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Effects of cooking methods on the nitrite level of different commercially available hotdogs

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Abstract: Hotdogs are processed meat popular in the Philippines particularly among children. This study aimed to investigate the effects of different cooking methods on the nitrite concentration of different commercially available hotdogs sold in the local market in Cogon, Cagayan de Oro City and Philippines. Three brands of commercially available hotdogs were used in this study. The study employed three cooking methods which are commonly used in cooking hotdog, namely; boiling, frying and pan grilling. Raw hotdog served as the control. Spectrophotometric method was used to determine the nitrite concentration of the different hotdogs cooked in different cooking methods. The nitrite concentrations (ppm) of raw, boiled, fried, and grilled hotdog for Brand A were as follows: 192, 181, 192, and 197; for Brand B, 82, 89, 74, and 98; for Brand C, 102, 113, 111, and 111, respectively. Results revealed that Brand A had the highest nitrite concentration among the three brands; nevertheless, the nitrite concentrations of the three brands were within the allowable limit set by the Philippine FDA. Moreover, the cooking process and the method of cooking did not significantly affect the nitrite concentration of the different hotdog brands. With the results of this study, it is recommended that other hotdog brands commercially available in the market be analyzed also to determine the nitrite level.

Key words: Hotdog; Nitrite concentration; Cooking methods; Allowable limit

1. Introduction

Hotdog consumption in the Philippines, particularly among children, is very much prevalent. The Phil Association of Meat Processors Inc. (PAMPI) reported that it has an estimated average industry growth of 20% in 2015 and projected a more than 20% growth on demand this year (Freeman News, 2016). Hotdogs fall under the processed cured meats category of food products. It is mainly made up of ground pork or beef and is highly appreciated because of its exceptional taste.

The addition of nitrates and nitrites stabilizes its flavor and gives the product its reddish color. The popularity of hotdog consumption in the Philippines can be attributed mainly to its very appealing red color as well as its good taste coupled with the very convincing advertisements by the hotdog companies in the mass media and availability of the product both in the rural and urban areas. This is manifested by the increasing number of fast-food establishments which are serving hotdogs in the country. Moreover, parents, particularly those who are working mothers, find it convenient to include hotdogs in meals especially breakfast, since very minimal cooking time is needed.

Cured meats like hotdogs have a characteristic color, unique taste and a longer shelf life over fresh meat products. Sodium nitrite is the compound that is responsible for the development of color and enhancement of taste in cured meat products. It possesses an antimicrobial property which primarily inhibits the growth and production of *Clostridium botulinum,* a potent anaerobic pathogen in packed meat products.

However, nitrites in processed meat are believed to form nitrosamines. With the rise of consumer demand for natural meat products due to health concerns of synthetic additives, the meat industry is focusing on the development of nitrite alternatives (Alahakoon, et al., 2015). Several studies have focused on alternatives to nitrite in meat preservation, one of these is the study conducted by Eskandari et al. (2013) which focused on nitrite free and low nitrite meat curing system using natural colorants. Results revealed that treatment with 0.015% cochineal, most closely resembled the 120 ppm $NaNO_2$ in producing cured-meat color. Butylated hydroxyanisole (BHA) was found to be a strong antioxidant at 30 ppm level in cooked sausages during storage. Reducing sodium nitrite to 40 ppm resulted in similar sensory attributes of frankfurter samples. Nonetheless, no single compound yet was found to perform the nitrite function in meat. Thus, nitrate and nitrite are still used as additives in the meat industry with controlled levels of application (Hospital et al., 2015).

Result of a national survey in the US reported that the nitrite/nitrate concentrations in cured meat

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products have remained low since the last national survey in 1997. This means that the USDA regulations and manufacturer's processing procedure are consistently controlling the levels of nitrite/nitrate in cured meat products and continue to be effective for minimizing their contribution to the dietary nitrite/nitrate load (Keeton et al., 2009).

Nitrosamines is a class of potent carcinogens found in cigarette smoke, which may explain why hotdog consumption has been associated with the two leading pediatric cancers-- brain tumors and childhood leukemia. Nitrosamine are powerful carcinogens and they may be mutagenic and teratogenic as well (Deman, 1999).

Carcinogenic nitrosamines are formed when amines that occur naturally in food react with sodium nitrite found in cured meat products.

