

EVALUATION OF THE INTAKE OF NITRATE, NITRITE, NITROSODIETHYLAMINE AND NITROSODIMETHYLAMINE BY FOOD CONSUMPTION

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Abstract

The aim of the present study was the evaluation of nitrate, nitrite, nitrosodiethylamine (NDEA) and nitrosodimethylamine (NDMA) intake through food consumption. We determined concentrations of nitrates, nitrites in 102 food samples (40 meat products, 15 the fermented cheese, 25 vegetables, 22 fruits) and the concentrations of NDEA, NDMA in 40 meat products. Nitrates and nitrites were determined using Peter-Griess method; nitrosamines were quantified by HPLC with UV detection. We designed vegetarian, vegetarian and conventional diets of about 2500 kcal/day. Based on the values found, we calculated the intake of nitrates, nitrites and nitrosamines. The obtained values fit into WHO's recommendations, except for vegetarian and conventional diet, in which the nitrate content was 3,46 respectively 1,64 times higher than the acceptable daily intake (157 mg NO₃⁻/day).

Keywords: *nitrates, nitrites, nitrosamines, food, diet*

Introduction

Natural occurrence of nitrates in plants is due to the nitrogen cycle, during which organic forms are converted by microorganisms to inorganic forms (nitrates, nitrites, ammonia) and assimilated by plants (Fytianos and Zarogiannis, 1999; Tamme et al., 2006). Nitrates and nitrites are used as additives; nitrite fixes color, contributes to the cured meat flavor, helps in the inhibition of the development of microorganisms and effectively controls rancidity by inhibiting lipid oxidation (Sindelar and Milkowski, 2012). Human exposure to N-nitroso compounds and their precursors (nitrates and nitrites), can occur through exogenous sources, such as diet, drinking water, occupation or environmental exposure, and through endogenous exposure resulting from the formation of N-nitroso compounds in the body. Legislation limits the concentrations of nitrates and nitrites in meat products; in food or consumer's body residual nitrite can form nitrosamines, compounds with carcinogenic potential (Bryan et al., 2012). The interaction between amines and nitrites may bring about a synthesis of N-nitroso compounds associated with gastric cancer (Iammarino et al., 2013). This work is a mere example of what could

be possible to ingest through three types of diets: vegetarian, vegetarian and conventional. The aim of the present study was the evaluation of nitrate, nitrite, NDEA and NDMA intake through food consumption.

Materials and Methods

Materials

The standards used are: sodium nitrite (43160-5, CAS 55-18-5), potassium nitrate (20411-0, CAS 7757-79-1), NDMA (Supelco Analytical 4-8552(1), CAS 62-75-9), NDEA (Sigma 057K1170, CAS 55-18-5). All the reagents were from Sigma Aldrich and were used as received: sulphanic acid, α -naphthylamine, cadmium powder (all of 99,9% purity), ascorbic acid, citric acid, disodium phosphate, anhydrous sodium sulfate, and dichloromethane were of analytical grade. HPLC grade acetic acid and acetonitrile were used for HPLC analysis. The size of Celite 545 particles was 0,02-0,1mm.

Equipment

JASCO V-550 UV-VIS Spectrophotometer, Shimadzu HPLC-2010 system (Shimadzu, Japan) equipped with a UV detector and a reverse-phase column (Welchrom-C18, 5 μ m, 250 \times 4,6 mm), BÜCHI rotaevaporator, magnetic stirrer IKAMAG[®]RT 15 power.

Foodstuff Analysis

Nitrates and nitrites. 102 samples of commercial food products (vegetables, fruits, processed meat, fermented cheese) were purchased from the marketplace and from a supermarket in Iasi. Each sample was analysed for nitrates, nitrites by Peter-Griess spectrophotometric method (JAOAC, 2000); calibration curves for the determination of nitrites and nitrates were achieved by the equations: ($y=0,0483x+0,0017$; $R^2=0,9977$) and ($y=0,048x-0,072$; $R^2=0,9985$). The yield of the reduction reaction, η , was calculated with Eq.1, UV evaluation of the yield of nitrate reduction to nitrite was achieved by the Eq.2 and the value obtained was 94,25%.

