

## Histamine, Nitrate, and Nitrite Content in Canned and Fresh Apple Products

Parisa Ziarati<sup>1\*</sup>, Estatira Sepehr<sup>2</sup> and Saeed Heidari<sup>3</sup>

<sup>1</sup>Department of Medicinal Chemistry, Pharmaceutical Sciences Branch, Islamic Azad University, (IAUPS) , Tehran, Iran.

<sup>2</sup>Food Toxicology Department, Division of Biochemical Toxicology, National Center for Toxicological Research, U.S. Food and Drug Administration, Jefferson, AR 72079, USA.

<sup>3</sup>Nutrition and Food Sciences Research Center, , Pharmaceutical Sciences Branch, Islamic Azad University, Tehran-Iran.

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Histamine, a biogenic amine, and inorganic nitrate and nitrite are nitrogenous compounds occurring in many foods. There has been increasing interest in determination of histamine, nitrate, and nitrite levels in fruits and canned products due to their potential adverse health effects on humans and animals. The aim of this study was to determine levels of nitrate and nitrite in commercially available canned apple products compared to fresh fruit collected from the Fars province of Iran. The nitrate content and histamine concentration in fresh and canned apples were determined by calorimetric methods and capillary electrophoresis, respectively. The histamine content of canned apples was determined at four different storage times. Also, physiochemical characteristics, such as pH and vitamin C content of the samples, were also determined. The results revealed that nitrate and nitrite levels were significantly higher ( $P < 0.05$ ) (up to four-fold) in canned apples compared to fresh apples from specific regions, which might be due to genotypic variations from different geographical sources. The histamine content in canned apples tested twelve months after the date of production was significantly higher ( $P < 0.003$ ) (up to three-fold) than levels in canned apples tested one month after production, suggesting that the length of storage may have an effect on histamine concentration.

**Keywords:** Canned Apple, Food Safety, Histamine, Genotype, Nitrate.

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Inorganic nitrate ( $\text{NO}_3^-$ ) and nitrite ( $\text{NO}_2^-$ ) are natural constituents of plant material commonly present in vegetables and plant based foods. People are mostly unprotected to nitrates and nitrites through their diet, drinking water, air, and soil. Up to 85% of total dietary nitrate intake comes from vegetables, fruits, and other plant-based foods as the most common sources of nitrate<sup>1</sup>. Normal amounts of nitrates and nitrites are necessary for physiologic roles in cardiovascular health and

immune function. Yet, little attention and screening has been paid to the sources of food by nitrates and nitrites as healthy dietary components<sup>2</sup>. Health prosperity of crops, vegetables and fruits may be attributed to their constituents such as vitamins, minerals, fiber, and their contributions to overall food patterns<sup>3-5</sup>. However, long term surveys have specifically identified that intake of green leafy vegetables with high nitrate concentrations play an extensive performance in diminishing the diabetic risk of aging in females and cardiovascular diseases in males<sup>6-7</sup>. Additionally, The Dietary Approaches to Stop Hypertension (DASH) studies found that diets which is rich in crops and vegetables and

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\* To whom all correspondence should be addressed.  
Tel: +98-21-22600037; Fax: +98-21-22633986  
E-mail: ziarati.p@iaups.ac.ir

products by low-fat dairy can decrease blood pressure to an extent similar to that achieved with a single hypotensive medication<sup>8-9</sup>. It is considered that the effect of lowering blood pressure of this diet can be attributed to the high calcium, fiber, potassium, polyphenol, low sodium, and animal protein contents<sup>10</sup>. Many findings claimed that the inorganic nitrate contents ( $\text{NO}_3^-$ ) in certain fruits and vegetables can present a proper physiological substrate for reducing to nitrite ( $\text{NO}_2^-$ ), nitric oxide, and some other form of metabolic products ( $\text{NO}_x$ ) give rise to decreasing blood pressure, vasodilation and enhancing cardiovascular function<sup>11</sup>.

