996). To control the amount of nitrite input in most of the products, and, for the possibility of in vivo nitrosamine formation, some regulations established limits for both the ingoing and residual nitrites. The European Parliament and Council published a revised directive (2006) regarding the use of nitrates and nitrites in cured products including residual nitrite regulations (Redondo-Solano, M. S, 2011).

Curing agents were established in the USA in 1926 (USDA, 1925; USDA, 1926), and the same rules are considered today, with slight modifications. The critical feature of these rules is that, a maximum use level of sodium nitrite is defined; but the meat processor may use less. Basically, no more than one-quarter ounce (7.1 g) may be used per 100 pounds (45.4 kg) of meat (resulting in 156 mg/kg or 156 ppm). While nitrate is still permitted, it is, in fact, not used by industries now. The regulations were changed for bacon, so that ingoing nitrite is targeted at 120 ppm, and the maximum use of ascorbates as (550 ppm) is mandated. The current routine use of ascorbates (ascorbic acid, sodium ascorbate, erythorbic acid and sodium erythorbate) by the meat processing industry is important not only because it accelerates and improves the curing process but also the use of ascorbates inhibits nitrosation reactions, which, might result in formation of carcinogenic nitrosamines. One possible chemical hazard involved in producing processed meats may be an error in the use of sodium nitrite. If too much is added there is a risk of illness, even death, of the consumer. United States Department of Agriculture (USDA) recognized this concern when the regulations permitting the direct use of sodium nitrite were established including the Levels of use and safeguards in handling (Borchert and Cassens, 1998). The

levels of analytically nitrite detectable in cured meat are greatly reduced from the amount added because; the nitrite reacts with the meat components during processing and storage. Modern-day cured meats at retail have a residual nitrite content of about 10 ppm. The National Institute of Occupational Safety and Health (NIOSH, 1997) reported that there are data indicating nitrite as a primary irritant, tumorigen, mutagen and it causes reproductive effects. The levels cited for toxic and lethal doses are in the range of those previously referenced. The National Academy of Sciences (1981) concluded that there is no definitive evidence to suggest that either nitrate or nitrite is carcinogenic. In animals, nitrate has not been shown to be directly carcinogenic or mutagenic. The limited data on nitrite indicate that, it may not act directly as a carcinogen, but that it is mutagenic in microbial systems. Also it is of interest, at this point, to understand what is considered an acceptable intake of nitrate and nitrite by humans. FAO/WHO Joint Expert Committee on Food Additives (JECFA) set an acceptable daily intake (ADI) of 0-5 mg/kg body weight for sodium nitrate and of 0-0.2 mg/kg body weight for sodium nitrite. In other words, they could be manufactured for different applications or for specific formulations, so as to make them optimally useful for a variety of products and applications. Germany and other European Community countries recognized the concern associated with keeping and handling a chemical such as sodium nitrite. They have mandated that only diluted forms of sodium nitrite such as those in pre-blends or curing salt mixtures be allowed in meat processing establishments (Borchert and Cassens July, 1998).

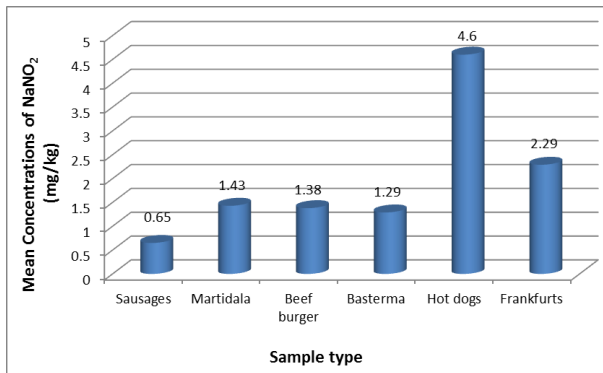
## **MATERIALS AND METHODS**

Thirty locally processed meat samples were collected from Khartoum state super markets. The samples include six types

of cured meats, namely Sausages, Martidala, Hambergar, Basterma, Hot dogs and Frankfurters. All chemicals used were of analytical grade. Uv- Vis spectrophotometer (Analytikjena Model 07745, single beam, Germany) was used for analysis. About 10 grams of each sample were accurately weighed in 250ml conical flask, 100 ml of hot water (80°C ),5 ml borax solution and 0.5 g activated charcoal were added .The mixture was transferred to a water bath at (70°C) for 15 min. After two hours, 1 ml of potassium ferrocyanide (10.6%), 1 ml of zinc acetate (21.6%) and 5 ml borax(5%) were added .Then the contents of the conical flask were transferred to a 250 ml volumetric flask and, allowed to stand for 30 min. then made up to the mark with distilled water, mixed and filtrate through a filter paper. 20 ml from each, sample filtrated were pipetted into a 50 ml volumetric flask, diluted to approximately 40 ml, 5 ml of sulphanilamide (0.5%) were added and allowed to stand for 3 mins. 2 ml of coupling reagent were then added. After 20 min the absorbance was measured at 540 nm in a 1 cm cell. The absorbance of blank solution was also measured.

## **RESULTS AND DISCUSSION:**

Sausages samples showed highest sodium nitrite concentration of 1.78 mg/kg and zero concentrations in tow samples. The mean value of concentrations was (0.66mg/kg). All concentrations are within the range according to the National Regulation for Food Additives (Sudan 2006) (Figure. 1).



