

DETERMINATION OF QUALITY PARAMETERS FOR THE VALIDATION OF NITRATE AND NITRITE METHODE CIDES VEGETABLES AND ANIMALS FOODSTUFFS

Bura Giani, Dale Laura

*University of Oradea , Faculty of Environmental Protection, 26 Gen. Magheru St. 410048 Oradea;
Romania e-mail buragiani@yahoo.com*

Universitatea de Științe Agricole și Medicină Veterinară, Cluj Napoca Facultatea de Agricultură,

Abstract

The aim of this research is the validation of nitrate and nitrite methode cides vegetables and animals foodstuffs, to say the condition of repetability and reproductibility, and determinate the uncertainty A and B. The practical work was made on salami from supermarket and with spectofotometric method. For nitrite and nitrate determination it was used: spectofotometru tip UV/VIS (Shimadzu tip UV-1700), as reference documents STAS 11581-83 (9065 /12-90). It was made tow series with 8 probes from animals origin matrice. The obtain value in repetability and reproductibility condition at nitrite and nitrate determination at matrice was in the maxim limits of STAS.

Key words: nitrite, nitrate, spectofotometric methods, repetability, reproductibility.

INTRODUCTION

Nitrates and nitrites are nitrogen mineral or natural forms, known as nitrogen and nitrates, which are used as fertilizers (manure, chemical fertilizers), where it can reach the groundwater and hence the human body.

Nitrates as such are not very toxic, but they, by the action of intestinal microflora turn into nitrites (nitrogen) and nitrosamine.

Sodium or potassium nitrites are used in current technology of meat due to their ability to combine with myoglobin and red complex (nitro-nitro-myoglobin and hemoglobin) is determined by heat (nitromiocromogen and nitrohemocromogen) . Nitrite is not combined as such (NO_2^-) with the meat pigment, but reduced form (NO^-). The meat that has been added nitrite, this role is fulfilled during the maturation of certain enzymes and natural reducing substances, but particularly so-called bacterial "denitrifiante".

Meat salted, dried, smoked, cured or stored, is a secondary source of nitrate (as plant), the intake of approximately 9.4 mg / day (what, 1975) and the main source for nitrite (2.38 mg / day) and average nitrate concentrations of 10 mg / kg and 1 mg nitrite / kg.

After Usher and Telling (1975) values for freshly meat can be between 0 and 49 mg / kg nitrates. With regard to horse meat, the only reference is made by Cantoni and Bianchi (1977) who reported very high concentration of nitrate. The preserved meat, White (1975) found the

estimated values ranging from 1.19 to 10 mg / kg sodium nitrate are, it depends very much on the processing method.

National Center of USA (1981) reported average concentrations of 40 mg / kg and 10 mg nitrate / nitrite kg for products considered being integral. For the corned beef, White (1975) found 60 mg / kg sodium nitrate, Howe and col. (1986) 141.6 mg / kg nitrate and 19.4 mg / kg nitrite, and Knight and col. (1987) 151.1 mg / kg nitrate and 19.9 mg / kg nitrite. For organs such as beef liver 10 mg / kg nitrate and 1 mg / kg nitrite. With regard to poultry meat data are not unequivocal.

The average **daily consumption** of nitrates, Jaqualine Cornea and cervical. (1992) reported in France consumption of 121 mg, 85% by intake of vegetables, preserved meat and 5% and 5% of processed grain products. These values are half the national inventory reports (1982) and 280 mg.

NO₃-NO₂-concentration in plant products depend on harvesting, preparation and storage methods, all of which significantly influences the difference between the results. For uniform data, the values for vegetable, fruit and fresh raw material parameters must be natural. For processed foods such as canned meat, salted fish and / or smoked, the concentrations can vary greatly depending on the methods used in processing and use of adjuvants (such as salt impurities).

The concentrations of nitrates and nitrites in food depend on external factors and can vary in large proportions for each food. The nitrates and the nitrogen, with NaCl are substances essential in making preparations of meat, including raw-dried. Production and distribution of food by many factors affect health first, they directly determine the nutritional status of populations and their resistance to disease, then working in this sector is associated with different diseases and finally, production, distribution, administration and preparation of food shall be accompanied the contamination of these products and water. Current capabilities and future food production and conservation is to ensure an adequate supply of food plant in healthy nutrition. At the same time will be needed to increase agricultural production and food industries to ensure sufficient food for seven billion people who will occupy Earth by 2010, avoiding environmental pollution and reducing infectious diseases (Hura C., 2003).

MATERIAL AND METHODS

Determination of nitrite in meat and meat products are made according to STAS site.

