

Nitrate and Nitrite Contents of Baby Foods

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ABSTRACT

Nitrate and nitrite are undesired compounds in foods. Nitrate ion is not directly toxic but it can readily be converted to harmful nitrite ion by microbial reduction. Nitrite can interact with hemoglobin to form methemoglobin by oxidation of ferrous iron (Fe⁺²) to the ferric state (Fe⁺³), a condition described as methemoglobinemia that is dangerous, especially in infants, and it is called as blue-baby syndrome. There have been a limited number of studies about nitrate and nitrite content of baby foods in literature. In this study, nitrate and nitrite analyses were made in 20 vegetable and fruit-based baby foods sold in Izmir, Turkey. Nitrate concentrations of baby foods changed from 3.32 to 99.73 mg/kg. Nitrite concentrations of baby foods changed from non-detectable values to 30.09 mg/kg. The results indicated that nitrate contents were all below the legislated value (200 mg/kg).

Key Words: Blue-baby syndrome, Nitrate, Nitrite, Baby food

Bebek Mamalarının Nitrat ve Nitrit İçerikleri

ÖZET

Nitrat ve nitrit gıdalarda bulunması istenmeyen bileşiklerdir. Nitrat iyonu direkt olarak toksik etkiye sahip değildir ancak mikrobiyal indirgenme ile zararlı nitrit iyonuna dönüşebilmektedir. Nitrit hemoglobin ile interaksiyona girmekte, Fe⁺² oksidasyon ile Fe⁺³'e dönüşmekte, 'methemoglobin' meydana gelmektedir. Bu olay "methemoglobinemia" olarak adlandırılmaktadır. Çocuklar için tehlikelidir ve "mavi bebek sendromu" olarak bilinmektedir. Literatürde bebek mamalarında nitrat ve nitritin belirlenmesi ile ilgili az sayıda çalışma mevcuttur. Bu çalışmada, İzmir'de marketlerde satılan sebze ve meyve bazlı 20 farklı bebek mamasında nitrat ve nitrit analizleri gerçekleştirilmiştir. Bebek mamalarının nitrat içeriği 3.32 ile 99.73 mg/kg arasında bulunmuştur. Bebek mamalarının nitrit içeriği ise tespit edilemeyen düzeylerden 30.09 mg/kg'a kadar değişen konsantrasyonlarda bulunmuştur. Örneklerin nitrat içeriği yasal sınır değerinin (200 mg/kg) altında bulunmuştur.

Anahtar Kelimeler: Mavi bebek sendromu, Nitrat, Nitrit, Bebek maması

INTRODUCTION

Concentrations of harmful nitrogen-containing compounds, such as nitrate and nitrite, have increased in foods and drinks. The increasing levels of these compounds result mainly from organic matter in the soil, the use of nitrogenous fertilizers and herbicides in industrial agriculture, livestock and human excrement

and other organic wastes from chemical industries, domestic wastes and septic tank effluents [1-3]. Nitrate is naturally present in leafy vegetables and nitrite is usually added to meat as a preservative in the form of sodium or potassium salt [4].

Nitrite has several adverse effects upon human health [5]. Nitrite can interact with hemoglobin to form

methemoglobin by oxidation of ferrous iron (Fe^{2+}) to the ferric state (Fe^{3+}), thus preventing or reducing the ability of blood to transport oxygen, a condition described as methemoglobinemia that is dangerous, especially in infants (the so-called blue-baby syndrome) [6]. In the stomach, the reaction between nitrite and secondary amines leads to the formation of nitrosamines (N-nitroso compounds), some of which are known as carcinogenic, teratogenic, and mutagenic and increase risk of cancer of the stomach and esophagus [7-10].

The potential hazard of vegetable-borne nitrate is from its conversion to methaemoglobin-producing nitrite before and/or after ingestion [11]. Methaemoglobin cannot bind oxygen and produces a leftward shift in oxygen-dissociation curve, causing hypoxaemia [12]. Infants under 3 months of age are particularly susceptible to methaemoglobinaemia, for several reasons [11, 13]. They have lower activity of red cell enzyme NADH-cytochrome b5 reductase, which converts methaemoglobin to haemoglobin. Their lower gastric pH results in proliferation of intestinal flora, which reduce the ingested nitrate to nitrite. Foetal haemoglobin is oxidised more readily by nitrite to methaemoglobin than adult haemoglobin [14].

Very high concentrations (>2500 mg/kg) of nitrate [16] in vegetables (especially leafy vegetables) have been reported in different countries [17, 18]. Oral reduction of nitrate is the most important source of nitrite, accounting for approximately 70–80% of the human total nitrite exposure. About 5–7% of all ingested nitrate is converted to nitrite at the base of the tongue, where nitrate reducing bacteria are present. For subjects with a high rate of conversion, this figure may be up to 20% [19].

