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Spectrophotometric determination of nitrite in curing meat samples basing on the nitrosation reaction

Chinar M. Rished, Nabil A. Fakhre
(Dept. of Chemistry, College of Science Education, Univ. of Salahaddine, Erbil, Iraq)

تعيين النتريت في نماذج اللحوم بواسطة تفاعل النترزة

جنار .م. رشيد ، نبيل عادل فخري

الخلاصة

لقد تم دراسة طريقة مطيافية ضوئية جديدة لتقدير النتريت. تعتمد الطريقة على تفاعل النترتة لأيون النتريت مع التربتوفان في وسط حامض الكبريتيك. حيث يتكون مركب اصفر اللون ذي أعلى امتصاصية عند الطول الموجي 440 نانوميتر. ويظهر منحني المعايرة بأنه يتم تطبيق بير ضمن مدى تركيز 0.5- 0.3 مايكروغرام/مل من ايون النتريت وبحد كشف 0.03 مايكروغرام/مل ومعامل امتصاص مولاري $1.3887*10^4$ لتر/مول.سم ومعامل ساندل 0.00331 مايكروغرام/سم^2. وتم دراسة الظروف المثلى والمتداخلات المؤثرة. وقد وجد بأن النتائج التي تم الحصول عليها لتقدير النتريت في عينات اللحم تتطابق مع تلك التي تم الحصول عليها بطريقة نفثيل اثلين ثنائي امين القياسية.

Abstract

A new Spectrophotometric method for the determination of nitrite ion was studied. The method is based on the nitrosation reaction of nitrite ion with tryptophan in sulphuric acid medium. During it's reaction with nitrite, an intensively yellow-coloured substance is formed, having maximum absorption at 440nm. The calibration curve showed that Beer's law obeyed over the range of 0.05-3.0µg/ml of nitrite, with a detection limit of 0.03µg/ml. The molar absorptivity and Sandell index were $1.3887*10^4$ L/mol.cm and 0.00331µg/cm^2 respectively. The optimum conditions affecting and interferences of the foreign ions were studied. The results obtained of the proposed method for determination of nitrite in meat samples were agreed with those obtained by the Naphthyl ethylene diamine NEDA standard method.

Introduction

Biomedical effects of nitrite have been drawing more attention recently, the nitrites assays in body fluids from the view point of clinical biochemistry was reviewed, a close relation between formation of nitrous compounds and nitrite has been demonstrated (1).

Nitrate and nitrite is present in foods naturally or may be present as a result of use of fertilizers on crops or from their uses as preservatives(2).

The main ingredients for curing the meat are salt and salts of nitrate and nitrite and vinegar, but there are other ingredients are added to accelerate the curing, stabilize the color (nitrites, nitrates and citrate) and to change the aroma and texture (phosphates, dextrose, salt, aromas and Soya protein) (3-6).

Nitrite acts as an antioxidant and most importantly, it is a very effective antimicrobial agent, unfortunately, nitrite can also react with amines, amides and amino acids in meats forming carcinogenic N-nitroso compounds(7,8).

Nitrite is a relatively strong reducing agent with antibacterial properties, although much of the preservation of the foodstuff is attributable to the high concentration of salts employed during the curing process, as is also the case for nitrate. In addition nitrate may act as a reservoir from which nitrite may be formed by microbiological reduction(6).

Among these ingredients the use of sodium and potassium salts of nitrite which plays a multi-faced role, due to their ability to give a particular color and taste to the meat as well as their preservative effect(9), which provides specific protection against Clostridium botulinum, a pathogenic micro-organism, responsible for numerous food poisonings(7,10). The color results from the nitrosation of the heme in myoglobin to form nitrsohemochrome.It also stabilizes the flavor of stored meats by preventing the formation of the undesirable products of oxidation, but meat is an extremely complex and variable system that offer an enormous number of constituents with capability for nitrosation.Consequently, it is possible to obtain a great variety of nitro sated compounds. Among them, the more unstable ones can undergo different kinds of chemical reactions (transnitrosation, oxidation, reduction and hydrolysis).Endogenous or added reductants (such as sodium ascorbate or eritorbate)play an important role(3).

Nitrates are reduced to nitrites in meats by naturally occurring bacteria and nitrites are reduced to nitric oxide, the compound that reacts with myoglobin to produce the typical color of cured meat(4).

Some urinary bladder and stomach has been associated with N-nitrosamines, there have been concerns over the presence of nitrate and nitrite in foods as they can be metabolized to potentially carcinogenic N-nitroso compounds. For this reason, Reports on the Scientific Committee for Food (1997) considered the
implications for human health of nitrate and nitrite in foods and has set Acceptable Daily Intake (ADI) for these compounds to be 0.06 mgKg-1 body weight for nitrite and 3.7 mgKg-1body weight for nitrate(2).