As plants absorb nitrate, it could be normally converted by the nitrate reductase system to nitrite. Environmental factors or genetic that interfere with or inhibit the responsibility of the nitrate reductase system allow nitrate to accumulate into the plant<sup>12-14</sup>. Nitrate accumulation in apples confined in some factors such as: agricultural considerations (soil type, the dose of nitrogen in different chemical forms, quantity of kind of herbicide application etc.), and environmental quality, like air humidity wind speed and temperature<sup>14-16</sup>.

Further differences in nitrate accumulation may arise from the time of harvest, vegetation season, and storage time<sup>14-16</sup>. According to consume large amounts of nitrate from a normal diet by animals and also human and the process of converting nitrate to nitrite which could be accumulated in body as toxic levels, it probably cause toxicity<sup>14-17</sup>. Toxicity would be arises by blood nitrite absorption that leads to oxidizes iron in hemoglobin.

The resultant methemoglobin has a very poor affinity for oxygen, which greatly reduces the oxygen-carrying capacity of red blood cells. Death from anoxia may occur if 70-80% of the hemoglobin is converted to methemoglobin<sup>18-22</sup>.

The Joint Expert Committee on Food Additives (JECFA) of the Food and Agriculture Organization of the United Nations/World Health Organization and the European Commission's Scientific Committee on Food have set an acceptable daily intake (ADI) for nitrate and nitrite of 0-3.7 and 0-0.07 mg nitrate ion/kg body weight, respectively<sup>19-23</sup>.

Histamine is a biogenic amine formed

mainly by de-carboxylation of the amino acid histidine. In food stuffs, such as fermented fish, meat, fruit, and vegetables, histamine could be found as reverberation of microbial activity. There are many aspects, such as storage conditions (temperature, humidity), manufacturing practices, pH of the product, additives, heat application, and raw material quality that can domination of histamine formation<sup>24</sup>. Histamine plays essential roles in human and animal physiological functions. It promotes growth and metabolic activity of the gut, and is active in nervous system. Despite these roles, the consumption of high histamine content foods can lead to adverse toxicological effects such as hypertension, headache, and abdominal cramps<sup>16</sup>. When the histamine content in the ingested food is higher than 500 mg/kg body weight, histamine poisoning will be occur<sup>25</sup>.

Apples are an important part of human diet as they are one of the most important sources of monosaccharides, mineral elements, dietary fiber and various biologically active compounds, such as vitamin C, some phenolic compounds which are known as natural antioxidants<sup>26</sup>. Apples are eaten not only fresh, but in many forms, such as juice concentrates, flavorings, vinegars, canned products, jams, juices, or extracts. Red fresh apple genotypes<sup>25</sup> contain high levels of antioxidants. The red to pink color of their flesh is due to anthocyanin production<sup>26</sup>. Recently, the red fleshed genotypes have been extensively studied not only for their anti-oxidative properties, but also for their remarkable marketing traits.

Due to the importance of apple production especially in the vast area in Iran and also the historical role of Persian apple, and the other hand high level of consumption apple (in fresh state or juice), the screening and monitoring its chemical composition and the quality of this popular fruit seems imperative.

The main aim of current study was to determine nitrate and nitrite concentrations in canned apple products commercially available in Iranian markets and compare them to nitrate and nitrite levels in fresh red apples collected from ten Iranian commercial cultivars (Haji Qermez, Arous Gousht Qermz, Shahroud-10, Gousht Qermez, Darab 1, 2, 3, 4, 5) from the Fars province in Iran.

The histamine content of canned apples was determined after four different lengths of

storage at 1, 3, 6, and 12 months. Physicochemical characteristics (pH and vitamin C content) of the samples were also determined.

## MATERIALS AND METHODS

### Study Area

Samples of fresh apples were collected from established farmlands in Darab (Figure 1) situated in the Fars province approximately 270 km south-east of the province capital Shiraz; (longitude 54° 55' N 53° 55' E and latitude 28° 20' to 29° 10' N). Darab has a total area of 11000 Km<sup>2</sup> of cultivable lands. Temperatures in this city are on average 38-46 °C in the summer and 15-25 °C in the winter<sup>27</sup>. Average yearly rainfall is nearly 300 mm<sup>27</sup>.