Spinach, lettuce, broccoli, cabbage, celery, radish, beetroot, etc. possess the tendency to accumulate nitrates. On the other hand, vegetables such as carrots, cauliflowers, French beans, peas and potatoes seldom accumulate nitrates. The nitrate content of vegetables may range from 1 to 10,000 mg/kg [15]. Baby foods have special functions in infant diets because they are major source of nutrients [20, 21] and a unique source of food during the first months of life [22]. According to Woese et al. [23], organic products contain fewer nitrates than their counterparts obtained with the conventional methods. On the other hand, De Martin and Restani [24] showed a content of nitrate independent from the agricultural practice used, with some organic leafy vegetables having very high nitrate content.

Consumption of vegetables containing high level of nitrates and incorrect storage of home-made vegetable purees has been found to be potential causes of infant methemoglobinaemia [25]. Processed infant foods may also contain increased levels of nitrites [26]. Commission Regulation (EC) No. 1881/2006 of 19 December 2006 [27], setting maximum levels for certain contaminants in food, establishes maximum levels for nitrates in spinach of 2000–3000 mg/kg, lettuce of 2000–4500 mg/kg and in processed cereal-based foods

and baby foods for infants and young children of 200 mg/kg. An acceptable daily intake (ADI) of 0–3.7 mg/kg body weight/day was established by the Scientific Committee of Food (SCF) in 1990 [28], retained in 1995 [29] and reconfirmed by the Joint FAO/WHO Expert Committee on Food additives (JEFCA) in 2002 [30]. The acceptable daily intake (ADI) for nitrite was set at 0.06 mg/kg body weight [31].

Considering the potential risks of nitrate and nitrite to health as well as the lack of occurrence data in Turkey, the aims of this study were to determine nitrate and nitrite levels in vegetable and fruit-based baby foods labeled as from organic and conventional origin available in Izmir, Turkey and to estimate the toxicological risk associated with the consumption of baby foods containing nitrate and nitrite.

MATERIALS and METHODS

Samples

Twenty different vegetable and fruit-based baby foods were examined. All samples were bought from markets in Izmir (the third biggest city in Turkey). Brand codes, main ingredients and production methods (organic or conventional) of all samples were shown in Table 1. Nine of the samples were from organic origin, 11 of the samples were from conventional origin. Nine of the samples were vegetable-based baby foods, 11 of the samples were fruit-based baby foods. Three of samples were belong to brand A, 7 of samples were belong to brand B, 6 of samples were belong to brand C, 4 of samples were belong to brand D.

Reagents

Analytical grade reagents were used. Sodium nitrite, sodium nitrate, cadmium acetate dihydrate, sulfanilic acid, N-(1-naphthyl)-ethylenediamine dihydrochloride, glacial acetic acid, ammonia, potassium ferrocyanide trihydrate, and disodium tetraborate decahydrate were purchased from Merck (Darmstadt, Germany). Zinc sulfate heptahydrate was purchased from Riedel-de Haen (Seize, Germany), and zinc powder from BDH (Poole, the U.K.).

Apparatus

Ultra pure distilled water used throughout the procedure was supplied from the distillation apparatus of Zener Power I ultra pure distilled water instrument (Human, Korea). Absorbance of the red-violet azo compound was measured with a Cary 50 UV-vis spectrophotometer (Varian, U.K.).

Sample Preparation

The method of Özdeştan and Üren [32] was followed. A 25 g amount of sample was homogenized with 80 mL of distilled water in a high-speed blender for 5 min and transferred into a 400 mL beaker; 100 mL of hot water (70-80°C) and 10 mL of 5% (w/v) $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$

solution were added and then mixed and heated for 15 min. The mixture was clarified by adding 5 mL of 23% (w/v) ZnSO₄.7H₂O solution and shaking for 15 s, then adding 5 mL of 15% (w/v) K₄Fe(CN)₆.3H₂O solution and shaking for 15 s. After cooling, sample was diluted to 250 mL with distilled water and filtered through a

Whatman (no. 40) filter paper, and the extract was analyzed immediately. Griess reagent was prepared by the method of Özdeştan and Üren [32].

