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Nitrate and Nitrite Content in Commercially-available Fruit Juice Packaged Products

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ABSTRACT

The concentration of nitrate and nitrite in fruit juice products varies depending on their origin, storage condition and processing technologies .The objectives of this study were: To determine the concentration of nitrate and nitrite levels in most known fruit juices available commercially in the form of packaged in Tehran market (Iran), by spectrophotometric methods, and to compare these results with the maximum admissible limit set in drinking water by different international organizations: United States Environmental Protection Agency (USEPA) and World Health Organization and also national standard. As the assessment of dietary nitrate and nitrite intake by consumption of fruit juice in accordance with acceptable daily intakes was the main objective of the present work, a descriptive – analytical and cross-sectional study was conducted for determination nitrate and nitrite by UV-Visible spectrophotometer in 540 samples of 6 varieties of Pineapple, Orange, Mango, Tropical, Cherry and Grape popular fruit juice packaged products (some were imported) commercially available in Iran market in three consecutive seasons of 2015. The result from this study show that all the fruit juices analyzed contained detectable amount of nitrate, nitrite. The mean nitrate and nitrite levels in the juices ranged from 14.02±0.02to 43.34±1. 42 and 4.23 ± 0.08 to 11.82±1.20 mg/L ± SE, respective ly. The consumption of two or more servings of these products or one serving along with supplement sources of nitrite in diet (such as vegetables, legumes, or even some water sources) could have the daily intake increased above ADI value as recommended by WHO.

Keywords: Fruit juice, Nitrate, Nitrite, Food safety, UV-Visible spectrophotometer.

INTRODUCTION

Nitrate and nitrite occurred naturally environmental and found in vegetable and foods .The most sources of these ions are nitrogen fertilizer in agriculture for the production of fruits and vegetable. Now, everybody care about not only the chemical compositions and nutritive value of vegetables, fruits and cereals but also to the amount of harmful or even toxics in them. Food additives such as nitrate salts (mostly Sodium and Potassium nitrate) have been used to preserves foods for years [1]. Nitrate ions have a low toxin in themselves but they would be converted to nitrites when consumed which have a higher toxic than nitrates [2-9]. Consuming water or food having nitrates is a health risk as it may result to an excess of methemoglobin in human body which is dangerous as it may itself cause toxic responses such as methemoglobinemia in infants and children, and N-nitroso-related carcinogenesis in adults.

Though normal amount of nitrates and nitrites is necessary for physiologic roles in vascular and immune function, little attention has been paid to the food sources of nitrates and nitrites as healthful dietary components [10]. In fact the health benefits of vegetables and fruit may derive from the contribution of their constituents to food patterns such as the Mediterranean-type pattern [11-13]. Recent research has found specific foods to be associated with a decreased risk of cardiovascular disease. Recent prospective epidemiologic studies have shown that green leafy vegetables are among the foods most protective against coronary heart disease and ischemic stroke risk [14-16]. The Dietary Approaches to Stop Hypertension (DASH) studies found that diets rich in vegetables and low-fat dairy products can lower blood pressure to an extent similar to that achieved with single hypotensive medications [17-18]. The blood pressure–lowering effect of this diet was hypothesized to be attributable to the high calcium, potassium, polyphenols, and fiber contents and low sodium and animal protein contents [19]. These and other findings point to a less widely acknowledged but biologically plausible hypothesis: the content of inorganic nitrate ($NO3^-$) in certain vegetables and fruit can provide a physiologic substrate for reduction to nitrite ($NO2^-$), nitric oxide, and other metabolic products (NOx) that produce vasodilation, decrease blood pressure, and support cardiovascular function [20-22]. Foods can increase the generation of nitric oxide in the gastrointestinal tract via the polyphenolic content of, for example, apples or red wine [23, 24]. If food contains high levels of nitrate, it is a potential risk if the conditions during storage or processing are conductive to conversion to nitrite [25, 26]. Most critical are vegetables that have been damaged, poorly stored, or stored for extended periods, pickled and fermented vegetables as well as raw vegetables juices.