$R_2NH + NaNO_2 \rightarrow R_2N\text{-}N=$ (amines) (sodium nitrite) nitrosamine)

Experimental animal studies have also shown that N-nitroso compounds (NOC) are potent carcinogens. However, epidemiological evidence of the carcinogenic potential of dietary NOC and precursor nitrates and nitrites in human remains inconclusive with regard to the risk of stomach, brain, esophageal, and nasopharyngeal cancers (Eichholzer and Gutzwiller, 1998). Recent epidemiological studies show a positive association between cancer incidence and high intake of processed meat. N-nitrosamines (NAs) in these products have been suggested as one potential causative factor (Herrmann et al., 2015).

Health risks associated with nitrites in foods begin when nitrates are converted to nitrites in the gut and saliva by bacterial nitrate reductase. The more processed meat consumers eat, the more they are at risk, and cancer may not be the only problem.

However nitrite can play a beneficial role in adult gastrointestinal and cardiovascular physiology. When nitrite is swallowed, some of it is converted to nitric oxide (NO) in the stomach and may then exert protective effects in the gastrointestinal tract and throughout the body (Kanady et al., 2012). A study conducted by Van Hecke et al. (2015), suggests that overcooking processed meat is likely to result in the formation of genotoxic compounds during digestion and should, therefore, be avoided.

Moreover, Li et al. (2012) found out that cooking by deep-frying or pan-frying significantly increase the concentrations of volatile N-nitrosamines, while boiling or microwave treatments, did not significantly differ from the raw. Boiling and microwave treatments decreased the total biogenic amines significantly ($P < 0.05$). Residual nitrite was significantly reduced by cooking treatments. The results suggest that boiling and microwave treatments were more suitable methods for cured meat. Moreover, Herrmann et al. (2015) reported that the levels of N-nitrosopiperidine (NPIP) increased after frying and baking of processed, while the levels of non-volatile nitrosamines, N-nitroso-thiazolidine-4-carboxylic acid (NTCA) and N-nitroso-2-methyl-thiazolidine4-carboxylic acid (NMTCA) decreased after frying and baking. Varying impacts were observed for N-nitrosoproline (NPRO), N-nitrosodimethylamine (NDMA), N-nitrosopyrollidine (NPYR), N-nitrosodimethyl-amine (NDEA) and N-nitrosodiethylamine (NDEA) and N-nitroso-methylaniline (NMA) depending on the type of product and/or the heat treatment. Hermann et al. (2014), found out that NDMA, NPYR, NMA, NPRO, NTCA, and NMTCA were found in one or several nitrite cured meat products, whereas none were detected in non-nitrite cured bacon.

Scientists also believe that nitrites are methemoglobinemia or "blue baby syndrome" stimulant. Nitrites when consumed in large quantity converts hemoglobin to methemoglobin which limits the red blood cells to carry oxygen. In view of the known carcinogenicity of N-nitrosocompounds, exposure to these compounds in food should be minimized by lowering the nitrite concentration in preserved foods to the minimum required to ensure microbiological safety and use of inhibitors of nitrosation like α-tocopherol or ascorbic acid (Walker, 2009).

This study focuses only in determining the nitrite concentration of the three different hotdog brands commonly found in wet markets in the Philippines, particularly in Cagayan de Oro City. Moreover, it also aims to determine whether cooking methods affect the nitrite concentration of different hotdog brands. The three commonly employed methods in cooking hotdogs are as follows; boiling, frying, and pan grilling.

2. Materials and methods

2.1. Sampling scheme

Three brands of commercially available hotdogs were used; Brands A, B, and C. One kilogram of each brand was obtained for the entire analysis in every sampling period. One-fourth kilo of each brand served as control and another one-fourth kilo for each cooking method-- boiling, frying, and grilling. Samples were bought from Cogon market, which is the nearest wet market from the University (approximately 5-minute ride). To ensure that samples were in good quality, expiration date was checked. The expiration of all the three samples was 2 months before the expiry date. Samples were then placed in an ice box and brought to the Food Science Laboratory. The samples were placed in a chiller prior to the sample preparation. There were three sampling periods for the whole duration of the study. Three trials were done per sampling period.

