DETERMINATION OF NITRITE IN PROCESSED MEAT

Abstract

A meat sample will be analyzed to determine its nitrite, NO₂⁻, content using a colorimetric procedure. Nitrite reacts with NEDA-Sulfa in acidic solutions to produce a red colored solution. Comparing the color intensity produced by known concentrations of nitrite with the color intensity produced by the nitrite in a processed meat sample, the amount of nitrite in the meat can be determined.

Introduction

Sodium nitrate, NaNO₃, and sodium nitrite, NaNO₂, are commonly added to meat products which are kept for an extended period in a cold, but not frozen state (e.g. bologna, hot dogs, salami, ham, sausage, etc). These additives have two purposes. One is "cosmetic", they give the meat a pinkish-reddish color and prevent it from turning brown. The other is that they prevent the growth of clostridium botulinum, the microorganism that produces the deadly botulism toxin.

The addition of nitrates and nitrites to foodstuffs are a mixed blessing. It has been found that feeding sizeable amounts of sodium nitrite to animals results in formation of compounds called *nitrosamines* which are suspected of being carcinogenic (i.e. cancer causing). Additionally, large amounts of nitrate or nitrite in the diet may induce the disorder known as methemoglobinemia, which can be fatal. In methemoglobinemia the ferrous ion, Fe^{2+} , in hemoglobin (red) is oxidized by nitrite to the ferric ion, Fe^{3+} , converting the hemoglobin to methemoglobin (brown) which does not transport oxygen as efficiently. The effects caused by nitrites and nitrates has lead the U.S. Public Health Service to specify that public water supplies shall not contain more than 45 ppm (parts per million) of nitrate from fertilizer run-off. Vermont state health authorities are more stringent and recommend that drinking water contain no more than 3 ppm nitrate, especially if it is to be used for the preparation of infant formulas. The limitation on sodium nitrite in meat is 200 ppm.

The reaction of nitrite with protein to produce a red/pink color is interesting. Muscle (meat) contains a red pigment, myoglobin, that is similar in many respects to hemoglobin in that it contains Fe^{2+} , and transports oxygen. Myoglobin and oxygen combine to form oxymyoglobin which reacts with nitrite in the presence of a reducing agent such as Vitamin C to yield nitrosomyoglobin, a stable red pigment. In the absence of this protection the Fe^{2+} in red myoglobin is converted to the brown methemoglobin. Nitrate effectively produces the same red color because it is reduced to nitrite in the tissues.

In order to produce the red color in meat, 10-20 times more nitrite is needed than is required for preservative effects. Thus, the quantity of these compounds in meats can be drastically reduced with only the loss of the "cosmetic" effects. Salami, hot dogs, ham, bologna, etc. might be slightly brown, but they would be safer.

The potential danger of nitrates and nitrites has lead to the development of a number of analytical methods that measure their concentrations in foodstuffs. In this experiment a colorimetric method will be used to determine the amount of nitrite in

processed meat. Since sodium nitrite is soluble in water it is easy to extract it from meat with hot water. After extraction, the nitrite is reacted with two reagents, sulfanilamide (sulfa) and naphthylethylenediamine (NEDA). These compounds react with nitrite to produce purple colored dye. The amount of dye formed is dependent on the concentration of nitrite ion present. The more intense the color of the solution the greater the quantity of nitrite present.

 $C_{12}H_{14}N_{2} + C_{6}H_{8}O_{2}N_{2}S + NO_{2}^{-} + H^{+} \longrightarrow C_{18}H_{19}O_{2}N_{5}S + 2H_{2}O$ NEDA sulfa nitrite acid purple color

Experimental

- 1. Weigh out about 1 g of meat in a large test tube.
- 2. Pipet 1.0 mL of water into the test tube and macerate the meat thoroughly using a glass stirring rod. Be sure to hold the test tube in paper towels in case it breaks in your hand while you mash up the meat.
- 3. Prepare a water bath by filling a 600 mL beaker half full with tap water and heat it to boiling on a hot plate.
- 4. Pipet 26.0 mL of **distilled** water into the test tube containing the meat and mix well. Then place the test tube in the boiling water for 20 min. Occasionally stir the mixture. While the mixture is heating, proceed to analysis of the standard nitrite solutions.

5.

⇒ This next part of the experiment requires the use of a spectrophotometer to determine the absorbance of the known samples. Four nitrite standards are provided: 0.3×10^{-3} , 0.6×10^{-3} , 1.2×10^{-3} , and 1.8×10^{-3} mg/mL of NaNO₂. Your instructor will have allowed them to react with the NEDA-sulfa reagent and transferred them to their corresponding cuvettes. The spectrophotometer will be set to 530 nm and the absorbance adjusted to zero using a NEDA-sulfa solution as a "blank". Two readings will be obtained for each standard, zeroing the instrument between each reading, and recorded on your data sheet.

