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Comparison of Two Nitrite UV-VIS Spectrophotometric Analysis Methods in Meat Processed Product

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Abstract: *In most countries, the use of nitrite in meat processed products either ingoing or the residual amount, is regulated by law. This regulation is continuously updated reflecting the newest and most relevant data after better understanding nitrite's potential harmful effects and its transformation pathways in organisms. Sodium nitrite added to meat products is partly converted to the heat stable NO-myoglobin and to nitrate by oxidation, acting this way as an antioxidant. These biochemical red ox reactions are not fully understood because they depend on many variables in meat matrixes. Nitrite residual content is limited at lower than 100 mg/kg product for most of the meat processed products, but because of the abovementioned biochemical often simultaneous reactions, its monitoring should be continuous based on a scientific sampling methodology and accurate methods of analysis. This paper aims to compare two UV-Vis spectrophotometric methods against the reference ISO method for nitrite analysis to recommend their use in specific cases. These methods mainly differ in the nitrite extraction procedures from the meat matrices. The SF UV-Vis AOAC method based on nitrite extraction in natural meat sample conditions and the method proposed by Merino L. in alkaline conditions are compared to the ISO method by plotting the interaction graph of the data obtained. The results obtained are satisfactory and the author recommends the Merino method when nitrate is to be analyzed in the same sample, otherwise, the AOAC method would be the choice as not much sample handling is required.*

1. INTRODUCTION

It is estimated that about 5% of NO_2^- exposure to human organisms results from its use as a food additive in meat and meat products (Zhang et al., 2023), while consumption of vegetables is responsible for approximately 85% of nitrate exposure and 80% of nitrite exposure (Zhang et al.,2023; Salehzadeh et al., 2020). It is estimated that about 25% of ingested nitrate is secreted in human saliva, of which about 20% is reduced to nitrite, i.e., about 5% of the overall dose of nitrate, clearly establishing mouth saliva as a major site of nitrite production in the body. (Merino et al., 2016). The remaining part of nitrite exposure to human organisms comes from polluted drinking water from nitrate use as fertilizer in agriculture.

Although these exposure ratios mentioned above may be only approximate, they demonstrate that by nitrate reduction, vegetables and water are the two major sources of nitrite exposure in humans followed by nitrite use as an additive in meat products (Zhang et al., 2023).

Pegg and Honikel (2015) who have studied nitrite and nitrate behavior in meat products in detail, also supported these findings. They have concluded that the intake of curing agents (nitrate and nitrite) from meat products in the daily diet is minor (only a few percent) in comparison with other foods.

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However, considering all of these different contributors, the total nitrite exposure to human organisms is not so easily estimated. This fact leads to the suggestion that a total daily diet should be undertaken for customers to have an overall estimation of all the potential exposure routes of the human organism to this chemical in every possible matrix, meat products included. But why is it so important to accurately measure the nitrite residual content in meat processed products? This importance derives primarily because of the different potential routes of nitrite in the organism as previously mentioned, and secondly, because there is a great concern about using chemicals in food, NO_2^- included. Whatever the source of nitrite entering the route in the organism is, some health risks are often correlated with its excessive consumption. The main health problem is associated with N-nitrosamine production. N-nitrosamines constitute a family of potent carcinogens that are readily formed from a diverse set of nitrogen-containing compounds and nitrite and its derivatives. In meat, they are produced by the reaction of secondary amines with nitrite at elevated temperatures (Pegg & Honikel, 2015). Although their research on this topic confirms that due to the exceptionally low levels of secondary amines in fresh meat, the low ingoing nitrite concentration, and the relatively high pH value (5- 6.5) usual for cooked meat products, the risk for nitrosamine formation during thermal processing is very low (Pegg & Honikel, 2015). However, further investigation is needed into the contributions of consuming meat or meat products treated with nitrite to cancer risks in humans (Zhang et al., 2023).

Despite this controversial dispute relating to the role of nitrite in human organisms, there are more factors to consider, all of them reinforcing the idea of an accurate, simple, fast, and reliable analytical method of NO_2 residual amount in meat matrices. For example, NO_2 is different from other food additives, especially in meat processed products, with a characteristic variable time degradation pattern dependent on storage time, pH, temperature, nature, and concentration of reagents (Zanardi et al., 2002), packaging mode, type of product, other additives present, etc. These suggest that the experimental conditions must be carefully controlled (Zanardi et al., 2002). To conclude with these arguments, it is the responsibility of official control laboratories to monitor and keep under continuous control the residual amount of nitrite by evaluating it accurately and through the production and distribution chain. It is, therefore crucial to have accurate, repeatable, linear, and sensitive analytical methods specifically apt for cured meat products (Zanardi et al., 2002).

