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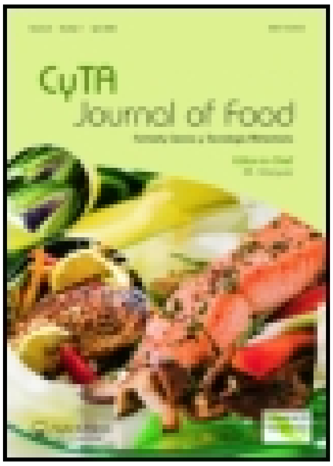

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Determination of nitrate and nitrite levels in infant foods marketed in Southern Italy

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SHORT COMMUNICATION

Determination of nitrate and nitrite levels in infant foods marketed in Southern Italy

Determinación de los niveles de nitrato y nitrito en la alimentación infantil comercializada en el sur de Italia

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Nitrate and nitrite concentrations in foods have aroused considerable interest because of their possible toxicity to humans, especially younger children. Since dietary exposure to these compounds includes sources other than vegetables, 104 infant foods consisting of animal, plant and mixed origin and available on sale to the public were examined using the spectrophotometric AOAC method to assess whether during the weaning period the use of commercial follow-on formulas could be a cause of concern for exceeding the ADIs set at international level. Nitrate values (mg kg^{-1}) ranged between 0.35 and 131.68, nitrite between 1.12 and 80.22. The highest mean values were found in foods of plant origin (45.5) for nitrate and in foods of mixed origin (12.48) for nitrite. Nitrate contents never exceeded the level of 200 mg kg^{-1} set by EC Regulation 1881/2006 whereas the ingestion of most of the samples would have been the reason for exceeding the ADI set for nitrites.

Keywords: nitrate; nitrite; infant foods

Las concentraciones de nitrato y nitrito en la alimentación han suscitado considerablemente el interés debido a su posible toxicidad para los humanos, especialmente para los infantes. Debido a que la exposición alimentaria a estos compuestos incluyen otras fuentes además de los vegetales, se examinaron 104 alimentos infantiles de origen animal, vegetal y combinado expuesto a la venta al público utilizando el método espectrofotométrico AOAC para evaluar si durante el periodo de destete el uso de fórmulas con fines comerciales podría ser motivo de preocupación a nivel internacional por su exceso de conjunto ADI. Se encontró que los valores de nitrato (mg kg^{-1}) estaban entre 0,35 y 131,68, los de nitrito entre 1,12 y 80,22. Los valores promedio más altos de nitrato se encontraron en alimentos de origen vegetal (45,5) mientras que en alimentos de origen combinado (12,48) los valores más altos fueron de nitrito. Los contenidos de nitrato nunca excedieron el nivel de 200 mg kg^{-1} determinado por la Regulación EC 1881/2006, mientras que la ingesta de la mayoría de las muestras habría estado la causa de exceder el conjunto de nitritos ADI.

Palabras clave: nitrato; nitrito; alimentación infantil

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Introduction

Humans have always ingested nitrates and nitrites, originating almost entirely from the consumption of vegetables in which these substances are naturally present. Smaller, even if not negligible, amounts come however from water and the foods in which these substances may be present as additives. Sodium and potassium nitrates are in fact used, sometimes together with nitrites, as food preservatives and antimicrobials, because they preserve the flavor and the color of foods and exert an antimicrobial effect, especially against *Clostridium botulinum*. In particular, they may be present in raw and processed meats, dairy products, fish and fish products and sometimes even in spirits and liqueurs (Lijinsky, 1999; Pannala et al., 2003; Santamaria, 2006).

It is widely accepted that the presence of nitrates in vegetables, as in other foods and in water, is a serious threat to human health. In reality, nitrates are not dangerous in themselves, as they are highly stable and non-toxic, but become so when they are reduced to nitrites. Approximately 25% of the ingested nitrate is secreted in saliva and about 20% of the nitrate of the salivary secretion is then converted into nitrite by microorganisms in the oral cavity. So, for normal individuals, about 5–7% of the ingested nitrate can be detected as nitrite in saliva (Chan, 2011; Pannala et al., 2003; Spiegelhalder, Eisenbrand, & Preussmann, 1976).

