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Nitrite Levels in Commercially Available Baby Purees Marketed in Turkey

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Pınar Erkekoğlu*, Ali Aşcı, Terken Baydar*,⁰**

Introduction

It is essential to take special care throughout the entire process of manufacturing foods for young children, as the fragility of them requires an increased safety. A contaminant is an impurity; any material of an extraneous nature associated with a chemical, a pharmaceutical preparation, a physiologic principle, or an infectious agent. Food consumption is an important route of human exposure to contaminants such as nitrites or industrial pollutants. Several contaminants, which can come from different stages of production and storage, may be present in infant formulas and baby food[1].

Nitrite and nitrate ions, which are widespread environmental contaminants present in drinking water, vegetables, fruits and meat are naturally occurring forms of nitrogen. Contamination of baby foods with nitrites can cause to detrimental effects on the baby's health[2-5]. Body converts nitrates to nitrites by reduction simultaneously and nitrites are responsible for most of the adverse effects observed after ingestion. Nitrites have strong oxidation ability of ferrohemoglobin and the final compound is methemoglobin, which is not capable of binding molecular oxygen[2, 6-8]. The primary adverse effect associated with human exposure to nitrite is introduced in clinic as methemoglobinemia[9]. High nitrate intake may also cause the same condition. In particular, infants are the most susceptible subpopulation to nitrite-induced methemoglobinemia. The possible reasons are as follows:

*Department of Toxicology, Faculty of Pharmacy, [1] Hacettepe University, Ankara,
**Atatürk University, Erzurum; Turkey
[0]Corresponding Author: Fax: 90312 311 47 77, e-mail: tbaydar@hacettepe.edu.tr

- The pH of the stomach and gut in infants and babies is normally higher than in older children and adults (pH>4). Higher stomach and gut pH enhances the conversion of nitrates to nitrites[10].

- The elevated levels of readily oxidized fetal hemoglobin and the immaturity of their NADH-dependent methemoglobin reductase system play an important role in their susceptibility[11-13].

- Congenital deficiency of glucose-6-phosphate dehydrogenase may also be a cause of methemoglobin reductase deficiency in some races (e.g. Mediterranean)[11-13].

- Frequent occurrences of infant gastroenteritis may favor the development of methemoglobinemia when the upper gastrointestinal tract becomes colonized with bacteria[11-13].

- The intake of fluid is higher relative to body weight in the early days of life[11-13].

Older children and adults are not as susceptible as the infants are to methemoglobinemia because of the induction of methemoglobin reductase in the rearing period[11]. There is little evidence that breast-fed infants develop methemoglobinemia from nitrites and nitrates ingested by nursing mother. Clinical manifestations of methemoglobinemia in infants and young children include cyanosis with unusual bluish- or brownish-grey skin color and it is termed as “Blue Baby Syndrome” generally. As the susceptibility of young children towards the adverse effects of toxic substances is higher than those of adults, these types of contaminations should seriously be taken into concern[6, 7, 9].

Therefore, the aim of the present study was to evaluate nitrite levels in baby purees by using a modified spectrophotometric method for providing information on nitrite status in baby purees produced and consumed in Turkey.

Materials and Methods

Sample Collection

Twenty-two baby purees in jars with sealed caps were randomly collected from different markets in Ankara, Turkey during the spring 2006.

The collected samples, which were distributed throughout the country, were of different brands within the groups and the limited number of samples was due to the limited number of different brands and sorts of baby purees in the country. The fruit-based purees belong to 4 different baby food producing companies while vegetable-based purees belong to 3 different companies. The shelf-life of the purees used in the study was maximum one month. The purees were immediately extracted after opening the cap of the jar. They were divided into two groups: fruit-based (n=14) and vegetable-based (n=8).