There are many analytical methods for NO_2 determination in meat matrices. Although spectroscopic methods are by far the most widely used for nitrite determination in food products, other methods have been reported in the literature such as HPLC, Chemiluminescence method, ion chromatography, capillary electrophoresis, differential pulse voltammetry, etc. (Della Betta et al., 2016; Scheeren et al., 2013). In general, four performance characteristics can be considered the main factors in deciding on the most suitable analytical method for nitrite/nitrate. These are selectivity, limit of detection, precision, and bias. Other factors such as speed, cost and safety, which are not directly related to the accuracy of analytical results, should also be considered by the analyst in the final selection of a method (Merino et al., 2017). Studies that compare the efficiency of the methods used for the determination of food additives, nitrite and nitrate in meat products, in particular, bring a significant contribution to the food industry. The search for the simplest, fastest, and most effective method remains a constant source of research in this field (Scheeren et al., 2013). Using a fast, reliable, and accurate method is also needed in the Albanian official laboratories which still lack the updated sophisticated equipment for additives analysis, nitrite and nitrate included. The use of different spectrophotometric methods for nitrite determination makes it necessary to estimate the validity of each of them because they constitute the first choice to be selected for use in the Albanian control laboratory. Until some years ago

the visual colorimetric method was used in Albanian official control labs, based on the complex of nitrite extracted from meat products with water and pink color development by Griess reagent. Recently AOAC method has been preferred to be used by official laboratories but the need to analyze simultaneously nitrate content in meat products except for nitrite has led to other alternatives for both these chemical analyses. Merino (2009) has proposed an interesting method for this purpose.

This paper aims to evaluate two SF UV-Vis analytical methods of nitrite ions determination in meat processed products as a contributor to total nitrite burden in human organisms. The AOAC method is simple, with fewer preparation and clarification steps, but the long extraction time (2h) at a relatively elevated temperature (80°C), leads probably to nitrite loss. The other recommended method validated by Merino (2009) is based on nitrite determination after extraction and clarification steps followed by complex measurement in alkaline conditions. Both these methods are compared to the reference ISO method which requires more reagents, and more sample preparation steps being laborious and time-consuming (Della Betta et al., 2016).

2. MATERIALS AND METHOD

2.1. Samples

The sample of a dry cooked meat processed product has been taken from the daily production of a meat processing company in Albania and immediately analyzed. Only sodium nitrite as a formulation named *Nitrisal* has been added. The sample divided into three independent units, is thoroughly ground to pass through 4mm diameter sieves. From each unit, analytical portions have been taken and simultaneously analyzed according to two recommended methods compared to the reference ISO method.

2.2. Reagents

All the reagents used in each method are of analytical grade. Sodium nitrite analytical reagent VWR CHEMICALS is used to prepare the standard solutions to plot the calibration curves. All the stock, intermediate and working standards were prepared in distilled water according to each method studied.

2.3. Apparatus

Except for ordinary laboratory equipment and glassware, a single-beam 6405 UV Vis Spectrophotometer JENWAY with a 1cm glass cuvette was used.

2.4. Methods

Two recommended methods are according to:

- Nitrite in cured meat, AOAC official method 973.31, 16thedition
- Development and validation of a method for determination of Residual Nitrite/Nitrate in Foodstuffs and Water after Zn reduction, Food Analytical Methods (Merino, 2009).

The AOAC method is based on the extraction of nitrite with hot water at 80°C for 2h, followed by filtering and by adding successively Color reagent 1 (Sulphanilic acid in acetic acid 15%) and

Color reagent 2 (alpha naphthylethylenediamine dihydrochloride in acetic acid 15%) to a pink color stable complex. Measurement of the complex absorbance in λ = 540nm and comparing to the calibration curve enable nitrite determination expressed in mg/kg product.

Spectrophotometric UV-Vis determination according to *Merino* is based on nitrite extraction with warm water at 60°C followed by clarification deproteination step, centrifugation, alcalinisation by ammonia buffer at pH=11 followed by diazotization and coupling, and color development by adding Color reagent 1 (Sulphanilic acid in hydrochloric acid 18%) and Color reagent 2 (alpha naphthylethylenediamine dihydrochloride in water) to a pink stable complex. The absorbance of the complex is again measured at 540nm.

