

**NITRITE CONTENT OF PROCESSED MEAT PURCHASED
IN PUBLIC MARKETS AND GROCERY STORES
AROUND ILOILO CITY**

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ABSTRACT

Nitrates and nitrites are used as preservatives, affects flavor and develop cured meat color. Too much nitrite from meat could produce nitrosamines which are related to spontaneous intrauterine growth restriction, abortions, birth defects and Methemoglobinemia or “blue baby syndrome”, and especially cancer. This study aimed to determine the concentration of nitrite in different processed meats purchased from markets and grocery stores around Iloilo City and to compare to the levels set by BFAD. Cured samples of hotdog, ham, longganiza, chorizo and tocino were purchased from different public markets and grocery stores around Iloilo City. The nitrite content of these cured meat products were analyzed using a UV-Vis spectrophotometer and the results were within the limits set by BFAD which is 416ppm for chorizo, longganiza and tocino; 134ppm for hotdogs and 168ppm for ham. The Acceptable Daily Intake (ADI) of 0.07 mg nitrite per kg body weight was set by JECFA-WHO/FAO. On the basis of a 65kg body weight, daily consumption must not exceed 42g, 117g, 170g, 262g, and 94g for chorizo, tocino, longganiza, ham and hotdog respectively. On the basis of a 25kg child, the hotdog consumption must not exceed 36g. It

is advised to eat cured meats in moderation to prevent the bad effects of nitrite consumption.

Keywords: nitrate, nitrite, nitrosamines, carcinogenic, processed meat, UV-Vis spectrophotometer, Acceptable Daily Intake (ADI)

INTRODUCTION

Background and Rationale

Meats are flesh taken from dead animals used as food. This product is perishable due to microorganisms like bacteria, yeasts, or molds which are present in the environment (Ray, 2017). Meat curing is the addition of salt, coloring ingredients, and seasoning in order to impart unique properties to the product. Nitrates and nitrites of either potassium or sodium are used to cure meat. They give a bright reddish or pink color which attribute for consumer acceptance (Cornforth and Jayasingh, 2004). More nitrites are required to prevent rapid fading and non-uniform curing while also maintaining its cured color throughout an extended shelf life (Sebranek and Bacus, 2007). In a series of normal reactions, nitrite is converted into nitric oxide which combines with myoglobin, the pigment responsible for the natural red color of uncured meat. They form nitric oxide myoglobin, which has a deep red color that changes to the characteristic bright pink normally associated with cured and smoked meat when heated during the smoking process. (Epley, Addis, and Warthesen, 1992). When nitrite is added to meat systems, it reacts with a number of chemical components such as protein (Cassens, 1997). Nitrites further affect flavor by acting as powerful antioxidants (Ray, 2017). Sodium nitrite prevents the growth of a microorganism known as *Clostridium botulinum*, the bacteria that causes botulism. Nitrites are considered to be more effective against gram- positive bacteria (Bauermann, 1979).

Nitrites must be used with caution during curing. Nitroso compounds have the potential to yield carcinogenic nitrosamines (Cassens, 1990, 1997). Nitrite is a toxic

inorganic contaminant that is hazardous to the health of humans and other organisms. High nitrite concentrations have been associated with methemoglobinemia or “blue baby syndrome” in infants (Titov and Petrenko, 2005), carcinogenic nitrosamine (Li, et. al, 2012), gastric cancer (Jakszyn and Gonzalez, 2006), spontaneous intrauterine growth restriction (Lyll et. al., 1996), abortions (Aschengrau, Zierler, and Cohen, 1989) and birth defects in the central nervous system (Brender et. al., 2004). The International Agency for Research on Cancer (IARC) found that eating 50 grams of processed meat every day will increase the risk of colorectal cancer by 18% and classified processed meat as carcinogen (IARC, 2015). Santarelli, Pierre and Corpet (2008) concluded that those eating processed meat have an increased risk of having colorectal cancer by 20-50% compared with non- processed meat eaters. Parnaud and Corpet (1997) showed a correlation between meat consumption and colorectal cancer incidence. According to UK Biobank, consuming processed meat may increase the risk of breast cancer (Anderson, et al. 2017). Larsson, Orsini and Wolk in 2006 concluded that increased consumption of processed meat can be associated with an increased risk of stomach cancer. Hu et al. (2011) found that processed meat was significantly related to the risk of the stomach, colon, rectum, pancreas, lung, prostate, testis, kidney and bladder, including cancer and leukemia. Zhu et al. in 2013 indicated that consumption of red meat may increase the risk of gastric cancer. Norat et al. in 2005 associated the intake of red and processed meat to colon cancer while inversely associated fish intake to colon cancer. Oba et al. in 2006 concluded that men with high consumption of processed meat may increase their risk of colon cancer compared to those with low processed meat consumption. A 14% colorectal cancer risk increase for every 100g/day

