# INFLUENCE OF SULPHITES PRESENCE IN NI TRITE AND NITRATE COLORIMETRIC DETERMINATION IN CURED MEAT PRODUCTS

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***Abstract***

*Chemicals currently used as preservatives in meat products are well known for their specific role and for their potential adverse effect on organisms if they are used in higher amount than regulated by law. Amongst these chemicals sodium/potassium nitrite (E249, E250) and sodium /potassium nitrate (E251, E252) are widely used, and both represent a class of most common preservatives used since a long time ago, in meat products. However, they are not the only chemicals to be used as allowable additives in food products. Sulfites in general such as metabisulphites E223, source of Sulphur dioxide limited in use only in some meat products, also present an inhibitive role against microorganism growth in meat matrices, thus acting as a preservative and antioxidant compound. It also poses some health concerns to allergic or asthmatic people. The use of all these chemicals in food products is regulated by Albania Directive No.16, dated 29.8.2011" Food Additives other than colorants and sweeteners", based on EU Directives, setting allowances to be monitored and kept under official control. However, research has confirmed the presence of nitrite, nitrate and sulfur dioxide even when they have not been intentionally used but deriving from external sources, microbial activity or redox reaction in meat matrices. The objective of this paper is to show the influence of incidental presence or intentional use of $SO_2$ derived from sulfites, in the nitrite and nitrate analysis. The findings indicate that the presence of $SO_2$ significantly diminishes the coloration of the diazo coupling complex formed in the process of nitrite/nitrate colorimetric determination. This highlights the need to explore alternative specific techniques for detecting these compounds in meat products in the presence of $SO_2$, as its presence may result in inaccurate negative outcomes.*

***Key words:*** *preservatives, nitrite, nitrate, sulfite, cured meat products.*

## *Përmbledhje*

*Kimikatet që përdoren aktualisht si konservantë apo antioksidantë në produktet e mishit, njihen për rolin e tyre specifik por njëkohësisht edhe për ndikimin e dëmshëm në shëndetin e njeriut kur ato përdoren mbi sasitë e rekomandura. Midis këtyre kimikateve të përdorura, nitriti i natriumit/kaliumit (E249, E250) si dhe nitrati i natriumit/kaliumit (E251, E252) përdoren më gjerësisht dhe të dy përfaqësojnë një klasë konservantësh të përdorur prej kohësh në shumë produkte ushqimore, përfshirë produktet e përpunara të mishit. Sidoqoftë nuk janë të vetmit kimikate të përdorura si shtesa te lejueshme në produktet ushqimore. Sulfitet në përgjithësi, si p.sh metabisulfiti i natriumit/kaliumit (E 223), burim i dioksidit të squfurit i kufizuar në përdorim për vetëm disa produkte ushqimore, gjithashtu kanë një rol pengues në zhvillimin e mikroorganizmave, duke vepruar kështu si konservant dhe antioksidant njëherësh. Sulfitet gjithashtu paraqesin shqetësim për shëndetin sidomos në personat alergjikë apo astmatikë. Për këto arsye, përdorimi i të gjithë shtesave ushqimore rregullohet në bazë të Udhëzuesit Nr.16 datë 29.08.2011: "Aditivët ushqimorë, përvec ngjyruesve dhe ëmbëlsuesve" i cili është hartuar në përputhje me Direktivat e BE. Direktiva përcakton sasitë maksimale të lejueshme të këtyre shtesave ushqimore të cilat duhet të monitorohen dhe ti nënshtrohen kontrollit zyrtar. Sidoqoftë studimet kanë treguar që prezenca e nitrateve, nitriteve dhe sulfiteve (shprehur si $SO_2$) mund të rezultojë edhe në rastet kur këta nuk janë përdorur si aditivë por, mund të rezultojnë nga burime të jashtme, apo nga reaksionet biokimike dhe aktiviteti mikrobial në produktet ushqimore. Ky artikull ka për qëllim të paraqesë ndikimin e dioksidit të squfurit që rezulton nga prezenca e rastësishme apo nga përdorimi si aditiv, në përcaktimin e përmbajtjes së nitriteve dhe nitrateve në produktet e përpunuara të mishit. Rezultatet tregojnë që dioksidi i squfurit, pavarësisht nga burimi i tij, ka një efekt të fortë dekolorues të kompleksit di-azocoupling që formohet gjatë përcaktimit kolorimetrik të nitriteve dhe nitrative, duke sugjeruar në këtë mënyrë që metoda të tjera selective duhen përdorur për të vlerësuar përmbajtjen e nitriteve dhe/apo nitrative në produktet e përpunuara të mishit në prezencë të dioksidit të squfurit.*