The only proven negative effect today is the danger of an excess of nitrates/nitrites in the diet of children below the age of 12 months or adults with a genetic predisposition. The first international evaluation of the risks associated with the ingestion of nitrate and nitrite was conducted by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1961. The Scientific Committee for Food (SCF) reviewed the toxicological effects of nitrate and nitrite and established an Acceptable Daily Intake (ADI) of 0–3.7 mg kg^{-1} b.w. for nitrate in 1990, retained the same ADI in 1995 and derived an ADI of 0–0.06 mg/kg for nitrite. The JECFA reconfirmed in 2002 an ADI of 0–3.7 mg kg^{-1} b.w. for nitrate and set an ADI of 0–0.07 mg kg^{-1} b.w. for nitrite. JECFA, in the general information on the two compounds, specifies that ADI does not apply to infants below the age of 3 months (Opinion SCF, 2008).

For infants and children there is a risk of ingesting a quantity of nitrates greater than ADI because the amount of food to b.w. is greater than that consumed by an adult. It is therefore important to define the limits of daily ingestion to prevent acute exposure to nitrate and the consequent risk of

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methemoglobinemia, which may even lead to death. Newborns are in fact very sensitive to this type of pathology, also known as "blue baby syndrome". unlike adults who have more protection against the low pH of the stomach reduction of nitrate to nitrite by bacteria Nitrites, which are much more unstable than nitrates, react with the haemoglobin of red blood cells to form methemoglobin. The presence of nitrite can also involve the formation of various N-nitroso compounds (nitrosamines and nitrosammides) by reaction with secondary amines and amides present in the food. It has been shown that this occurs both with nitrites ingested and with those present in the saliva (Gangolli et al., 1994; Pannala et al., 2003; Speijers & Van Den Brandt, 2003).

The European Union has always paid attention to food components and contaminants, nitrate and nitrite included, as shown by the directives and the regulations on the subject. In 2008 and 2010 EFSA was asked additional questions about the dangers of exposure to nitrates in order to revise, if necessary, the current legislative framework relating to food contaminants. With the advent of industrial agriculture and the heavier fertilization and changes in consumption habits, the assumption levels might be reasonably supposed to have been higher than in the past (Chan, 2011; Hardisson, González Padrón, Frias, & Reguera, 1996; Santamaria, 2006; Vasco & Alvito, 2011).

The number of recent studies on nitrate and nitrite contents in baby foods is rather limited (Özdestan & Üren, 2012; Pardo-Marín, Yusà-Pelechà, Villalba-Martín, & Perez-Dasí, 2010; Vasco & Alvito, 2011). Since dietary exposure to these compounds includes sources other than vegetables, the aim of the present study was to research the levels of nitrates and nitrites in infant foods of different composition produced and/or sold in Italy to assess whether, in the weaning period, from 4 to 12 months, the use of commercial follow-on formulas could be a cause of concern for nitrate and nitrite levels exceeding the ADI parameters.

Materials and methods

N. 104 infant foods (Table 1), from all of the 6 trading brands (A, B, C, D, E, F) found on the market, were bought at retail outlets in Southern Italy. The samples analyzed were divided into the following categories:

(1) N.38 foods of animal origin, both homogenized and freeze dried;
(2) N.33 foods of plant origin, both homogenized and freeze dried;
(3) N.33 samples of homogenized baby foods of mixed origin, including n. 10 homogenized full meals.

The samples were transported to the laboratory and analyzed for nitrates and nitrites using the spectrophotometric AOAC official method (1995). Freeze-dried foods were reconstituted according to the manufacturers' instructions before analysis.

Analytical grade reagents were used. Sodium nitrite, sodium nitrate, cadmium sulfate, sulfanilic acid, N-(1-naphthyl)-ethylenediamine dihydrochloride, glacial acetic acid solution 20%, ammonia hydroxide 25%, sodium tetraborate decahydrate, zinc sulfate heptahydrate and zinc metal powder were purchased from Acros Organics (Geel, Belgium). Distilled water used throughout the procedure was supplied from the distillation apparatus of Purelab Option Elga (UK).