Principle of the method and scope: Determination of nitrites, by measuring the intensity of color compound formed following reaction of nitric diazotare of acid and nitrites sulfanilinic of aqueous extract of the

sample and coupling alfanafilamina. Nitrite content is calculated using a calibration curve.

Sampling and conservation / Prepare samples of meat and meat preparations and mechanically homogenize small. In membrane preparations, it is removed in advance. The first slice sausage removed from the surface of section as terminal portion of the full bar and finely grind. The sample is analyzed up to 24 hours after mixing. If products are not cooked, the sample analyzed immediately after mixing.

Specificity / Interferences / Sensitivity: The sensitivity of the method is 0.1 mg per 100 g product

Reagents: The solution to precipitate proteins:

I - Ferrocyanide potassium, 10.6% solution: in a 1000 cm³ volumetric flask is placed 106 g of crystallized potassium ferrocyanide, weighed to 0.01 g is dissolved [K₄Fe(CN)₆·3H₂O], lved in water and Bring flask to the mark with water;

II - Zinc acetate solution 22%: in a 1000 cm³ volumetric flask 2H₂O, is placed 220 g of crystallized zinc acetate Zn(CH₃COO) weighed 0.01 g and 30 cm³ of glacial acetic acid is dissolved in 300 ... 400 cm³ water and bring to volume with water;

- ❖ Saturated borax solution: 50 g crystalline sodium tetraborate 10H₂O is inserted into a 1000 cm³ volumetric flask, Na₂B₄O₇ dissolve in hot water (40 ... 50°C), allow to cool at room temperature environment and bring to volume with water;
- ❖ Griess reagent mixture of equal volumes of solution I and solution II. The mixture is prepared in time use.

Solution I

- Dissolve, by heating the water bath, 6 g sulphanilic acid (NH₂C₆H₄SO₃H · 2H₂O), weighed 0,001 g, 200 cm³ glacial acetic acid and 400 cm³ water. Cool and add 200cm³ solution 10% sodium chloride and dilute with water to 1000cm³.

Solution II

- Dissolve, by heating the water bath, 0.3 g alpha-naphthylamine hydrochloride C₁₀H₇NH₂NCl) in 100 cm³ water filter - if necessary - cool and add 200 cm³ glacial acetic acid. Bring to volume with water in a volumetric flask of 1000.

Solutions I and II are kept in brown bottles, sealed, no more than a week.

Note: Solution II is handled with care, avoiding contact with skin - Standard solution of sodium nitrite prepared as follows: 0.1 g sodium nitrite weighed to ± 0.001 g, quantitative cross with water in a 1000 cm³ volumetric flask, bring to volume with water and shake. From this solution

take Pipette 10cm³ is inserted in a 1000cm³ volumetric flask, bring to volume with water and shake. Prepare the solution at the time of use.

-1 cm³ solution containing 0.0010 mg of sodium nitrite.

Measuring and test equipment and materials:

- Spectrophotometer type Spekol series 733291IU16
- Analytical balance type WA 44781 series 31 UI 10
- Test tubes
- Beaker 100 ml

Procedure Preparation filtrate for determination of the samples for testing weigh about 10 grams, with precision of 0, 001 g, quantitative cross in a 200 cm³ volumetric flask with approximately 100 cm³ of hot water (70 ... 80 ° C) . Add 5 cm³ saturated solution of borax and heat the dish for 15 minutes in boiling water bath, stirring occasionally. Allow to cool to room temperature and successively added 2 cm³ of potassium ferrocyanide and 2 cm³ of zinc acetate, is shaking after each addition. Allow to stand 20 ... 30 minutes and then bring to volume with water. Content shake flask and filtered through a filter paper with high porosity, iron. The filtrate is collected in a dry Erlenmeyer flask. The filtrate obtained should be clear.

Determination of nitrites from the filtrate obtained taking 10 cm³ is placed in a beaker of 50 cm³, 10 cm³ add Griess reagent, stir and allow to stand at least 20 minutes but not more than 4 hours in the dark at room temperature for color development.

From the solution obtained is placed in a cuvette with thickness of 1 cm and measure the color intensity in spectrophotometer at wavelength of 520 nm from a control solution made with reagent for replacing the test sample with water (10 cm³). It shakes out the two determinations of the same sample.

If extinction colored solution obtained is greater extinction of the most concentrated standard solution, dilute the appropriate amount of extract taken it, supplementing the volume to 10cm³ of water and then adding the Griess reagent 10cm³. The calculation result will take into account the dilution made.

In six glasses Erlenmayer 50 cm³ are introduced, in turn, by pipette, standard solution of sodium nitrite, Griess reagent water in.