$$\eta = C_2 \cdot 100 / C_1 \quad [1]$$

$$y = 0,0238x + 0,0169 \quad R^2 = 0,9929 \quad [2]$$

Nitrosamines were determined only in meat products. Nitrosamines analysis (Mitacek et al. 1999) was carried as follows: (a) the minced sample was homogenized with phosphate-citrate buffer pH=6,5 and ascorbic acid and filtered, then the extract was purified on Celite 545 column, the resulted eluate was extracted with dichloromethane, the organic solution was dried with anhydrous sodium sulphate and evaporated to dryness; (b) building the calibration curves using the results of HPLC analysis on solutions of pure nitrosamines of known concentrations. HPLC conditions: injection volume 20 μ L, column temperature 25°C, eluent acetonitrile: 1% acetic acid in water (20:80, v/v), at 0,8 mL/min, UV detection. Retention time for NDMA and NDEA was 4,56 min and 9,11 min,

respectively; (c) Sample analysis by HPLC, using similar conditions, except for introduction of a gradient elution after 10 min, raising the acetonitrile content from 20 % to 90% during 30 min. They were obtained by HPLC analysis of NDEA and NDMA standard solutions (Fig.1).

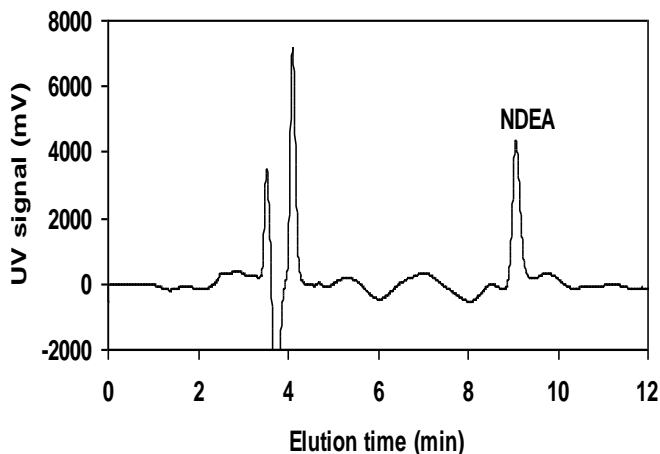
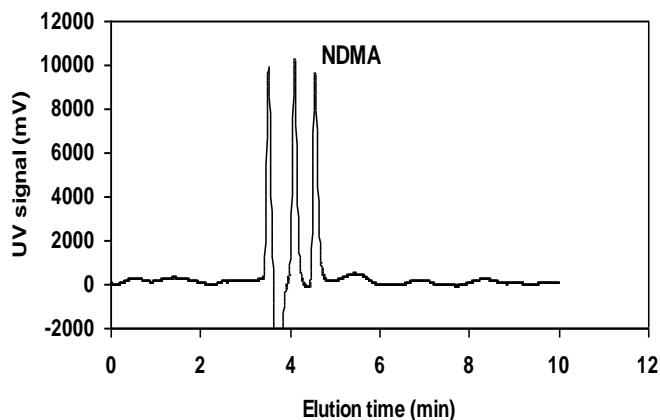


Figure 1
The chromatogram of a 0,4 µg/ml NDEA and of a 0,4 µg/ml NDMEA standard solution



Due to different retention times, the two nitrosamines can be determined simultaneously in the samples (Fig. 2).

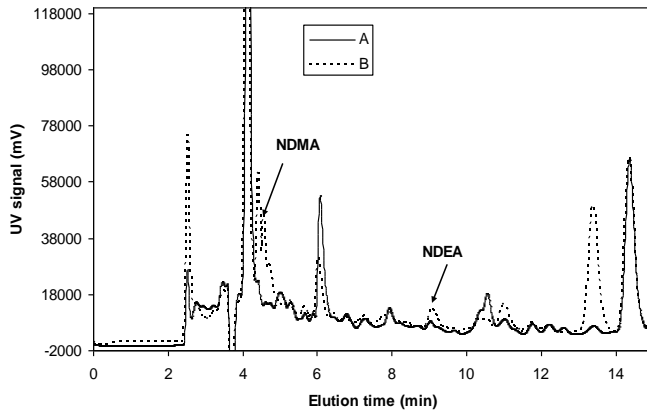


Figure 2
The chromatogram for ham-type processed meat products loin fillet (A) and pork pastrami (B)