***Fjalë kyçe****: konservantë, nitrite, nitrate, sulfite, produkte mishi.*

## Introduction

According to the Albanian Legislation (*Directive No16, dated 29.08.2011"Food additives, other than colorants and sweeteners"*), the limit for addition of nitrate (E 251 = $NaNO_3$, E 252 = $KNO_3$ expressed as $NaNO_3$) during meat processing is 300mg/kg for cured meat products with a residual nitrite amount lower than 250mg/kg. While the limit on the use of nitrite (E 249 = $KNO_2$, E 250 = $NaNO_2$; expressed as $NaNO_2$) in cured meat products is 150 mg/kg with a residual amount lower than 50-100 mg/kg for specific meat products. Nitrites are commonly used in dry cured meat products while nitrates are mainly used in some long shelf-life fermented meat products acting this way as nitrites reservoir. Their use as common food additives is a controversial issue widely discussed in scientific panels, considering their potential positive and negative impact on human health.

The same Directive dictates the maximum allowable content of sulfites or sulfur dioxide (*E 220=$SO_2$, E223=$Na_2S_2O_5$ expressed as $SO_2$*) at 450 mg/kg for specified meat products at a residual content lower than 10mg/kg. Sulfites are also used as additives in food products, acting as preservative and antioxidant compounds. This way their principal role is to extend the shelf life of some meat products retaining their characteristic fleshy color. When used in specific products, sulfites may occur together with nitrites. Although the positive effect of nitrite on human health has already been indicated (*Capillas. et al., 2008) (Merino. 2017*) (*Iammarino. et al., 2023*), it is still under continuous review. Nitrite and nitrate residual contents due to their dual relationship and time dependence is already well studied and understood (*Peg & Honnikel, 2014*) *(Merino, et al., 2016*).

But quite otherwise nitrite, sulfite toxicity is generally low, consequently its presence in food products is not widely discussed and given attention to, though its use poses some adverse impact to asthmatic and allergic people and leads to vitamins loss in foods (*D'Amore. et al., 2020*), (*Koricanac, et al., 2017*). When sulfites are used to meat products, it reacts with water instantly and about 50-55% of its amount (*Feiner. 2006*) can be found quantitatively as $SO_2$. Some other parts are lost in some unspecified chemical reaction in meat products and cannot be detected any longer (*D'Amore T. et al., 2020*). Because of the abovementioned facts one can hardly detect more $SO_2$ amount than its MRL. Except for its intentional use in food products, FDA specifies referring to *D'Amore T.,* that sulfites may also be considered as "incidental additives", for example, when some meat formulation (E223, E262, E330 metabisulfite, sodium acetate and citric acid) containing sulfite is added to meat (spices, wine, etc.), for longer shelf life. This is the case with cooked sausages and cooked salami as well as fermented sausages, but on condition that the final concentration is less than 10 mg/kg. The presence or detection of $SO_2$ in meat products may also happen because of microbial reduction of sulfate to $SO_2$ in fermented meat products. (*D'Amore T. et al., 2020).* It can also be present because of microbial activity towards S containing proteins. These facts suggest that level of $SO_2$ at or lower than 10mg/kg does not prove the use of sulfite in food products in meat products included. Therefore, the value of 10 mg kg−1 may be considered as the maximum residual limit (MRL). A $SO_2$ content of not more than 10 mg/kg is not considered to confirm its presence even according to our legislation (*Albanian Directive No16, 23.08.2011 on Food Additives*). Considering the importance of the use of all the food additives abovementioned to guarantee safe meat products on condition to respect their allowable maximum limits, it can be said that determining them accurately is essential for the purpose of legislation and consumer safety (*Capillas R C et al., 2011*). There are many analytical methods for $NO_2$/$NO_3$ determination in meat matrices.

Although spectrophotometric 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, flow injection analysis etc. (*Della Betta et al., 2016), (Scheeren et al., 2013) (Capillas et al., 2008*). Like nitrite, UV Vis spectrophotometric remains the most common analytical method for nitrate determination after its reduction to nitrite by using Cd or Zn in alkaline condition. This colorimetric UV Vis method for nitrite and nitrate is based on forming a diazo coupling complex by Griess reagent, whose optical density is measured in 540 nm. This method is commonly used in Albanian official food control laboratories which are in lack of sophisticated equipment to enable other instrumental methods.