Both methods are compared to each other and to SF UV-Vis reference method ISO 2918:1975, which is similar to the method proposed by Merino (2009) regarding sample handling such as deproteination, and nitrite extraction except for the alcalinisation step by ammonia buffer (pH=11). In reference ISO method conditions of analysis are acidic.

Two kinds of analysis have been performed for each recommended method:
- Nitrite standard solutions - *for linearity, method sensitivity and limit of detection.*
- Sample without/with standard solution added (spiked sample)-*for recovery and matrix effect.*

3. RESULTS AND DISCUSSION

3.1. Linearity

Two calibration curves have been plotted by using nitrite standard solutions as described in detail in each colorimetric method. Four working solutions range between 0.2-0.8mg NO_2/L expressed as NO_2^- ion served to plot the calibration curves for each method. Three replicate analyses have been made.

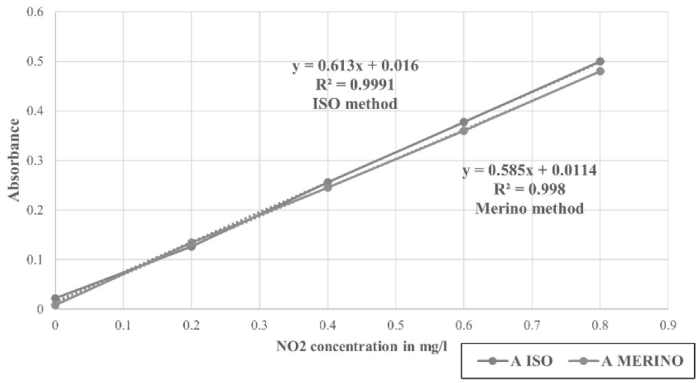

Figure 1. Calibration graphs according to the ISO method and Merino
Source: Own research

Both calibration curves are compared to each other to reference the ISO method, in order to evaluate the linearity range, sensitivity and limit of detection. Figures 1, 2, and 3 show the calibration curves for each method used.

From the regression analysis, it can be seen that the slope of calibration curves for both recommended methods compared to the reference ISO method, show quite a low difference between each other, the highest in the reference ISO method and the lowest in the AOAC method. Table 1

provides the equations of the calibration curves for the two methods compared to the ISO method. Coefficients of the correlation are given as well in Table 1.

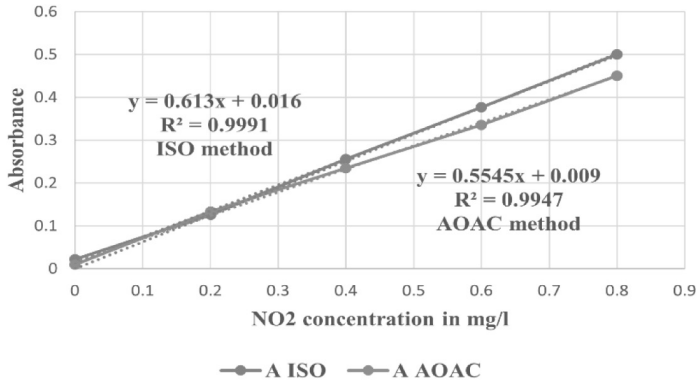

Figure 2. Calibration graphs according to ISO and AOAC method

Source: Own research

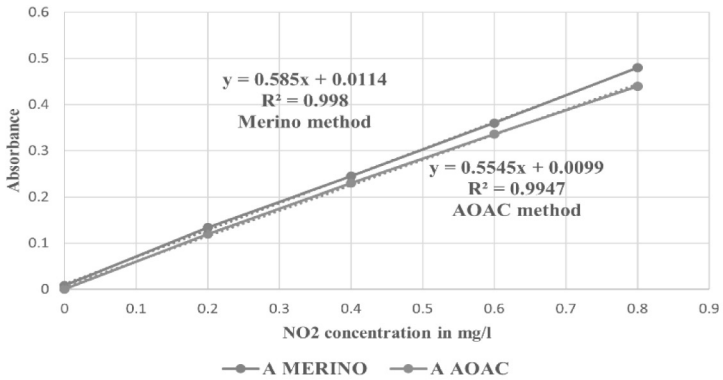

Figure 3. Calibration graphs according to Merino and AOAC method

Source: Own research

3.2. Recovery

Fiddler and Fox (1978) suggest that spiked meat samples could not be used in comparing nitrite analysis methods because results are misleading. Three levels of nitrite standard solutions were used in 3 blank sample aliquots to evaluate recovery in different concentration nitrite levels.