Fig. 1: Hotdog samples

2.2. Cooking methods

Three different commercially available hotdogs were used in the study and were cooked using three different cooking methods, namely, boiling, frying, and pan grilling. One kilogram of hotdog per brand was obtained from Cogon market, Cagayan de Oro City, Philippines. It was divided equally into four portions. One portion for each cooking method was allotted and the last portion served as the control. Boiling was done by adding 1 ½ cup of water to the hotdog in a frying pan and was done for 6 minutes. The hotdog was, then, drained and cooled. In another pan, frying was done by adding 1 cup of oil to another portion of hotdog for 6 minutes. The fried hotdog was also drained and cooled. The third portion of the hotdog was pan grilled for about 8-10 minutes. The same procedure was done for the other two brands of hotdogs. In all the cooking methods, moderate heat was employed and cooking was done simultaneously. The last portion served as the control. Both the raw and cooked hotdog samples were homogenized and extracted using saturated borax solution, potassium hexacyanoferrate (II), and zinc acetate. The figures below show the different cooking methods for hotdog.

Fig. 2: Frying method

Fig. 3: Boiling method

Fig. 4: Pan grilling method

2.3. Sample extraction

Both the raw and cooked hotdog samples were homogenized first by the use of mortar and pestle. Ten grams (10g) of the homogenized samples was placed in a 500 mL beaker. Five mL of saturated borax solution was then added to it, followed by 100 mL of hot water at 70^o C. The solutions were then heated on boiling water by the use of water bath for 15 minutes with constant stirring. The flask and its content were then cooled at room temperature. After cooling, 2 mL of potassium hexacyanoferrate was then added, followed by 2 ml of zinc acetate. The solution was mixed thoroughly after each addition. The contents were transferred to a 200 mL volumetric flask and were diluted with water up to its mark and then were mixed. The flask was allowed to stand for thirty minutes at room temperature. Afterwards, the supernatant liquid was carefully decanted and filtered through fluted filter paper to obtain clear extracted solution. The extracted solution was used for analysis of nitrite.

2.4. Preparation of standard nitrite solution

A 1.000 g of pure nitrite was dissolved in distilled water and diluted to 1L volumetric flask. One hundred mL of the stock solution was pipetted to a 1L volumetric flask and diluted to its mark. For the working solution, 10 mL of the intermediate solution was pipetted to a 1L volumetric flask and was diluted with distilled water up to its mark. Working solution was equivalent to 1 ppm.

2.5. Preparation of standard calibration curve

A 5 mL, 10 mL, 15 mL, and 20 mL of nitrite working solutions were pipetted to a 50 mL volumetric flask. Two point five (2.5) mL of sulphanilamide reagent was added and the solution was left in the dark for 5 minutes. Then 2.5 mL of NED reagent was added and left again in the dark for another 5 minutes. Portion of the solution was transferred to a photometer cell and its absorbance was measured at 538nm against blank of solution of 45ml distilled water, added with 2.5 mL sulphanilamide reagent and 2.5 mL of NED (naphltyl ethylenediamine) solution.

Table 1: Standard nitrite calibration curve

Volume of standard, mL	Absorbance	Concentration (mg/g)
Blank	0.000	0.0
5	0.079	0.1
10	0.147	0.2
15	0.232	0.3
20	0.297	0.4

Table 1 shows the different absorbance readings, as well as the corresponding nitrite concentration of the different volumes of standard (Fig. 5).

The concentration of nitrite in all sample solutions was determined through the use of equation of the line of this standard calibration curve.

2.5. Nitrite determination

Ten (10) mL of the extracted solution was pipetted into a 50 mL volumetric flask. Twenty (20) mL of distilled water was then added, followed by 5 mL sulphanilamide and 3 mL concentrated HCl. The solution was left in the dark for five minutes.