On the other side the main method for sulfite determination expressed as sulfur dioxide remains optimized Monier Williams method (*AOAC official method 16$^{th}$ edition)* which is considered as a reference method. Quite otherwise colorimetric method for nitrite, this is a classic, $SO_2$ selective method based on its distillation followed by subsequent volumetric titration (*D'Amore T. et al., 2020*). The problem of the sulfite presence in some meat products leads to a suspiciously low level of nitrite presence in meat products, because of the strong interference of sulfite with nitrite determination. This fact is noticed during the nitrite analysis, and it leads to a wrong presumption that nitrite level is too low in meat products. Using reliable and accurate methods for food additives analysis such as nitrite, nitrate and sulfur dioxide included makes necessary to clarify the influence of their simultaneous presence in colorimetric determination because this technique constitutes the first choice to be selected for use in Albanian control laboratories.

The aim of this paper is to draw attention to the sulfite influence in nitrite and nitrate analysis in cured meat products by UV Vis spectrophotometric method by using Griess reagent before and after nitrate reduction to nitrite. Whichever the source of sulfite presence is, its role in nitrite determination by colorimetric method is evident and leads to suggestion that other methods should be used for nitrite or nitrate analysis in cured meat products, when sulfites are present.

**Material and method**

To show the influence of sulfur dioxide in nitrite/nitrate determination, two different samples of cured meat products-sausages- have been taken for analysis.

One of them is free from sulfites or saying it correctly, does not contain added sulfites. The other sample was formulated with an addition of a commercial mixture preparation containing (citric acid E 251, metabisulphite E 223, sodium acetate E 252). Both samples are formulated with potassium nitrite added at 100mg/kg product. The samples were analyzed for nitrite and nitrate content even though no nitrate was added. The reason is that the literature confirms that the fate of nitrate and nitrite is complex especially in meat processed matrices. Nitrate and nitrite are ions which co-exist in most foodstuffs and analysts rarely find one without the other. (Peg & Honnikel 2014). For this reason, the analysis results for both nitrites and nitrates are considered here. The only difference between the two kinds of sausages analyzed is the presence of sodium metabisulphite in the meat preparation in the second sample, which is completely absent at the first sample. Photo of the two samples is given in fig.1.

**Figure 1.** Two sample os sausages: left side sulphite free sample, right side containing sulphite

The colorimetric method based on Griess reagents after deproteinization and clarification step for nitrite determination was conducted. This method is based on diazo coupling complex formed by successive adding of *sulfanilamide* and *N-1-naphtyl-ethylenediamine dihydrochloride* reagents. It is a widely used quantitative method of a first choice regarding its simplicity, not requiring sophisticated equipment or any dangerous chemicals or specific work conditions. The same procedure was followed for nitrate determination after nitrate reduction by Zn powder as it is described in detail by *Merino, 2009*. Calibration curves for both nitrites and nitrates are given in fig.2.

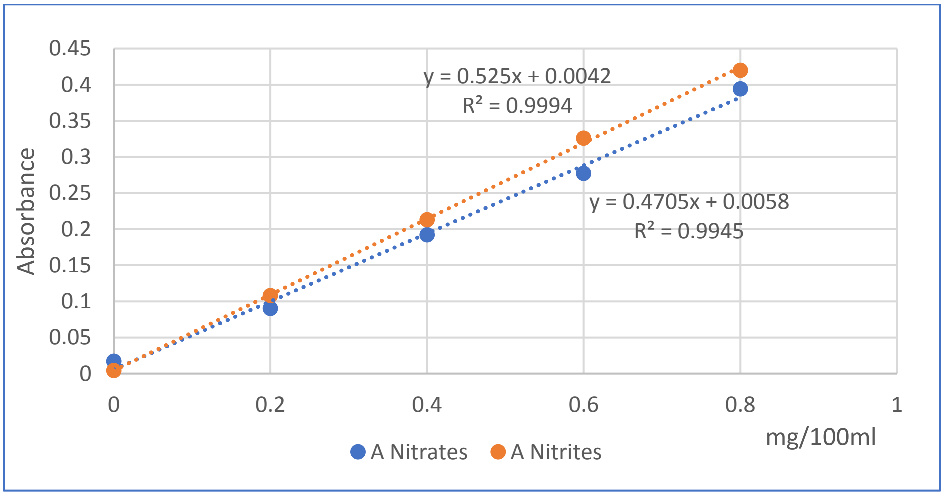


**Figure 2.** Calibration curves of nitrites and nitrates

Though in the food industry, the co-addition of two or more additives is very common, the co-treatment of sulfites/NOx is not recommended because of inactivity of their derivatives (*D'Amore et al., 2020*). Despite this, in some specific meat products can often be used some sulfite containing preparation which make necessary to assess its contribution to other additives determination such as nitrite and nitrate content. Monier-Williams method was used to determine sulfur dioxide residual amount based on distillation step followed by subsequent titration with a standardized sodium hydroxide solution. Three different sample units were used to get analytical results.