Table 1. Brand codes, main ingredients and production types of baby foods

Sample Code	Brand Code	Main Ingredients	Organic
BF1	A	patato, spinach	+
BF2**	A	apple, carrot	+
BF3	A	carrot, patato, pea, cauliflower	+
BF4	B	broccoli, patato, grape juice, pea	+
BF5**	B	grape juice, apple, carrot, banana, apricot	+
BF6	B	parsley, carrot, leek, patato, pea, broccoli, celery root	-
BF7**	C	peach, banana, apple	-
BF8	C	patato, apple puree, bean, carrot, pea, celery root, concentrated tomato juice, onion	-
BF9	D	carrot, celery root, patato, grape juice	-
BF10	B	bean, tomato, apple juice, patato	+
BF11	D	carrot, apple, apple juice	-
BF12**	B	apricot, banana, carrot, apple juice	+
BF13**	B	apple, apple juice, carrot	+
BF14	D	carrot, patato, cauliflower	-
BF15**	D	grape juice, banana, carrot, peach	-
BF16**	C	apple, grape, orange, banana	-
BF17**	C	pomace, banana puree, concentrated apple juice	-
BF18**	C	pear, peach, pineapple, concentrated grape juice	-
BF19**	C	banana, mandarin orange, pear, apple, carrot	-
BF20**	B	apple, apple juice, banana, peach, carrot	+

** Fruit based baby foods

Nitrate and Nitrite Analyses by Spectrophotometric Method

Nitrate and nitrite analyses were performed according to the method of Özdeştan and Üren [32]. The principle of the method was reduction of nitrate to nitrite with cadmium acetate solution and zinc powder and then colorimetric determination of nitrite with Griess reagent. Absorbance of the red-violet azo compound was read at 538nm against a reagent blank using a spectrophotometer. This optical density was a measure of both nitrate and nitrite in the sample due to contribution of the nitrite content, which might occur in the food sample being analyzed. Therefore, nitrate concentration of the sample was determined after subtraction of the corresponding optical density of nitrite from the above-mentioned optical density. Nitrate concentration was calculated by using a nitrate calibration curve. A nitrate calibration curve was prepared by treating 8 mL portions of standard nitrate solutions with nitrate concentrations ranging from 0.5 to 6.0 mg/L, with 1 mL of glacial acetic acid and 1 mL of Griess reagent and plotting absorbance values against nitrate concentrations.

To evaluate the recovery rate of nitrate determination, 2 mL of standard nitrate solution (20 mg of nitrate/L) and 5 mL of distilled water were added to an 8 mL aliquot of sample extract in a 50 mL volumetric flask, and the analysis was completed with the same procedure by reducing nitrate to nitrite. Nitrite concentration was calculated by using a nitrite calibration curve. A nitrite

calibration curve was prepared by treating 8 mL portions of standard nitrite solutions, with nitrite concentrations ranging from 0.025 to 0.400 mg/L, with 1 mL of glacial acetic acid and 1 mL of Griess reagent and plotting absorbance values against nitrite concentrations treatment with Griess reagent. To find the recovery rate of nitrite determination, 5 mL of standard nitrite solution (2 mg of nitrite/L) was added into 5 mL of sample extract, and the solution was diluted to 50 mL with distilled water. The analysis was completed by treatment with Griess reagent.

Dietary Exposure Estimates

Considering the absence of consumption data regarding Turkish infants and young children, the estimated intake of nitrates through the consumption of commercial baby foods was calculated by multiplying different exposure scenarios including the mean, highest and lowest nitrate concentrations in all baby foods analyzed [33] by the mean consumption of baby foods per day per body weight (bw) provided by the DONALD (Dortmund Nutritional and Anthropometrical Longitudinally Designed) study [34]. The worst case, concerning girls' weight was used. The DONALD study provided separate data for the mean consumption of baby foods (without consideration of infant formulae, follow-on formulae and dry cereal-based foods), from infants of 3, 6, 9 and 12 months, that was directly used to estimate the exposure to nitrate and nitrite in the commercial products analyzed in this study [35].

Statistical Analysis

Some of the statistical analyses were performed with the SPSS 16.0 statistics package program. Statistical analysis of data was achieved by using one-way analysis of variance (ANOVA) and Duncan post-test. XLSTAT Version 2012.4.02 statistics package program was used for principal component analysis (PCA). PCA was used to discriminate vegetable-based and fruit-based baby food samples according to their nitrate and nitrite concentrations.