We added 0.5, 0.75, and 1 ml respectively from the 1µg/ml working nitrite standard solution in three blank meat sample solutions. Recovery for the three levels resulted in the range of 60-90% for the AOAC method, and 90-110 % for the ISO method, while Merino (2009) reported in his study an average recovery of 102% for meat products. The recovery values are given in Table 1.

Table 1. Comparison of the three methods

Variables	Equation	R^2	Recovery %
Merino Method	y=0.5867x+0.011	0.998	102*
AOAC Method	y=0.5545x+0.010	0.995	60-90
ISO Method	y=0.6126x+0.016	0.999	90-110

** for meat products (Merino, 2009)*

Source: Own research

3.3. Matrix Effect

Merino (2009) shows that the meat matrix does not present any bias effect on the calibration graph, at least for nitrite analysis. However, we evaluated the matrix effect on blank meat samples spiked with nitrite solution for both methods. A volume of nitrite standard solution was added to the blank meat ground sample during the extraction process. The procedure then followed the same as for sample analysis.

Table 2 shows the calibration curve parameters for *Merino* and AOAC methods plotted by using nitrite standard solutions as well as for the fortified blank meat sample by adding nitrite standard solution.

Table 2. Comparison of matrix effect on calibration curves of two recommended methods

NO_2 mg/kg	Slope of calibration curve According to Merino	Slope of calibration curve According to AOAC
Nitrite standard solution	0.585	0.555
Blank sample with nitrite standard solution added	0.531	0.421

Source: Own research

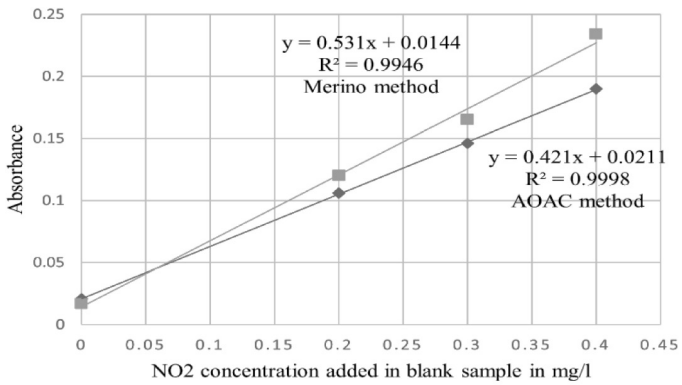

Figure 4. Comparison of matrix effect for AOAC and Merino method

Source: Own research

The data presented in Table 2 show that there is a more considerable matrix effect by using the AOAC method compared to the Merino method. This may probably be explained by the long extraction time in higher temperatures used in the AOAC method which as the literature says may lead to nitrite loss from acidic pH meat samples (Sen et al., 1979) but maybe even from the matrix influence that is more considerable in AOAC method by the lack of sample processing steps such as deproteination and extract centrifugation which avoid matrix interference but in the same time requires more excessive work to do by the analyst.

3.4. Limit of Detection

This parameter for both recommended methods is estimated from the intercept of the calibration curves. The intercept values are presented in Table 1. It can be seen that as there is no considerable difference in the intercept values as well as in the calibration curves slopes for both recommended analytical methods, the Limit of Detection for both methods results comparable at 4.3mg/kg for the Merino method and 4.5 mg/kg for the AOAC method.

4. CONCLUSION

The two SF UV Vis methods for nitrite determination in meat products gave satisfactory results regarding linearity range, sensitivity, limit of detection and recovery.

The AOAC method is quite simple to use, with no excessive reagents for protein precipitation without many sample preparation steps, but the long time of 2h at 80°C leads to eventual nitrite loss, which can be noticed in the lower recovery. The matrix effect is more evident in this method compared to the method proposed and validated by Merino.

The method according to Merino generally presented the same satisfactory results as AOAC, but it needs more reagents and sample preparation, and it requires attentive procedure, especially regarding the pH of ammonia buffer to give reliable and persistent results. It is worth using the Merino method when NO_3^- ion determination is required as well as following NO_2^- ion analysis in the same sample. Recovery showed better results compared to Merino maybe because the exact value of pH=11.0 of the ammonia buffer is important to give reliable and consistent results.

Anyway, for routine analysis the AOAC method being simple, with no hard work or excessive analysis steps, can be recommended.

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