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FIA evaluation of nitrite and nitrate contents of liver pâtés

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A comparative study on the nitrite and nitrate contents of 15 liver pâté brands, in a total of 45 samples (three batches per brand) is presented. The study was conducted on two different kind of pâtés: pork liver pâtés and fowl liver pâtés. For the simultaneous determination of nitrite and nitrate, an automated flow injection system with spectrophotometric detection was used. The manifold was based on the splitting of the flow after injection and subsequent confluence of the flow before reaching the detector, allowing the reduction of nitrate to nitrite in part of the sample plug on an on-line copper cadmium reductor column. Spectrophotometric determination was made after a diazotization coupling reaction. The levels of nitrites and nitrates ranged from 1.07 mg $NaNO_2\,kg^{-1}$ ± 0.43 to 15.9 mg $NaNO_2\,kg^{-1}$ ± 5.2 and from 24.5 mg $NaNO_3\,kg^{-1}$ ± 2.7 to 207 mg $NaNO_3\,kg^{-1}$ ± 14, respectively. The levels of concentration of these constituents were below the allowable limits. A significant dispersion in the results was observed between different brands and within some of the brands. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Nitrite and nitrate have been commonly used in food industry as additives, namely in meat products, in order to confer them their characteristic colour, flavour and texture, properties well-recognised by consumers. In addition, a microbiological preservative effect is attained–a factor of considerable importance in view of the present concern about foodborne pathogens; for example, nitrite provides specific protection against *Clostridium botulinum* (Report of the Scientific Committee for the Human Nutrition, 1990; Casas *et al.*, 1991; Cassens, 1995). Nevertheless, owing to their toxicity, these compounds are reactive chemicals to be used with caution.

With respect to nitrite, its most toxic effect is metahemoglobinemia. In addition, it has been proved that nitrite is an important precursor of N-nitrosamines, which are potential carcinogens.

Nitrates are considered compounds of lower toxicity, representing a danger only when ingested in excessive doses or when converted to nitrites.

Residual nitrite and nitrate levels are the analytically detectable amounts found in a food product and are considerably lower than the amount added. The European Community legislation for liver pâtés allows 100 mg kg^{-1} for residual nitrites (expressed as $NaNO_2$) and 250 mg kg^{-1} for residual nitrates (expressed as $NaNO_3$), (95/2/EC Directive).

The official methods used for the quantification of nitrite and nitrate (International Standard Organisation, ISO 2019 and ISO 3001, 1975; Official Methods of Analysis, 1980), recommend the application of spectrophotometric analytical methodologies. These are, however, of tedious execution and economically less favourable because the use of a high number of reagents coupled with their intense handling and consumption is involved. In contrast, Ferreira *et al.* (1996) developed a very accurate, rapid and precise methodology by FIA, in which a multidetection flow injection system is used for the automatic simultaneous determination of nitrate and nitrite. The manifold consists of splitting the sample plug after injection with a subsequent confluence of the flow before reaching the spectrophotometric detector. This enables the reduction of nitrate to nitrite in part of the sample plug, due to an on-line copper-cadmium reductor column. Each channel has a different residence time and therefore two peaks are obtained which correspond to either nitrite or nitrite plus nitrate. Spectrophotometric detection of either peak is performed after diazotization-coupling reaction. This method allows the simultaneous determination of nitrite and nitrate present in different concentration ranges in the same sample using a unique detector, and also provides an alternative method of analysis to perform the conventional instrumental methods automatically with considerable time saving.

The objective of our study was to evaluate the nitrite and nitrate contents of liver pâtés available on the retail market using the foregoing FIA methodology in order to: (i) compare their concentration levels with respective allowable limits; (ii) determine the differences between their detectable levels in pork liver and fowl liver pâtés; (iii) assess the uniformity of manufacture within the same brand. Such information is useful as it may provide nutritionists and consumers with a more complete perception of the food product's safety and quality.

MATERIALS AND METHODS

Reagents and chemicals

All reagents were analytical grade and deionised water had a specific conductivity inferior to 0.1 $\mu S\,cm^{-1}$. N-(1-naftyl)ethylenediammonium dichloride, phosphoric acid and sulphanilamide were obtained from Sigma Chemical Company.