Table 1

Serial number of glasses Erlenmayer	1	2	3	4	5	6
Soluție etalon cu conținut de nitrit de sodiu cm ³	0	2	4	6	8	10
Water, cm ³	0	8	6	4	2	0
Griess reagent, cm ³	10	10	10	10	10	10
Containing sodium nitrite, mg	0	0,002	0,004	0,006	0,008	0,01

After the addition of Griess reagent, homogenize and leave at room temperature in the dark, 20 min., To develop color.

Measure the extinction of the standard sample Spectrophotometer at wavelength 520 nm of the sample solution girl I.

Plot a calibration curve, scoring on ordered extinctions values obtained, and the abscissae the corresponding sodium nitrite content in mg.

Expression of results and accuracy:

Nitrate content, expressed in milligrams sodium nitrite per kilogram produced, is calculated as:

$$\text{Nitrites (NO}_2\text{Na)} = \frac{c \cdot V}{m \cdot V_1} \cdot 1000 \quad [\text{mg/Kg}] \text{ (ppm)}$$

$$\text{Nitrites (NO}_2\text{Na)} = \frac{c \cdot V}{m \cdot V_1} \cdot 100 \quad (\text{mg}/100\text{g})$$

C = amount of sodium nitrite read the calibration curve in mg;

V = total volume of extract obtained in cm³;

V1 = volume of extract taken for determination, in cm³ ;

M = sample mass, g

Accuracy: Repeatability: difference between results of two determinations made by the same operator in the same laboratory should not exceed 10% of the nitrite content

RESULTS AND DISCUSSION

Food security will eliminate or reduce priority problem contaminants in food. To protect consumer health, the acceptable daily intake (ADI) are established for food additives, pesticides and veterinary drugs (or international), whether by ingestion ADI is not exceeded, then the substance is not considered harmful. With strict regulations will assess and monitor chemicals in food, but must consider the scientific evaluation of risks and benefits of using agricultural production methods, techniques, conservation and distribution of foods and food consumption reported everyday risks.

Nitrates and nitrites are natural components of soil from the mineralization of nitrogenous substances of plant and animal, a part of it is absorbed by plant roots and used in protein synthesis and nitrogen compounds, and another is coached by surface water or passing soil.

Use of nitrates and nitrites is due first to their antibacterial effect immediately and intervention in germ metabolism, inhibiting the formation of toxins, and on the other hand, forming a red color as close to fresh meat during salting process.

Use of nitrates and nitrites is allowed, but in all cases using low doses, established to ensure the desired effects and avoid the danger of harm. Toxic action is sodium nitrite, but nitrate reduction by reactions be transformed into nitrite. Given the toxicity of these substances fixed dose of

WHO for nitrate is 5 mg / kg / body for an adult and 0.2 mg / kg / body to nitrite.