consumption of processed meat, 25% in colon cancer, and 31% in rectal cancer was analyzed by Chan et al. in 2011. English et al. in 2004 concluded that consumption of red and processed meat is associated with an increased risk of rectal cancer while consumption of chicken and fish has no risk increase. In an episode of *Salamat Dok* (2015), Dr. Earle Castillo of UP-PGH reiterated that consuming three hotdogs is like having the effects of smoking one pack of cigar. Demeyer et al. (2016) found that when heme protein is broken down in the gut, N-nitroso chemicals are formed. These chemicals have been found to damage the cells in the bowel lining, leading to bowel cancer. These N-nitroso chemicals also form when processed meat is digested. In addition, the nitrite and nitrate added as meat preservatives produce these same N-nitroso chemicals. Pure nitrite, if consumed at levels of 3-5 grams can be very dangerous and can cause death because it binds to oxygen in the body stronger than the hemoglobin-oxygen binding in the blood thus, not allowing oxygen to reach several important organs (Sindelar & Milkowski, 2011).

Due to the damage caused by nitrite to human health, the World Health Organization (WHO) recommends an acceptable daily intake of nitrite concentration in meat products at 200 ppm. The JECFA-WHO/FAO (2002) Acceptable Daily Intake is 0.07 mg nitrite per kg body weight. A 50 kilogram person should not consume more than 3.5 mg nitrite a day from foods containing nitrite. The Bureau of Food and Drugs under the Department of Health indicated that for tocino, beef tapa, longganisa and chorizo, the maximum allowable limit is 416 mg/kg of nitrite. For ham, the BFAD has approved an allowable daily limit of 168 mg/kg. Hotdog, corned beef, Vienna sausage, luncheon meat, beef and meat

loaf have a BFAD allowable daily limit of 134 mg/kg (DOH-BFAD, 2006).

Significance of the Study

This study will be beneficial to all consumers who are eating processed meats as they will be aware of the amount of nitrites present in these foods. They could adjust their consumption of processed foods based on this study. In the future, government agencies like BFAD may tap the University Research Center for other researches regarding processed meat products.

Objectives

This study determined the levels of nitrite in different processed meat purchased in markets and grocery stores around Iloilo City and compare them to the levels of nitrite set by DOH-BFAD for allowable consumption.

Hypothesis

1. There is no significant difference in the amount of nitrites from the processed meat samples purchased in public markets and grocery stores around Iloilo City.
2. The cured meat samples purchased in public markets and grocery stores around Iloilo City passed the limits set by DOH-BFAD.

Scope and Limitations

This study only measured the concentrations of nitrites in different brands of processed meat products purchased around Iloilo City. The samples were limited only to processed pork meats that are locally produced and commercially available in the markets and in grocery stores around Iloilo

City. Chorizo, ham, hotdog, longanisa and tocino were considered as samples. The concentration of nitrites in these cured meat samples were measured using a UV-Vis Spectrophotometer. This study did not include the common practices for the preparation and processing of cured meat as it is against research ethics.

MATERIALS AND METHODS

Collection of Samples

The cured pork meat samples were taken from different markets and grocery stores located within Iloilo City based on Figure 1. Sampling was done in the month of December 2018. One kilogram of cured pork meat was purchased for every kind of product in all markets and grocery stores included in the study.