Models menus: vegetarian diet consists of fruits and vegetables, vegetarian diet has added their fermented cheese and conventional diet includes fruits, vegetables, fermented cheese, meat products. Estimation took under consideration the daily intake of drinking water (1.5 L with a content of 50 mg NO₃⁻/L, for all diets), and bread (200 g of black bread containing 4.3 mg NO₂⁻/kg and 20 mg NO₃⁻/kg, included in the vegetarian diet, as well as 200 g of white bread containing 3.4 mg NO₂⁻/kg and 13 mg NO₃⁻/kg, included in the vegetarian and conventional diets).

Statistical data were processed by Stats Direct software version 2.7 and Microsoft Excel 2007. Data calculated for each group are expressed as mean ± standard error of the mean and range.

Results

Nitrate and nitrite concentration are presented in Table 1. Based on nitrates, nitrites and nitrosamines contents determined in foodstuffs, we could estimate their intake for each of the three types of the chosen diets (vegetarian, vegetarian and conventional).

Table 1. Nitrate, nitrite and nitrosamines content (results of this variable are presented as mean ± standard deviation and range) in foodstuffs

Samples	Sample number	mg NO ₃ ⁻ /kg	mg NO ₂ ⁻ /kg	µgNDEA/kg	µgNMEA/kg
Processed meat products	40	27.54-146.36	0.74-32.24	nd-6.14	nd-4.0
Fermented cheese	15	0.91-16.84	0.29-1.16	-	-
Vegetables	25	14.39-1363.80	0.29-9.65	-	-
Fruits	22	3.98-21.27	0.55-3.08	-	-

nd=not detectable

Using all these values, the nitrite, nitric and nitrosamines doses were calculated either as mg/day, or expressed in mg/kg body weight/day (for an adult with a body mass of 60 kilograms, who would take any of the diets mentioned in the study) (Table 2).

Type of diet		Vegetalian diet	Vegetarian diet	Conventional diet
NO ₂ ⁻	mg/day	2,93	1,49	8,42
	mg/kg bw/day	0,048	0,024	0,140
NO ₃ ⁻	mg/day	543,47	113,24	258,84
	mg/kg bw/day	9,057	1,887	4,314
NDEA	µg/day	-	-	0,202
	µg/kg bw/day	-	-	0,0033
NDMA	µg/day	-	-	0,036
	µg/kg bw/day	-	-	0,0006

Table 2
The daily doses of nitrate, nitrite, NDEA and NDMA

Discussion

Human exposure to nitrates and nitrites is associated with three food sources: vegetables, processed meat and drinking water. According to Table 1, the smallest nitrate concentrations were determined in frankfurtes, Parmesan cheese, pepper and watermelon; the highest concentrations of nitrate were found in pork ham, goat cheese with herbs, lettuce and blackberries. In terms of nitrite concentration, the smallest values were determined in sausages, cheese, papper and watermelon; the highest values were determined in chicken frankfurtes, cheese, red cabbage and blackberries. Nitrosamines concentration were below the limit of detection of the method in 6% of the samples; the highest concentrations were determined in sausages and 20% of the samples had concentrations of nitrosamines greater than 1 µg/kg. European Food Safety Authority (EFSA) assessed a daily permissible dose at 3,7 mg nitrate/kg body, which is the equivalent of 222 mg nitrate ingested by an adult with 60 kg body weight. A person eating 400 g vegetables/day, according to WHO's recommendations, would ingest 157 mg nitrate/day (Tamme et al., 2010) and 0,5 mg nitrite/kg (EFSA, 2008). According to the measurements performed during the present study, the chosen vegetalian, vegetarian and conventional diets would result in a daily intake of nitrite of 0,048 mg/kg body, 0,024 mg/kg body and 0,140 mg nitrite/kg body, respectively. For the same diets, the daily intake of nitrate would be 9,057 mg/ kg body, 1,887 mg/ kg body and 4,314 mg/ kg body. It is worth mentioning that the vegetalian and conventional diets used in the present study exceed the ADI 3,46 and 1,64 times, respectively. A diet containing vegetables with a high content in nitrates might increase the risk for gastrointestinal cancer in humans (Bryan et al., 2012). The possible intake of nitrosamines during a conventional diet would be as high as 0,036µg NDMA/day and 0,202 µg NDEA/day.