The concentration of nitrate and nitrite in fruit juice products varies depending on their origin, storage condition and processing technologies [27]. High levels of nitrate and nitrite in drinking water and fruit juices has become one of the serious issues of public health concern, but still has not received much research attention in many developing countries such as Iran especially in case of fruit juices. The nitrate and nitrite levels of fruit juices may be expected to be influenced by the nature of the fruit, fertilizers: the agricultural practices such as the types and amounts of fertilizers used,, the weather conditions, the mineral composition of the soil from which it originated according to the geographical conditions, the composition of the irrigation water, and other factors such as water used in the fruit juices reconstruction in the manufacturing process [28]. The ingestion of 8–15 g nitrate can cause abdominal pain, blood in stool and urine, weakness and collapse . Chronic ingestion of smaller doses can cause dyspepsia, mental depression and Headache stomach cancer was associated with the consumption of well water containing nitrate (12.5-30.9 ppm), in particular if the intake of such waters occurred during the first [29] general the epidemiological evidence for nitrate and nitrite playing a role in stomach cancer etiology is at present inadequate. A recent study of the total extractable N-nitroso compounds in human gastric juice showed that the concentrations were highest in patients with carcinoma of the stomach and pernicious anemia, which is in line with a role for nitrosamines as a risk factor for stomach cancer [1,8]. The present paper examines cancer risk in an area with long-term evidence of nitrate in its drinking water. The admissible concentration of nitrates and nitrites in drinking water in the majority of countries controlling these parameters is 50 mg/ L [30] . Technological processing to control the nitrite levels is necessary to the harvested vegetables and fruit destined for consumption the results showed that moderate aqueous ClO2 solutions (30 and 45 mg/ L, 01 min) treatment could effectively reduce the nitrite levels in fresh lettuces during storage[31]. The reduction of the residual nitrite levels could be an acceptable alternative to reduce the intake of nitrite in processed meats. More recently, it has been observed how the incorporation of citrus co-products can reduce the residual nitrite con centration, which is a reason enough to encourage further study into these products. The aim of this article is to describe the latest advances concerning the use of citrus co-products in meat products as a potential ingredient to reduce the nitrite level [32]. Calcium di-hydrogen phosphate CaHPO4 used as Buffering agent, firming agent, sequestrate, leavening agent, dough conditioner, texturizer, yeast food, and nutrient. Calcium hydroxyapatite is the major inorganic phase of bones and a member of the apatite comprise, the mineral phases of calcite tissues. Calcium di-hydrogen phosphate (CaHPO4) major inorganic phase of bones, is a member of the apatite family produced calcium hydroxyapatite in physical and mechanical properties methods in which the consolidation of fine Ca-P particles to form a thin films are applied using reagents consisting of a colloidal suspension solution of inorganic particles or as metal or other organic precursors [33-35] . Ca_3 $(PO4)_2$ for the removal of Pb (supplied as nitrate) resulted in 97% immobilization of Pb in food [33]. The formation of bio inspired calcium phosphate nano composites on nanostructured self-assembling polymeric. This polymer reduce metal and nitrate, nitrite in water [35].

The changes of nitrite by nitrate reeducates in plants have been recognized as a biochemical process. As the activity of NR could lead to the conversion of nitrite levels in plants, it can be hypothesized that some processing methods which could inactivate NR or kill the microbes may influence on the nitrite levels of fresh vegetables. Some technological processing is necessary to the harvested vegetables destined for consumption,

The objectives of this study were:
1-To determine the concentration of nitrate and nitrite levels in most known fruit juices available commercially in the form of packaged in Tehran market (Iran), by spectrophotometric methods.

2-To compare these results with the maximum admissible limit set in drinking water by different international organizations: United States Environmental Protection Agency (USEPA) and World Health Organization (WHO) and also national standard. The assessment of dietary nitrate and nitrite intake by consumption of fruit juice in accordance with acceptable daily intakes (ADI) was the other objective of the present work**.**

EXPERIMENTAL SECTION

***Sampling method*:**
A descriptive – analytical and cross-sectional study was conducted for determination nitrate and nitrite in 540 samples of 6 varieties of Pineapple, Orange, Mango, Tropical, Cherry and Grape popular fruit juice packaged products (some were imported) commercially available in Iran market in three consecutive seasons (winter, spring and summer)of 2015.