Numerous Spectrophotometric method have been proposed for the determination of nitrite using nitrosation method(11-13), in which secondary amine, both aliphatic and aromatic (tryptophan in the present work) reacts with sodium nitrite in acid medium to yield N-nitrosamines.

Experimental

Reagents

All chemicals used were of analytical reagent grade.

Stock nitrite solution (100 μg/ml): Sodium nitrite (0.1500 g) was dissolved in distilled water, 1.0 ml of chloroform and a pellet of sodium hydroxide were added (9), and the solution was diluted with distilled water to 1.0L. Working standard solutions were freshly prepared by diluting the stock solution with distilled water.

Tryptophan solution (0.02M): 2.0424g of the compound was dissolved in 500ml of 0.1M sulphuric acid.

Concentrated sulphuric acid.

NEDA method: Naphthyl ethylene diamine method

N-(1-naphthyl) ethylene diamine dihydrochloride solution (0.0034M): 0.5g of the compound was dissolved and diluted to 500ml with distilled water.

Sulfanilamide solution (0.058M): 5.0g of the compound was dissolved in a mixture of 50ml conc. hydrochloric acid and 300ml distilled water then diluted to 500ml with water.

Apparatus

The spectral were carried out on a CECIL CE 3021 UV/Vis double beam spectrophotometer. While absorbance measurements were carried out on JENWAY 6305 UV-Vis spectrophotometer.

Sample preparation

Meat samples were treated according to the process recommended by the International Standard Organization (ISO 2019 and ISO 3001, 1975). Samples were extracted with hot water and then purified by protein precipitation with each of carrez reagents followed by filtration (4, 14).

General procedure

To a series of 25ml volumetric flasks containing 1.25-75 μg of nitrite, 1.3ml of tryptophan soln.(dissolved in 0.1M H_2SO_4) and 4.0ml of Concentrated sulphuric acid were added, and the volume was made up to the mark with distilled water. The reaction mixture was seted aside for 15min., and then the

absorbance was measured at 440nm against a reagent blank prepared in the same manner but containing no nitrite in 1.0-cm cell.

Results and discussion

When nitrite ion was treated according to the previous procedure, the absorption spectra shown in Fig.1 was obtained .The maximum absorption was 440nm, whereas, the blank has no absorbance in this region.

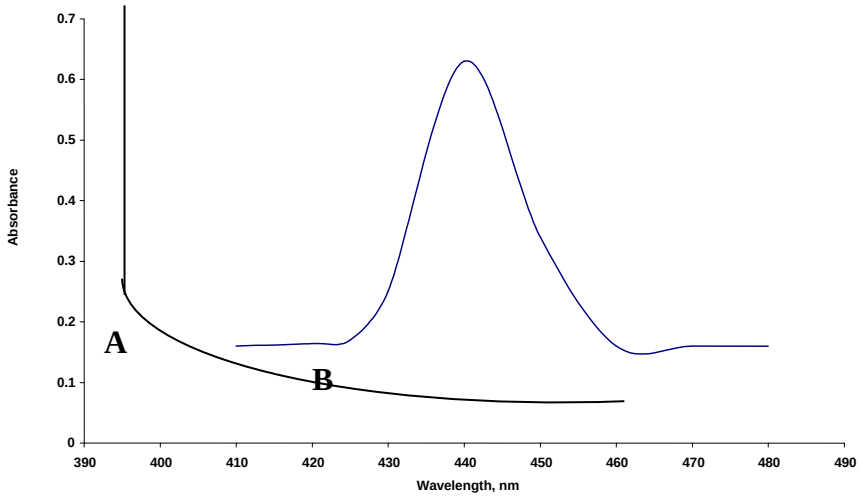

Fig.(1): Asorption spectra of : A. 2.0u g/ml of nitrite treated according to the recommended procedure against blank B.Blank against distilled water

Different 0.1M acids (hydrochloric, nitric, sulphuric and acetic acids), 0.1M bases (potassium hydroxide, sodium hydroxide and sodium carbonate), hot water and ethanol have been investigated for dissolving and preparing 0.02M of tryptophan solution to obtain the maximum absorption .Results showed that hydrochloric acid gives 90% of the intensity given by sulphuric acid, while nitric acid gives 50% of that of sulphuric acid. Therefore, 0.1M sulphric acid was used for preparation of tryptophan solution.

It was found that 1.3ml of 0.02M composite reagent gives the maximum absorption.

To study the effect of the types of mineral acids, concentrated acetic, hydrochloric, nitric and sulphuric acids were tested for obtaining the maximum

absorption. concentrated Sulphuric acid was selected as a medium in this work, and 4.0ml gave the most useful results.

