



Sensitive and Selective Spectrophotometric Assay of Nitrite in Canned Meat and Vegetable Offered in the Mosul Market

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ABSTRACT

Twelve different canned food samples were used to assess the amount of nitrite using two distinct spectroscopic methods. A: Follow the absorbance and B: Follow the peak area; these two methods are predicated on the oxidation of nitrite by iron (III) ion, and the reduced iron ion reacts with the bathophenanthroline (BTN) to form a red soluble complex. The method is sensitive with $676.13 \text{ l.mol}^{-1}.\text{cm}^{-1}$ as a molar absorptivity, and Beer's law is followed over the concentration range of 3–45 $\mu\text{g/ml}$ with a maximum wavelength at 534 nm., limit of quantitation (LOQ) value is 0.5956 $\mu\text{g/ml}$, and limit of detection (LOD) value is 0.1786 $\mu\text{g/ml}$. Nitrite has been extracted from meat samples by hot distilled water in the presence of mercuric chloride, and from vegetables by hot basic aqueous solution, the validation of the method exhibits good ability for routine examination analysis of nitrite in quality control units of foods. Ferric chloride has been added and optimized to eliminate the effect of ascorbic acid which is already added as an antioxidant through the production of canned meats.

Keywords: Nitrite, spectrophotometry, indirect determination, oxidation – reduction.

INTRODUCTION

Nitrite is already used to preserve canned meat and give it a pink and refreshment appearance. As nitrite adds a taste and a distinctive flavor (Sun *et al.*, 2023; Sun *et al.*, 2017). Nitrite intoxication is the cause of severe methemoglobinemia, as the efficiency of methemoglobin in transferring oxygen is lower than hemoglobin (Katabami *et al.*, 2016) by an oxidation reaction in which electrons are transported from one reactant to another (Skoog *et al.*, 2013). One common way of oxygen consumption in water is the oxidation of nitrite (Beman *et al.*, 2021), and the other oxidation of nitrite to nitrate naturally occurs by the effect of bacteria (Salazar *et al.*, 2023). As higher concentrations of nitrite lead to methemoglobinemia it is also caused by blue baby syndrome in human infants, and produces carcinogenic N-nitrosamines by reacting with secondary or tertiary amines present in the human body (Singh *et al.*, 2019).

Diazotization reaction is commonly used for determination of nitrite in different samples using many reagents (Moldovan, 2012; Narayana and Sunil, 2009; Tarafder and Rathore, 1988). A reduction reaction of nitrite to nitric oxide by iron (III), followed by subsequent complexation of iron (II) with thiocyanate in acidic medium was used to determine nitrite at 460 nm (De Andrade *et al.*, 2003). Many flow injection analyses have been used for the determination of nitrite (Ribas *et al.*, 2024; De Oliveira, 2023). In contrast the area under the absorption curve has been used for the determination of such samples (Karajgi *et al.*, 2016; Tanveer and Kalyane, 2016) but not for the determination of nitrite yet.

Iron (II) and a few other ions can form stable complexes with 1,10-phenanthrolines, and its derivatives (Ali, and Nabeel, 2023; Nabeel, *et al.*, 2017; Thura, 2020). In this study o-phenanthroline the derivative of 1,10-phenanthrolines has been used in a simple and rapid spectrophotometric method for the determination of nitrite in the canned food offered in Mosul/Iraq markets.

EXPERIMENTAL

Apparatus

1. Spectrophotometer: Shimadzu UV-VIS 1900i.
2. Balance (Sartorius BL 20 S, Germany).

Materials

NaNO₂ (BDH), Ferric Chloride (Fluka), 4,7-Diphenyl-1,10-phenanthroline (Bathophenanthroline) (SEARLE Company), Ethanol (99%) (Cristalco/FranceAlcools).

Solutions

NO₂⁻ solution (100 µg/ml): This solution was prepared by dissolving 0.015 g of pure solid of NaNO₂ salt in 100 ml of distilled water in a calibrated flask, Ferric Chloride solution (5x10⁻³M) this solution was prepared by dissolving 0.0811 g of pure solid of FeCl₃ in 5 ml of acetic acid (1M) then diluting the solution with distilled water in a 100 ml volumetric flask; Bathophenanthroline (BTN) solution (5x10⁻³M) was prepared by dissolving 0.1661 g of pure solid of 4,7-Diphenyl-1,10-phenanthroline in 10 ml of ethanol then diluting the solution with ethanol in a 100 ml volumetric flask.

RESULTS AND DISCUSSION

Preliminary investigation

1ml of nitrite solution (100 µg/ml) is added to 1.5 ml of ferric chloride followed by addition of 0.5 ml BTN. Oxidation reduction reaction occurs after heating for 15 min in water bath, the solution is diluted to make exactly 10 ml, The absorption spectrum has a maximal response at 534 nm, and a red color appears in comparison to a colorless blank solution made in the same manner Fig. (1).