RESULTS and DISCUSSION

Nitrate and Nitrite Contents of Baby Foods

Nitrate and nitrite concentrations found, expressed in mg/kg (w/w), in the different baby foods monitored are summarized in Table 2. According to the results, nitrate concentrations of baby foods changed from 3.32 to 99.73 mg/kg. Average nitrate content of baby foods was 37.12 mg/kg. Results were all below the legislated value (200 mg/kg) for nitrate content. Nitrite concentrations of baby foods changed from non-detectable values to 30.09 mg/kg. Average nitrite content of baby foods was 7.90 mg/kg. BF6 contained the highest amount of nitrate and BF4 contained the highest amount of nitrite. Nitrate contents of samples were higher than the nitrite content of samples. Baby foods from organic and conventional origin showed mean nitrate contents of 29.33 and of 35.15 mg/kg, respectively. Organic baby foods contained less amount of nitrate compared with conventional products. Baby foods from organic and conventional origin showed mean nitrite contents of 8.92 and of 7.88 mg/kg, respectively. Average nitrate content of baby foods belong to brand A, B, C and D was 25.03, 41.23, 23.11 and 37.08 mg/kg, respectively. Average nitrite content of baby foods belongs to brand A, B, C and D was 6.63, 9.26, 6.02 and 9.32 mg/kg, respectively. Baby foods, which belong to B and D brand, contained the highest amount of nitrate and nitrite. Average nitrate and nitrite content of fruit-based baby foods was 25.04 and 1.90 mg/kg, respectively. Average nitrate and nitrite content of vegetable-based baby foods was 41.69 and 15.45 mg/kg, respectively. Nitrate and nitrite content of vegetable-based baby foods were higher than the nitrate and nitrite content of fruit-based baby foods.

When the overall mean nitrate and nitrite levels in all baby foods were compared, there were statistically significant differences ($P < 0.05$). When the overall nitrate and nitrite concentrations of organic and conventional baby foods were compared, there were no statistically significant differences between nitrate and nitrite contents of samples ($P > 0.05$; $P > 0.05$). When the overall mean nitrate levels in different brand baby foods were compared, there were no statistically significant differences between different brands ($P > 0.05$). According to the results of ANOVA, there were no statistically significant differences between the nitrite contents of different brand baby foods ($P > 0.05$). When the overall mean nitrate and nitrite levels of fruit and vegetable-based baby foods were compared,

statistically significant differences were obtained. Among the baby foods, vegetable-based baby foods had the higher nitrate and nitrite contents ($P < 0.05$; $P < 0.05$). The result of PCA for vegetable and fruit-based baby foods was seen in Figure 1.

Table 2. Nitrate and nitrite contents (mg/kg) and standard deviation values of baby foods^a

Sample code	Nitrate	Nitrite
BF1	25.01 ^{fg} ±0.97	9.70 ^e ±0.64
BF2	31.81 ^{cdef} ±5.49	1.11 ^f ±1.00
BF3	18.26 ^{hi} ±0.52	9.07 ^e ±0.88
BF4	35.31 ^{cd} ±7.76	30.09 ^a ±6.82
BF5	29.10 ^{def} ±6.09	2.25 ^f ±1.21
BF6	99.73 ^a ±3.87	13.26 ^d ±0.38
BF7	37.37 ^c ±2.10	8.64 ^e ±0.09
BF8	34.26 ^{cde} ±2.33	23.17 ^b ±1.15
BF9	28.06 ^{ef} ±6.39	13.45 ^d ±0.22
BF10	50.80 ^b ±1.89	16.46 ^c ±0.43
BF11	46.31 ^b ±1.71	0.51 ^f ±0.48
BF12	17.80 ^{hi} ±1.97	1.88 ^f ±0.37
BF13	25.03 ^{fg} ±1.24	0.04 ^f ±0.08
BF14	37.48 ^c ±5.26	23.30 ^b ±0.51
BF15	36.46 ^c ±0.78	ND
BF16	21.50 ^{gh} ±1.72	ND
BF17	13.87 ⁱ ±0.94	0.44 ^f ±0.47
BF18	3.32 ^h ±0.99	1.18 ^f ±0.61
BF19	28.33 ^{ef} ±2.50	2.69 ^f ±0.66
BF20	30.87 ^{cdef} ±4.12	0.82 ^f ±0.43

ND: Not detected

^aDifferent letters within a column mean significant differences according to the Duncan test ($P < 0.05$).

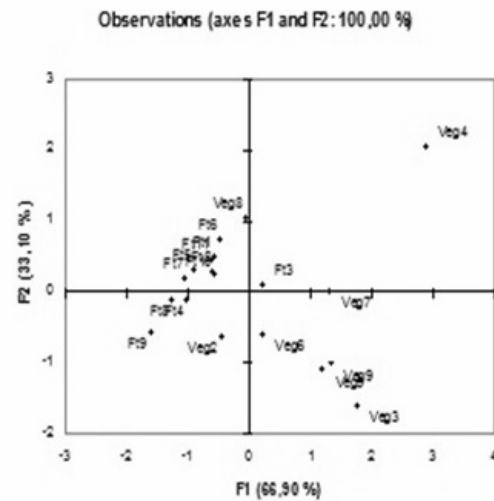


Figure 1. Principle component analysis (PCA) plot for vegetable and fruit-based baby foods

