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## Physicochemical Characteristics and Nitrate Content in Fresh and Canned Pears Products

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#### ABSTRACT

In recent years, an increasing interest concerning determination of nitrate levels in food products has been observed, essentially due to the potential reduction of nitrate to nitrite, which is known to cause adverse effects on human and animal health. The main goal of this study was measuring nitrate content of pear and apple derived canned products samples commercially available in Iran market in comparison on fresh fruits. Series analyses were performed with 80 canned pear samples produced at a large-scale local enterprise and purchased at recognized market and simultaneously fresh and 150 untreated samples was collected by going to the chosen areas of recognized farms in region of Fouman in Giulan province, Iran. In order to conduct a comparison between the content of nitrate in the studied samples, dry matter content was determined according to the association of office analytical chemists (AOAC). A ten gram sample of the prepared pear and apple was blended with 50ml distilled water in a home blender. The mixture was filtered and was passed through a glass 39 column fitted with a tape and filled with Activated alumina, in order to separate the color of Chlorophyll and get a transparent solution. The eluted solution by water filtered using 0.45um filter paper in order to eliminate the turbidity and get a clear solution. Nitrite concentration in pear samples was determined by spectrophotometric methods at a wavelength of 538 nm, after reducing nitrate to nitrite by using cadmium column. Nitrate content in analyzed fresh pears ranges between 63.22 - 143.76 mg/kg FW with an average of 101.743 mg/kg. The highest nitrate content was found in 'Abkhoj' (143.76 mg/kg) and the lowest content was in Amrud' genotypes (63.22 mg/kg FW). For canned pear the nitrate level ranges between 166.11 - 374.02 mg/kg with an average of 266.08 mg/kg. All the nitrate values in canned food samples are higher than those for corresponding fresh fruits. The nitrate levels in pear compotes are much higher than those for corresponding fruit due to the concentration process that takes place during pear processing. It is clear that the result of our study shows a variation in the nitrate and nitrite levels in the different genotypes samples and processing products.

Key words: Nitrate, Nitrite, Fresh fruit, Canned Pears.

#### INTRODUCTION

Food is a primary need of a human being for his or her survival and welfare. Nowadays, more attention has been paid not only to the chemical composition and nutritive value of vegetable products, but also to the content of harmful or toxic compounds for human. As a food additive, sodium and potassium nitrate or nitrite salts have been used for centuries to preserve foods<sup>1</sup>. The toxicity of nitrate ion by itself is relatively low, but nitrates up-taken by a human organism are converted into nitrites, which are much more toxic than nitrates<sup>2-8</sup>. Nitrate in food is considered a primary health problem because it may create an excess of methemoglobin in human body and later may possibly lead to toxic responses such as methemogobinemia for infants and small children.

The presence of nitrates and nitrites in food is associated with an increased risk of gastrointestinal cancer and, in infants, methemoglobinemia. Despite the physiologic roles for nitrate and nitrite in vascular and immune function, consideration of food sources of nitrates and nitrites as healthful dietary components has received little attention<sup>9</sup>. Whereas the health benefits of vegetables and fruit may derive from the contribution of their constituents to food patterns such as the Mediterranean-type pattern<sup>10-12</sup>. 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<sup>13-15</sup>. 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<sup>16-17</sup>. 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<sup>18</sup>. 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<sup>19-21</sup>. Foods can increase the generation of nitric oxide in the gastrointestinal tract via the polyphenolic content of, for example, apples or red wine<sup>22,23</sup>. Pomegranate juice has been shown to protect nitric oxide from oxidation while enhancing its biological activity<sup>24</sup>. The metabolic activity of commensal bacteria in the gastrointestinal tract and probiotic bacteria also provide nitric oxide from nitrite, and to a lesser extent, from nitrate<sup>25,26</sup>.

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<sup>27</sup>. 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. In such circumstances, nitrite levels of up to 400 mg kg-1 have been found<sup>28</sup>. In fermented maize on storage in water at room temperature over period of 8 days, nitrate levels decreased 80% in average while the nitrite content increased about 200% in average of its initial level<sup>29</sup>.

Pear (*Pyrus communis* L.) is an important fruit of temperate regions of Iran and grown for its desirable taste and commercial value. According to recent statistics the area of pear culture in Iran consists of 19,219 hectares with production of about 180,000 tones. Most pear orchards in Iran are located in Tehran (Karaj), Khorasan, Isfahan, East and West Azarbayjan and Ghazvin province. More than 26% of the pear orchards are located in Tehran province with 64,741 tonnes annual production. More than 70 pear cultivars are available in the Kamal-Abad collection orchard, located at the Seed and Plant Improvement Institute (SPII), Ministry of Agriculture 50 km west of Tehran<sup>30</sup>.