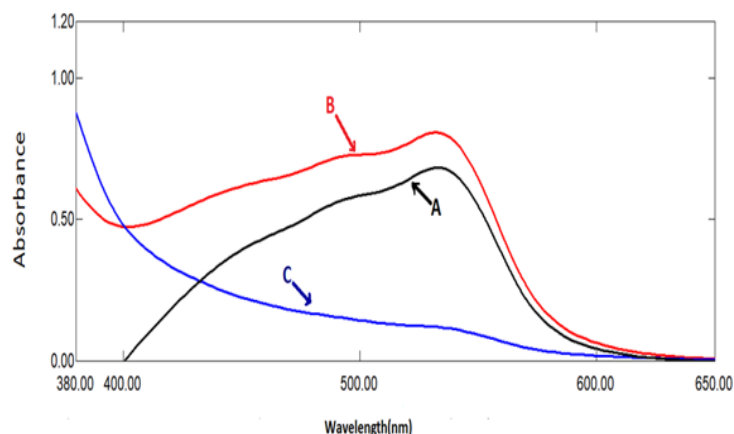


Fig. 1: The spectrum of the reaction, A=sample against blank, B= sample against distilled water and C=blank against distilled water.

Study of the reaction conditions

Different conditions have been examined and optimized to obtain maximum sensitivity for the determination of nitrite.

Effect of temperature with effect reaction time

The effect of different temperatures and different oxidation times on the absorption of the resulting product was studied, as in the (Table 1). It was observed that absorption increased with increasing temperature. The results indicate that the optimal condition is 50°C as oxidation temperature and 15 minutes as the oxidation time. The reactions of phenanthroline and its derivatives are endothermic, so, an increase in temperature increases the formation of the colored product by shifting the reaction to forward direction (Nkole *et al.*, 2021), in the other side quantitative analysis at temperature higher than 50°C is unpreferable refer to the effect of heat on the concentration.

Table 1: Effect of temperature with effect reaction time

Time(min)	Absorbance/Temperature			
	R.T(20)	30	40	50
5	0.004	0.076	0.096	0.172
10	0.007	0.090	0.155	0.228
15	0.012	0.173	0.205	0.364
20	0.001	0.078	0.199	0.268

Effect of acid and the amount of acid

After studying the effects of five acids (1 ml, 1 M), on the reaction process, 0.5 ml of CH₃COOH (1M) produced the maximum absorbance as in (Table 2), but studying the amount of acetic acid show that there is no significant difference in the presence or absence of the acid at low (0.5 and 1 ml) while it is decreased at high amount of the weak acid which is indicate that the complex form in neutral to slightly acidic medium.

Table 2: Effect of different acid

Acid (1M)	Absorbance
CH ₃ COOH	0.375
H ₂ SO ₄	-0.067
H ₃ PO ₄	-0.046
HCl	-0.071
HNO ₃	-0.041
In the absence of acid	0.364

Effect of the amount of oxidizing agent

To study the influence of the amount of oxidizing agent, 0.5 to 2 ml of ferric chloride ($5 \times 10^{-3} \text{M}$) have been added to solutions containing varying concentrations of NO_2^- (10–50 $\mu\text{g/ml}$) in the presence of fixed amount of (0.5 ml, $5 \times 10^{-3} \text{M}$) of BTN and heating for 15 min at 50°C in water bath. The results listed in (Table 3) show that the addition of 1.5 ml of ferric chloride sensitivity and best determination coefficient.

Table 3: Effect of the amount of oxidizing agent

MI of ($5 \times 10^{-3} \text{M}$) FeCl_3	Absorbance/ $\mu\text{g/ml}$					
	10	20	30	40	50	R^2
0.5	0.115	0.122	0.127	0.132	0.141	0.9877
1	0.492	0.513	0.534	0.552	0.568	0.9965
1.5	0.740	0.759	0.775	0.797	0.817	0.9976
2	0.699	0.734	0.760	0.790	0.808	0.9898

Effect of the amount of reagent

0.5 ml of Bathophenanthroline (BTN) solution ($5 \times 10^{-3} \text{M}$) gave high absorbance, as shown in (Table 4), and the effect of the amount of reagent was investigated on solution absorbance. The NO_2^- concentration ranged from (10 to 50 $\mu\text{g/ml}$).

Table 4: Effect of the amount of reagent

MI of BTN (5×10^{-3})	Absorbance/ $\mu\text{g/ml}$ of NO_2^-					
	1	2	3	4	5	R^2
0.25	0.309	0.338	0.346	0.375	0.406	0.9725
0.5	0.734	0.753	0.771	0.785	0.801	0.9967
1	0.434	0.501	0.525	0.567	0.584	0.9526
1.5	0.432	0.477	0.520	0.553	0.583	0.9918

Effect of the additions sequence

When the effect of the additions' order was examined, it was found that the absorptions were the same.