Sampling was replicated twice within each month at intervals of two weeks. To evaluate variability of nitrate and nitrite content within sub-samples, (1620 sub-samples) on the whole were analyzed separately.

***Quantitative Determination*:**
a) Quantitative determination of nitrate: Fifty to seventy mg of sub-sample was selected and deionized water was added to the samples (nine times than exact the sample weight) and the water and sub-sample were homogenized for 15 minutes [36-40]. A 30 gram sample of homogenate was placed in a centrifuge tube, and 0.5 ml of H_2O_2 was added and the tube was capped and shaked well by the hand after adding H_2O_2. All samples were centrifuged at 3500 rpm for 3 min. The supernatant was then separated and filtered with filter paper wattman ≠ 1 and nitrate concentration in the filtrate was determined calorimetrically by a flow injection analysis system [41-43]. Nitrate content was expressed as mg nitrate per kg on a fresh weight basis (mg NO3/kg FW) unless otherwise stated. Nitrate concentration in celery was calculated from nitrate content in leaves and petioles separately on the weight of each part.

b) ***Quantitative Determination of nitrite***: with the AOAC official Methods 973/31. A portion of solution containing nitrite was transferred into a 25 mL volumetric flask. Then 2.5 mL sulfonamide were added, followed by addition of 2.5 mL NAD (N−(1−naphthyl) ethylenediamin.2HCl).The volume was complete with water and left 15 minutes in order to give time for color development. The absorbance was measured at 545 nm against a blank solution. The nitrite concentration was determined using the calibration curve solutions of 0.1, 0.2, 0.4, 0.6, and 0.8 ppm NaNO2. The absorbance values were measured at 545 nm. The calibration curve was constructed by plotting the absorbance vs. the concentration [38-40].

Experimental Method
A Shimadzu (Model No: UV-2550) The double mono-chromator UV-Visible spectrophotometer with 1 cm matching quartz cells were used for the absorbance measurements.

***Statistical Analysis*:**
Values were expressed as the mean (mg/L) ± standard deviation (SE). Nitrate content differences on the basis of the type of fruit were determined by student t-test. The brand and manufactures differences were calculated by one way ANOVA and for analysis of the role of multiple factors univariate analysis was used by SPSS 20 .Probability values of <0.05 were considered significant.

RESULTS AND DISCUSSION

The different brands and manufactures in this research shows a significant effect on the nitrate and nitrite content samples tested ($p \leq 0.001$) as nitrate content in mango and tropical fruit juice samples were significantly higher (

$p < 0.05$) and the highest nitrate content was determined in tropical (winter season) samples. The mean content of nitrate and nitrites and their ranges in fruit juice studied samples were determined as fresh weight ± standard error of the mean and shown in Table 1.

Table 1- Mean nitrate (NO_3) content (mg/L) in the fruit juice packaged products available commercially in Iran market in 2014-2015.