Nitrate stock solution (1000 mg L^{-1})

Dissolve 137.1 mg of primary standard sodium nitrate, previously dried for 24 h at 105°C, in sufficient water and then dilute to 100 mL. For preparing standard nitrate solutions dilute nitrate stock solution with distilled water.

Nitrite stock solution (1000 mg L^{-1})

Dissolve 150.0 mg of primary standard sodium nitrite, previously dried for 1 h at 110°C, in sufficient water and dilute to 100 mL. For preparing standard nitrite solutions dilute nitrite stock solution with distilled water.

Color reagent

Color reagent was prepared according to the official method of AOAC International (993.03). Dissolve 600 mg of sulfanilic acid ($C_6H_7NO_3$ S) in 50 mL hot water. Let it cool; add 20 mL glacial acetic acid and dilute to 100 mL with water. Dissolve 20 mg N-(1-naphthyl)-ethylenediamine dichloride ($C_{12}H_{16}C_{12}N_2 \cdot CH_3OH$) in 20 mL glacial acetic acid and dilute to 100 mL with water. Mix equal volumes of sulfalinic acid solution and N-(1-naphthyl)-ethylenediamine dichloride reagent immediately before use. Discard any unused color reagent.

Sample preparation

Weigh a sample of 3–10 g into a 200 mL volumetric flask. Add 150 mL hot water and 10 mL of 5% (w/v) borax solution ($Na_2B_4O_7 \cdot 10H_2O$) to precipitate protein. Then mix and heat for 15 min. Clarify the mixture by adding 4 mL of 30% (w/v) $ZnSO_4 \cdot 7H_2O$ solution and shaking for 15 s. After cooling to room temperature, dilute the sample to volume with distilled water, mix and filter through a Whatman (no. 40) filter paper. Prepare blank without sample material.

Reduction of nitrate to nitrite

For each sample, weigh ca 600 mg Zn powder into separate 50 mL volumetric flasks and spread powder over bottom of flask. Prepare additional flask for standards. Carefully add 4 mL of 10% (w/v) cadmium sulfate ($3CdSO_4 \cdot (H_2O)$) solution to zinc powder in flask to obtain homogeneous mixture. Let newly formed spongy metallic cadmium stand for 10 min without moving. Add 2 mL 25% NH_4OH and 10 mL sample solution to 1 flask. Prepare standard nitrate concentrations (containing 0, 50, 100, 150 and 200 μg $NaNO_3$) by adding 10 mL of each standard solution to separate volumetric flasks prepared with spongy cadmium. Shake flasks for exactly 1 min to loosen spongy cadmium; then let stand for 10 min. Dilute to volume with H_2O and filter.

After use, pour contents of volumetric flasks into waste bottle. Dissolve possible residues in volumetric flasks with concentrated HCl to another waste bottle. Collect waste from color reaction in another bottle. Arrange for proper disposal of waste bottles.

Nitrite determination

Pipet 10 mL clear filtrates of samples and standard solutions (equivalent to 0, 10, 20, 30 and 40 μg $NaNO_2$) to separate glass-stoppered mixing cylinders. Add 10 mL color reagent to each, mix for 1 min by hand, and record absorbance at 530 nm using Spectrophotometer UV/VIS V-530 Jasco (Japan) and water blanks.

Table 1. Composition of the infant foods examined for nitrate and nitrite levels divided according to the producers (A,B,C,D,E,F).

Tabla 1. Composición de los alimentos infantiles examinando los niveles de nitrato y nitrito divididos según los productores (A,B,C,D,E,F).