Effect of surfactant solution

One milliliter of each surfactant (S) positive and negative (CPC, CTAB and SDS) was added to the reaction solutions in a different order to show how the surfactants affected the subsequent absorption, as in (Table 5) which show no significant difference in absorbance in the presence or absence of surfactant and this indicates no ternary mixture is formed in the presence of the examined types of surfactants.

Table 5: Effect of surfactant solution

Surfactant solution ($1 \times 10^{-3} \text{M}$)	Absorbance / Order of addition		
	$\text{NO}_2^- + \text{S} + \text{Fe}^{+3} + \text{BTN}$	$\text{NO}_2^- + \text{Fe}^{+3} + \text{S} + \text{BTN}$	$\text{NO}_2^- + \text{Fe}^{+3} + \text{BTN} + \text{S}$
SDS*	0.568	0.582	0.680
CTAB**	0.707	0.712	0.716
CPC***	0.720	0.721	0.724

*Sodium dodecyl sulphate, **Cetyltrimethylammonium bromide, ***Cetylpyridinium chloride

Time's impact on the product's absorption

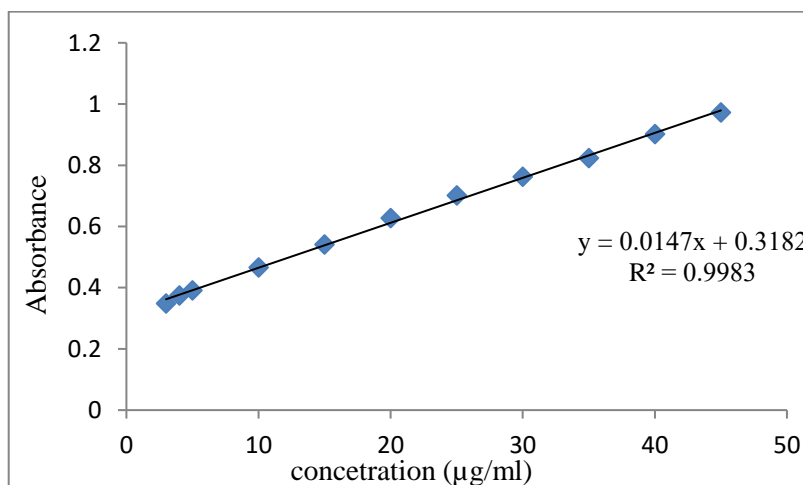
Different times were studied to examine the impact of time on the colored product's absorption. At a wavelength of 534 nm, the absorbance of a solution was measured against the blank solution, and at 50°C . The stability of the resultant product was also measured. An hour of stability was observed in the final product, as in (Table 6).

Table 6: Time's impact on the product's absorption

Time (min.)	Absorbance /at 50 °C
Immediately	0.760
5	0.767
10	0.769
15	0.778
20	0.778
25	0.778
30	0.779
35	0.782
40	0.784
45	0.785
50	0.786
55	0.788
60	0.789

The calibration curve of the nitrite

Various concentrations of nitrite are added to a 10-ml series volumetric flask (3–45 $\mu\text{g/ml}$). Next, 1.5 ml of ferric chloride (5×10^{-3} M) and 0.5 ml of the reagent BTN (5×10^{-3} M) are added, and the flask is placed in a water bath at 50 °C for 15 min, the solution is then diluted to make 10 ml exactly. After five minutes, the absorbance at 534 nm. for each solution is measured. Fig. (2) shows the calibration curve, (Table 7) shows the characteristics of the calibration curve, and (Table 8) show the calculated (Christian, 2004) accuracy and the relative standard deviation.

**Fig. 2: The calibration curve of the nitrite****Table 7: The information of the calibration graph**

The parameter	The value
Correlation coefficient R	0.9991
Determination coefficient R ²	0.9983
Slope	0.0147
Intercept	0.3182
Molar absorptivity ($\text{l.mol}^{-1}.\text{cm}^{-1}$)	676.13
LOD*	0.1786
LOQ*	0.5956

Table 8: Accuracy and precision

Concentration ($\mu\text{g/mL}$)	RSD%*(10)	Error%*
10	1.6	1.27
30	1	0.078
45	0.25	0.03

*Average of three determinations

Effect of dilution solvents

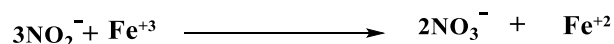
By continuing the procedure but substituting these organic solvents for water in the reaction solutions' dilution, and then measuring the absorbance of those solutions, the impact of the organic solvents on the spectrum characteristics of the colored complex was examined, the result given in (Table 9). The table exhibits that the maximum absorbance is resulted by using water as solvent.