Apparatus

The absorbance was measured with a Jenway 6105 UV/VIS spectrophotometer, equipped with an 8 μl Hellma type 178.713 flow cell, connected to a Gilson recorder. An Ismatec model S 820 peristaltic pump was used in this FIA system for propelling the samples. The insertion of samples and standards into the system was carried out with a Rheodyne 5020 six port valve. Omnifit Teflon tubings (0.8 and 0.3 mm i.d.), Gilson end fittings and connectors were used to connect the different components of the manifold.

FIA manifold

Figure 1 shows the FIA manifold used in the simultaneous biparametric determination of nitrite and nitrate in liver pâtés. The cadmium column used in the reduction of nitrate to nitrite was prepared as described elsewhere (Henriksen and Selmer-Olsen, 1970) by using a glass tube (3 mm i.d.) filled with cadmium–copper filings, held in position by glass wool plugs.

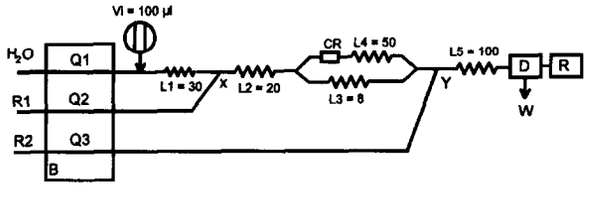

Fig. 1. FIA manifold used in the simultaneous multiparametric determination of nitrite and nitrate in liver pâtés. Vi — injection volume; B — peristaltic pump; Li — length of the reaction coils (cm); Qi — flow ($ml\,min^{-1}$), Q1 = 3.0, Q2 = 0.5, Q3 = 1.0; CR — cadmium-copper reductor column; D — spectrophotometric detector; X and Y — confluences; R1 — a solution containing 100 g ammonium chloride, 20 g sodium tetraborate, 1 g Na_2EDTA in 1 l; R2 — a solution containing 20 g sulphanilamide, 1 g N-(1-naftyl)ethylenediammonium dichloride and 100 ml of 80% phosphoric acid diluted to 1 l with water. W — waste; R — recorder.

This manifold, enabled to perform analysis within a linear response range from 0.02 to 2 ppm for NO_2-N and from 0.1 to 5 ppm for NO_3-N, with a sampling rate of 40 samples per hour, thus corresponding to 80 determinations per hour.

Calibration and quantification

Standard solutions of nitrite (1 ml = 1000 mg NO_2-N) and nitrate (1 ml = 1000 mg NO_3-N) were prepared by dissolving 4.92 g and 6.07 g of $NaNO_2$ and $NaNO_3$ (dried for 1 h at 100°C), respectively, carefully weighed and diluted to 1000 ml. The nitrite solution was standardised against a 0.1 N permanganate solution. The referred solutions were treated with some chloroform drops to prevent the development of micro-organisms and were stored in a refrigerator. Working standard solutions containing nitrite and nitrate were prepared by appropriate dilution, in the following way: standards of 0.03; 0.05; 0.1; 0.5; 2.0 ppm of NO2-N and standards of 0.1; 0.5; 1.0; 2.0; 3.0; 4.0; 5.0 ppm of NO_3-N all of them mixed with 0.05 ppm of NO_2-N. All analyses were performed in duplicate.

Samples

This study was performed on 15 brands of pâtés (two Portuguese brands and 13 imported from European Community) including 10 brands of pork liver pâtés and