Chemicals and Standards

All chemicals used in this study were obtained from Sigma Co. (St. Louis, MO, USA) and from Merck Co. (Darmstadt, Germany). Ultra-high pure distilled water was used in the analytical work. Stock nitrite solution was prepared by dissolving 50 mg sodium nitrite per dl of ammonia. A working solution containing 5 g ml^{-1} sodium nitrite was prepared daily by proper volume of stock solution. Standard solutions containing 0.25, 0. 50, 0. 75, 1.0, 2.0, 4.0, 6.0, 10 g ml^{-1} sodium nitrite were obtained with dilution.

Extraction Procedure

The procedure was applied as in our last study on baby foods with slight modifications[3]. Briefly, 10 grams of each sample was used for the nitrite analysis. 40 ml hot water and 12 ml sodium hydroxide (2% w/v in water) was added on the sample and blended for 5 minutes. 50 ml water was then added and mixture was blended again for another five minutes. Later, 10 ml of zinc sulfate (7.2% w/v in water) was added and mixture was blended for 5 minutes. The next step was to add 5 ml sodium hydroxide and the mixture was blended for another five minutes. 83 ml of water was added and mixed for 5 minutes. The mixture was filtered through filtered paper (preferably Whatman No.1) until the filtrate was completely clear.

Nitrite Determination

Nitrite was detected and analyzed by the Griess Reaction, involving the formation of a deep red-colored azo dye upon treatment of nitrite-containing sample with sulfanilic acid and N-(1-naphtyl) ethylenediamine[14].

The clear 10 ml-filtrate was used as spectrophotometric measurement. 9 ml of ammonia buffer and 5 ml of 60% acetic acid was added on the filtrate tubes. 5 ml of sulfanilic acid (1% w/v in 30% acetic acid) and 5 ml of Marshall's Reagent (N-(1-naphtyl) ethylenediamine hydrochloride; 0.1% w/v in 60% acetic acid) was added, and then diluted to 50 ml by water and the mixture was vortexed for 30 seconds. The mixture was let in the dark for 25 minutes. The same procedure was applied to standard solutions and the absorbance was determined at 550 nm using Shimadzu 160 UV spectrophotometer (Kyoto, Japan).

Statistical Analysis

The nitrite content of the sample was expressed as microgram nitrite per gram of puree wet weight. All of the results were expressed as mean±standard error of mean (SEM). Comparisons between two independent groups were made by the Mann-Whitney U-test considering p at 0.05.

Results

The detection limit of the method was found 0. 25 g ml^{-1}. A linear concentration-absorbance relationship with nitrite standards (0.25, 0.50, 0.75, 1.0, 2.0, 4.0, 6.0, 10 g ml^{-1}) was obtained as shown in Figure 1. The recovery average was 98.82% on four occasions for supplementation with 0.50 g ml^{-1} of nitrite. The coefficient of variations at 0.50 g ml^{-1} concentration of nitrite were 0.25 and 0.71 for with-in and between-run precisions, respectively.

The mean nitrite contamination of all samples (n=22) was found 31.30 ± 10.10 g g^{-1}. The mean nitrite levels in the tested purees were found 23.05 ± 6.10 and 39.29 ± 16.57 g g^{-1} in fruit- and vegetable-based purees, respectively. The individual results were shown in Figure 1. Though vegetable-based baby foods had higher nitrite content, we could not find any difference between the groups ($p>0.05$). On the other hand, it was observed that the group of vegetable-based purees has 58.66% higher nitrite than fruit-based samples.