Table 9: Effect of organic solvents

Solvent	λ_{max}	Absorbance
Water	534	0.769
Acetone	534	0.700
Ethanol	532	0.709
Methanol	530	0.772
Acetic acid	534	0.691

Chemical reactions

Step 1: Oxidation of nitrite and reduction of ferric to ferrous (Merino *et al.*, 2017).



Step 2: Complexation of ferrous with bathophenanthroline reagent (Sulthana *et al.*, 2017).

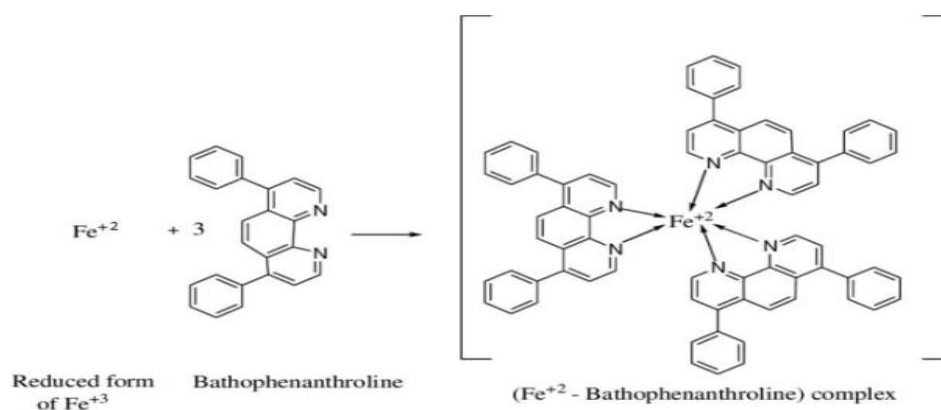


Fig. (3): The chemical reactions of the proposed method.

Application of the method

Nitrite extraction from canned beef

After crushing 10 g various meats, the mixture was put in a 50 ml beaker and 10 ml of hot (80 °C) distilled water was added with smooth stirring, the mixture was transferred to a 250 ml beaker, filled with 70 ml of hot distilled water and placed in a water bath for two hours. One milliliter of saturated mercuric chloride (1 g of mercuric chloride dissolved in ten milliliters of distilled water) was added to precipitate the protein and release nitrite (Bousset, 1980). The solution was filtered and transferred to a 100 ml volumetric flask after chilling to laboratory temperature, finally the solution was diluted to be exactly 100 ml (Bilal and Bashir, 2013).

Nitrite extraction from food samples

Many canned vegetable and mushroom samples were chopped, weighed, and then 10 grams of each sample were added to a 25 ml beaker along with 20 ml of distilled water and 1 ml of 4% sodium hydroxide. After heating the mixture for 20 minutes at 80 °C in a water bath while sometimes stirring, it was cooled. After bringing the mixture to room temperature, strain it, pour it into a 100 ml volumetric flask, and fill it up to the correct volume (Manas *et al.*, 2006). The studied samples are displayed in (Table 10).

Table 10: Samples under the study

Meat samples			Food samples		
Type	Expire date	Country of origin	Type	Expire date	Country of origin
M1/ Bordon	2023/1/8 2025/1/17	Brazil	V1/ Peas-Sutura	2022/10/2 2024/10/8	Turkey
M2/ Bordon	2023/5/22 2025/5/21	Iraq	V2/ peas-Abu Al-ward	2023/8/1 2025/1/1	Jordan
M3/Altunsa, chicken luncheon	2022/11/7 2024/11/6	Turkey	V3/ yellow corn-Dohha	2022/11/26 2024/11/26	China
M4/Gozde, chicken franks	2023/9/21 2024/11/20	Turkey	V4/ Mushroom-Warda	2023/7/20 2025/7/19	Iraq
M5/rawaby, lunchone, chicken	2022/10/25 2024/10/24	Turkey	V5/ Beans-Pemina	2023/3/4 2025/3/3	Iran
M6/Altaghziah, beef luncheon	2023/11/29 2024/12/29	Lebanon	V6/ Okya	2023/3/22 2024/3/21	Egypt

Use of FeCl₃

Ascorbic acid is already added to canned foods to prevent the oxidation of nitrite, in order to remove the effect of ascorbic acid an increasing amount of FeCl₃ has been added, the effect of FeCl₃ has been studied and 1ml of ferric chloride in this step was sufficient to eliminate the effect of ascorbic acid, while the added amount of the reagent BTN has been optimized to be equivalent to the nitrite within the Beer's law range.

A) Determination through absorbance measurement

Using spectrophotometric techniques, the amount of nitrite was determined by measuring the absorbance of specific meat and canned food samples that were sold in the Iraqi market, Using oxidation-reduction reaction, as in (Table 11).