### Sampling Method

All samples were analyzed in quintuplicate. Fresh apples purchased from local markets were washed in warm water, mechanically brushed, and peeled. Raw juices were produced with a commercially available juicer. Different brands of canned products were purchased from local supermarkets. After opening each can, apple and juice were ground in a food blender with stainless steel cutters to make a completely homogenized sample. Aliquots of fresh and canned samples were refrigerated at (4-6 °C) up to two days before analysis.

### Histamine Determination

#### Chemicals

Analytical reagent grade chemicals, as well as deionized water, were used in all experiments. The following chemicals, with the indicated specifications, were used for histamine determination: hydrochloric acid (HCl) 0.1 mol/L; sodium hydroxide (NaOH) 1.0 mol/L; phosphoric acid (H<sub>3</sub>PO<sub>4</sub>) 1.2 mol/L (dilute 12.2 ml of 85% acid in a 100 ml flask with water); orthophthalate aldehyde (1% solution); and sodium citrate buffer (20 mM, pH 2.5). Histamine standard (e<sup>+</sup> 97.0% purity) was purchased from Sigma Aldrich (Steinheim, Germany). A stock standard (1 mg/mL) was prepared in 0.1 M HCl and kept at 4°C. Working standards (0.005, 0.01, 0.02, 0.04, 0.06, 0.08, 0.1 and 0.2 mg/mL) were prepared by appropriate dilutions of the stock solution in 0.1 M HCl.

#### Apparatus

a) Electrophoretic separations were performed using an Agilent (Agilent Technologies, Waldbronn, Germany) Capillary Electrophoresis (G1600A model 3D) system and Agilent uncoated fused-silica capillary (50 μm x 41 cm).

b) Photo diode array detector (PDA) 1200 series model G1315B (Agilent Technologies, Waldbronn, Germany).

At first, a histamine solution (1 mg/mL) was prepared by dissolving 167.4 mg of histamine

**Table 1.** Average nitrate (NO<sub>3</sub><sup>-</sup>) and nitrite (NO<sub>2</sub><sup>-</sup>) content (mg/kg FW) in fresh apple genotypes and canned apples available commercially in Iran

Crops	No. of Samples	Mean(NO <sub>3</sub> <sup>-</sup> ) mg/kg ± S.E*	Range (mg/kg FW)	Mean(NO <sub>2</sub> <sup>-</sup> ) mg/kg ±S.E*	Range (mg/kg FW)	mg vitamin C/100 grams**
Haji Qermez	18	150.67 ± 15.46 <sup>b</sup>	112.86- 224.18	0.92 ± 0.09 <sup>d</sup>	0.09 – 2.01	16.8-21.2
Arous Gousht Qermz	15	59.44 ± 3.01 <sup>c</sup>	51.73 -85.11	0.78 ± 0.07 <sup>d</sup>	0.06 – 2.36	27.1 -33.3
Shahroud-10	20	70.33± 2.66 <sup>c</sup>	65.34 -92.16	1.52 ± 0.27 <sup>c</sup>	0.41 – 3.68	18.4-24.1
Gousht Qermez	22	78.44 ± 8.42 <sup>c</sup>	65.39- 110.81	1.89 ± 0.62 <sup>b</sup>	0.56 – 4.02	————
Darab 1	21	130.64± 18.42 <sup>c</sup>	102.76- 182.29	1.82 ±0.66 <sup>b</sup>	0.34 -3.52	15.2-18.4
Darab 2	20	129.71± 10.21 <sup>c</sup>	100.49-142.76	1.78 ±0.38 <sup>b</sup>	1.03– 3.86	15.4-19.0
Darab 3	18	89.42 ± 6.73 <sup>d</sup>	60.76- 129.42	1.64±0.44 <sup>c</sup>	0.99-2.88	15.0-19.5
Darab 4	16	142.22 ± 5.11 <sup>b</sup>	120.72- 142.08	1.88 ± 0.56 <sup>b</sup>	1.03-3.66	16.1-20.8
Canned Apple Product	80	301.22 ± 25.67 <sup>a</sup>	201.44 – 412.26	3.97±1.43 <sup>a</sup>	1.14- 4.41	5.2-8.5

\* S.E : standard error of the mean

Apples picked and examined straight from the tree (without peeling).