With more than 10 species, Iran is one of the important genetic resources of the Pyrus in the world. For example, it has been reported that 12 species of pear are grown in the Iranian plateau. This genus is an important element of Irano-Turanian region (species such as *P. syriaca, P. glabra*, and *P.oxyprion*)<sup>30</sup>. The distribution zone of the Pyrus in Iran is Alborz and Zagros Mountains, south and some elevations in the east of Iran<sup>31,32</sup>. In addition, native cultivars in Giulan province especially in fouman cultivate different genotypes including 'Latanz', 'khalshekan', 'Amrud', 'Arbakhoj', 'Abkhoj', 'Rashtehkhoj', 'Sangsar' and 'Zizaling'.

The aim of the present work was to study the changes of nitrate and nitrite levels in selected raw and fresh Iranian genotypes of pear under refrigerated and ambient temperature storage conditions for a period of 2 days and compare these by canned pear commercially available in order to find the source of nitrate content and comparing nitrate contents between fresh fruit and its derived canned product. The assessment of dietary nitrate and nitrite intake by consumption of raw and fresh fruits and canned food in accordance with acceptable daily intakes (ADI) was the second objective of the present work.

#### MATERIAL AND METHODS

All samples were analyzed in five replications. Canned Series analyses were performed with samples produced at a large-scale local enterprise and purchased at recognized market. The series 2 consisted of raw and fresh fruit. Briefly, samples of eight species of pear were collected from different outlets of Giulan province. The fruits were washed with warm water, mechanically brushed and peeled. Raw juices were produced with commercially available squeezer. All the raw and fresh samples were stored at two different temperatures, refrigerated (4-6 °C) and ambient (20-22 °C) for two days. The nitrate and nitrite contents, Histamine and pH were determined after opening the commercial canned packages and immediately after preparation of the homemade fruit as well as after 24 and 48 hours of storage.

#### **Sampling Method**

This study was carried out on 150 samples of local pear fruits of different genotypes during spring and summer 2015 and including 'Latanz', 'khalshekan', 'Amrud', 'Arbakhoj', 'Abkhoj', 'Rashtehkhoj', 'Sangsar' and 'Zizaling' which were different in terms of flavor from 10 recognized farmlands. 30 fruits were chosen randomly from harvest of 5 trees which was physiological maturity (peel color changes from green to yellow) from every farmland in the region of Fouman in Giulan province (37°132 263 N 49°182 453 E) [figure 1] and then were transported to the laboratory of Food Science & Technology od Pharmaceutical Sciences Branch, Islamic Azad University in Tehran. Quantitative indices of fruit were measured including length, diameter and length ratio to diameter ratio (L/D) using a digital caliper, fruit weight using a digital scale with 0.01g, fruit juice soluble solids (TSS) by rfraktometer, pH using pH meters, fruit juice, organic titratable acid (TA) by titration with a solution of the 0.1normal. In the other part of this study 80 canned pear were purchased from recognized markets in Tehran due to comparing nitrate contents between fresh fruit and its derived canned product.

#### **Experimental Method**

All chemicals used were of analytical reagent grade, and doubly distilled water was used in the preparation of all solutions in the experiments. Nitrate solution (1000  $\mu$ g/ml) was prepared by dissolving 0.7220 g potassium nitrate in water and diluting to 100 ml.

According to AOAC official method 993.03[33] spectroscopic method, 3.0 g of homogenized canned and fresh fruit samples were taken into a 200 ml volumetric flask and 150 ml hot water and 10 ml standard borax solution were added in order to precipitate protein.

Warm volumetric flask in boiling water bath was taken for 15 minutes and 4 ml zinc sulfate solution slowly added with shaking, and cooled to room temperature in cool water bath. The test solutions were diluted with water, mixed and filtered by nitrate and nitrite free qualitative grad: 20 µm pore size (Whatman No.4). Appropriate aliquots of 15 ml of the solution was transferred into a 10 ml calibrated flask and analyzed according to the official procedure o method 993.03 due to the principle of reducing nitrate to nitrite by treatment with spongy cadmium and their nitrite contents were determined calorimetrically by absorbance at 530 nm, and compared with standard curve using H<sub>2</sub>O blank standard nitrate concentrations containing 0, 10, 20, 30, 50 and 100 µg NaNO, , and mg NaNO, contents have been calculated as :

mg NaNO<sub>3</sub> / Kg test portion =  $b \times 100/m$ where b = sodium nitrate from standard curve (µ g ) and m = weight of test portion homogenate (g) [ J. AOAC Int. 77,425 (1994)].