The calculation for samples of 04/04 nitrous

To prove 19.1

$$\text{NITRITES} = \frac{0.01 * 200}{10 * 10} * 1000 = 20 \text{ mg/ kg}$$

For sample 20-1

$$\text{NITRIES} = \frac{0.01 * 200}{10 * 10} * 1000 = 20 \text{ mg/ kg}$$

For sample 21-1

$$\text{NITRITES} = \frac{0.01 * 200}{10 * 10} * 1000 = 20 \text{ mg/ kg}$$

For sample 22-1

$$\text{NITRITES} = \frac{0.01 * 200}{10 * 10} * 1000 = 20 \text{ mg/ kg}$$

For sample 23-1

$$\text{NITRITES} = \frac{0.01 * 200}{10 * 10} * 1000 = 20 \text{ mg/ kg}$$

For sample 24-1

$$\text{NITRITES} = \frac{0.01 * 200}{10 * 10} * 1000 = 20 \text{ mg/ kg}$$

For sample 25-1

$$\text{NITRITES} = \frac{0.01 * 200}{10 * 10} * 1000 = 20 \text{ mg/ kg}$$

For sample 26-1

$$\text{NITRITES} = \frac{0.0095 * 200}{10 * 10} * 1000 = 19 \text{ mg/ kg}$$

Calculation for nitrite samples of 14/04

For sample 19-2

$$\text{NITRITES} = \frac{0.01 * 200}{10 * 10} * 1000 = 20 \text{ mg/ kg}$$

For sample 20-2

$$\text{NITRITES} = \frac{0.01 * 200}{10 * 10} * 1000 = 20 \text{ mg/ kg}$$

For sample 21-2

$$\text{NITRITES} = \frac{0.01 * 200}{10 * 10} * 1000 = 20 \text{ mg/ kg}$$

For sample 22-2

$$\text{NITRITES} = \frac{0.01 * 200}{10 * 10} * 1000 = 20 \text{ mg/ kg}$$

For sample 23-2

$$\text{NITRITES} = \frac{0.01 * 200}{10 * 10} * 1000 = 20 \text{ mg/ kg}$$

For sample 24-2

$$\text{NITRITES} = \frac{0.01 * 200}{10 * 10} * 1000 = 20 \text{ mg/ kg}$$

For sample 25-2

$$\text{NITRITES} = \frac{0.0095 * 200}{10 * 10} * 1000 = 19 \text{ mg/ kg}$$

For sample 26-2

$$\text{NITRITES} = \frac{0.01 * 200}{10 * 10} * 1000 = 20 \text{ mg/ kg}$$

Measurement uncertainty ESTIMATION of PI-04
DETERMINATION nitrites MEASURING: nitrite content;
MEASUREMENT UNIT g/1000g

sample: 19 to 26: sausage commercial

a) Uncertainty type A

The mean humidity is:

$$\bar{x}_1 = 19,875 \text{ mg/kg}$$

$$\bar{x}_2 = 19,875 \text{ mg/kg}$$

$$\text{Standard deviation: } S = \sqrt{\frac{\sum_{i=1}^8 (x_i - \bar{x})^2}{8 - 1}}$$

$$S_1 = 0,060381 \%$$

$$S_2 = 0,060381 \%$$

Calculation of the relative uncertainty of type A: $u_{rA} =$

$$\frac{u_{xi}}{|x_i|} = \frac{S}{x} = 0,0303$$

$$u_{rA1} = 0,0303$$

$$u_{rA}^2 = 0,918 \times 10^{-3}$$

$$u_{rA2} = 0,0303$$

$$u_{rA}^2 = 0,918 \times 10^{-3}$$

b). Type B uncertainty

b1) EC Analytical balance

Operating: 10 g weighing expanded uncertainty: $U = 0.05$ g for $k = 2$ and $P = 95\%$.

Calculation composite uncertainty: $u_{cB1} = \frac{U}{k} = \frac{0,05 \text{ g}}{2} = 0,025 \text{ g}$

Calculation of the relative uncertainty: $u_{rB1} =$

$$\frac{u_{cB1}}{x} = \frac{0,025 \text{ g}}{10 \text{ g}} = 2,5 \times 10^{-3}$$

$$u_{rB1}^2 = 6,25 \times 10^{-6}$$

b2) CE pipette Nr. 404-079/2007

Operating: pipetting 10ml expanded uncertainty: $U = 0.1 \times 10^{-2}$ ml for $k = 2$ and $P = 95\%$.

Uncertainty Calculation compound: $u_{cB2} = \frac{U}{k} = \frac{0,1 \times 10^{-2} \text{ ml}}{2} = 0,05 \times 10^{-2} \text{ ml}$

Calculation of the relative uncertainty: $u_{rB2} =$

$$\frac{u_{cB2}}{x} = \frac{0,05 \times 10^{-2} \text{ ml}}{10 \text{ ml}} = 0,5 \times 10^{-4}$$

$$u_{rB2}^2 = 2,5 \times 10^{-9}$$

b3) volumetric flask - as specified by the producer

Operating: 200 ml measurement expanded uncertainty: $U = 0.16 \times 10^{-2}$ ml for $k = 2$ and $P = 95\%$.

Calculation of compound uncertainty: $u_{cB3} =$

$$\frac{U}{k} = \frac{0,16 \times 10^{-2} \text{ ml}}{2} = 0,08 \times 10^{-2} \text{ ml}$$

Calculation of the relative uncertainty : $u_{rB3} =$

$$\frac{u_{cB3}}{x} = \frac{0,08 \times 10^{-2} \text{ ml}}{200 \text{ ml}} = 0,4 \times 10^{-5}$$

$$u_{rB3}^2 = 0,16 \times 10^{-8}$$

b4) Device Spectrophotometer - as specified by the producer

Operating: UV measurement. Expanded uncertainty: $U = 0.7 \times 10^{-2}$ ml for $k = 2$ and $P = 95\%$.