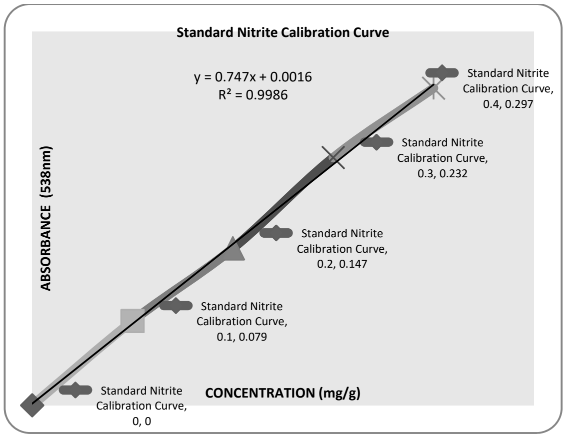

Fig. 5: Standard nitrite calibration curve

Then one mL of NED (naphltyl ethylenediamine) was added and left again in the dark for 15 minutes. Afterwards, it was diluted with water up to its mark. The absorbance was measured using HACH DR500 UV-Vis spectrophotometer at 538nm wavelength. Equation 1 is the formula in calculating the nitrite concentration based on the absorbance at 538nm.

$A=\varepsilon bc$; $c=A/b\varepsilon$

Where: A=Absorbance

ε=molar absorptivity (slope of the standard curve)

b=path length (1 cm)

c=concentration

Slope of the plot: $y=mx + b$

$y=0.747x+0.0016$

$C=0.107/(0.747 \times 1)+0.0016$

3. Analysis of data

The data obtained was analyzed using the two-way Analysis of Variance to determine whether there was a significant difference in the nitrite concentrations of the three different commercially available hotdogs cooked in different cooking methods. To further locate significant means if the F values are significant, the Duncan's New Multiple Range test was used.

4. Results and discussions

The determination of nitrite in hotdogs was done through the use of ultra-visible spectrophotometer. Samples were reacted with NED (naphltyl ethylenediamine hydrochloride) and Sulphanilamide to form a purple color in which the intensity of the color was proportional to its nitrite concentration. Absorbance of sample was read at 538 nm wavelength. The average nitrite concentrations of the different hotdog brands cooked in different cooking methods for three sampling periods are shown in Tables 2-4. Table 5 shows the result for the two way Analysis of Variance.

Table 2: Average nitrite concentration of brand A.

Cooking method	Absorbance	Concentration (ppm)
Raw	0.142	192
Boiling	0.134	181
Frying	0.140	189
Pan grilling	0.146	197

The above Table shows that the nitrite concentration Brand A hotdog did not exceed the allowable nitrite content for cured meat which is 200 ppm (Philippine National Standards, 2006). Among the cooking methods employed in cooking hotdog, pan grilled hotdog had the highest nitrite concentration, while boiled hotdog contained the least nitrite concentration.

Table 3: Average nitrite concentration of brand B.

Cooking method	Absorbance	Concentration (ppm)
Raw	0.060	82
Boiling	0.065	89
Frying	0.054	74
Pan grilling	0.072	98

Table 3 shows that the nitrite concentration of Brand B hotdog did not exceed also the allowable nitrite content for cured meat. The result also indicates that this brand had the lowest nitrite concentration among the brands. Moreover, pan grilled hotdog also had the highest nitrite concentration, while fried hotdog contained the least nitrite concentration.

Table 4: Average nitrite concentration of brand C.

Cooking method	Absorbance	Concentration (ppm)
Raw	0.075	102
Boiling	0.083	113
Frying	0.082	111
Pan grilling	0.082	111

The nitrite concentration of brand C hotdog is shown in Table 4. The result also indicates that the nitrite concentration did not exceed the allowable nitrite for cured meat. The result shows that this brand had lower nitrite concentration than brand A but a little higher than brand B. Boiled hotdog had the highest nitrite concentration, while raw hotdog contained the least nitrite concentration.

Table 5: F-values for the different brands and cooking methods

Source of variations	F-calculated	F-critical
Brands	228.9414	5.143253
Cooking methods	3. 058565	4.757063

Table 5 shows the result for the two-way analysis of variance. The calculated F value for the different brands is significantly higher than the tabular F value. This indicates that the nitrite content of the different brands significantly differed. However, the calculated F value for the different cooking methods is lower than the tabular F value. This indicates that cooking and cooking methods did not significantly affect the nitrite content of the hotdog,

Tables 2 to 4 show that brand A contained the highest nitrite concentration for both the raw and cooked hotdog, while brand B had the lowest nitrite concentration. The significant difference in the nitrite concentrations among the different brands of hotdogs is reflected in the result of the two-way analysis of variance. The calculated F value is much higher compared to the critical F value. Furthermore, Duncan's Multiple Range Test (DMRT) result shows that brand A hotdog differed significantly from brands B and C; while brand B did not differ significantly from brand C.

