COMPARISON OF DIFFERENT METHODS FOR NITRITE AND NITRATE DETERMINATION IN MEAT PRODUCTS

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Abstract – It is very important to have a control regarding the amount of food preservatives in the meat because of their risk to form N-nitrosamines. Studies which compare the efficiency of the methods used for the determination of these substances bring a significant contribution to the food industries. The search for the simplest, faster and more effective method is a constant source of research. The objective of this study was to compare two different methods (chromatography and chemioluminescence determination after reduction to nitric oxide) for nitrite and nitrate measurements. For this purpose, nitrite and nitrate were extracted with hot water from ham samples and determined by HPLC with reversed phase columns (C18 and Acclaim PA) and also by NO Analyzer. The results obtained from both methods were plotted in an interaction graph to compare the values. The results showed that quantification of nitrites and nitrates ions through the analysis of nitric oxide production has proven to be the quickest and most sensitive method.

Key Words – Nitrate, Nitrite, Determination, Comparison

I. INTRODUCTION

The potassium and sodium salts of nitrite and nitrate are used in curing mixtures for meat to inhibit microorganism growth, to develop and fix the color and characteristic flavors and to retard the lipid oxidation. Several studies have reported the potential toxicity of excess nitrite to the human body [1,2]. The residual nitrite can react with secondary amines and form carcinogenic compounds [1,3,4]. Therefore, taking into account the wide use of these additives in processed meat and the high consumption of these products, it is very important to have a control regarding the amount of these substances in food. Classical methods [5] commonly used to determine the nitrite and nitrate involve laborious colorimetric measurements, and suffer interference from the matrix [6]. Then, simpler, faster, more sensitive and selective methods are being investigated, including spectroscopic determination after enzymatic reduction [7], differential pulse voltammetry [8], capillary electrophoresis [9], liquid chromatography [6] and chemioluminescence determination after reduction to nitric oxide [10].

Because food preservatives are important for the technological and also to public health point of view, studies which compare the efficiency of the methods used for the determination of these substances in meat products bring a significant contribution to the food industries. The search for the simplest, faster and more effective method is a constant source of research in this field. The objective of this study was to compare two different methods (chromatography and chemioluminescence determination after reduction to nitric oxide) for nitrite and nitrate measurements.

II. MATERIALS AND METHODS

Samples - Ham samples bought at a local market were cut in small pieces, frozen with $N_{2(l)}$ and triturated in a blender. The samples were kept at -20°C until the extraction moment.

Extraction - For the extraction, 10 g of sample were weighed and 20 mL of hot (50-60°C) high purity water and 1 g of activated charcoal were added. The mixture was kept under agitation (magnetic stirrer - 900 RPM) during 15 minutes at 50-60°C. After that, the mixture was filtered through filter paper (Whatman #1) and through a membrane filter (0.4 μm) to a centrifugation tube. Then, 5 mL of acetonitrile was added to precipitate the peptides which could interfere in the High Performance Liquid Chromatography (HPLC) analysis and this mixture was centrifuged (10 min, 5000 rpm). The supernatant

was collected and stored at -20°C. In order to check the amount of nitrate and nitrite recovered and if there was any interference, the extraction was performed (1) in the samples, (2) adding a known amount of standard in the sample (15 mg . L^{-1}), and (3) without sample but with a known amount of standard (15 mg . L^{-1}).

Quantification – Two methods were evaluated. The first one was based on liquid chromatography analysis - adapted from Ferreira *et al.* [6], where a solution of 0.01M n-octylamine/5 mM of tetrabutylammonium hidrogenosulphate (pH 6.5) was eluted through a reversed phase column (C18 and Acclaim PA, both with dimensions of 5.0 μm, 4.6x250 mm were tested) in an isocratic elution (flow rate: 1 mL/min). The volume of sample injected was 20 μL, and the detection was performed spectrophotometrically (λ = 208 nm). The second method evaluated was standardized by Samouilov *et al.* [10] and consists in the nitrite and nitrate determination by NO Analyzer (NOA), where the sample was injected in a reaction chamber. In this measurement, nitrite was decomposed to nitric oxide (NO) with iodide, while "nitrate + nitrite" was decomposed to NO with vanadium (III) chloride. The NO generated was transported by the gas flow until the detector, where was determined by chemioluminescence (NO reacts with ozone, forming excited state NO_2 which emits light).