Sample	Animal origin (n.38)^{AB}	Sample	Plant origin (n.33)^{A}	Sample	Mixed origin (n.3)^{B}
A2H	Horse	A1H	Carrots	A3H	Horse with vegetables
A4H	Rabbit	A13–14–15H	Early legumes	A5H	Mixed legumes and poultry
A6H	Beef	A16H	First sauces	A7H	Beef, ham and vegetable
A25F	Poultry	A17H	First sauces with tomato and vegetables	A8H	Hake with vegetables
A26F	Lamb	A22–23H	Mixed vegetables	A9H	Sea bream with vegetables
A27F	Turkey	A24H	Cream of courgettes with little macaroni	A10H	Peas and carrots with veal
A28F	Veal	A30–32F	Vegetable creams	A11–12H	Plaice with vegetables
A29F	Rabbit	A31F	Vegetable broths	A18H	Ham, potatoes and carrots
B38H	Lamb	B39H	Cream of carrots, potatoes and courgettes	A19–20H	Whey cheese with vegetables
B40H	Horse	B46–47H	Legumes and vegetables	A21H	Sea bass with vegetables
B41H	Rabbit	B54–55–56H	Peas and courgettes	A33FM	Vegetables, pasta and processed cheese
B44H	Processed cheese	B66–67H	Mixed vegetables	A34FM	Vegetables, pasta and beef
B45H	Processed cheese with fontina	B70H	Pumpkin and chickpeas	A35FM	Vegetables, pasta and veal
B48–49H	Pork	B71–72H	Cream of courgettes and peas	A36FM	Vegetables, creamed rice and trout
B50–51H	Beef	B73H	Cream of pumpkin and carrots	A37FM	Vegetables, poultry and semolina
B52H	Beef and poultry	B76–77–78F	Vegetable broth	B42–43H	Processed cheese with potatoes and carrots
B57–58H	Poultry	B74–79F	Vegetable cream	B53H	Hake with potatoes
B59H	Poultry and veal	E101H	Courgettes with potatoes	B61H	Salmon with vegetables
B60H	Cooked ham	F102F	Vegetable broths	B64–65H	Trout with vegetables
B62–63H	Turkey	F103F	Cream of legumes	B80FM	Vegetable, pasta and beef
B68H	Veal	F104F	Cream of vegetables	C82H	Veal with vegetables
B69H	Veal and cooked ham			C83H	Poultry with vegetables
B75F	Rabbit			C88FM	Vegetable, pasta and beef
C81H	Poultry and veal			C89FM	Vegetables, rice of cream and veal
C84–85H	Beef			D90H	Beef with vegetables
C86H	Turkey			D91H	Veal with vegetables
C87H	Poultry			D92H	Poultry with vegetables
E95–96H	Cooked ham			D93FM	Vegetables, pasta and ham
E97H	Beef			D94FM	Vegetables, pasta and beef
E98H	Veal				
E99H	Poultry				
E100H	Turkey				

Notes: H = homogenized; F = freeze-dried; FM = full meal A, B, AB = P < 0.01.
Nota: H = homogeneízado; F = liofilizado; FM = comida completa A, B, AB = P < 0,01.

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Nitrate and nitrite concentrations were calculated using nitrate and nitrites calibration curves.

Statistical analysis

The data were subjected to analysis of variance (ANOVA) using NCSS software. The comparison of the means was carried out using the Tukey-Kramer multiple comparison test.

Results

The nitrate values (mg kg^{-1}) ranged between 0.35 and 83.2 in homogenized samples of animal origin and from 2.01 to 80.26 in the freeze-dried ones (Figure 1); from 4.82 to 131.68 in homogenized samples of plant origin (Figure 2) and from 19.41 to 85.03 in the freeze-dried ones (Figure 2); from 3.77 to 67.31 in homogenized samples of mixed origin and from 1.1 to 93.42 in full meals (Figure 3). The highest contents, for each of the three types of food, were found in samples B49 (pig homogenate), B55 (peas and courgettes homogenate) and C89 (full meal of vegetables, rice cream and veal).