Fruit Juice sample	Mean(NO_3) mg/L ± S.E*	Range of Nitrate content (mg/L)	Mean(NO_2) mg/L ± S.E*	Range of Nitrite Content (mg/L)
Mango1	23.14 ± 1.05	19.86-23.83	8.47 ± 0. 52	6.26 - 9.56
Mango2	26.44± 2.06	24.23 - 27.54	10.34± 0. 76	8.25-11.55
Mango3	27.65 ± 1.01	25.44- 28.33	9.42±1.08	8.36 -10.53
Mango4	27.14 ±1.09	26.24 – 28.78	10.58 ± 0.08	8.32- 11.62
Mango 5	27.78±0.78	19.87- 28.87	10.05 ±0. 25	8.54-12-26
Mean	^{c}26.43 ±1.20	19.86-28.87	^{b}9.77±0.54	6.26-11.62
Pineapple 1	23.66±0.89	18.56- 25.08	6.36± 0.15	4.18- 8.41
Pineapple 2	18.34±0.91	15.44- 25.09	5.39± 0.82	4.06- 8.89
Pineapple 3	26.71±0.76	24.09-35.19	7.30± 1.02	5.03-9.23
Pineapple 4	25.34±0.54	20.17-28.16	7.11 ± 0.67	5.67-7.98
Pineapple 5	27.20±0.52	19.65-29.89	8.01± 0.27	5.02-8.39
Mean	^{c}24.25±0.72	15.44-35.19	^{d}6.83 ±0.59	4.06-9.23
Orange 1	18.02±0.37	16.11-19.44	4.23 ± 0.08	3.78-6.80
Orange 2	25.06±0.12	18.21-29.63	7.89 ±0.22	5.89-10.46
Orange 3	17.55±0. 21	15.44-24.36	6.32 ± 0.42	5.19-9.65
Orange 4	20.11±0.14	13.29-24.63	6.39 ± 0.39	5.21-9.89
Orange 5	17.07±0.11	15.09-19.03	7.78 ± 0.27	5.11-9.82
Mean	^{d}19.56 ± 0.19	13.29-29.63	^{d}6.52±0.28	3.78-10.46
Cherry 1	14.02±0.02	10.24 -18.73	5.04 ± 0.05	3.18 - 8.82
Cherry 2	16.31±0.08	15.31-18.68	6.04 ± 0.02	4.29-6.92
Cherry 3	15.73 ± 0.05	14.42-19.32	7.32± 0.08	4.76-9.06
Cherry 4	19.32±0.12	18.02-22.67	8.02 ± 0.03	6.78-5.20
Cherry 5	17.32 ±0.08	16.22-19.50	7.31± 0.03	5.59-8.01
Mean	^{d}16.54 ±0.07	10.24-22.67	^{d}6.75±0.04	3.18-9.06
Grape 1	30.19 ± 0.25	20.55-33.06	9.74± 0.65	7.52-10.44
Grape 2	29.88± 0.63	25.67-30.54	7.88 ± 0.27	5.44-9.12
Grape 3	32.76±0.93	28.72-39.48	8.62± 0.23	6.19-10.73
Grape 4	27.83±0. 15	25.39-30.05	7.39±0.18	6.49-8.94
Mean	^{b}30.16 ±0.49	20.55-39.48	^{c}8.34±0.33	5.44-10.73
Tropical 1	42.38±0.83	30.91-45.73	11.82±1.20	10.76-13.32
Tropical 2	43.34±1. 42	38.73-49.05	10.89 ± 1.8	9.44-15.02
Tropical 3	42.76±1.07	37.29-45.20	10.92±0.32	8.39-13.02
Tropical 4	38.78±0.78	34.21-47.03	10.21±0.41	8.43-13.65
Tropical 5	39.32±0.43	31.06-42.58	10.04 ± 0.92	8.32-14.01
Mean	^{a}41.32±0.91	30.91-49.05	^{a}10.78±0.93	8.32-15.02

** S.E : standard error of the mean*

The result from this study show that all the fruit juices analyzed contained detectable amount of nitrate, nitrite. The mean nitrate and nitrite levels in the juices ranged from 14.02±0.02 to 43.34±1. 42 and 4.23 ± 0.08 to 11.82±1.20 mg/L ± SE, respectively.

The different between mean values of nitrate in different juice samples is statistically significant. The mean concentration of nitrate in tropical fruit juice sample is significantly higher than other juices while mean nitrate content in orange and cherry juice are the same and has no meaningful differences. Nitrate contents in all samples were lower than maximum permissible level. Tropical juice samples had the highest content of nitrate while cherry had the lowest.

Mean of No_3 level in the fruit juices ranged from 16.54 ±0.07 to 41.32±0.91 (mg/l ± SE) (figure 1).