In spite of the rapid color development the reaction mixture has to stand for 15min. to attain maximum absorbance after the absorbance remained constant for about 1.5h., and then a slight decrease took place.

Calibration curve

Using the optimum conditions obtained previously, a straight line of calibration curve was obtained, and the colored system followed Beer's law over the concentration range of 1.25-75 μg of nitrite ion in a final volume of 25 ml (i.e. 0.05-3.0 μg/ml) with a detection limit of 0.03 μg/ml. The molar absorptivity, Sandell index and correlation coefficient were $1.388*10^4$ L/mol.cm, 0.00331μg/cm^2 and 0.9994 respectively.

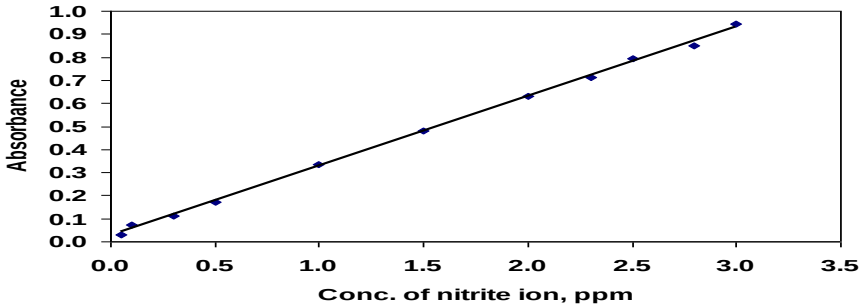

Fig.(2): Calibration curve of the method

Precision and accuracy

The precision and accuracy of the determination of nitrite ion were studied depending upon the values of the relative standard deviation percentage (RSD %), and relative error percentage (Error %) for five replicates, respectively. Table (1) shows the results.

Table (1): Precision and accuracy of the proposed spectrophotometric method.

Concentration of nitrite (μg/ml)	RSD%	Error %
0.1	0.11	0.56
1.5	0.42	0.33
3.0	0.11	0.16

The t-test calculated of the method was 0.79 and the t-test of the table is 2.78 therefore, there is no significant difference between the two results.

Interferences

In order to apply the proposed method for the determination of nitrite in cured meat samples, the interference effect of several anions and cations were evaluated by adding various concentration of the foreign ions to 2.0ppm nitrite solution upto amounts where the relative error (error %) reached a value of about ≤ ± 5%.Results are summarized in Table (2).Most of the ions are not interfere in this study, except ascorbic acid and this was oxidized by adding 0.2ml of 0.5% iodine.

Table (2): Effect of interfering ions on 50μg of nitrite determination in a final volume of 25ml.

Ions	Acceptable amount to be added to nitrite(μg)	Error %
Chloride	**3750**	**+3.96**
Bromide	**2500**	**+2.38**
Iodide	**2000**	**-4.76**
Nitrate	**1375**	**-1.58**
Acetate	**1250**	**+1.58**
Lead(II)	**250**	**-2.38**
Ascorbic acid	**100**	**+3.17**
Citric acid	**100**	**+4.20**

Application of the method

The proposed method was applied to the determination of nitrite in cured meat samples .In the present investigation, 5.0ml of meat extract was taken, 0.2ml of 0.5% iodine was added and the recommended procedure was applied. The results of the proposed method were compared with those of the standard NEDA method[(12)]. Table (3) shows the results.

Table (3): Determination of nitrite in cured meat samples

Sample	Nitrite(ppm)	
	Present method	NEDA method
Farm	48.7	50.5
Bordon	120.0	122.8
Neirs	126.3	125.9
Cow brand	128.5	131.0
Al-Taghzea	130.6	128.2

Comparison with other methods

The main features of the present method are the use of non toxic and easily soluble compound, i.e. tryptophan in comparison with other reagents. The present method has low detection limit in compare with other methods and has a wide range of determination limit. Results are shown in Table (4).

Table(4): Comparison of the present method with other nitrosation method

Reagent	λ_{max} (nm)	Optimum acidity	Beer° law(ppm)	Reference
Rhodamine 6G	445	Acidic medium	0-3.0	(7)
Pararosaniline color	540	pH 2.0	0.0064-0.32	(9)
Acridine red	525	6M HCl	0.025-0.5	(10)
Tryptophan	440	Strong acidic medium(H_2SO_4)	0.05-3.0	Present work

Conclusion

The proposed method for nitrite determination has good sensitivity and wide applicability .It can be used to determination of nitrite to a level of 1.25µg .As the results of the interferences study showed that there is no significant interference arose from the foreign species, so the present work was applied successfully to the determination of nitrite in cured meat samples.

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