Mean in a column with different superscript alphabets differ significantly (P < 0.05)

hydrochloride with a 0.1 mol/L HCl in a 100 mL volumetric flask. The capillary electrophoresis instrument was programmed to run a voltage gradient between 5–30 kV over 15 min, with replenishment of the sodium citrate buffer after every injection. The following rinses were utilized after each sample: water 1 min, aqueous NaOH 1 min, water 1 min, and sodium citrate buffer 1 min. Histamine was detected at 212 nm, with an operating temperature of 35 °C. Samples and standards were hydrodynamically injected (50 mbar for 10 s)<sup>16,27-28</sup>. A calibration curve consisting of eight concentrations of histamine ranging from 0.005-0.2 mg/mL was prepared daily. Each calibration point was analyzed in triplicate and used to calculate histamine levels in the study samples. The entire system from sample injection to data acquisition was computer-controlled with ChemStation software (Agilent Technologies, Waldbronn, Germany).

#### Ascorbic acid Determination

##### Chemicals

L-Ascorbic, meta-phosphoric acid, 2,6-dichloroindophenol, xylene, and acetic acid were obtained from Sigma Aldrich (Madrid, Spain). A stock solution of ascorbic acid (10 mg/mL) was prepared by dissolving 1000 mg of ascorbic acid in 100 mL water and stored in a glass-stoppered bottle at 4 °C in the dark. Working standard solutions were freshly prepared by appropriate dilution of stock solutions in water or 3% (w/v) meta-phosphoric acid-8% (v/v) acetic acid solution.

##### Apparatus

Dual-beam UV-visible spectrophotometer model UV-1601, with 1 cm quartz cell and UV probe software version 2.21 (Shimadzu, Japan).

Five grams of fresh or canned apple sample were homogenized until uniform consistency in a meta-phosphoric acid and acetic acid solution. This solution was used for the

ascorbic acid extraction after quantitative reduction of 2,6-dichlorophenolindophenol dyestuff by ascorbic acid and extraction of the excess dyestuff using xylene. The excess of ascorbic acid was measured at  $\lambda = 578$  nm and compared with a vitamin C reference standard according to International Organization for Standardization ISO/6557-2-1984 method<sup>29</sup>.

##### pH Determination

The pH of fresh and canned apple samples was measured by direct insertion of the electrode of the digital pH meter (Inolab, Weilheim, Germany) into the sample. The sample pH was recorded after the pH meter provided a final reading. Prior to analyses of apple samples, the pH meter was calibrated using standard buffers pH 6.0 and 9.0.

##### Nitrate and Nitrite Determination

##### Chemicals

- Zinc sulfate solution- Dissolve 30 g zinc sulfate ( $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ ) in 100 ml water.
- Saturated borax solution- Dissolve 5 g sodium tetraborate ( $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$ ) in 100 mL hot water
- Sodium Nitrate solution- Dry sodium nitrate 2 h at 105 °C. Standard sodium nitrate solutions in water in a concentration range 0, 10, 20, 30, 50 and 100  $\mu\text{g/mL}$  was used for standard curve
- Cadmium sulfate solution-Dissolve 10 g cadmium sulfate ( $3\text{CdSO}_4 \cdot 8\text{H}_2\text{O}$ ) in 100 mL water
- N-(1-Naphthyl)ethylene di-ammonium dichloride reagent-Dissolve 20 mg N-(1-naphthyl)-ethylene diammonium dichloride ( $\text{C}_{12}\text{H}_{16}\text{C}_{12}\text{N}_2 \cdot \text{CH}_3\text{OH}$ ) in 20 mL glacial acetic acid and dilute to 100 mL with  $\text{H}_2\text{O}$
- Sulfanilic acid solution-Dissolve 600 mg sulfanilic acid ( $\text{C}_6\text{H}_7\text{NO}_3\text{S}$ ) in 50 ml hot water. Let cool; add 20 mL glacial acetic acid and dilute to 100 mL with  $\text{H}_2\text{O}$
- Color reagent-Immediately before use