Özdeştan and Üren [32] developed a new method for the determination of nitrate and nitrite content of green leafy vegetables. The developed method was applied to a range of baby foods. The regression equation for nitrate was $y = 0.1438x + 0.009$ with a correlation coefficient of 0.9957. The regression equation for nitrite was $y = 0.2298x + 0.0002$ with a correlation coefficient of 0.9996. The limit of detection was calculated to give a

signal-to-noise ratio of 3 and found as 31.4 mg of nitrate/kg of food [32]. Recovery rates of nitrate determination were calculated for samples. Nitrate recovery for baby food was satisfactory and 99.29%. Recovery rates of nitrite determination were calculated for baby foods. Nitrite recovery was satisfactory and 99.43%.

Table 3 shows the estimated nitrate intake values calculated using consumption information provided by the DONALD study. The estimated nitrate intake through baby foods for the mean, highest and lowest nitrate concentration ranged between 0.43 (11.60% of ADI) and 1.01 mg/kg bw/day (27.30% of ADI), 1.15 (31% of ADI) and 2.71 mg/kg bw/day (73.2% of ADI) and 0.04 (1.08% of ADI) and 0.09 mg/kg bw/day (2.43% of ADI), respectively, all below the ADI level (3.7 mg/kg bw/day).

Table 3. Estimated nitrate intake from commercial baby foods calculated using consumption information provided by DONALD study [34, 35] and percentage of acceptable daily intake (ADI) for the different exposure scenarios using the mean, highest and lowest nitrate concentrations in all baby foods in this study (n=20)

Consumption scenarios			Estimation of nitrate intake (mg kg ⁻¹ bw day ⁻¹)			% of ADI		
Age (months)	Body weight (kg)	Mean consumption of baby foods (g/day)	Mean (37.12 mg/kg)	Highest value (99.73 mg/kg)	Lowest value (3.32 mg/kg)	Mean (37.12 mg/kg)	Highest value (99.73 mg/kg)	Lowest value (3.32 mg/kg)
3	5.80	67.0	0.43	1.15	0.04	11.60	31.10	1.08
6	7.50	195.0	0.97	2.59	0.086	26.20	70.00	2.32
9	8.60	234.0	1.01	2.71	0.09	27.30	73.20	2.43
12	9.40	208.0	0.82	2.50	0.07	22.20	59.50	1.89

Pardo-Marin et al. [36] analyzed the vegetable-based baby foods to determine nitrate contents. The mean nitrate content in vegetable-based baby foods (taking into account the samples with concentrations above the minimum reporting levels) was 60.4 mg/kg with a standard deviation of 38.6 mg/kg [36]. The nitrate levels were lower the maximum level proposed by European Union legislation. The estimated nitrate daily intake through baby foods for infants between 0–1 and 1–2 years of age were 13 and 18%, respectively, of the acceptable daily intake. This level is lower than those in Spain [37] and Estonia [38], where mean levels of 92 and 88 mg/kg were reported. All these values are higher than our results. Tamme et al. [38] detected the highest levels in baby foods containing carrot and pumpkin (62-148 and 124-162 mg/kg, respectively). Similar results have been reported in a Spanish study [37], where nitrate concentration exceeding the regulatory limit was found in baby foods in which the main ingredient was carrot. In our study, all the samples were in mixed form so we couldn't find a similar result. The baby food sample, which contained the highest amount of nitrate, contained parsley, carrot, leek, potato, pea, broccoli, celery root as main ingredients. The baby food sample, which contained the highest amount of nitrite, contained broccoli, potato, grape juice, pea as main ingredients. But it was obvious that vegetable-based baby foods contained higher amount of nitrate and nitrite compared with fruit-based baby foods.

Nitrite intake values were also calculated using consumption information provided by the DONALD study. The estimated nitrite intake through baby foods for the mean and highest nitrite concentration ranged between 0.09 and 0.21 mg/kg bw/day, 0.35 and 0.82 mg/kg bw/day, respectively, above the ADI level (0.06 mg/kg bw/day).

According to the results of Vasco and Alvito [35] nitrate contents ranged from 5 to 230 mg/kg with a mean concentration of 102 mg/kg for vegetable-based baby foods, and a median of 5 mg/kg for both fruit purees and juices. One sample of vegetable-based baby food was higher than the legislated value (200 mg/kg) [35]. All of our results were found below the legislated value (200 mg/kg) for nitrate contents. There was no legislated value for nitrite contents.

Vasco and Alivito [35] revealed that there were no significant differences in nitrate content when comparing baby food products from organic and conventional origin for each product group analyzed. Similar result was obtained in this study, no significant difference was obtained between the nitrate and nitrite contents of organic and conventional baby foods (P<0.05).