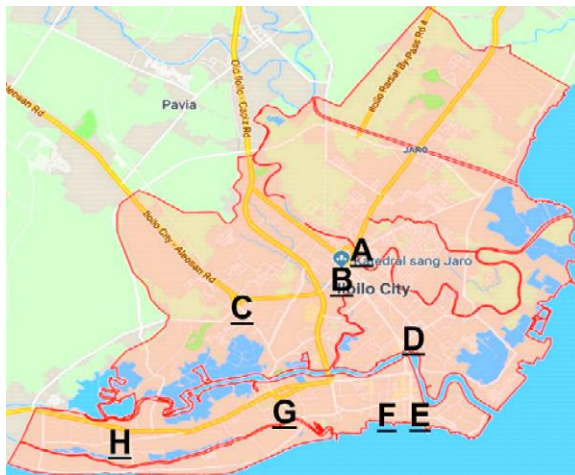


Figure 1. Iloilo City Map where cured samples were collected from different public markets.

(Retrieved from <https://www.google.com.ph/maps/search/iloilo+city+map+pdf/@10.7289411,122.5522316,13z>)

The samples that were taken from grocery stores and local public markets around Iloilo City were either branded or locally made. Locally made cured meat samples produced and packed locally within Iloilo City may have no product labels and can be considered as having less quality control. Processing may be done manually and are usually being sold using a weighing balance in the wet markets. Branded cured meat samples have proper labeling, are produced within the Philippines, and are packed in definite weights. Machines and instruments are used to process branded samples, and they undergo with strict quality control. Branded cured meat samples are usually sold in the grocery stores and some in the public markets. Cured samples with proper packaging and made within Iloilo City were considered as branded even though they were locally made. Chorizo, longganisa and tocino samples used in the experiment were either branded or

locally produced. Ham and hotdog samples used in the experiment were all branded as there are no locally produced products being made in Iloilo City.

Preparation of the Sample/Test Portion

A one kilogram sample was cut into smaller pieces and quartered. From the quartered sample, five grams of the homogeneous sample was used for deproteinization and color reaction. The remaining samples were kept in an air-tight, closed container under refrigeration for 4°C for storage.

Deproteinization

The test portion was quantitatively transferred into a 250-ml beaker and added with 5 ml of saturated borax solution and 100 ml of hot water. The beaker was heated for 15 min on a boiling water bath and shaken repeatedly. The beaker and its contents were allowed to cool to room temperature and added with 2 ml each of Carrez I and Carrez II solutions. It was mixed thoroughly after each addition and then filtered. The filtrates were placed into a 250 ml volumetric flask, diluted to the mark with distilled water, and mixed. The flask was allowed to stand for 30 minutes at room temperature (ISO 2918-1975, 2007).

Determination of nitrite

Principle

The nitrite in the sample was determined through formation of a reddish purple azo dye produced at pH 2.0 to 2.5 by coupling diazotized sulfanilic acid solution with Alpha-

naphthylamine. The method for nitrite analysis was based on ISO 2918-1975 (2007) Meat and meat products – Determination of nitrite content and on AOAC 973.31 Nitrite in Cured Meat – Colorimetric Method (2000).

Color Reaction and Instrumentation

Five milliliters portion of the filtrate was pipetted into a test tube. A 0.2 ml of Alpha-naphthylamine solution and 0.2 ml of Sulfanilic acid solution were then added. The solution was mixed and allowed to stand for 10 min at room temperature. The absorbance of the solution was measured in a 1 cm cell using a spectrophotometer set at a wavelength of 538 nm. The UV-Vis spectrophotometer was used to determine the concentration of nitrite in cured meat samples. It required standards with known nitrite content to establish the relation between the measured absorbance and the nitrite concentration which relied on the Beer-Lambert Law.