**Table 2.** Histamine content (mg/kg FW) in the canned apples available commercially in Iranian market

	Minimum $\pm$ SE	Maximum $\pm$ SE	Mean $\pm$ SE
Histamine Content(1 month after production date)	45.56 $\pm$ 2.23	201.46 $\pm$ 14.83	165.86 $\pm$ 24.22 <sup>c</sup>
Histamine Content(3 months after production date)	103.44 $\pm$ 10.73	430.24 $\pm$ 40.28	287.63 $\pm$ 18.92 <sup>b</sup>
Histamine Content(6 months after production date)	220.87 $\pm$ 14.76	398.11 $\pm$ 23.17	298.78 $\pm$ 18.42 <sup>b</sup>
Histamine Content(12 months after production date)	290.46 $\pm$ 13.11	505.82 $\pm$ 42.67	432.09 $\pm$ 25.67 <sup>a</sup>

Mean in a column with different superscript alphabets differ significantly ( $P < 0.05$ )

mix equal volumes of sulfanilic acid solution, and N-(1-naphthyl)ethylene diammonium dichloride reagent

**Apparatus**

- a) Filter papers, nitrate- and nitrite-free, qualitative grade 20 µm pore size (Whatman No.4)
- b) Double mono-chromator UV-Visible spectrophotometer Model UV-2550 with 1 cm matching quartz cells capable of measuring absorbance at 530 nm Shimadzu (Tokyo, Japan)

Nitrate and nitrite in fresh and canned apple were measured according to the Association of Analytical Communities (AOAC) official spectroscopic method (993.03). Briefly, 3.0 g of homogenized canned or fresh fruit sample was extracted with 150 mL of hot water and protein was precipitated by adding 10 mL of a saturated-borax solution. The aqueous extract was clarified with 4 mL of zinc sulfate solution. Nitrate is reduced to nitrite on a spongy cadmium column (the nitrite originally present is unaltered); sulfanilic acid is diazotized by the nitrite and coupled with N-(1-naphthyl) ethylene diamine dihydrochloride to form a pink azo dye, the absorbance of which is measured colorimetrically at 530 nm and compared with a standard curve constructed with standard sodium nitrate solution in water. Concentration/contents of nitrate were calculated as follows:

$\text{mg NaNO}_3 / \text{Kg test (sample) portion} = b \div 100 / m$ , where:

$b$  = concentration of standard sodium nitrate solution from curve (µg)

$m$  = weight of test portion homogenate (g)

For those samples containing both nitrite and nitrate, the nitrite content is first determined from the unreduced filtered sample followed by determination of total nitrite content (existing nitrite and nitrite formed from nitrate) from the column eluate. The nitrate concentrations next would be calculated by difference<sup>22</sup>.

**Statistical Analysis**

All data were expressed as mean (mg/kg FW) ± standard error (SE). Differences in the nitrate content based on the genotype variations were determined using student t-test. Nitrate and histamine changes in fresh and canned apples were analyzed using one way ANOVA. The role of multiple factors was determined by Statistical Package for Social Sciences (SPSS) version 20. A probability value of  $P < 0.05$  was considered statistically significant.

**RESULTS AND DISCUSSION**

The different agricultural areas in Darab city show a significant effect on the nitrate and pH in the tested red fresh apple samples ( $P < 0.05$ ).