Calculation of compound uncertainty: $u_{cB4} =$

$$\frac{U}{k} = \frac{0,7 \times 10^{-2} \text{ ml}}{2} = 0,35 \times 10^{-2} \text{ ml}$$

Calculation of the relative uncertainty: $u_{rB4} =$

$$\frac{u_{cB2}}{\bar{x}} = \frac{0,35 \times 10^{-2} \text{ ml}}{19,875} = 0,017 \times 10^{-2}$$

$$u_{rB2}^2 = 0,0289 \times 10^{-6}$$

$$u_{rB}^2 = \sum_{i=1}^3 u_{rBi}^2 = 6,25 \times 10^{-6} + 2,5 \times 10^{-9} + 0,16 \times 10^{-8} + 0,0289 \times 10^{-6}$$

$$u_{rB}^2 = 6,283 \times 10^{-6}$$

$$\left[\frac{u_{c(y)}}{|y|} \right]^2 = u_{rA}^2 + u_{rB}^2$$

$$u_{c(y)} = |y| \times \sqrt{u_{rA}^2 + u_{rB}^2}$$

$$|y| = 19,875$$

Combined uncertainty:

$$u_{c(y)} = 19,875 \times \sqrt{0,918 \times 10^{-3} + 6,283 \times 10^{-6}}$$

$$u_{c(y)} = 0,602$$

Expanded uncertainty $k=2$, $P=95\%$ $U = k \times u_{c(y)} = 2 \times 0,602 = 1,204$

Result: $Y = \bar{x} \pm U$

$Y = 20 \pm 1.204$ mg nitrite / kg product

CONCLUSIONS

Determinations were made of the content of nitrites and nitrates in terms of repeatability and reproducibility in animal matrices "paprika salami - Trade and vegetable matrix" apples - Golden Romanian "absolutely necessary in order to validate the method for determination of nitrites in matrix of animal origin and of determination of nitrites and nitrates in the matrix of plant origin.

For the matrix of animal origin, the average content of nitrate resulted in the matrix of animal origin is: 19.875 mg / kg.

For determination of nitrite in animal matrix were calculated following types of uncertainties:

$$\text{Uncertainty type A: } u_{rA}^2 = 0,918 \times 10^{-3}$$

$$\text{Uncertainty type B: } u_{rB}^2 = 7,0181 \times 10^{-6}$$

And expanded uncertainty:

$$U = k \times u_{c(y)} = 2 \times 0,604 = 1,208$$

The resulting value for the real content of nitrites in animal matrix is:

$$Y = 19.875 \pm 1.208 \text{ mg nitrite / kg product}$$

Future research should consider the following:

- Periodic verification of calibration curves;
- The quality determinations by analyzing the concentrations of the calibration curve before each set of determinations;
- It is necessary to determine the recovery coefficient, to quantify the losses they cause sample preparation;

Acknowledgments

This work is a part of a research project, we thank for ICAR laboratory from USAMV Cluj, because a lot of analyses we made there.

REFERENCES

1. Banu C., Preda N., Vasu S.S., 1982 – *Produsele alimentare și inocuitatea lor*. Edit. Tehnică, București, 216-245;
2. Bibicu Miruna, 1994 – *Cercetări metodologice privind determinarea nitraților și nitriților din țesuturi vegetale și nivelul de acumulare în produsele horticoale*. Teză de doctorat, București;
3. Bibicu Miruna, Mărgineanu Liana, 1997 – *Poluarea cu nitrați, factor respectiv în comercializarea legumelor de seră*. Hortinform 1/53;
4. Boor Gabriela, Alexandrescu Adriana, 1977 – *Influența nivelului de fertilizare asupra acumulărilor de nitrați și nitriți la salată și gulioare și posibilitățile de reducere a acestora*. Hortinform 12/64;
5. Derache, R. si colab., 1986 - *Toxicologie et sécurité des aliments*, Edit. Technique et Documentation- Lavoisier, Paris, 281-298;
6. Green, L. C., D. A. Wagner, J. Glogowski, P. L. Skipper, J. S. Wishnok, and S. R. Tannenbaum. 1982. *Analysis of nitrate and [15N] nitrate in biological fluids*. Anal. Biochem. 126:131–138.
7. I.Onyesom, P. N. Okoh, *British Journal of Nutrition*, 96, 902–905, (2006).
8. J. Susin, V. Kmecl, A. Gregorcic, *Food Addit. Contam.*, 23(4), 385390, (2006). Association of Official Analytical Chemists, Official Methods of Analysis, 13th ed., pp. 653–664. Benjamin Franklin Station, C: Association of Official Analytical Chemists, 1975
9. Michael Wootton, Siu H. Kok, Ken A. Buckle, (2006) *Determination of nitrite and nitrate levels in meat and vegetable products by high performance liquid chromatography*, School of Food Technology, The University of New South Wales, P.O. Box 1, Kensington, New South Wales 2033, Australia, [Volume 36 Issue 4](#), Pages 297-304.

***2006 – Colecția de Standarde editată de IRS până în 2006