Analysis of Nitrite Using UV-Vis Spectrophotometer

The instrument was warmed up for 15 min for electrical current stability. The instrument parameters for the nitrite analysis were set. The wavelength was set at 538nm. Distilled water was added with 0.2ml of Alpha-naphthylamine solution and 0.2ml of Sulfanilic acid solution. This solution was used to auto zero the instrument and used as 0 ppm. The standard solutions were measured according to the increasing order of concentration. The instrument gave an absorbance result when a standard or a sample solution was introduced to it via a 1 cm cuvette cell. These absorbance results were recorded. Plotting the prepared concentration with its corresponding

absorbance produced a straight line. This line followed the line equation: $y=mx+b$ where y is the absorbance given by the instrument, m is the slope of the line, b is the line intercept and x is the unknown concentration. Rearranging this equation to determine the concentration of the unknown would have: $x=(y-b)/m$. Once a standard curve was established, the blank and the sample were measured. The nitrite content of the cured meat samples and allowable daily intake were determined using this formula:

$$\text{ppm NO}_2^- = \text{concentration obtained from line equation in } \mu\text{/ml} \times \frac{250 \text{ ml total volume}}{\text{weight of sample in grams}}$$

$$\text{Allowable daily intake of nitrite} = \frac{0.07\text{mg NO}_2^-}{\text{kg body weight}} \times \text{kg body weight} \times \frac{\text{kg food}}{\text{value of NO}_2^- \text{ in mg}}$$

Data Processing and Analysis

All analyses were carried out in triplicates. The mean, standard deviation and significances of each data were determined. The comparisons of nitrite concentration on different processed meat purchased in markets around Iloilo City were analyzed using One-Way ANOVA (LSD and Duncan’s Multiple Range Test) while the data comparison with the acceptable values by BFAD and WHO values were analyzed using t-test. All data analyses were done using Statistical Package for Social Science (SPSS) version 17.0.0 for Windows. Statistical tests were performed at 95% confidence level.

RESULTS AND DISCUSSION

The nitrite contents of chorizo, tocino and longganisa are shown in Table 1. Results showed that all of the chorizo, tocino and longganisa samples purchased on the local markets and on the grocery stores around Iloilo City were within the limits set by BFAD.

Chorizo is a Filipino sweet sausage, brought by the Spaniards and originated from Mexico. It is typically made with a highly seasoned ground pork. It is usually fermented, cured or smoked and wrapped in a casing of pig's intestines. In the Philippines, the chorizo is rounded and it contains spices that were mixed with the ground pork. The color of the product depends on the amount of spices placed inside and the cured meat. Based on the results obtained in Table 1, Brand K contained more nitrite compared to other chorizo brands while Brand B contained the least amount of nitrite. Eventhough Brand K is a branded product which underwent strict quality control, its ingredients indicated that it contained more nitrite salts than other chorizo products. It can be noted that only two locally made products have nitrite concentrations above ten ppm while all the branded samples have concentrations higher than 10 ppm. All of the chorizo products were within the safe limits imposed by BFAD which is 416 ppm.

Tocino means bacon in Spanish. It is made of pork fat, salt, vinegar, minced garlic, onion powder, soy sauce; sodium nitrite as preservative and food coloring as optional (Joven, 2011). Based on the result, Brand J had the highest nitrite content of 39.28ppm while Brand C had the lowest nitrite content of 3.96ppm. Compared to locally made tocino which can be bought in the markets around Iloilo City, Brand J added

more nitrite salts to their products compared to other tocino products. Tocino samples from the markets have very low nitrite content except for Brands C and D which have concentrations above 10ppm. Only one brand from the grocery store had a concentration of less than 10ppm while other brands were above 10ppm. All of the tocino samples purchased were within the limits of BFAD.

Longganisa is also a Filipino sweet sausage which originated from Spain. The difference between longganisa and chorizo is that longganisa is longer, made with minced meat and seasoned with paprika, cinnamon, aniseed, garlic and vinegar (Tan, 2016). Based on Table 1 results on longganisa, Brand N had the highest nitrite content at 26.83ppm while Brand E had the lowest nitrite content at 2.13ppm. Brand N has added more nitrite salts in its longganisa than other longganisa products that were sold in Iloilo City. The concentrations of nitrite in all markets and branded longganisa products were almost equal. From the result, all of the longganisa products purchased were within limits set by BFAD.

It can be noted that tocino, chorizo and longganisa branded samples contain more nitrite salts compared to their locally made counterparts. Adding more nitrite salts would increase the shelf life as they are sold around Philippines. Local products of tocino, longganisa and tocino samples have an average nitrite concentrations of less than 10ppm while their branded samples have an average nitrite concentrations of more than 10ppm.

Table 1. Nitrite Content in Chorizo, Longganisa and Tocino.