Table 11: The determination of nitrite in meat and food samples

Sample	Absorbance in the presence of excess amount of FeCl ₃	Concentration of nitrite (µg/ml)	Concentration of nitrite in samples (mg/g)	RSD%*
M1	0.2566	17.45	0.1745	2.2
M2	0.3418	23.25	0.2325	1
M3	0.2569	17.74	0.1774	0.76
M4	0.3255	22.14	0.2214	0.30
M5	0.2424	16.4	0.164	0.33
M6	0.3898	26.5	0.265	0.46
V1	0.2971	20.2	0.202	0.33
V2	0.2874	19.5	0.195	0.35
V3	0.3025	20.5	0.205	0.24
V4	0.4344	29.5	0.295	0.27
V5	0.2410	16.3	0.163	0.56
V6	0.1951	13	0.13	0.43

*average of three determinations

As (Table 11) show, that nitrite in meat samples is ranged from 0.265 mg/g in M6 sample (Altaghziah, beef luncheon of Lebanon origin) to 0.164 mg/g in M5 sample (rawaby, lunchone, chicken of Turkish origin). About the vegetables, sample V4 (Mushroom-warda of Iraqi origin) contain the highest concentration of nitrite (0.295 mg /g) and V6 (Okya of Egyptian origin) contain the lowest (0.13 mg/g).

Method validation

The standard addition method has been used to validate the method of measuring absorbance for the purpose of determining the amount of nitrite in food samples. The results of standard addition method show the recovery of nitrite in meat samples ranged from 103.15 to 103.7, and in vegetable sample 95.04 (Table12).

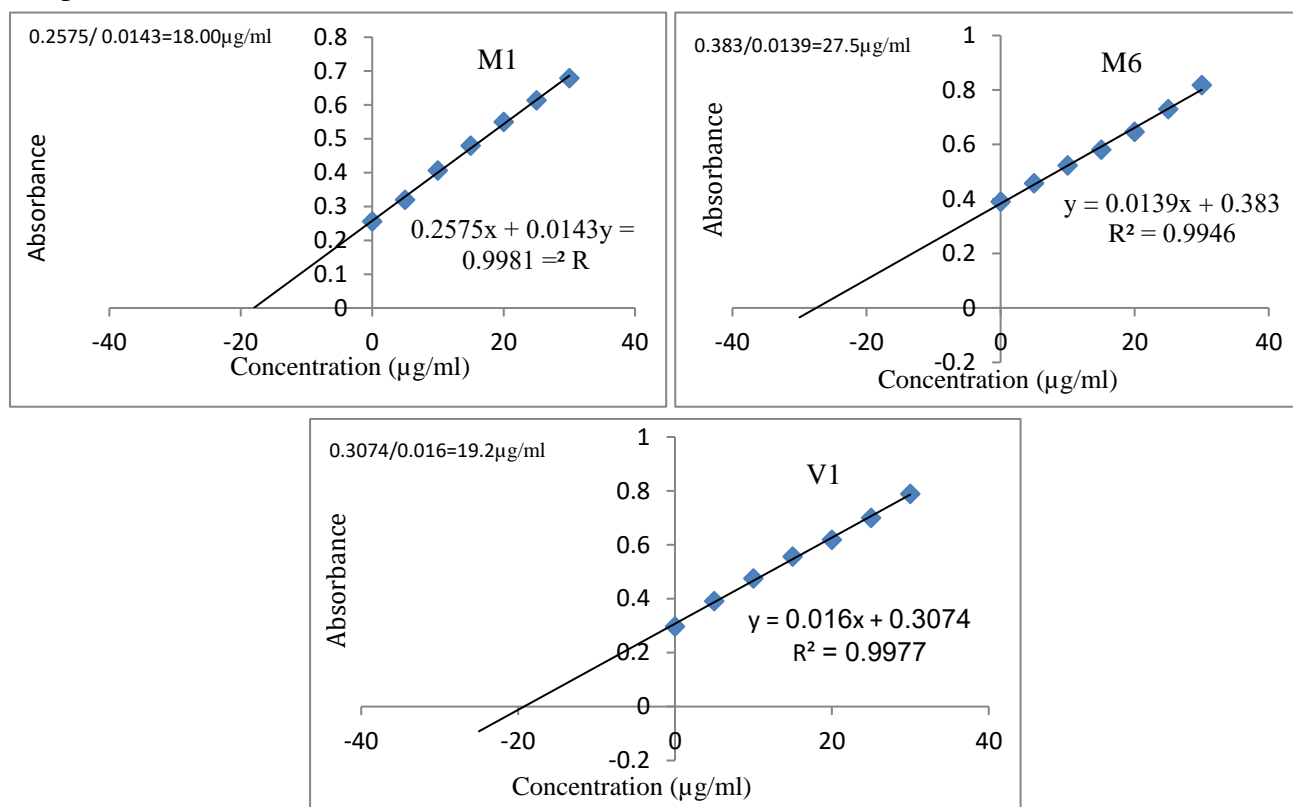


Fig. 4: Standard addition method of samples M1, M6 and V1.