Conclusion

Human exposure to nitrates by food consumption fits to WHO's recommendations only in the case of a vegetarian diet, which leads to a daily ingestion of less than 157 mg nitrate. As concerning the nitrite intake determined for the three proposed diets (vegetarian, vegetarian, conventional), it was 16, 8, and 46 times higher than the permissible limit given by EFSA (0,003 mg nitrite/kg body/day, calculated for an adult weighing 60 kg). Taking into account the well recognized nitrosamine carcinogenic risk, the present study highlights the decisive consequence of choosing the right diet for limitation of the nitrite intake.

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L'ESTIMATION DE L'APPORT DES NITRATES, DES NITRITES, DE LA DIÉTHYLNITROSAMINE ET DE LA DIMETHYLNITROSAMINE PAR LA CONSOMMATION D'ALIMENTS

Résumé

Le but de cette étude a été d'évaluer la contribution des nitrates, des nitrites et des nitrosamines dans la consommation. Nous avons déterminé les concentrations de nitrates, de nitrites dans 102 échantillons d'aliments (40 produits à base de viande, 15 produits fromage, 25 légumes, 22 fruits). Les nitrates et les nitrites ont été déterminés par la méthode Peter-Griess. Les nitroso diméthylamine et nitroso diéthylamine ont été déterminés dans 40 produits à base de viande; la détermination des nitrosamines a été effectuée par HPLC. Les régimes végétalien, végétarien et conventionnel ont été conçus pour une valeur énergétique d'environ 2500 kcal/jour. Sur la base des valeurs obtenues, on a calculé l'apport de nitrates, nitrites, nitrosamines. Les résultats sont conformes aux recommandations de l'OMS, sauf l'alimentation végétalienne et l'alimentation conventionnel ou l'apport de nitrates est 3,46 respectivement 1,64 fois plus élevé que la dose journalière admissible (157mgNO₃⁻/jour).

Mots-clés: *nitrates, nitrites, nitrosamines, alimentation*

STIMA DELL'ASSUNZIONE DI NITRATI, NITRITI, DIETILNITROSAMMINA E DIMETILNITROSAMMINA ATTRAVERSO IL CONSUMO DI ALIMENTI

Riassunto

Lo scopo di questo studio è stato di valutare l'apporto di nitrati, nitriti, NDEA e NDMA attraverso l'alimentazione. Abbiamo determinate le concentrazioni di nitrati e nitriti in 102 alimenti (prodotti a base di carne 40, formaggio 15, verdure 25, frutti 22). Nitrati e nitriti sono stati determinati con il metodo spettrofotometrico Peter-Griess. Nitroso dimetilammina e nitroso dietilammina sono stati determinati in prodotti a base di carne; la determinazione di nitrosammine è stata eseguita mediante HPLC con rivelazione UV. Le diete alimentari vegetalia, vegetariana e convenzionale, sono state programmate per garantire un apporto energetico di circa 2.500 kcal/giorno. Sulla base dei valori trovati, abbiamo calcolato l'assunzione di nitrati, nitriti e nitrosamine. I risultati ottenuti sono coerenti con le raccomandazioni dell'OMS, con l'eccezione di dieta vegetaliana e dieta convenzionale, l'assunzione di nitrati è 3,46 rispettivamente 1,64 volte superiore a dose giornaliera accettabile (157 mgNO₃⁻/giorno).

Parole chiave: *nitrati, nitriti, nitrosamine, diete alimentari*