Market	Chorizo	Tocino	Longganisa
	NO ₂ ⁻ in mg/Kg	NO ₂ ⁻ in mg/Kg	NO ₂ ⁻ in mg/Kg
	Mean ± SD	Mean ± SD	Mean ± SD
Brand A	8.17 ± 0.25 ^c	3.99 ± 0.16 ^a	5.92 ± 0.04 ^h
Brand B	3.58 ± 0.13 ^a	3.96 ± 0.05 ^a	2.30 ± 0.08 ^b
Brand C	7.24 ± 0.12 ^b	16.01 ± 0.15 ^f	4.66 ± 0.03 ^f
Brand D	12.74 ± 0.09 ^f	12.48 ± 0.07 ^e	10.88 ± 0.02 ^k
Brand E	8.63 ± 0.22 ^d	5.93 ± 0.07 ^b	2.13 ± 0.06 ^a
Brand F	7.97 ± 0.21 ^c	6.56 ± 0.14 ^c	2.14 ± 0.06 ^a
Brand G	11.71 ± 0.29 ^e	7.56 ± 0.04 ^d	2.80 ± 0.03 ^c
Grocery Store			
Brand L	36.96 ± 0.12 ^j	24.81 ± 0.50 ^j	
Brand P	7.03 ± 0.25 ^h		
Brand N	26.80 ± 0.04 ⁱ	25.32 ± 0.19 ^j	26.83 ± 0.06 ^m
Brand Q	15.87 ± 0.17 ^g		
Brand K	108.83 ± 0.92 ^k	24.11 ± 0.080 ^j	
Brand H		7.99 ± 0.11 ^d	7.21 ± 0.06 ⁱ
Brand M		18.59 ± 0.22 ^g	4.01 ± 0.08 ^e
Brand I		24.79 ± 0.44 ^j	10.58 ± 0.04 ^j
Brand J		39.28 ± 0.53 ^k	17.93 ± 0.06 ^l
Brand R			4.84 ± 0.05 ^g
Brand AB			3.28 ± 0.07 ^d
Brand O		20.66 ± 0.32 ^h	
BFAD Allowable Limits	416	416	416

Note: Values are mean ± standard deviation. Values having different superscripts within a column are significantly different at $p < 0.05$. Lowest value are assigned with a superscript a.

Table 2 shows the nitrite contents of ham and hotdog. Results shows that all of the ham and hotdog samples purchased from the local markets and on the grocery stores around Iloilo City were within the limits set by BFAD.

Ham is made from a bone-in pork shoulder which was cured and added with brine for salting effect. From the results in Table 2, it shows that Brand J had a nitrite content at 17.35ppm which was highest among the other brands of ham

while Brand M had the lowest nitrite content at 1.84ppm. All of the ham brands passed the requirements and did not exceed the BFAD limits.

Hotdog in the Philippines is colored red while in other countries it is usually tan-colored. According to San Miguel Purefoods Company, they added food coloring to the casing. The bright red color adds a dose of fun and vibrancy for eating enjoyment, as well as helping distinguish Filipino hotdogs from the blander, more common franks and sausages. The ingredients of the Purefoods TJ hotdogs were separated turkey, pork, water, modified food starch, salt, sodium phosphates, spice, sodium erythorbate, flavoring, sodium nitrite, Oleoresin of Paprika and Red 40 as coloring (Ligaya, 2017). Other hotdog brands may have more or less the same ingredients. Based on Table 2, Brand V had the highest nitrite content among the different brands of hotdogs with a nitrite concentration of 48.58ppm while Brand Z had the lowest nitrite concentration of 7.16ppm. Hotdogs had color ranges of light red to slightly bright red in color. The tendency of nitrite accumulation in hotdogs is high based on their texture. Tocino and hams are made from a cut meat while chorizo and longganisa are made from the same grounded or minced pork meat. Hotdogs are made from different trimmings of pork meat which makes them porous in texture compared to other processed meat. The porosity of hotdogs could absorb more nitrite salts compared to other cured meat samples. All of the hotdog brands purchased did not reach the limits of nitrite content set by BFAD.