An ion chromatographic method with post-column derivatization and spectrophotometric detection is presented for the determination of nitrate and nitrite in baby food by Casanova et al. [39]. Nitrate was reduced to nitrite online by post-column reduction using vanadium (III) chloride and heat. Nitrite reacted with Griess reagent to produce a dye that was detected at 525 nm. The proposed method provides simultaneous determination of nitrate and nitrite. Average recoveries of nitrate and nitrite residues ranged from 82 to 107% for fortification levels of 25-400 ppm.

An ion chromatographic method was developed for the determination of nitrate and nitrite in vegetable and fruit baby foods by McMullen et al. [40]. In this method, nitrate and nitrite were separated on a hydroxide-selective anion exchange column using online electrolytically generated high-purity hydroxide eluant and detected using suppressed conductivity detection. Average recoveries of spiked nitrite residue ranged from 91 to 104% and spiked nitrate residue ranged from 87 to 104% [40]. Recovery rates for our method were found

similar with the literature values. Our method is accurate, simple, fast and cost-effective spectrophotometric method. The principle of the method is the reduction of nitrate to nitrite with cadmium acetate solution and zinc powder and then spectrophotometric determination of nitrite with Griess reagent. This method is much more reliable than the cadmium column reduction method. The proposed method is easily applicable, and it is not necessary to monitor the reducing capacity of the cadmium acetate solution-zinc powder system. In addition, preparation of a cadmium column, which is a difficult and time-consuming procedure, is not necessary. Also, the method does not need any sophisticated instruments like HPLC.

Erkekođlu and Baydar [41] aimed to detect whether there was any nitrite contamination in infant formulas and baby foods marketed in Turkey and to estimate possible toxicological risks in this sensitive physiological period. For this purpose, the samples were randomly collected and divided into four groups: milk-based, cereal-based, vegetable-based, and fruit-based. The average nitrite contamination was found to be $204.07 \pm 65.80 \mu\text{g/g}$ in 42 samples, with $1,073 \mu\text{g/g}$ maximum. These values were very high compared with the literature values. According to the results, baby and infant formulas include various nitrite levels. These results were higher than our results. We found less amount of nitrite compared with the results of Erkekođlu and Baydar [41]. In our study, only fruit and vegetable-based baby foods were analyzed so the samples were not the same with the other study.

According to the results of Vasco and Alvito [35], the estimated nitrate intake through baby foods for the mean and for the 90th percentile of nitrate concentration ranged between 0.5 (15% of ADI) and 1.3 mg/kg bw/day (35% of ADI) and 1.4 (38% of ADI) and 3.3 mg/kg bw/day (89% of ADI), respectively, all below the ADI level (3.7 mg/kg bw/day). These values were higher than those reported by Pardo-Marin et al. [36] and Tamme et al. [38], with nitrate intake mean values of 13 and 22%, respectively. In this study, the estimated nitrate intake through baby foods for the mean nitrate concentration ranged between 0.43 (11.60% of ADI) and 1.01 mg/kg bw/day (27.30% of ADI). These results were found similar with the literature values. The estimated nitrite intake through baby foods for the mean and highest nitrite concentration ranged between 0.09 and 0.21 mg/kg bw/day, 0.35 and 0.82 mg/kg bw/day, respectively, above the ADI level (0.06 mg/kg bw/day). For 11 of the samples (all of them fruit-based except one sample), the estimated nitrite intake were below the ADI level.

CONCLUSIONS

The described method is adequate for the analysis of nitrates and nitrites in baby foods (vegetable-based and fruit-based baby foods). The present study reports the first data on nitrate and nitrite occurrence in 20 commercial baby foods marketed in Izmir. Nitrate concentrations of baby foods changed from 3.32 to 99.73 mg/kg. Nitrite concentrations of baby foods

changed from non detectable values to 30.09 mg/kg. Nitrate contents were all below the legislated value (200 mg/kg) in Turkey. Nitrate levels were lower than the maximum limits established by European Union legislation. To date, there are no reports comparing nitrate content of baby foods from this origin. The estimated nitrate intake through baby foods for the mean nitrate concentration was found below the ADI level (3.7 mg/kg bw/day). For 11 of the samples, the estimated nitrite intake was below the ADI level. It is essential to take special care throughout the process of manufacturing food for infants and children. Nonetheless, since food consumption data for risk assessment should be based on real intakes, it is recommended that research be conducted on actual food consumption by Turkish infants and young children. Attention should be paid to ensure nitrate and nitrite levels are as low as reasonably achievable, since vegetables have an essential nutritional function and play an important role in health protection.