The results were compared graphically using Microsoft Office Excel 2010.

III. RESULTS AND DISCUSSION

The amount of nitrate and nitrite found in market hams are shown in Table 1. It is possible to observe that the amount of nitrite and nitrate intentionally added at the beginning of the extraction process (H+STD and STD) had a good recuperation when NOA method was used. This shows that the chosen extraction method was efficient to extract nitrates and nitrites from the samples. However, the recuperation of nitrate using the chromatographic analysis (both columns) was lower than 15 mg . L^{-1}, indicating that this method is less sensible when compared to the NO Analyzer.

Table 1 Nitrite and nitrate concentration found in ham samples analyzed by HPLC and NO Analyzer. H: ham extracts; H+S: extracts of ham added a known amount of standard; and STD: extract without sample but with a known amount of standard (*mg . L^{-1}).

Samples	$NaNO_2$ (mg . Kg^{-1})			$NaNO_3$ (mg . Kg^{-1})		
	HPLC Acclaim PA	HPLC C18	NOA	HPLC Acclaim PA	HPLC C18	NOA
H1	6.91	1.38	3.15	6.24	8.27	6.77
H2	5.55	3.87	2.79	5.32	4.40	4.34
H3	9.88	3.96	4.11	3.83	3.29	3.20
H+S1	20.93	14.64	17.13	15.45	16.86	19.35
H+S2	24.54	12.97	16.00	19.16	22.06	21.60
H+S3	25.76	9.74	15.62	20.63	19.60	21.46
STD*	13.87	14.93	15.65	11.62	11.40	14.31

Table 2 shows the comparison between the three methods studied. By analyzing the data, is possible to see that when the nitrite results by HPLC-Column Acclaim PA analysis are related to other methods, the slope (a) values of the straight lines is further than 1.0 than the slope values obtained from HPLC-C18 Column x NOA line. How further the slope is from 1.0, less similar are the related data between two methods. This indicate that the results obtained by HPLC - Acclaim PA column method had different values of nitrite compared to other methods. However, when nitrate is analyzed, the results of the three methods are similar. Therefore, it is preferable do not use the HPLC method with Column Acclaim PA for the quantification of nitrites.

Table 2 Linear equation (y=ax+b) obtained when the nitrite and nitrate by different methods (HPLC - C18 column, HPLC- Acclaim PA column and NOA) are compared.

		Comparison		
		HPLC (C18 *vs.* Acclaim PA)	HPLC (C18) *vs.* NOA	HPLC (AcclaimPA) *vs.* NOA
Nitrite	a	0,493	1,149	0,722
	b	1,203	0,539	0,451
	R^2	0,5356	0,9069	0,7855
Nitrate	a	1,058	1,077	1,169
	b	-0,1642	-0,209	-0,738
	R^2	0,9577	0,9626	0,9708

This occurs, probably, because the peak of nitrite is not completely separated from the peak of another compound present in the sample (possibly peptides) and this cause a problem to the peak integration (Figure 1).

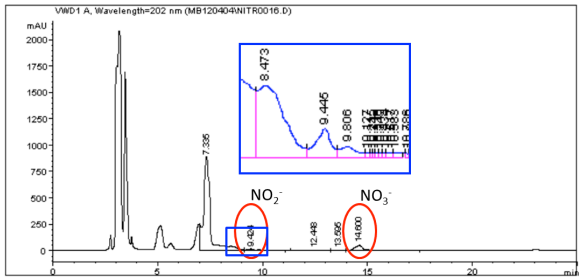

Figure 1 Chromatogram obtained with the Column Acclaim PA for a ham sample.

The results obtained in this study contribute to the choice of a simple, effective and fast for the determination of nitrite and nitrate in meat products.

This is the first study where the chromatographic and the chemioluminescence method are compared. The results obtained on this work contribute to the choice of a simpler and more sensitive method for the determination of food preservatives in meat products.

IV. CONCLUSION

The chosen method of extraction proved to be simple and efficient to extract nitrates and nitrites of ham. The quantification of nitrites and nitrates ions through the analysis of nitric oxide production has proven to be the quickest and most sensitive method.

More research should be conducted to compare other methods of nitrites and nitrates determination and to contribute to the best choice of the most effective method and the one which better fits the working conditions.

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