**Figure (1): Mean concentrations of NaNO<sub>2</sub> (mg/kg)**

Figure (1) showed that Martidala samples show highest concentration as 3.00 mg/kg and lowest concentration as zero value in one sample. The mean of nitrite content was within the permissible range (1.44mg/kg).

Beef bergar samples shows highest sodium nitrite concentration of 1.94mg/kg and lowest concentration of 0.97mg/kg. The concentrations of sodium nitrite in beef burger samples were within the accepted range with a mean value of (1.42 mg/kg).

Nitrite concentration in Basterma had highest value as 1.62mg/kg and lower value as 0.91mg/kg. All Basterma samples showed nitrite concentrations in the permissible range, with a mean value of (1.29 mg/kg).

Hot dog samples shows highest sodium nitrite concentration of 12.74mg/kg and lowest concentration of 1.01mg/kg. The concentrations of sodium nitrite in all hot dog samples were within the accepted range with a mean value of (4.5 mg/kg).

Frankfurters samples showed higher sodium nitrite content as 3.4mg/kg. The lowest concentration as 1.18mg/kg, and mean value as (2.29 mg/kg)



The mean concentrations for the six analyzed types of samples indicated considerable nitrite residue content in processed meats in Khartoum state (Fig 1).

## CONCLUSIONS

- . Most of the analyzed samples showed a presence of sodium nitrite residue.
- . From consumer's safety point of view, the presence of nitrite in food is a hazardous factor, regardless of its permissible range.

## REFERENCES

1. Benjamin N. and Collins J. (2003). Food Preservatives (2<sup>ed</sup>). Russell N. and Gould G (Eds.). New York, Kluwer Academic. Page 102.
2. Erich Lück and Gert-Wolfhard von Rymon Lipinski, (2002), "Foods, 3. Food Additives" in *Ullmann's Encyclopedia of Industrial Chemistry*, Wiley-VCH, Weinheim
3. Gary Anthony Sullivan, (2011). Naturally cured meats: Quality, safety, and chemistry, Iowa State University.
4. Grosse, Y., R. Baan, K. Straif, B. Secretan, F. El Ghissassi, V. Cogliano, (2006), on behalf of the WHO International Agency for Research on Cancer Monograph Working Group. Carcinogenicity of nitrate, nitrite, and cyanobacterial peptide toxins. Original Text. *Lancet Oncol.* **7**:628–629.
5. Honikel K, (2008). The use and control of nitrate and nitrite for the processing of meatproducts. *Meat Science.* **78**: 68-76.

6. Jeffrey J. Sindelar and Andrew L. Milkowski, (2011). Sodium Nitrite in Processed Meat and Poultry Meats: A Review of Curing and Examining the Risk/Benefit of Its Use.
7. Jeff J. Sindelar, Ph.D, (2012). Extension Meat Specialist. University of Wisconsin. Meat Laboratory.
8. Karl-Otto Honikel, (2008). The use and control of nitrate and nitrite for the processing. Federal Research Centre for Nutrition and Food, E.C. Baumann Strasse 20, 95326 Kulmbach, Germany
9. Lundberg, J. O., Weitzberg, E. & Gladwin, M. T, (2008). The nitrate-nitrite-nitric oxide pathway in physiology and therapeutics. *Nature Reviews Drug Discovery*, **7**, 156-167.
10. L.L Borchert and R.G. Cassens, (1998). Chemical hazard analysis for sodium nitrite in meat curing. University of Wisconsin.
11. Mauricio Redondo-Solano, (2011), Effect of Sodium Nitrite, Sodium Erythorbate and Organic Acid Salts on Germination and Outgrowth of *Clostridium perfringens* Spores in Ham during Abusive Cooling, University of Nebraska.
12. Montville T. and Matthews K, (2008). *Food Microbiology: An introduction* (2 ed). Washington D.C., ASM Press. Pages 221-222.
13. National Academy of Sciences, (1981), *The Health Effects of Nitrate, Nitrite and N-Nitroso Compounds*. Natl. Acad. Press, Washington, DC.
14. Richard J. Epley, Paulb. Addis and Joseph J. Warthesen (1992). *Nitrite in meat*, University of Minnesota
15. Roberts T. A. and Dainty R. H. 1996. *Nitrates and nitrites in Food and Water*. Hill M. J (Ed.). Cambridge, Woodhead Publishing. Pages 117-130.

16. Tompkin, R. B, (2005). Nitrite. In Davidson, P.M., Sofos, J.N. and Branen, A.L. (eds.), *Antimicrobials in Food*, CRC Press, Boca Raton, Florida, pp. 169-236.
17. Wentworth, E. N, (1956). Dried meat: Early man's travel ration. *Agricultural History*, **30**, 2-10.
18. World Cancer Research Fund (WCRF), (2007), *Food, Nutrition, Physical activity, and the Prevention of Cancer*, American Institute for Cancer Research, Washington  
(<http://preventcancer.aicr.org/site/PageServer?pagename=research>)
19. Wong, D. W. S. (1989). *Mechanisms and Theory in Food Chemistry*, Van Nostrand Reinhold, New York, New York, pp. 428.