## Results and discussion

From the photo of both samples Fig.1, it can be clearly seen the effect of sulfite presence in external product appearance. The sample with sulfite shows a more like meat bright color compared to the other more brownish sample. Despite this qualitative assessment of the sulfite presence in one sample, the analysis showed quite different results relating to nitrites and nitrates contents between two sample types.

The data given in Table 1 are the average values of nitrites, nitrate, and sulfites in both samples for three samples analyzed for each product according to the analytical methods described above.

The results show that all the target compounds are well below the permissive norms as dictated by legislation. (*Albanian Directive No16, 23.08.2011 on Food Additives*). The amount of $SO_2$ at 34.12 mg/kg show that any kind of sulfite compounds may have been used though its presence is very low to predict its presence from any incidental source. While this can be said for the first sample with $SO_2$ amount at 14 mg/kg. There are some findings arguing that the level of 10mg/kg is too low to serve as a threshold value for $SO_2$ use in meat products (*D'Amore, et al., 2020).*

***Table 1.*** *The average results obtained for nitrites, nitrates, and sulfites.*

| Parameter | Sample sulfite free | Sample with sulfite | LD* |
|---|---|---|---|
| Nitrites (*expressed as mg/kg* $NaNO_2$) | 58.4 | 3.04 | 2 |
| Nitrates (*expressed as mg/kg* $KNO_3$) | 72.2 | 11.56 | 5 |
| Sulfite (*expressed as mg/kg* $SO_2$) | 14 | 34.12 | 10 |

**Limit of Detection*

Although introduction of metabisulfite into cooked meat products is occasionally practiced, because of the lack of free water available to convert metabisulfite to sulfur dioxide (*Feiner G 2006*), it happens to find its presence in some meat preparation mixtures. Just this presence of sodium sulfite acting as decolorant of azo dye complex is the cause of much lower nitrite level measured in the second sample compared to the first one even if the same initial sodium nitrite amount is used in both samples. The decrease ratio of nitrites and nitrates at approx. 85% is quite evident between the two samples.

Another fact is to be underlined. Although the residual content of sulfur dioxide on the day of testing is comparable to the maximum residue limit (MRL) (10 mg/kg) for the first sample, it leads to the confirmation of findings from different authors (*D'Amore, et al., 2020) (Feiner G 2006*), that this amount may be due to sulphate reducing bacteria present in meat products or S containing proteins. Some authors suggest that this limit of 10 mg/kg sulfite expressed as $SO_2$, which is the limit of detection of the Monier Williams method, is too low to predict weather sulfite is intentionally added when concentrations higher than 10 mg/kg are detected. However, this point is not the objective of this paper. It is widely discussed elsewhere, and it is worth investigating in depth (*D'Amore,. et al., 2020*).

Another fact is to be noticed from the data obtained. As it is already said no nitrate was used in any of samples. Different authors argue this finding. Complex matrix, pH, redox system condition, microbial activity are only some of the factors influencing the nitrite to nitrate conversion (*Pegg & Honikel, 2014), (Merino. et al., 2016*). The authors of this paper have also presented their work on this topic elsewhere, showing the influence of time and cooking methods in this inner conversion process (*Boci I. et al., 2023*). Relating to this paper it should be noted that nitrate amount obtained from spontaneous redox reactions in meat matrices, except of the factors already mentioned above, has also reflected the influence of sulfur dioxide in its final decreased residual amount by comparing both samples.

## Conclusions

Nitrites, nitrates, and sulfites are common additives used in food products, included cured meat, while sulfites are allowed only in some specific meat products. Their use is dictated by law and their residual amount should be the object of continuous control. Sulfite interferes chemically during the colorimetric determination of nitrite and nitrate in meat products, regardless of the source of its presence. While the simultaneous presence of both sulfite and these compounds is infrequent, it occurs selectively in certain types of meat products. The results obtained for nitrite in two samples with and without sulfite presence show a considerable difference between them, showing much lower levels for nitrite even in sulfite presence somewhat higher than its MRL. The same can be concluded for nitrate amount derived only from redox nitrite reactions with meat ingredients or other stimulating favorable conditions such as pH, microbial activity, etc. The results of this study suggest that other selective methods should be used for nitrite and nitrate determination to accurately estimate their final residues in those products where sulfite is intentionally used or incidentally present.

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