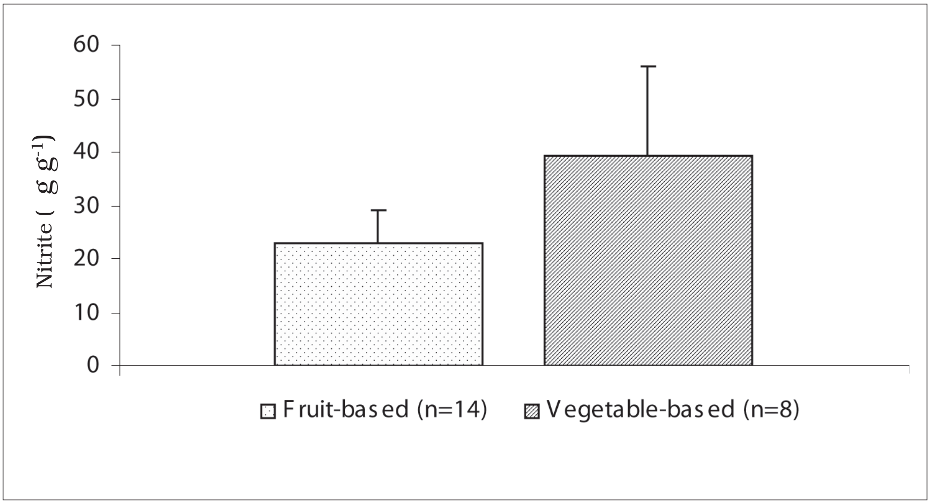

Figure 1
Comparison of the nitrite levels in baby puree samples
($p>0.05$, Mann–Whitney U-test).

Discussion and Conclusion

Infant formula, like no other food, is regulated by its own law, the Infant Formula Act of 1980 as amended in 1986[15]. EU Commission has set regulations (Commission Regulation 466/2001 and Commission Regulation 466/2005 Amendment) on the maximum levels of nitrates, mycotoxins, heavy metals and polycyclic aromatic hydrocarbons (PAHs) in infant food[16-18].

Vegetables are usually rich from nitrites and they are the main source of uptake of an organism. The presence of nitrates and nitrites in agricultural products has been in concern of public in the last years[19]. There are many factors that influence the level of nitrite and nitrate in plants: kind of vegetable/fruit, the nitrogen content of the soil, the amount of sunlight to which the plant is exposed to and seasonal conditions[20]. As green-leaf vegetables (spinach, collard, greens), turnips, carrots and beets accumulate high amounts of nitrite, American Association of Pediatrics recommends that their consumption below the age of 6 months should be avoided as they may contain sufficient nitrate/nitrite to cause methemoglobinemia[21].

Thompson et al. performed a study on 200 foodstuff and 1021 water samples from 561 different supplies[22]. They found that green vegetables had the highest nitrite content as expected. A study by Chung et al. showed that winter and summer nitrite content of vegetables show differences[23]. It is known that, fruits have substantially lower content of nitrite than vegetables. Apple and pear seem to have the lowest content of nitrite among other fruits[19, 24]. As expected in our study we found that fruit-based purees had less nitrite content than vegetable-based purees though the difference between them was not statistically important. This can be due to high standard of deviation with-in the groups.

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) and Scientific Committee on Food (SCF) have proposed an ADI for nitrite of 0-0.07 mg kg^{-1} (body weight, b.w.) per day while EPA has set a reference dose (RfD) of 0.10 mg nitrite nitrogen per kg b.w. per day (equivalent to 0.33 mg nitrite ion kg^{-1} b.w. per day)[25]. However, these values are not applicable to infants and young children; we can assume that the nitrite limit to an 8 kg baby is only 0.56 mg of nitrite in one day according to FAO/WHO. As shown in Table 1, and also according to the results of the present study, a baby is about to take nitrite mean 3.46 mg in 150 g of fruit-based baby puree and a baby is about to take nitrite mean 5.89 mg in 150 g of vegetable-based baby puree. Therefore, the nitrite intake of a baby from both fruit-based and vegetable-based baby purees would be

TABLE I

The nitrite content and estimated intake of nitrite from baby purees for one meal

Puree sample	Nitrite content of the sample (g nitrite per gram, wet weight)		Estimated nitrite intake for one meal (mg nitrite 150 per gram)	
	Maximum	Mean	Maximum	Mean
Fruit-based	84.60	23.05	12.69*	3.46*
Vegetable-based	145.90	39.29	21.88*	5.89*

-For an 8 kg baby the ADI is 0.56 nitrite kg^{-1} day^{-1} according to FAO/WHO.