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/ kg FW) ± standard deviation (SD). Nitrate content differences on the basis of the type of genotype were determined by student t-test. Fresh and canned processing changes 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

The different agricultural areas in the different farms in Fouman city in Gillan province in this research shows a significant effect on the nitrate and pH in pear samples tested (p  $\leq$  0.002) as nitrate content in east part farmlands in this city

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Crops	No. of Samples	Mean(NO <sub>3</sub> ) mg/kg $\pm$ S.E*	Range (mg/kg FW)
Latanz	21	132.44 ± 13.22	103.11- 148.76
khalshekan	20	69.89 ± 5.11	60.56-107.89
Amrud	22	63.22± 4.28	61.09-78.92
Arbakhoj	20	82.18 ± 6.78	73.78- 104.48
Abkhoj	18	143.76± 1.14	105.62- 178.43
Rashtehkhoj	15	107.32± 18.90	100.06-139.02
Sangsar	16	76.54 ± 10.21	64.26- 116.98
Zizaling	18	130.54 ± 8.72	128.16- 139.55
Canned Pear Produc	t 80	266.08± 32.67	166.11 – 374.02

Table 1: Average nitrate  $(NO_3)$  content (mg/kg FW) in the fresh pear and canned pear available commercially in Iran market in 2014-2015

\* S.E : standard error of the mean

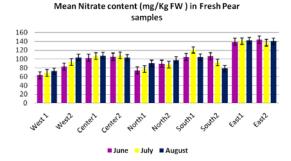


Fig. 1: Location map of the studied area indicating sample points

was significantly higher. The mean content of nitrate and its ranges in fresh fruit according to the locations of sites were determined as fresh weight and shown in figure 2.

The results concerning nitrate contents in 8 pear genotypes and derived product in 3 consecutive months of summer 2015 are shown in table- 1 and reveals that nitrate content in analyzed fresh pears ranges between 63.22 – 143.76 mg/ kg FW with an average of 101.743 mg/kg. The highest nitrate content was found in 'Abkhoj' (143.76 mg/kg) in August and the lowest content was in'Amrud' genotypes (63.22 mg/kg FW) in June 2015. For canned pear the nitrate level ranges between 166.11 – 374.02 mg/kg with an average of 266.08 mg/kg.

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# Fig. 2: The mean content of NO<sub>3</sub> in fresh Iranian pear samples collected from Gilan province

The highest nitrate content was found in T3 in the east of Fouman city in Gilan province (Farmland 10) and the lowest was in west location Farmland 1): 63.22, 68.54, 72.65 mg/kg Fresh weight in June, July and August 2015.

It is clear that the result of our study shows a variation in the nitrate levels in the different genotypes especially in Abkhoj and Sangsar variety and also processed samples due to different brands ( $p\leq0.03$ ).

#### CONCLUSION

Due to the variability in nitrate and nitrite concentrations of foods reported in other researches<sup>2-9</sup> we conducted nitrate analyses on a convenience sample of fresh pear genotypes in Iran as a commercial fruit consumed vastly in Iran as well as its canned processed product. The pear genotype with the mean nitrate concentrations in our sample included Abkhoj (143.76± 1.14mg/kg FW), Latanz (132.44 ± 13.22 mg/kg FW), khalshekan (69.89±5.11mg/kg Fw), Amrud (63.22± 4.28 mg/kg FW), Arbakhoj (82.18 ± 6.78/kg Fw), Abkhoj (107.32± 18.90mg/kg Fw), Rashtehkhoj (107.32± 18.90 mg/kg FW), Sangsar (76.54 ± 10.21mg/kg Fw), and Zizaling ( $130.54 \pm 8.72$  mg/ 100 g FW) while the mean content of nitrate in processed canned pear was 266.08± 32.67 which was about two times higher than fruit samples.

The accumulation of nitrates in fruit depends on many factors; genetic, agricultural (e.g. type of soil, the dose and chemical forms of nitrogen, availability of other nutrients, herbicide application etc.)[34,35] and those environmental such as air humidity. Further differences in nitrate accumulation may arise from the time of harvest, vegetation season and storage time. Finding from this research reveals that for the processed pear products, by arranging the results of mean content of nitrate level, the following order : pear fresh fruit < canned pear is remarkable ( $p \le 0.001$ ). A toxicological endpoint of concern for nitrate is nitrosamine formation and the potential for tumour formation. However, when nitrate is consumed in a normal diet containing vegetables, other bioactive substances concomitantly consumed, such as the antioxidant vitamin C, may inhibit the endogenous formation of nitrosamines. Dietary exposure estimates showed that the ADI for nitrate would not be exceeded by an adult eating 400 g of mixed vegetables. However, high level consumers, of vegetables grown under unfavorable local production conditions may exceed the ADI approximately two fold. In these calculations the nitrate concentrations were not corrected for mitigation factors e.g. fruit consumption and processing and may overestimate exposure. Consumption of more than 53 g of canned pear at the median nitrate concentration would lead to an excursion above the ADI without taking into account any other sources of nitrate exposure.

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