Analysis of nitrite and nitrate in the corned beef and smoked beef by Using Visible Spectrophotometry method

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Abstract. Nitrite and nitrate are food additives that can be used as preservatives permitted with the maximum limit in foods, because nitrite can react with alkylamines to form carcinogenic nitrosamines. To measure nitrite and nitrate contents in corned and smoked beef, the samples purposively collected four corned beef products and three smoked beef products. Determination of nitrite was carried out by using visible spectrophotometry method N-(1-naphthyl) ethylene diamin dihydrochloride (NED) in acetic acid solution as color reagent and measured at maximum wavelength of 536 nm. Nitrate was reduced into nitrite by Zincum (Zn) powder in acidic condition and then analyzed as nitrite, then converted into nitrate. The results showed that nitrite and nitrate in samples found were to be lower than maximum permitted levels. Nitrite and nitrate levels in corned beef samples were greater than smoked beef samples. Nitrite content obtained in the range of 13.95-34.40 μg/g in corned beef, and 6.63-17.19 μg/g in smoked beef. While the nitrate content was found in the range of 1.69-32.53 μg/g in corned beef and 4.73-6.37 μg/g in smoked beef. Based on the results from the present research, the levels of nitrite and nitrate in corned beef and smoked beef was lower than maximum limit permitted by regulation based on Permenkes No. 722/Menkes/IX/1988.

Keywords: corned, beef, nitrite, nitrate, visible spectrophotometry

1.   Introduction

Food additives are ingredients added and mixed during food processing to get better product. Food additives usually used as coloring, flavor and aroma flavoring agent, stabilizers, antioxidants, preservatives, emulsifiers, thickener, etc [1]. Preservatives are used to prevent or to inhibit spoilage or other decomposition of food that is caused by microorganisms [2,3]. Nitrite and nitrate are used as preservative in the form of potassium and sodium salt, it can inhibit microbial growth in processed meat and fish products. Both are also often used to maintain the color of the meat to keep it looks like fresh[3]. As a preservative, nitrite can inhibit the growth of bacteria, especially pathogenic bacteria Clostridium botulinum [4].

The use of nitrite in foods needs to be limited because nitrate may be converted in to nitrite by bacteria then nitrite can react with primary and secondary amines, in order to form carcinogenic nitrosamines [5]. Nitrosamines are formed through chemical reactions between nitrosation agents and alkylamines which are easily nitrosated. In general, amine components that may generate nitrosamines are secondary and tertiary amines [4].

Based on Permenkes No. 722/Menkes/IX/1988, the maximum limit of nitrite is 125 μg/g (processed meat and preserved meat) and 50 μg/g (canned corned beef), while the maximum limit of nitrate is 500 μg/g. Based on the research findings conducted by Govari and Pexara on sausages and beef burgers still meet the requirements, while on corned beef contained nitrite exceeds the permitted maximum level [6].

The aim of this study was to determine the levels of nitrite and nitrate in corned beef and smoked beef by using visible spectrophotometry method. The visible spectrophotometry method used was based on the diazotation reaction of nitrite with aromatic primary amine compound is coupled with N-(1-Naphthyl) and ethylene diamin dihydrochloride (NED). Nitrate is reduced to nitrite and then determined as nitrite [7].

2.   Materials and methods

The research method was a descriptive study to measure the levels of nitrite and nitrate in corned and smoked beef. Sampling purposively collected, namely sampling chosen that the samples analyzed representative of samples in the markets. The samples used in this study were four corned beef products and three smoked beef products from Brastagi Supermarket and Lotte Mart in Medan, Indonesia.

The tools used in this study were a UV-VIS spectrophotometer (Shimadzu UV mini 1240), analytical balance (Mettler Toledo), water bath (Griffin), cuvette, filter paper, rubber ball, spatula, thermometer, mortar, and pestle and necessary ware glass. Chemicals used in this study were E-Merck of pro-analysis grade, namely sodium nitrite, sulfanilic acid, N-(1-naphthyl) ethylenediamine dihydrochloride, hydrochloric acid, concentrated sulfuric acid, glacial acetic acid and non- pro-analysis grade, namely distilled water and Zincum (Zn) powder, ferrous sulfate,concentrated sulfuric acid.

The reagents used in this study were 15% acetate (v/v), NED solution, and sulfanilic acid solution. Solution of acetic acid 15% (v/v) was prepared by diluting 75 ml of glacial acetic acid withdistilled water in a 500 mL volumetric flask. The NED solution was prepared by dissolving 0.350 g N-(1-naphthyl) ethylenediamine dihydrochloride in 250 mL of 15% (v/v) acetic acid. Filtered with filter paper and stored in a brown bottle. Sulfanilic acid solution was prepared by dissolving0.850 g of sulfanilic acid in 250 mL of acetic acid 15% (v/v). Filtered with filter paper and filtrate was stored in abrown bottle[8].