- 6. After the meat extract has been heated for about 20 min, remove the test tube from the boiling water bath and cool it in a beaker of ice water for 10 min with occasional stirring. Separate the solid material from the mixture by gravity filtration. Keep the liquid portion.
- 7. Pipet 2.00 mL of the filtrate (liquid portion) into a clean 13 x 100 mm test tube and then pipet 4.00 mL of NEDA-sulfa reagent into the test tube. Cover the test tube with parafilm and gently shake to mix.
- 8. Prepare a new "blank" solution by pipetting 2.00 mL of the meat filtrate and 4.00 mL of water into a cuvette and gently shaking.
- 9. Obtain two absorbance readings of the "blank" meat solution at 530 nm.

10. Obtain two absorbance readings of the meat sample at 530 nm.

 \Rightarrow Dispose of all NEDA containing waste in the waste bottles (in the fume hood), not down the drain.

Calculations

The absorbance readings obtained are directly related to the intensity of the color, which in turn is directly related to the concentration of nitrite present. The absorbance of the standard solutions provide a direct measure of the amount of nitrite present in the **known solutions**. By comparing the absorbance of the meat solution to the absorbances of the known concentrations, the amount of nitrite in the meat solution may be estimated.

- 1. Using graph paper, plot a working curve of absorbance versus concentration. The data obtained from the known solutions is used for this plot. Average the absorbance readings obtained for each of the known concentrations. After plotting the points, draw a trendline ("line of best fit") through the points on the graph. If done correctly, your plot should produce a straight line that passes through the origin.
- 2. Use this graph and the absorbance of the meat solution to estimate the amount of nitrite present in the meat solution.
- 3. When determining the amount of nitrite in the meat sample, remember the nitrite from the meat was extracted in 26 mL of water. To obtain the total amount of nitrite extracted from the meat, multiply the value you read from the graph by 26.

#g NaNO₂ = 26 mL (#mg NaNO₂ / mL) x (1 x 10⁻³ g / mg)

% NaNO₂ = (# g NaNO₂ / # g meat sample) x 100

Experimental Observations & Data

A. Absorbance of Nitrite Standard Solutions

mg/mL NaNO ₂		0.3×10^{-3}	$0.6 \ge 10^{-3}$	1.2 x 10 ⁻³	1.8 x 10 ⁻³
Vol. of Std Soln (mL)		2.0	2.0	2.0	2.0
Vol. NEDA-sulfa rgt (mL)		4.0	4.0	4.0	4.0
Absorbance (530 nm)	Trial 1				
	Trial 2				
Average Abso	rbance				

 \Rightarrow Using the absorbance values obtained from the standard solutions, make a plot of absorbance versus concentration of NaNO₂. Use graph paper provided for the plot.

B. Nitrite in Meat

1. Mass of test tube		g		
2. Mass of meat & test tube	g			
3. Mass of Meat		g		
4. Volume of water added to meat mL				
5. Absorbance of meat sample	e			
	Trial 1		Trial 2	
Absorbance (530 nm)				
Average Absorbance				
NaNO ₂ per mL of extract (from plot)		mg/mL	,	

Calculations (show work)

A. mass of NaNO₂ in meat sample: _____ g

B. Percent (%) of NaNO₂ in the meat sample: ______%

<u>Questions</u>

1. Use your plot of absorbance versus concentration of NaNO₂ to predict the absorbance that would be observed from mixing 2.0 mL of a solution containing 1.4×10^{-3} mg/mL of NaNO₂ with 4.0 mL of NEDA-sulfa reagent.

2. Why are different "blank" solutions used to zero the spectrophotometer when measuring the absorbance of the standard solutions and when measuring the absorbance of the meat extract?

3. Briefly explain how each of the following errors would affect the accuracy of the experimental data collected. State whether, or not, the error would result in an artificially inflated mass of $NaNO_2$ in the meat sample.

- a. The meat was not completely ground up when it was mixed with the water prior to the extraction step.
- b. The standard sodium nitrite solutions did not stand long enough for complete color development to take place, but the meat did stand long enough.

c. The experimenter got a large, greasy finger print on the cuvette containing the meat extract and NEDA, and did not wipe it off prior to inserting it into the spectrophotometer.

BONUS: Suppose instead of adding 2.0 mL of meat extract to the spectrophotometer tubes containing your sample and "blank" you actually added only 1.0 mL. From the absorbance reading could you calculate the amount of NaNO₂ in the meat? Explain briefly how you would accomplish this. Assume that for your standard solutions you followed the correct procedure, using 2.0 mL. (Hint: be sure to remember that the total volume in the cuvette will be different for the meat sample and the standards).