Table 12: Validation of the method by standard addition curves and comparison with the results of the determination

Sample	Concentration of nitrite (µg/mL)		Recovery %
	Direct application	Standard addition method	
M1	17.45	18	103.15
M6	26.5	27.5	103.77
V1	20.2	19.2	95.04

(Table 12) show that the detected amount of nitrite in the selected samples M1, M6, and V1 is identical using the standard addition methods which indicates that there is no interfering effect caused by sample components, it is give the validation of the method.

B) Determination by measurement of the peak area

Nitrite has been also determined by following the increase in the area under the peak against the concentration. Three calibration curves were prepared to follow the area under peak at three

different ranges in which the absorption maximum of the reaction within the selected ranges (530-540, and 528-543, and 532-552 nm) Fig. (6).

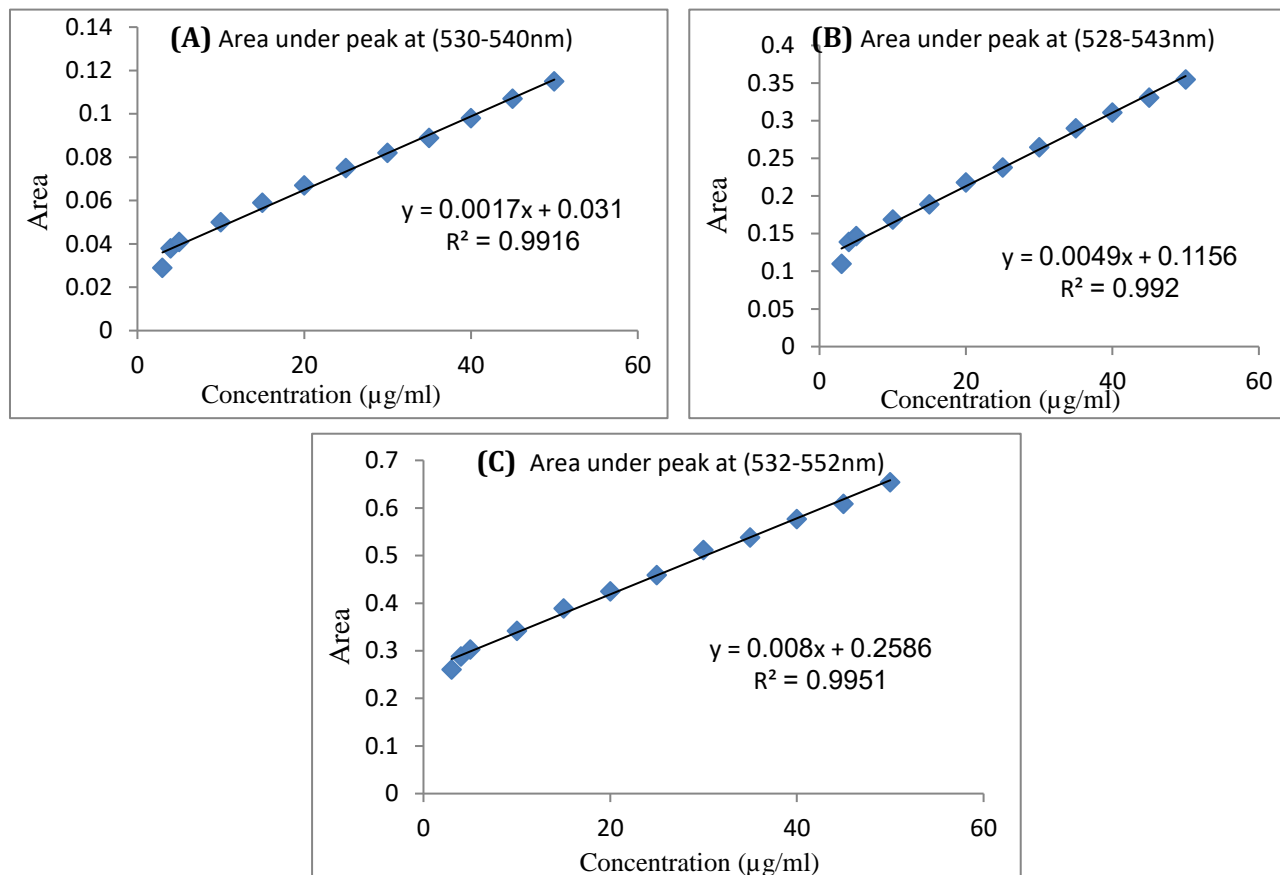


Fig. 5: Calibration curves of peak area method

As Fig. (5) show, the determination coefficient of all selected peaks are good, and 528-543 nm. exhibits low absorbance of the blank as shown in (Table 13), with high accuracy and precision as shown in (Table 14) therefore, it is selected for the determination of nitrite in meat samples M1, M6, and in vegetable samples V1 and V6. Fig. (6).