* Indicates that the intake for one meal is higher than the ADI given by FAO/WHO.

higher than the limits given by regulatory authorities. This may cause mild to moderate methemoglobinemia in children below six moths of age and the susceptibility of the children may vary from one to other. The critical points are the age of the child, methemoglobin reductase level and intake amount of the food. In our previous study[3], we found that baby foods and infant formulas marketed in Turkey (n=42) have a mean nitrite level of 204.07 g g^{-1}. In another study we conducted on the nitrite levels of commercially available soups, we found that the mean±SEM nitrite content was 551±259 (median: 108), 189±35 (median: 136), 57±15 (median: 46.8), 118±23 (median: 109), 58±14 (median: 37.95), 87±48 (median: 39.6), 509±127 (median: 389) g g^{-1} in tomato-, chicken-, yoghurt-, mushroom-, lentil-, vegetable- and meat-based soups, respectively[26]. From these studies, we can clearly demonstrate that these levels are very high compared to different studies conducted in different countries on several different foodstuff[22, 24, 28].

As time is very important for mothers today, they chose to buy baby food and baby purees rather than preparing them at home. Though generally commercially prepared infant foods are thought to be safe, sanitary, and nutritious alternatives for a caregiver to use when not preparing an infant's foods at home, several contaminants or several detrimental ingredients that come from the nature of the product, which it is prepared from, may be present. The important thing is to give baby a balanced diet that has vegetables and fruits, during the first years of life. When introducing infants to complementary foods, it is essential for the caregivers to introduce the right food for the right month and determine the infant's acceptance to each food. Though the manufacturers put the right month to be chosen on the label, mothers may still buy an inappropriate food or puree by mistake. For instance, consumption of green-leaf vegetables or vegetable-based baby purees is the potential cause of acquired infant-methemoglobinemia, this may cause serious consequences, and there are reports in the literature that these babies are hospitalized and needed medical help[19, 20, 27]. Therefore, we can demonstrate that both home-made and commercially available baby foods may pose serious hazards.

Manufacturers of baby foods with vegetable or fruit should select to produce these in areas of the country that do not have high nitrate levels in the soil and monitor the amount of nitrate/nitrite in the final product[21]. Furthermore, the nitrite contamination in baby foods, infant formulas and baby purees should be monitored routinely for safety especially for infant formulas and baby foods to reduce food-borne hazards in infants and young children. Ultimately, surveillance should be continuous, widespread, and must be conducted by government and related authorities.

Summary

This study was aimed to detect whether there was any nitrite contamination in baby purees and to estimate the possible toxicological risks. For this purpose, twenty-two baby purees were randomly collected from the local markets. A modified spectrophotometric method was used to detect nitrite levels in the samples. It was found that the vegetable-based purees had 58.66% higher nitrite concentration than fruit-based purees. According to the data, it can be concluded that commercial baby purees should be monitored routinely because of the nitrite content and surveillance should be continuous in order to reduce of nitrite consumption.

Key words: Nitrite, baby puree, food toxicity

Özet

Türkiye'de Satılan Bebek Pürelerinde Nitrit Düzeyleri

Bu çalışmada bebek pürelerinde nitrit kontaminasyonunun olup olmadığının saptanması ve olası toksikolojik risklerin belirlenmesi amaçlanmıştır. Bu amaçla yerel marketlerden rastgele yirmi iki bebek püresi toplanmıştır. Örneklerdeki nitrit düzeylerinin belirlenmesinde modifiye edilmiş bir spektrofotometrik metot kullanılmıştır. Sebze bazlı pürelerdeki nitrit kontaminasyonunun meyve bazlı olanlardan %58,66 daha fazla olduğu bulunmuştur. Bu verilere göre, nitrit içeriğinin belirlenmesi amacıyla ticari bebek püreleri rutin olarak izlenmeli ve nitrit tüketiminin azaltılması için izlemenin sürekliliği sağlanmalıdır.

Anahtar sözcükler: Nitrit, bebek püresi, gıda toksisitesi

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