The nitrite values (mg kg^{-1}) ranged between 6.6 and 48.87 in homogenized samples of animal origin and from 1.3 to 74.74 in the freeze-dried ones (Figure 1); from 2.26 to 20.71 in homogenized foods of plant origin and from 1.34 to 6.62 in the freeze-dried ones (Figure 2); from 1.98 to 80.22 in homogenized samples of mixed origin and from 1.12 to 30.11 in full meals (Figure 3). The highest contents, in each type of food, were found in samples B75 (freeze-dried rabbit), A1 (carrots homogenate) and B65 (trout with mixed vegetables). Nitrite levels were higher than those of nitrates in 27 samples (15 of animal, 5 of plant and 7 of mixed origin).

On the whole the highest mean (mg kg^{-1}) for nitrate was found in foods of plant origin (45.5), followed by those of animal (27.39) and mixed origin (24.19) whereas the highest means for nitrite were found, in the order, in foods of animal origin (14.82), mixed (12.48) and plant origin (8.2).

Nitrate levels resulted significantly different ($P < 0.01$) only for samples of baby foods of plant and mixed origin whereas foods of animal origin were not significantly different from the other two types of products. No significant difference was found for nitrites.

Discussion

The presence of nitrates and nitrites, in agricultural products in particular, has attracted much attention in recent years as many factors influence the level of these compounds in plants, such as the species of vegetable or fruit, the soil nitrogen content, the amount of sunlight to which the plant is exposed and the seasonal conditions (Murone, Stucki, Roback, & Gehri, 2005).

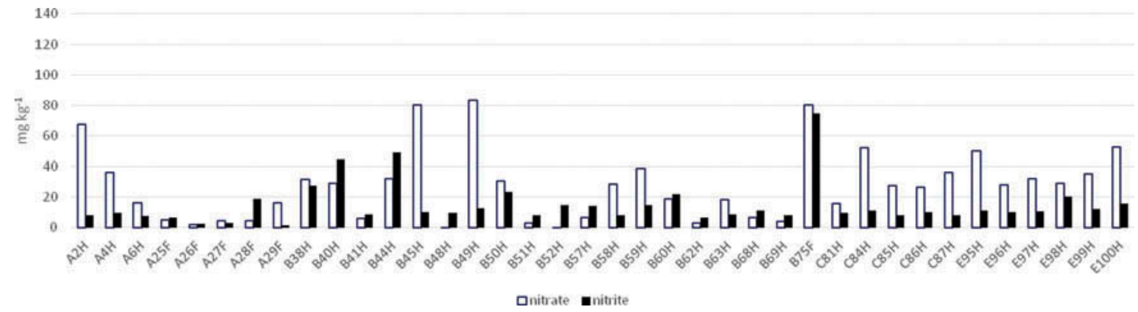

Figure 1. Nitrate and nitrite levels (mg kg^{-1}) in infant foods of animal origin.
LOD (limit of detection) = 0 LOQ (limit of quantification) = 8.
Note: Maximum level for nitrate in processed cereal-based foods and baby foods for infants and young children according to (EC) Regulation 1881/2006 is 200 mg kg^{-1}.

Figura 1. Niveles de nitrato y nitrito (mg kg^{-1}) en alimentos infantiles de origen animal.
LOD (límite de detección) = 0 LOQ (límite de cuantificación) = 8.
Nota: Nivel máximo de nitrato en alimentos a base de cereales procesados y alimentos infantiles y para bebés según la Regulación (EC) 1881/2006 es de 200 mg kg^{-1}.