Table 13: The absorbances of blank at the selected peak area

λ_1 - λ_2 (nm.)	Absorbance of blank
530-540	0.011
528-543	0.010
532-552	0.039

Table 14: Accuracy and precision for (528-543nm)

Concentration of nitrite ($\mu\text{g/mL}$)	RSD*%	Error*%
10	3.5	3.4
30	3.2	2.5
45	3.1	3.7

* Average of three determinations.

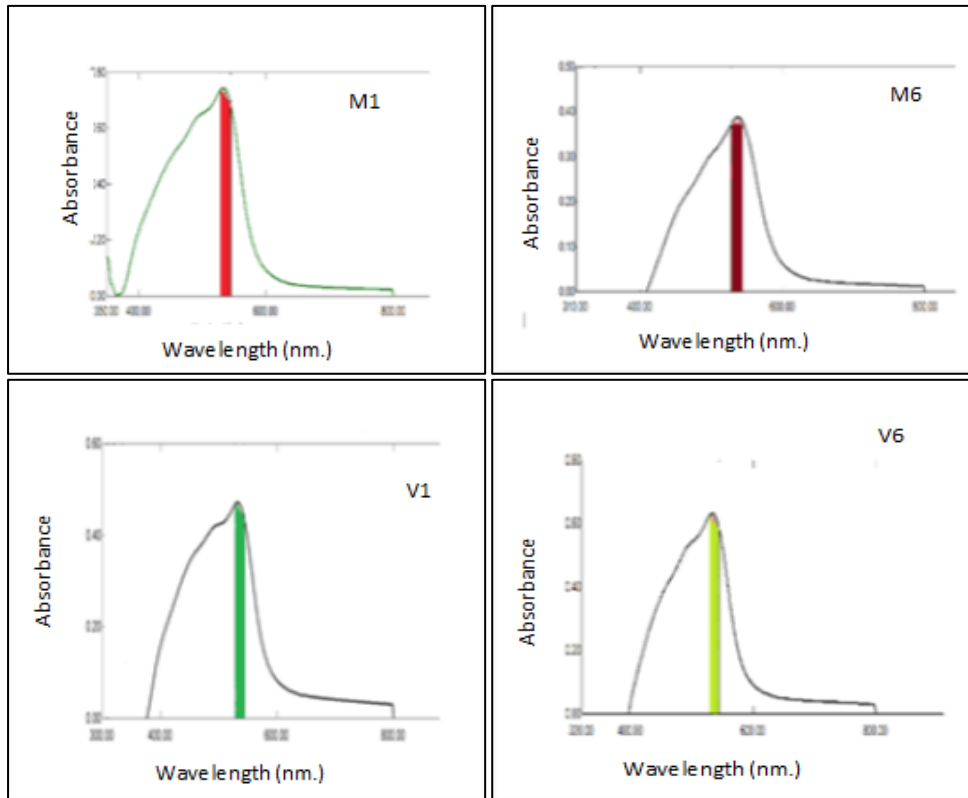


Fig. 6: Determination of nitrite in meat samples M1, M6, and in vegetable samples V1 and V6.

Method validation

Using the standard addition method, the peak area measurement technique for determining the amount of nitrite in food samples has been validated (Table 15).