As much as 100 mg of sodium nitrite was transferred into a 100 ml volumetric flask and dissolved in distilled water, then added distilled water to volume (C = 1 mg/mL). One (1) ml from this solution was transferred into 100 mL volumetric flaks dissolved in distilled water and made to volume with distilled water until the mark line (C =10 μg/mL).

Ten (10) g sample of each product was grounded and then placed in beaker glass, and added distilled water, heated on water bath and shaken for a while, then cooled and filtered. The filtrate was collected and transferred into a test tube to identify nitrite and nitrate in samples. Nitrite was tested by addition of sulfanilic acid and NED solution, and allowed to stand for a while. The presence of nitrite was indicated by the appearance of violet color [9]. Identification of nitrate was done by addition of several drops ferrous sulfate solution and then added few drops of concentrated sulfuric acid slowly through the inner wall of the test tube. The formation of brown ring indicated the presence of nitrate[10].

Four (4) mL of nitrite standard solution (10.0 μg/mL) was transferred into 50 mL volumetric flask, then 2.5 mL sulfanilic acid solution was added and shaken, then allowed to stand for 5 minutes. Furthermore, 2.5 mL NED reagent was added and then made to volume with distilled water and

homogenized by shaking (0.8 μg/mL). Absorbance of prepared standard solution was measured at wavelength of 400-800 nm. Then, absorbance curve was made by plotting absorbance versus wave length. This absorbance curve used to determine wave length of maximum absorbance. Maximum absorbance was found at wavelength of 536 nm, and then used in the analysis procedure [11].

Four (4) mL of standard solution of nitrite (10.0 μg/mL) was placed into 50 mL volumetric flask, to which 2.5 mL of sulfanilic acid added and homogenized and allowed to stand for 5 minutes, then 2.5 mL NED was added and made to volume of 50 mL by adding distilled water and homogenized. Absorbance was determined at maximum absorbance wave-length obtained from absorbance curve (536 nm), and stability of absorbance was determined by measuring absorbance at every minute for 1 hour. The absorbance was shown to be relatively unchanged within minute 8 to 12, and used for analysis [11].

Different volume (1;1.5; 2;2.5;3 mL) of nitrite standard solution of (10.0 μg/mL) of was put into separated 25 mL volumetric flasks, then 2.5 mL sulfanilic acid solution was added and homogenized by stirring. After 5 minutes, 2.5 mL NED solution was added, and finally distilled water was added to volume of 25 mL and homogenized. These standard solutions with the series of concentration were of 0.4 μg/mL; 0.6 μg/mL; 0.8 μg/mLl; 1.0 μg/mL; 1.2 μg/mL. Absorbance of this solution was measured at wave-length of 536 nm in minute 8 to 12. Absorbance versus concentration of each solution was plotted to form calibration curve, then linearity of regression equation ($Y = aX + b$) and correlation coefficient determined. From the values obtained the regression equation was $Y = 0.569965X + 0.007057$, and correlation coefficient (r) was 0.9997[11].

Ten (10) g of each mashed sample was put in a 250 mL beaker glass. Then hot distilled water was added ($\pm$ 80°C) to a volume of 150 mL. Mixed and homogenized it with a stirring rod and heated in a waterbath for 2hours, thenmixed it again. Then, it was cooled down to room temperature and transferred quantitatively into a250 mL volumetricflask. Distilled water was added to the mark line, homogenized and filtered, and thefirst filtrate as much as 10 mL was discarded, and the next filtrate was collected. The next filtrate of 10 mL transferred into a 50 mL volumetric flask,then added 2.5 mL of sulfanilic acid reagent and shaken. After 5 minutes, added 2.5 mL of N-(1-naphthyl) ethylenedihydrocloride (NED) reagent and finally distilled water was added until the mark line andhomogenized. Absorbance was measured in the minute 10 at the wavelength of 536 nm. Nitrite levels in the sample was calculated by using the regression equation ;$Y = 0.569965X + 0.007057$.
Note:Y = absorbance, X=concentration
The equation to calculate nitrite level:

$$C = \frac{X \, x \, V \, x \, Fp}{sample \; weigt \; (g)} \qquad (1)$$

Note:
C = Nitrite content in the sample (μg/g)
X = Nitrite content in the sample solution after dilution calculated from regression equation
V = Volume of sample (mL)
DF = Dilution factor

Each sample of 10 g mashed was placed into a 250 mL beaker glass. Then hot distilled water was added ($\pm$ 80°C) to a volume of 150 mL. Mixed it to homogeneous with a stirring rod and heated in a water bath for 2 hours. After that, it was cooled down to room temperature and transferred quantitatively into a250 mL volumetricflask. Distilled water was added to the mark line, homogenized and filtered, and thefirst filtrate as much as 10 mL was discarded. Then 10 mL of next filtrate was pipettedinto a 50 mL volumetric flask, and thennitrate was reduced into nitrite by adding 1 g zincum (Zn) powder, and allowed to stand for 10 minutes, then analyzed as nitrite as above procedure. Nitrite concentration from reduction of nitrate into nitrite was calculated:

$$A = B - C \qquad (2)$$

Notes:
A = Nitrite concentration from reduction of nitrate into nitrite

B = Total nitrite content
C = Initial nitrite concentration in samples
Nitrate content was converted into nitrate;

$$D = A \times \frac{MW\ nitrate}{MW\ nitrite}$$ (3)

Notes:
A = Nitrite concentration from reduction of nitrate into nitrite
D = Concentration of nitrate
MW= molecular weight

3. Results and Discussion

3.1. Identification of Nitriteand Nitrate in the Samples
The results of qualitative test for nitrite using sulfanilic acid and N- (1-naphthyl) ethylenediamine dihydrochloride (NED) reagent, and brown ring formation reaction for nitrate is presented in Table 1.