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REFERENCES

- [1] Johnson, C.J., Kross, B.C., 1990. Continuing importance of nitrate contamination of groundwater and wells in rural areas. *Am. J. Ind. Med.* 18(4): 449–456.
- [2] Hallberg, G.R., 1989. In: Follet, R.F. (Ed.), Nitrate in Groundwater in the United States: Nitrogen Management and Groundwater Protection. Elsevier, Amsterdam, Netherlands, pp. 35–74.
- [3] Van Leeuwen, F.X.R., 2000. Safe drinking water: the toxicologist's approach. *Food Chem. Toxicol.* 38: S51–S58.
- [4] Cammack, R., Joannou, C.L., Cui, X-Y., Martinez, C.T., Maraj, S.R., Hughes, M.N., 1999. Nitrite and nitrosyl compounds in food preservation. *Biochim. Biophys. Acta* 1411: 475–488.
- [5] Connolly, D., Paul, B., 2001. Rapid determination of nitrate and nitrite in drinking water samples using ion-interaction liquid chromatography. *Anal. Chim. Acta* 441: 53–62.
- [6] Cemek, M., Akkaya, L., Birdane, Y.O., Seyrek, K., Bulut, S., Konuk, M., 2007. Nitrate and nitrite levels in fruit and natural mineral waters marketed in western Turkey. *J. Food Comp. Anal.* 20: 236–240.
- [7] Amr, A., Hadidi, N., 2001. Effect of cultivar and harvest date on nitrate (NO_3^-) and nitrite (NO_2^-) content of selected vegetables grown under open field and greenhouse conditions in Jordan. *J. Food Comp. Anal.* 14: 59–67.
- [8] Hsu, J., Arcot, J., Lee, N.A., 2009. Nitrate and nitrite quantification from cured meat and vegetables and their estimated dietary intake in Australians. *Food Chem.* 115: 334–339.