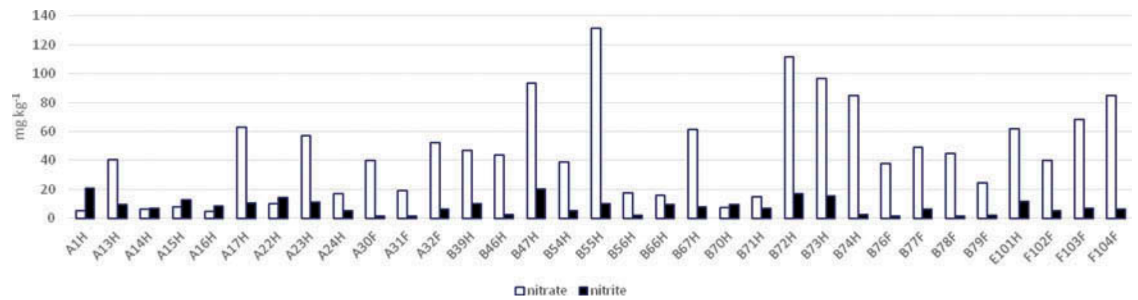

Figure 2. Nitrate and nitrite levels (mg kg^{-1}) in infant foods of plant origin.
LOD (limit of detection) = 0 LOQ (limit of quantification) = 15.
Note: Maximum level for nitrate in processed cereal-based foods and baby foods for infants and young children according to (EC) Regulation 1881/2006 is 200 mg kg^{-1}.

Figura 2. Niveles de nitrato y nitrito (mg kg^{-1}) en alimentos infantiles de origen vegetal.
LOD (límite de detección) = 0 LOQ (límite de cuantificación) = 15.
Nota: Nivel máximo de nitrato en alimentos a base de cereales procesados y alimentos infantiles y para bebés según la Regulación (EC) 1881/2006 es de 200 m g kg^{-1}.

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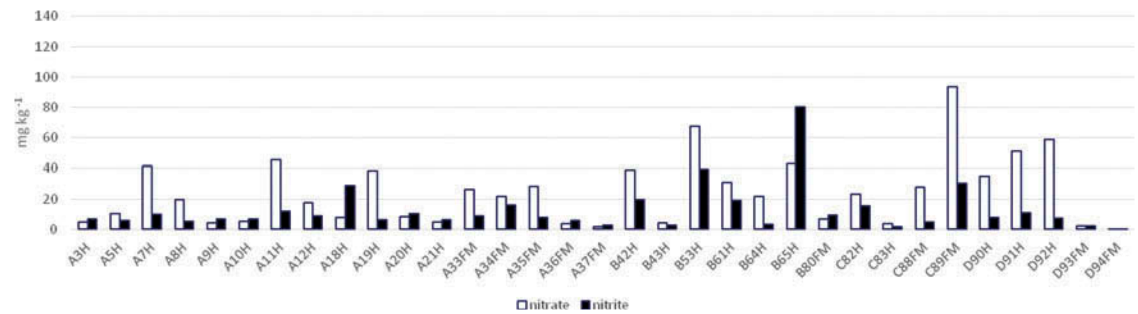

Figure 3. Nitrate and nitrite levels (mg kg^{-1}) in infant foods of mixed origin.
LOD (limit of detection) = 0 LOQ (limit of quantification) = 12.
Note: Maximum level for nitrate in processed cereal-based foods and baby foods for infants and young children according to (EC) Regulation 1881/2006 is 200 mg kg^{-1}.

Figura 3. Niveles de nitrato y nitrito (mg kg^{-1}) en alimentos infantiles de origen combinado.
LOD (límite de detección) = 0 LOQ (límite de cuantificación) = 15.
Nota: Nivel máximo de nitrato en alimentos a base de cereales procesados y alimentos infantiles y para bebés según la Regulación (EC) 1881/2006 es de 200 mg kg^{-1}.

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With the European Commission Regulation No. 1881/(2006), the Commission established maximum levels for nitrates, mycotoxins, heavy metals and polycyclic aromatic hydrocarbons (PAHs) in baby foods. Since nitrates and nitrites may be present, for different reasons, in foods of both plant and animal origin, also infant foods of mixed composition were considered.

None of the examined samples exceeded the maximum admitted level of 200 mg kg^{-1} set for nitrate by the Regulation 1881/2006 for processed cereal-based foods and baby foods intended for infants and young children, as defined in European Commission Directive No 96/5/EC. The notes of the Regulation specify that a) an infant is a child under the age of 12 months, young children are those aged between 1 and 3 years and b) the established limit refers to the foods ready to use, marketed as such or after reconstitution as instructed by the manufacturer.