Ham and hotdog samples were all branded because those that were sold in the markets were all branded. No locally manufactured ham and hotdogs are sold within Iloilo

City. On the average, ham products contain less nitrite salts as compared to hotdogs. An average of more than 10ppm nitrite concentrations can be found in hotdogs.

Table 2. Nitrite Content in Ham and Hotdog.

Brand	Ham	Hotdog
	NO ₂ ⁻ in mg/Kg Mean ± SD	NO ₂ ⁻ in mg/Kg Mean ± SD
Brand L	4.97 ± 0.05 ^e	35.50 ± 0.07 ^f
Brand H	3.66 ± 0.05 ^b	8.87 ± 0.05 ^c
Brand N	12.43 ± 0.06 ^g	
Brand R	6.79 ± 0.03 ^f	
Brand I	13.47 ± 0.08 ^h	
Brand P	4.00 ± 0.04 ^c	
Brand S	4.64 ± 0.06 ^d	
Brand J	17.35 ± 0.08 ^j	
Brand T	14.80 ± 0.05 ⁱ	
Brand M	1.84 ± 0.08 ^a	
Brand U		11.53 ± 0.05 ^d
Brand V		48.58 ± 0.06 ^h
Brand W		29.58 ± 0.08 ^e
Brand X		48.28 ± 0.06 ^g
Brand Y		8.38 ± 0.07 ^b
Brand Z		7.16 ± 0.03 ^a
BFAD Allowable Limits	168	134

Note: Values are mean ± standard deviation. Values having different superscripts within a column are significantly different at p<0.05. Lowest value are assigned with a superscript a.

Based on the results, all of the cured meat samples passed the BFAD approved limits. It can be noted that consuming more than the recommended amount may have adverse effects on our body. Locally produced cured meat products contain less nitrite salts than branded ones as they are usually meant to be consumed within a week. More nitrite salts are added for branded products for the purpose of having a longer shelf life while being sold around the Philippines.

The comparison between the allowable daily intakes of cured meat on adult and on a child with specific weights are shown in Table 3. On the basis of a 65 kg adult, the daily consumption must not exceed 42g, 117g, 170g, 262g, and 94g for chorizo, tocino, longganiza, ham and hotdog, respectively. On the basis of a 25 kg child, the hotdog consumption must not exceed 36g, while for chorizo, tocino, longganisa and ham are 16g, 45g, 65g and 101g, respectively. Based on the table, the allowable daily intake of the different cured pork meat samples are proportional to the consumer's body weight. Comparing the values of Table 3, it is safe and recommended to eat less chorizo compared to other cured pork meat samples. The computation is based on the highest concentration of nitrite obtained on different cured pork meat samples.

Table 3. Allowable Daily Intake of Cured Pork Meat Sample Depending on Specific Body Weight.

	25 kg body weight	65kg body weight
Chorizo	16 g	42 g
Tocino	45 g	117 g
Longganisa	65 g	170 g
Ham	101 g	262 g
Hotdog	36 g	94 g

CONCLUSION AND RECOMMENDATION

Based on the study conducted, it can be concluded that all of the processed meat purchased locally around Iloilo City had lower nitrite content and were within the limits set by BFAD. Branded samples of chorizo, longganisa and tocino

contain an average higher amount of nitrite compared to locally made products. There were significant differences among groups of processed meats in terms of nitrite content and the BFAD allowable limits.

The allowable daily intake of nitrite from cured pork meat samples depends on the person's body weight. Chorizo must be consumed in moderation and awareness as it has the highest level of nitrite content compared to other cured pork meat samples.

It is recommended to notify the Regional Office of BFAD about this study that the processed meat purchased in public markets and grocery stores around Iloilo City are within the limits set by their department. Since the Regional Office of BFAD does not monitor nor test any processed food which may contain nitrite, it can be recommended that the University Research Center may be tapped by BFAD and have a memorandum of agreement that we will be the one to analyze the nitrite content of any processed meat submitted to them.

The nitrite content of these processed meats should not be the only basis for food safety, but it is recommended that other parameters like percent fat, calorie content, and among other tests be made for awareness and food safety. It is advised to eat cured meats in moderation to prevent the bad effects of nitrite consumption. It is recommended that any food intake must be done in moderation.

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To GOD be the glory!