Table 1. Nitrite and nitrate identification in the samples

Group.	Sample		**Nitrite** [Sulfanilic acid and N- (1-naphthyl) ethylenediamine dihydrochloride (NED)]	**Nitrate** [Ferrous sulfate and sulfuric acid]
1.	Corned Beef	Pronas®	Purple – red	Brown ring
		Cip®	Purple – red - strong	Brown ring
		Ajib®	Purple – red	Brown ring
		Baliko®	Purple – red – weak	Brown ring
2.	Smoked Beef	Kimbo®	Purple – red – weak	Brown ring
		Bernard®	Purple – red	Brown ring
		Farmhouse®	Purple – red - weak	Brown ring

Based on the Table 1, it can be seen that when the color intensity produced was be brighter in the qualitative test will be correlated with the nitrite content. These results indicate that all samples (corned beef and smoked beef) found to contain nitrite and nitrate used and added as preservatives in the processed meat to prevent the growth of bacteria especially clostridium botulinum and also to polong storage time and to keep the red colour.

3.2. Nitrite and Nitrate Levels in Corned Beef and Smoked Beef Samples
Nitrite and nitrate levels in corned and smoked beefsamples can be seen in Table 2.

Table 2. Nitrite and Nitrate Levels in Corned Beef and Smoked Beef

Group.	Sample		Nitrite (µg/g) (n=3)	Nitrate (µg/g)
1.	Corned beef	Pronas®	16.6051 ± 0.1336	10.0344 ± 0.2757
		Cip®	34.4024 ± 0.3216	32.5343 ± 0.2166
		Ajib®	15.9665 ± 0.0962	4.6456 ± 0.2438
		Baliko®	13.9531 ± 0.0284	1.6856 ± 0.0634
2.	Smokedb eef	Kimbo®	14.0588 ± 1.3198	4.7297 ± 1.4191
		Bernard®	17.1886 ± 0.2285	4.9753 ± 0.2918
		Farmhouse®	6.6328 ± 0.0706	6.3709 ± 0.2088

As shown in the Table 2, it can be seen that in corned beef samples, Baliko contains the lowest

levels of nitrite and nitrate, while Cip® contains the highest levels of nitrite and nitrate. In smoked beefsamples, the lowest levels of nitrite and nitrate were found in Farmhouse and Kimbo, while the highestlevels of nitrite and nitrate were in Bernard and Farmhouse. Nitrite functions as a preservative andto retain the meat withbrighter color. As a preservative, nitrite used to inhibit the growth of several bacteria, but especially pathogenic bacteria Clostridium botulinum [15]. In all corned beef samples and smoked beef has a different brightness leveldue to the differences in the formulation of each product produced by different manufacturers (factories).

From the pressent study, nitrate was found, although in all the labels of corned beef and smoked beef samples were not mentioned the addition of nitrate. But, nitrate can be formed from the result of oxidation of nitrite to nitrate or nitrate from raw materials during the production process of corned beef and smoked beef [13]. Nitrite and nitrate salts areusually used in meat curing to obtain good colors and to prevent microbial growth. In meat, nitrite will form nitroxide which with flesh pigment will form bright red nitrosomyoglobin [14].

The results of previous research conducted by [6] nitrite in sausages and beef burgers still meetthe requirements, while the research conducted on corned beef contained nitrite level exceeded the maximum limit of 70.34 - 109.75 μg/g. Based on Permenkes No. 722/Menkes/IX/1988, the maximum limit for using nitrite is 125 μg/g (processed meat and preserved meat) and 50 μg/g (canned corned beef), while the use of nitrate in processed meat and preserved meat has a maximum limit of 500 μg /g[12]. From the present results, the data show that all of samples analyzed contain nitrite and nitrate at levels are lower than permitted maximum limitmentioned in the applicable regulations.

4. Conclusions

Based on the results of the present study, it can be concluded that nitrite and nitrate were found in the samples of corned beef and smoked beef. Nitrite levels found in the samples were in the range of 13.95-34.40 μg / g (corned beef) and 6.63-17.19 μg / g (smoked beef). While the nitrate content found in the sample was in the range of 1.69-32.53 μg / g (corned beef) and 4.73-6.37 μg / g (beef smoke). Thus, the levels of nitrite and nitrate obtained were safe because the level still lower than the maximum limit based on Permenkes No. 722 / Menkes / IX / 1988.

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