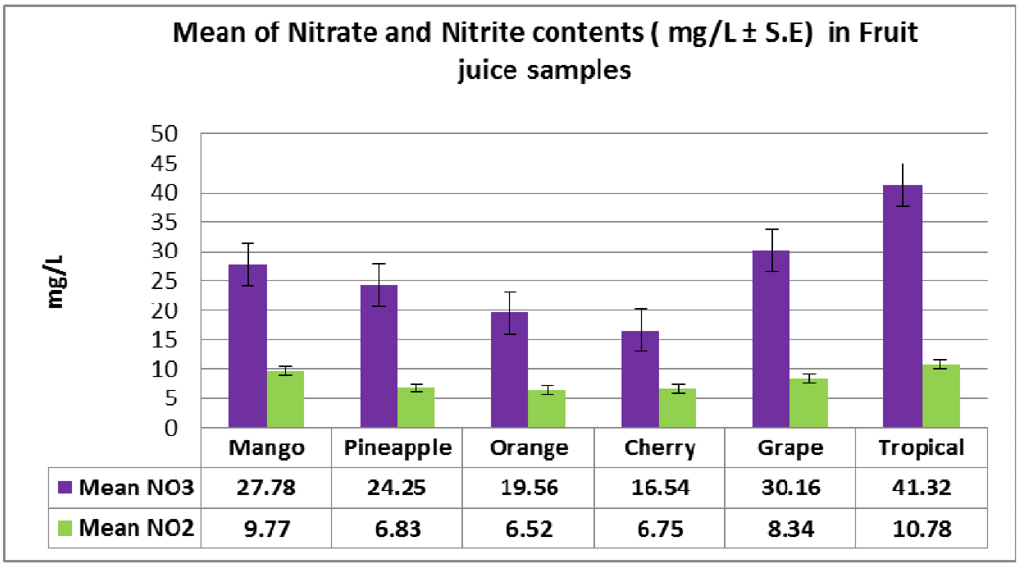

Figure 1- The mean content of nitrate and nitrite (mg/L ±SE) in studied fruit juice samples

Concentrations of nitrate in tropical and mango juice and nitrite in tropical and grape and mango juice were relatively high. Tropical juice by 10.78±0.93 had the significant difference ($p < 0.01$) among all kind of studied juice samples while the pineapple and orange juices has the lowest and meaningful differences by other samples ($p < 0.01$). The mean of nitrate and nitrite contents according to analyzing of 1620 sub-samples were shown in figure 1. The concentration of nitrate in the samples fall below the WHO's Acceptable Daily intake (AD1) which is set at 5 mg/kg body weight. However, the risk to human with respect to methaemoglobinaemia and conversion of nitrate to nitrite in oral cavity and stomach leading to the possible formation of nitrosamines cannot be ignored. Chronic ingestion of small dose of nitrate can also cause dyspepsia, mental depression, and headache [39].

For the children under 10 kg body weight, the levels of nitrite from those fruit juices are higher than the WHO's (1978) recommended AD1 of nitrite which is stipulated at 0.2 mg/kg body weight [40-43].

The levels of nitrite in the total volume pack (200 ml) for the brand with least level of nitrite (4.23 ± 0.08 mg/L) will be 1.68 mg and 3.26 mg for brand with the highest concentration (11.82±1.20 mg/L). Children of 15 to 20 kg of body weight consuming one serving of these products will be ingesting about 42.53 to 57.12 % respectively of ADI (for brand with least nitrite level) and 94.32 to 124.67% of ADI (for brand with highest level of nitrite). Furthermore, the consumption of two or more servings of these products or one serving along with supplement sources of nitrite in diet (such as vegetables, legumes, or even some water sources) could have the daily intake increased above ADI value as recommended by WHO. Thus, some of these fruit drinks could be said to be unsafe for children and infants and for elderly people.

CONCLUSION

Children might be especially susceptible to the toxic effects of these compounds as they have low body weight, immature enzymatic system (especially for xenobiotic metabolism) and low gastric acidity (a good condition for Nnitrosamine formation). Due to the poor screening and monitoring system in developing countries like Iran, it is very difficult to evaluate how the consumer can be affected by taking fruit juices. While most juice quality with low price sold in the market, in addition to having the properties of the fruit, is harmful to health risks as well.

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Conflict of Interest
The authors have no affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this publication.

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