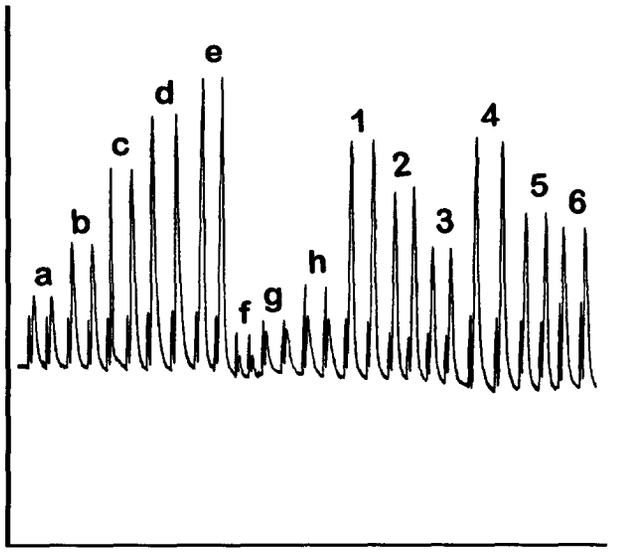

Fig. 2. Record of a typical calibration loop and of a series of samples: (a) 0.05 ppm NO_2 – N; +0.5 ppm NO_3 – N; (b) 0.05 ppm NO_2 – N; +1.0 ppm NO_3 – N; (c) 0.05 ppm NO_2 – N; +2.0 ppm NO_3 – N; (d) 0.05 ppm NO_2 – N; +3.0 ppm NO_3 – N; (e) 0.05 ppm NO_2 – N; +4 ppm NO_3 – N; (f) 0.03 ppm NO_2 – N; (g) 0.05 ppm NO_2 – N; (h) 0.1 ppm NO_2 — N; 1 to 6 samples.

5 brands of fowl liver pâtés, in order to have a working sample as representative as possible of the Portuguese liver pâtés retail market. Three lots of each brand in a total of 45 samples were randomly purchased from the retail market.

Sample preparation

Samples were pre-treated by homogenisation according to the process recommended by the International Standard Organisation (ISO 2019 and ISO 3001, 1975). Samples were extracted with hot water and then purified by protein precipitation with Carrez I (kaliumhexacyanoferrat(II)) and Carrez II (zinkacetat) followed by filtration.

Recovery study

The recovery study was performed by standard addition method for all 45 samples in order to verify the validity of the results obtained. The analyses, carried out in duplicate, consisted of adding a known amount of nitrite to one of the sample aliquots and of nitrate to the other, and will be denoted hereafter as control assays. The recovery percentages of nitrite and nitrate were obtained after subtracting the previously quantified endogenous nitrite and nitrate contents from total values.

Statistical analysis

Data are represented as the mean ± standard deviation. The results were statistically analysed by analysis of variance (ANOVA) methodology followed by Fisher's PLSD test. Differences were considered significant for $p < 0.01$.

RESULTS AND DISCUSSION

Under optimal conditions for nitrite and nitrate analysis, the standards and extracts were injected directly into the FIA system and a FIA record as shown in Fig. 2 was obtained. The analytical methodology used proved to be quite adequate for the simultaneous quantification of nitrite and nitrate in liver pâtés. The detection limit, calculated as the concentration corresponding to three times the SD of the background noise, obtained with 10 determinations was 0.01 ppm of NO_2-N and 0.1 ppm of NO_3-N.

On the control assays the recovery percentages ranged between 90.0 and 106% and between 81.7 and 109% for nitrite and nitrate, respectively. The average values were 99.0 ± 3.1% for nitrite and 98.9 ± 6.1% for nitrate, thus confirming the adequacy of the analytical methodology chosen.

Table 1 summarises the results of nitrite and nitrate contents of the 15 commercial brands of liver pâtés

Table 1. Contents of nitrite and nitrate of 15 commercial brands of liver pâtés in the total of 45 samples