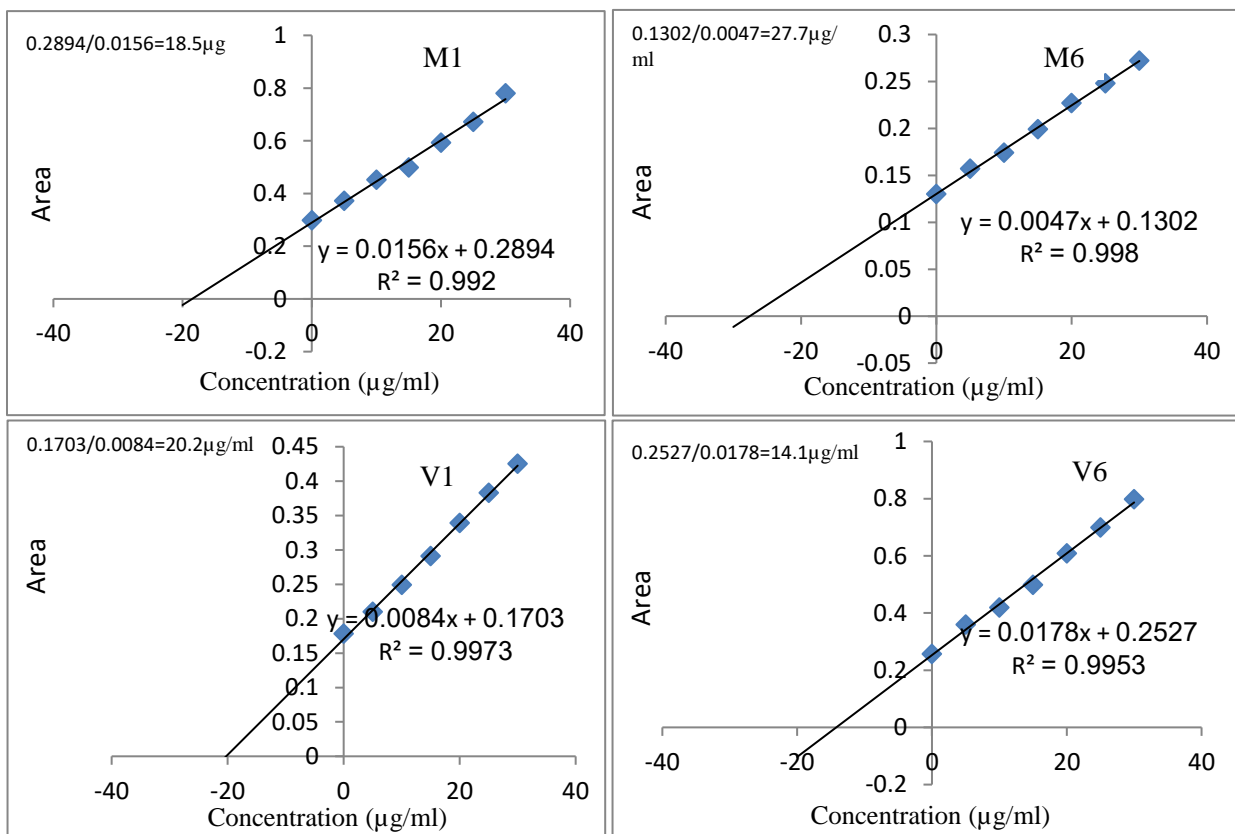


Fig.7: The standard addition method for the determination of M1, M6, V1, and V6 samples.

Table 15: Validation of peak area method by standard addition curves and comparison with the results of the direct determination

Sample	Concentration of nitrite ($\mu\text{g/mL}$)			Recovery %
	Direct application	Standard addition method*	Standard addition method**	
M1	17.45	18	18.5	102.70
M6	26.5	27.5	27.7	100.72
V1	20.2	19.2	20.2	105.20
V6	13	-----	14.1	108.45

* of the first method, ** of the peak area method

CONCLUSIONS

There is an increase in canned foods that entering the country, associated with an increase in the consumption of this type of foods due to its ease of preparation. This research provides a way to estimate nitrite in canned foods precisely and accurately, the selected foods are meats and many kinds of vegetables of different origins. The study shows that nitrite in meat samples is ranged from 0.164 mg/g in M5 sample (rawaby, lunchone, chicken of Turkish origin) to 0.265 mg/g to in M6 sample (Altaghzhiah, beef luncheon of Lebanon origin). In canned vegetables, V6 sample (Okya of Egyptian origin) contain the lowest amount of nitrite (0.13 mg/g), and sample V4 (Mushroom-warda of Iraqi origin) contain the highest (0.295 mg /g). The method exhibits good ability for routine examination analysis of nitrite in quality control units of foods.

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طريقة طيفية حساسة وانتقائية لتقدير النترت في اللحوم والخضروات المعلبة والمعروضة في اسواق الموصل

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الملخص

تم تقدير النترت في اثني عشرة عينة مختلفة من الأغذية المعلبة باستخدام طريقتين طيفيتين متميزتين. الطريقة أ: القياس المباشر لامتصاص معقد الحديد الثنائي الناتج عن اكسدة النترت في هذه العينات. الطريقة ب: قياس المساحة تحت منطقة الذروة لطيف امتصاص المعقد.

تعتمد هاتان الطريقتان على أكسدة النترت بواسطة أيون الحديد الثلاثي ثم مفاعلة الحديد المختزل (الثنائي) مع كاشف الباثوفينانثرولين (4 و7-ثنائي فينيل 10,1-فينانثرولين) لتكوين معقد أحمر. أظهرت الطريقة حساسية مقبولة إذ كان معامل الامتصاص المولاري 676.13 لتر. مول⁻¹. سم⁻¹، وتبعية لقانون بير في نطاق التركيز من 3 إلى 45 جزء في المليون عند طول موجي أقصى يبلغ 534 نانومتر، وقيمة حد التقدير الكمي (LOQ) تبلغ 0.5956 ميكروغرام / ملتر، وقيمة حد الكشف (LOD) تبلغ 0.1786 ميكروغرام / ملتر. تم استخلاص النترت من عينات اللحوم بواسطة الماء المقطر الساخن وبوجود كلوريد الزئبق، ومن الخضروات بواسطة محلول المائي الساخن، ويظهر التحقق من صحة الطريقة قدرة جيدة على التحليل الروتيني للنترت في وحدات متابعة الجودة الخاصة بالأغذية.

الكلمات الدالة: النترت، الطرائق الطيفية، تقدير غير مباشر، اكسدة واختزال.