Average nitrates content found in vegetable foods during the present study (45.5 mg kg^{-1}) are lower than those reported by Hardisson et al. (1996), Pardo-Marín et al. (2010), Tamme et al. (2006), and Vasco and Alvito (2011) who found average nitrate contents of 60, 59, 88 and 102 mg kg^{-1}. Moreover, all samples examined in this study were lower than the established limit (200 mg kg^{-1}), the highest level being 131.68 mg kg^{-1}. Also Özdestan and Üren (2012) report that no sample out of 20 vegetable and fruit-based baby foods exceeded the maximum admitted level whereas concentrations above the limit were detected in a broccoli and rice sample (230 mg kg^{-1}) by Vasco and Alvito (2011), in fresh carrot juice (251 mg kg^{-1}) by Tamme et al. (2006) and in a carrot and rice sample (382 mg Kg^{-1}) by Hardisson et al. (1996).

The time factor has become an increasingly important element in modern society and the percentage of commercial products given to children aged one year and younger, as reported in the Donald study (Kersting, Alexy, Sichert-Hellert, Manz, & Schöch, 1998), is about 51% at 3 months, 62% at 6 months, 53% at 9 months and 37% at 12 months, when infants are often given home-cooked meals. According to the data of the same study, children ingest daily about 142/203 g of follow-on formulas containing meat, fish and vegetables around the 6th month, 165/248 g at the 9th month and 125/196 g at the age of 1 year. If the mean amounts of commercial foods consumed by children at these ages (i.e.172.5, 206.5 and 160.5 g) and the mean nitrate and nitrite contents of samples examined during this research are considered, results show that an infant might ingest levels of nitrate (mg kg^{-1}) ranging from 0.06 to 22.71 at 6 months; from 0.07 to 27.19 at 9 months and from 0.05 to 21.13 at 1 year whereas nitrite levels would range from 0.19 to 13.83 at 6 months; from 0.23 to 20.89 at 9 months and from 0.17 to 12.86 at 1 year. On the whole, even if an occasional exceeding of the ADI does not arouse particular concern, the ingestion of none of the examined samples should have been cause of exceeding, and in most cases not even approaching, the ADI for nitrate at each of the considered ages. Different considerations must be made for nitrites since only a small percentage of the samples would not be cause of exceeding the ADI. In fact nitrites levels ranged from 1.12 to 80.22 mg kg^{-1}. These levels are higher than those reported by Özdestan and Üren (2012) who found concentrations from non-detectable to 30.09 mg kg^{-1} in 20 different vegetable- and fruit-based baby foods bought in Izmir (Turkey).

Critical points for the onset of methemoglobinemia are the age of the child, the level of methemoglobin reductase and the amount of ingested food. The American Association of Pediatrics recommends that consumption of green leafy vegetables (spinach, cabbage), turnips, carrots and beets, which accumulate high amounts of nitrites, should be avoided in infants under 6 months because they can contain nitrate/nitrite levels sufficient to cause methemoglobinemia (United States Department of Agriculture [USDA], 2006).

Even if producers put the age in months on the label when the food can be given, errors regarding age or other factors cannot be excluded. Santamaria (2006) reports two cases of blue baby syndrome in infants who became ill in Wisconsin (USA) after being fed a formula that had been reconstituted with water from private wells with nitrate concentrations of 22.9 and 27.4 mg L^{-1}.

In any case it cannot be ignored that studies on nitrates have demonstrated that they have also potential beneficial health effects, such as the prevention of microbial infections, reduction of hypertension and cardiovascular diseases, and reduction in the risk of gastric cancer (Santamaria, 2006).

The results obtained during this research appear to confirm that a great deal of attention is paid to the preparation of baby foods. Surveillance must be widespread and regularly performed by competent authorities and food business operators. Producers of infant foods should continue to select the raw materials suppliers and monitor the amount of nitrate and nitrite in the final product in order to reduce the risks posed by these foods for young consumers.

Disclosure statement

No potential conflict of interest was reported by the authors.

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