Brand A hotdog was classified as premium hotdog while brands B and C were classified as non-premium hotdogs since the percent meat component was lower than that of Brand A. Subsequently, the price per kilo of brand A was much higher compared to the prices of brands B and C. The addition of nitrates in cured meat like hotdogs was based on percent meat content of the product. This was probably the reason why brand A contained the highest nitrite concentration.

The Philippine FDA standard for nitrite concentration for cured meat should not exceed 200 ppm (Philippine National Standards, 2006). All the hotdog samples contained nitrite that is within the allowable limit set by the Philippine FDA at the time of sampling. However, it does not take into account possible reactions that may take place from the time of curing to storage until sampling and possible reactions from consumption to digestion. Results in the study conducted by De Mey et al. (2014) on the screening of the residual sodium nitrite and nitrate contents, biogenic amines and volatile N-nitrosamine concentrations of the dry fermented sausages revealed that the biogenic amine accumulation remained low at the end of shelf life. It was only in one product that the amount of biogenic amines reached intoxicating levels. Biogenic amines can function as precursors for the formation of carcinogenic N-nitrosamines when nitrite is present.

In this study, the different cooking methods did not significantly change the nitrite content of the three brands of hotdog after cooking. The result is shown in Tables 2-4 in which the nitrite concentration of the different hotdog brands, both raw and cooked, using the three cooking methods were similar. This was further confirmed by the calculated F-value which was lower than the critical F value, hence indicated a non-significant difference. This means that the original nitrite content of the raw hotdog was more or less the same in nitrite content in the cooked hotdog.

When nitrites are exposed to high heat, in the presence of amino acids, nitrosamines can be formed. In a study conducted by Li et al. (2012), on the influence of various cooking methods on the concentrations of volatile N-nitrosamines and biogenic amines in dry-cured sausages, it was found out that cooking by deep-frying and pan-frying resulted in products with the highest nitrosamine contents, while boiling and microwave treatments did not differ from the raw product. However, boiling and microwave treatments decreased the total biogenic amines significantly. Moreover, the study concluded that residual nitrite was significantly reduced by cooking treatments.

In this study, the possible reason why cooking methods did not significantly change the nitrite concentration of the hotdogs was because the cooking was done in moderate heat for a very short time. This is the usual method of cooking hotdogs in the Philippines.

5. Conclusion

Based on the findings of the study, the following are the conclusions (1) Premium hotdogs have higher nitrite content than non-premium hotdogs. (2) Hotdog manufacturing companies adhere to the Philippine FDA standard for nitrite content of hotdog. (3) Cooking does not significantly decrease nor increase the nitrite concentration of the different commercially available hotdogs. (4) The nitrite concentrations of the hotdog cooked in different cooking methods do not significantly differ from one another.

Based on the findings and insights of this study, a follow up study must be done to include other commercially available hotdogs to determine whether different cooking methods do not significantly increase or decrease the nitrite level of hotdogs. Other cured meat products commonly consumed must also be explored. Nitrosamine and other nitroso compounds can be also determined from hotdogs and other cured meat products. Moreover, a comparison can also be done among premium hotdogs and non-premium hotdogs.

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References

A. Alahakoon, D. Jayasena, S. Ramachandra and C. Jo. (2012). Alternative to nitrite in processed meat: up to date. Trends in food science and technology. Vol. 45(1) pp. 37-49.

A. Crujido, etl. (2010). Determination of nitrates in selected local spices. An unpublished undergraduate thesis. Mindanao University of Science and Technology. Lapasan, Cagayan de Oro City. Philippines.

American Meat Institute. (2009). Nitrites and nitrates offer cardiovascular health benefits. Regulatory update. Vol. 2, issue 13.

E. De Mey, K. De Klerck, H. De Maere, L. Dewulf, G. Derdelinckx, M. Peeters, I. Fraeye, Y. Vander Heyden, , and H. Paelinck (2014). The occurrence of N-nitrosamines, residual nitrite and biogenic amines in commercial dry fermented sausages and evaluation of their occasional relation. Meat Science 2014 vol: 96 (1) pp: 821-828

F. Bruning, and J. Kaneene (1993). The effect of nitrates, nitrites, and nitroso compounds on human health: a review. PMID: 8303822. http:// europepmc.org.