Samples[a] brand/lot	Nitrite mg/kg (x±sd)	Nitrate mg/kg (x±sd)	Samples[a] brand/lot	Nitrite mg/kg (x±sd)	Nitrate mg/kg (x±sd)	Samples[a] brand/lot	Nitrite mg/kg (x±sd)	Nitrate mg/kg (x±sd)
A	4.95 ± 0.13	40.0 ± 6.2	A	4.99 ± 0.01	57.1 ± 0.3	A	12.47 ± 0.26	32.0 ± 3.5
1 B	5.12 ± 0.36	63.8 ± 4.8	6 B	5.23 ± 0.08	53.6 ± 0.3	11 B	12.18 ± 0.34	23.2 ± 0.6
C	5.26 ± 0.09	38.1 ± 4.5	C	4.79 ± 0.13	49.3 ± 1.1	C	22.93 ± 0.11	38.0 ± 3.8
A	14.4 ± 0.38	116 ± 11	A	9.59 ± 0.08	34.6 ± 0.1	A	3.19 ± 0.29	104 ± 1.5
2 B	13.5 ± 0.40	119 ± 4	7 B	10.16 ± 0.59	37.2 ± 0.7	12 B	2.61 ± 0.38	126 ± 0.8
C	13.3 ± 0.34	124 ± 7	C	12.48 ± 0.39	33.5 ± 0.7	C	3.00 ± 0.21	108 ± 2
A	10.0 ± 0.4	23.7 ± 1.8	A	5.96 ± 0.20	52.0 ± 2.0	A	3.94 ± 0.15	222 ± 1
3 B	10.3 ± 0,1	24.2 ± 1.1	8 B	5.25 ± 0.11	61.4 ± 0.9	13 B	4.45 ± 0.25	212 ± 4
C	9.08 ± 0.66	39.1 ± 0.1	C	4.99 ± 0.44	78.9 ± 1.6	C	5.41 ± 0.19	190 ± 4
A	8.52 ± 0.41	59.9 ± 0,9	A	1.45 ± 0.10	50.5 ± 1.9	A		
4 B	10.9 ± 1.3	56.3 ± 4.5	9 B	0.52 ± 0.13	42.4 ± 7.8	14 B	N.D.	N. D.
C	15.1 ± 1.1	56.3 ± 4.4	C	1.25 ± 0.14	29.2 ± 0.5	C		
A	5.72 ± 0.56	25.8 ± 0.7	A	8.13 ± 0.12	53.2 ± 0.7	A	5.89 ± 0.11	21.4 ± 1.0
5 B	6.59 ± 0.36	24.6 ± 1.0	10 B	6.02 ± 0.72	48.1 ± 1.9	15 B	5.90 ± 0.21	26.2 ± 2.0
C	6.47 ± 0.13	25.4 ± 0.4	C	10.0 ± 0.40	45.3 ± 1.7	C	5.54 ± 0.24	26.0 ± 0.8

(a) 1 to 10 are different brands of pork liver pâtés; 11 to 15 are different brands of fowl liver pâtés, the letters A, B, C represent different lots: A—lot with oldest production date; C—lot with most recent prodution date. N.D.—not detected.
Significant differences between the results were determined by ANOVA methodology followed by Fisher's PLSD test. Differences were considered significant for $p < 0.01$.

analysed in a total of 45 samples, including pork liver pâtés and fowl liver pâtés.

The levels of nitrite and nitrate ranged from 1.07 ± 0.43 to 15.9 ± 5.2 mg $NaNO_2\,kg^{-1}$ and from 24.5 ± 2.7 to 207 ± 14 mg $NaNO_3\,kg^{-1}$, respectively. Only one brand had nitrite and nitrate below the detection limit (0.25 mg $NaNO_2\,kg^{-1}$ and 2 mg $NaNO_2\,kg^{-1}$), which was in compliance with the respective label (no addition of nitrite and nitrate). It should be mentioned that in spite of its storage under strong vacuum, this brand had no attractive organoleptic characteristics. The general detection of these compounds, possibly implies that most manufacturers make use of these additives in the preparation of liver pâtés. However, the statistically significant ($p < 0.01$) differences observed for the mean values of either compound in some samples within and between brands suggest a certain discrepancy in the quantities added during manufacture; for example, brand 9 has as little as 0.5–1.5 mg $NaNO_2\,kg^{-1}$ against 12–13 mg $NaNO_2\,kg^{-1}$ for brand 11. The large variability reported for mean values of nitrite and nitrate pertaining to samples within the same brand may indicate a lack of uniformity during manufacturing. This can be partially explained by the difficult homogenisation of small quantities of additives in a large quantity of matrix and also by the diversified solubility characteristics of nitrite and nitrate (water-soluble) and of the pâté matrix (with high level of fat, 30.53 Me 7.00, $n = 45$; determined by soxhlet extraction A.O.A.C. 24.005). Despite the wide variation in concentration levels of nitrite and nitrate, when expressed as averages all levels found were, for all samples analysed, within the same limits.

As can be seen from our sample collection, pork liver pâtés prevail in the retail market comparatively to fowl liver pâtés. No significant differences ($p > 0.01$) with respect to nitrite and nitrate contents were reported between both types of liver pâtés.

CONCLUSIONS

In terms of nitrite and nitrate contents, the liver pâtés analysed may be considered adequate for consumption, despite the wide variation in results observed between different brands. Although recognised as satisfactory, a moderate consumption of these pâtés is recommended.

In addition, the great variability pertaining to nitrite and nitrate contents of samples within the same brand suggest the need for improvement of the manufacturing process.

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