- [9] Kazemzadeh, A., Ensafi, A.A., 2001. Sequential flow injection spectrophotometric determination of nitrite and nitrate in various samples. *Anal. Chim. Acta* 442: 319–326.
- [10] Prasad, S., Chetty, A.A., 2008. Nitrate-N determination in leafy vegetables: study of the effects of cooking and freezing. *Food Chem.* 106: 772–780.
- [11] Greer, F.R., Shannon, M., 2005. American Academy of Pediatrics Committee on Nutrition, American Academy of Pediatrics Committee on Environmental Health, Infant methemoglobinemia: the role of dietary nitrate in food and water. *Pediatrics* 116: 784–786.
- [12] Chan, T.Y.K., 1996. Food-borne nitrates and nitrites as a cause of methemoglobinemia. *Southeast Asian J. Trop. Med. Public Health* 27: 189–192.
- [13] Savino, F., Maccario, S., Guidi, C., Castagno, E., Farinasso, D., Cresi, F., Silvestro, L., Mussa, G.C., 2006. Methemoglobinemia caused by the ingestion of courgette soup given in order to resolve constipation in two formula-fed infants. *Ann. Nutr. Metab.* 50: 368–371.
- [14] Chan, T.Y.K., 2011. Vegetable-borne nitrate and nitrite and the risk of methaemoglobinaemia. *Toxicol. Lett.* 200: 107–108.
- [15] Ximenes, M.I.N., Rath, S., Reyes, F.G.R., 2000. Polarographic determination of nitrate in vegetables. *Talanta* 51: 49–56.
- [16] Santamaria, P., 2006. Nitrate in vegetables: toxicity content, intake and EC regulation. *J. Food Agric.* 86: 10–17.
- [17] Du, S.T., Jin, C.W., Zhang, Y.S., 2010. Current situations and research progress of nitrate pollution in vegetables and their regulating strategies. *Sci. Agric. Sin.* 43: 3580–3589.
- [18] EFSA, 2008 European Food Safety Authority. Nitrate in vegetables – scientific opinion of the panel on contaminants in the food chain. *Eur. Food Saf. Assoc. J.* 689: 1–79.
- [19] Eisenbrand, G., Spiegelhalter, B., Preussmann, R., 1980. Nitrate and nitrite in saliva. *Oncology* 37: 227–231.
- [20] Bermejo, P., Pena, E., Dominguez, R., Bermejo, A., Fraga, J.M., Cocho, J.A., 2000. Speciation of iron in breast milk and infant formulas whey by size exclusion chromatography–high performance liquid chromatography and electrothermal atomic absorption spectrometry. *Talanta* 50(6): 1211–1222.
- [21] Ikem, A., Nwankwoala, A., Oduyungbo, S., Nyavor, K., Egiebor, N., 2002. Levels of 26 elements in infant formula from USA, UK, and Nigeria by microwave digestion and ICP–OES. *Food Chem.* 77(4): 439–447.
- [22] Rodriguez, R.E.M., Sanz, A.M., Diaz, R.C., 2000. Concentrations of iron, copper and zinc in human milk and powdered infant formula. *Int. J. Food Sci. Nutr.* 51(5): 373–380.
- [23] Woese, K., Lange, D., Boess, C., Bogl, K.W., 1997. A comparison of organically and conventionally grown foods - results of a review of the relevant literature. *J. Sci. Food Agric.* 74: 281–293.
- [24] De Martin, S., Restani, P., 2003. Determination of nitrates by a novel ion chromatographic method: occurrence in leafy vegetables (organic and conventional) and exposure assessment for Italian consumers. *Food Add. Contam.* 20: 787–792.
- [25] Sanchez-Echaniz, J., Benito-Fernandez, J., 2001. Methemoglobinemia and consumption of vegetables in infants. *Pediatrics* 107: 1024–1028.
- [26] Hill, M., 1996. Nitrates and nitrites in food and water. Cambridge: Woodhead.
- [27] EC, 2006. European Commission. Commission Regulation (EC) No. 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. *Off. J. Eur. Commun. L.* 364: 5–24.
- [28] EC, 1992. European Commission. Opinion on nitrate and nitrite. Reports of the Scientific Committee for Food (SCF) 26th Series, 21–28.
- [29] EC, 1997. European Commission. Opinion on nitrate and nitrite. Reports of the Scientific Committee for Food (SCF) 38th Series, 1–33.
- [30] FAO/WHO, 2003. Food and Agriculture Organisation of the United Nations/World Health Organization. Nitrate (and potential endogenous formation of N-nitroso compounds). WHO Food Additive series 50. Geneva: World Health Organisation.
- [31] EU Scientific Committee for Food Opinion on nitrate and nitrite, 1995. European Commission DG III Brussels *Expressed on 22 September 1995-Annex 4 to document III/56/95, CS/CNTM/NO3/20-FINAL.*
- [32] Özdestandan, Ö., Üren, A., 2010. Development of a cost-effective method for nitrate and nitrite determination in leafy plants and nitrate and nitrite contents of some green leafy vegetables grown in the Aegean Region of Turkey. *J. Agric. Food Chem.* 58: 5235-5240.
- [33] EFSA, 2005. European Food Safety Authority. Opinion of the Scientific Committee on a request from EFSA related to a harmonised approach for risk assessment of substances which are both genotoxic and carcinogenic opinion. *EFSA J.* 282: 1–31.
- [34] Kersting, M., Alexy, U., Sichert-Hellert, W., Manz, F., Schoch, G., 1998. Measured consumption of commercial infant food products in German infants: results from the DONALD study. Dortmund Nutritional and Anthropometrical Longitudinally Designed. *J. Pediatr. Gastroenterol. Nutr.* 27: 547–552.
- [35] Vasco, E.R., Alvitoa, P.C., 2011. Occurrence and infant exposure assessment of nitrates in baby foods marketed in the region of Lisbon. Portugal. *Food Add. Contam. B4(3):* 218–225.
- [36] Pardo-Marin, O., Yusa-Pelecha, V., Villalba-Martin, P., Perez-Dasi, J.A., 2010. Monitoring programme on nitrates in vegetables and vegetable-based baby foods marketed in the Region of Valencia, Spain: levels and estimated daily intake. *Food Add. Contam.* 27(4): 478-486.
- [37] Hardisson, A., Gonzalez-Padron, A., Frias, I., Reguera, J.I., 1996. The evaluation of the content of nitrates and nitrites in food products for infants. *J. Food Comp. Anal.* 9: 13–17.

- [38] Tamme, T., Reinik, M., Roasto, M., Juhkam, K., Tenno, T., Kiis, A., 2006. Nitrates and nitrites in vegetables and vegetable-based products and their intakes by Estonian population. *Food Add. Contam.* 23: 355–361.
- [39] Casanova, J.A., Gross, L.K., McMullen, S.E., Schenck, F.J., 2006. Use of Griess reagent containing vanadium (III) for post-column derivatization and simultaneous determination of nitrite and nitrate in baby food. *J. AOAC Int.* 89(2): 447-451.
- [40] McMullen, S.E., Casanova, J.A., Gross, L.K., Schenck, F.J., 2005. Ion chromatographic determination of nitrate and nitrite in vegetable and fruit baby foods. *J. AOAC Int.* 88(6):1793-1796.
- [41] Erkekoğlu, P., Baydar, T., 2009. Evaluation of nitrite contamination in baby foods and infant formulas marketed in Turkey. *Int. J. Food Sci. Technol.* 60(3): 206-209.
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