Food standards australia new zealand. Survey of nitrates and nitrites in food and beverages in Australia.Freeman News. January 19, 2016.

J. Sindelar (2006). Investigating uncured no-nitrate-or-nitrite-added processed meat products. A dissertation at Iiowa State University.

J. De Man (1999). Principles of Food Chemistry. 3rd ed. Aspen Publishers, Inc. Gaithersburg, Maryland.

J. Kanady, A. Aruni, J. Ninnis, A. Hopper, J. Blood, B. Byrd, L. Holley and M. Staker (2012). Nitrate reductase activity of bacteria in saliva of term and preterm infants. Nitric oxide: biology and chemistry/ official journal of the nitric oxide society. Vol 27 (4) pp. 193-200.

J. Keeton, W. Osburn, M. Hardin, and N. Bryan (2009). A national survey of the nitrite/nitrate concentrations in cured meat products and non meat foods available at retail-NPB #08-124. Research report. Research.pork.org

J. Santarelli, J. Vendeuvre, N. Naud, and S. Tache, (2010) Meat processing and colon carcinogenesis: cooked nitrite treated, and oxidized high-heme cured meat promote mucin-depleted foci in rats. American association for cancer research. Doi:10.1158/1940-6027. CARP-09-0160.

J. Sebranek and J. Bacus (2007) Cured meat products without direct addition of nitrate or nitrite what are the issues? 53rd International congress of meat science and technology. Vol.77 (1): 136147.http://sciencedirect.com.

L. Li, P. Wang and X. Xu, (2012). Influence of various cooking methods on the concentrations of Volatile N-Nitrosamines and Biogenic Amines in Dry-Cured Sausages. Journal of Food Science. Vol. 77(5).

M. Eichholzer, And F. Gutzwiller (1998). Dietary nitrates, nitrites, and N-nitro compounds and cancer risk: a review of the epidemiologic evidence.http://m.nutritionreviews.oxford journal. org.

M. Liu, J. Callahan, and M. Solomon (2009). Meat products, processed meat ingredients. Kirk-Othmer Encyclopedia of chemical technology. 1-5.

O. Fennema, 2008. Food Chemistry. 4th ed.New York: CRC Press Tailor and Francis group.

Philippine National Standards. (2006). Bureau cicular No. 2006-016. Updated List of food. Additives. http://www.fda.gov.ph.

R. Cammack, C. Joaanou, X-Y. Cui, C. Martninez, S. Maraj, and M Hughes (1998). Nitrite and nitrosylcompounds in food preservation. Biochimica et Biophysica Acta 1411 (1999) 475-488 . Elsevier.

R. Cassens (1997). Residual nitrite in curedmeat. Food technology, 51:53-55.

R. Walker (2009). Nitrates, nitrites, and nitroso-compounds. A review of the occurrence in food and diet and the toxicological implications. Food additives and contaminants. Vol 7. Issue 6. Pp. 717-768..

S. Herrmann, L. Duedahl-Olesen, and K. Granby(2015). Occurrence of volatile and non-volatile N- nitrosamines in processed meat products and the role of heat treatment. Food Control vol: 48 pp: 163-169. Elsevier Lt

S. Herrmann, L. Duedahl-Olesen, T. Christensen, P. Olesen, and K. Granby, (2015). Dietary exposure to volatile and non-volatile N-nitrosamines from processed meat products in Denmark. Food and Chemical Toxicology vol: 80 pp: 137-143. Pubmed.

T. Van Hecke, E. Vossen, L. Hemeryck, JV. Bussche, L. Vanhaecke, and S. De Smet (2015). Increased oxidative and nitrosative reactions during digestion could contribute to the association between well-done red meat consumption and colorectal cancer. Food Chemistry. vol: 187 pp: 29-36. DOI: 10.1016/j.foodchem.2015.04.029. Pubmed.

X. Hospital, J. Carballo, M. Fernandez, J. Arnau, M. Gratacas and E. Hierro (2015). Technological implications of reducing nitrate and nitrite levels in dry fermented sausages: typical microbiota, residual nitrate and nitrite and volatile profile.Food control vol. 57: 275-281, do i:10.1016/j.foodcont.2015.04.024