**Fig. 1.** The map of sampling localities, including the studied province

The mean content of nitrate and nitrite in fresh and canned apple products are presented in Table-1. The nitrate and nitrite content in canned apples were significantly ( $P < 0.05$ ) higher (up to four-fold) than in fresh apples from (Arous Gousht Qermz, Shahroud- 10) and (Arous Gousht-Qermz, Haji Qermez), respectively. Nitrate and nitrite content in fresh apples ranged from 51.73 –224.18 mg/kg FW and 0.06-4.02 mg/kg FW, respectively. Canned apples contained 201.44 – 412.26 mg/kg FW of nitrates and 1.14-4.41 mg/kg FW of nitrites. The nitrate content in center farmlands in this city was significantly higher than in farmlands of the east and west, which is probably related to the presence of industrial factories that produce flowers with rose water and oil<sup>16,22</sup>.

Date from table 1 declared that canned apple product has remarkably much higher nitrate and nitrite in depends on fresh ones and much less Vitamin C.

Results from table 1 show a wide range of nitrate and nitrite contents. In table 2, the effect of storage of canned apples is shown. Additionally, it depends on consumption amounts and storage conditions of the canned products. The daily doses are exceeded by adults even by consumption of freshly apple juices as well as by using the canned apples after definite time of storage should be considered.

The nitrate content in center farmlands in this city was significantly higher than in farmlands of the east and west, which is probably related to the presence of industrial factories that produce flowers with rose water and oil<sup>20,21</sup>.

In accordance with our data, it is obvious that only freshly apple from Arous Gousht Qermz; Shahroud-10; Gousht Qermez; Darab 3, as a raw juice can be utilized for infants because the ADI values of nitrate and nitrite were 8.23% and 0.2% , respectively<sup>15</sup>. Consumption of the fresh and even refrigerated temperatures stored can be recommended, but for canned apples more than one month after producing due to increased histamine daily intakes to 21.1% of 3 months, 32.7% after 6 months and 38.7% after 12 months storage at ambient temperatures the histamine contents were higher compare to raw apple which resulted with the mean values of much higher than maximum permissible value. Around 83% of fresh fruit samples had a pH of approximately 4.6, while only

17% had a pH between 4.3-4.5. The pH values of the canned apple samples in the first month post production were lower than 4.2, and fall within acceptable U.S. Food and Drug Administration (FDA) levels (pH at or below 4.6)<sup>15</sup>. The vitamin C content in fresh apples was significantly ( $P < 0.05$ ) higher (up to three-fold) than in canned apples. The highest concentration of vitamin C (27.1 -33.3 mg/100g FW) was found in fresh apples produced by the Arous Gousht Qermz region (Table-1).

The histamine content in canned apple samples stored for twelve months after production date was up to three-fold higher when compared to canned apples tested one month after production (Table-2). This suggests that the length of storage time may have an effect on histamine concentration. Due to our expectation by passing time the histamine contents would be increased and the best recommendation for taking canned apple is one month after production and in fact the best suggestions is taking fresh apple instead of cane apples and as some patients should not take this useful fruit as fresh, it is better to purchase only up to one month products.

## CONCLUSIONS

Due to the variability in nitrate and nitrite concentrations of foods reported in other studies<sup>2-9</sup>, nitrate and nitrite analyses were conducted on samples of fresh and canned apples.

The findings from this research reveal that nitrate levels in canned apples are significantly higher than those found in fresh apples ( $P < 0.001$ ). 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. However, high level consumers of vegetables grown under unfavorable local conditions may exceed the ADI by approximately two fold. In this report, the nitrate concentrations were not corrected for mitigating factors, such as fruit consumption and processing effect, which may overestimate exposure. The consumption of more than 38.2 g of canned apples at the median nitrate concentration would lead to an excursion above the ADI without taking into account any other sources of nitrate exposure. As histamine is potentially toxic, controlling and reducing the concentration

of histamine in canned food is recommended. In summary, in order to maximize nutritional quality and health benefits of fresh and canned apples, measures should be taken to reduce the histamine, nitrate, and nitrite exposure by examining the farm waters as well as other sources of contamination.

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