Original Article

Nitrate Reduction in Canned Apples and Pears Using Calcium Hydrogen Phosphate (CaHPO4)

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ABSTRACT

Background: The aim of this study was to introduce a new and economical method for reduction of nitrate content in canned apples and pears.

Methods: The nitrate content was determined before and after treatment with $CaHPO_4$ ranging from 0.01% to 0.1% using spectroscopic method in 2015 in Pharmaceutical Sciences Research Center, Pharmaceutical Sciences Branch, Islamic Azad University, Tehran- Iran. The effect of treatment time at three different time points (30, 60, 90 min) was determined. Sensory evaluation was performed using five-point hedonic scales.

Results: Nitrate content in fresh fruit is significantly (P<0.05) lower than canned products; this may be explained by the effect of water for washing fruits during processing. The mean value of nitrate in canned apples were significantly (P<0.05) higher compared to the canned pears; this may be related to the type of fruits and its texture and composition. Nitrate content of canned apples and pears were decreased from 233.24±24.90 to 128.80±0.423 and 195.11±20.32 to 118.804±0.634 mg/kg, respectively. Different concentration of $CaHPO_4$ did not influence sensory attributes of canned apples while overall acceptance of canned pears decreased only in 0.1% $CaHPO_4$ (P<0.05). The most efficient time for treating by $CaHPO_4$ was 90 min, but the most practical one is recommended 30 min. Addition of $CaHPO_4$ did not change pH of canned samples.

Conclusion: Application of $CaHPO_4$ is suggested as a novel, safe and economical method for removal of nitrate in canned products.

Keywords: CaHPO4, Canned Apples, Canned Pears, Food Safety, Removing Nitrate.

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INTRODUCTION

Nitrate concentration in water and food products has increased in recent years due to the use of nitrate as food preservative, nitrogen fertilizers and industrial pollutants [1, 2]. Nitrate is mainly transferred into the human body through water, and food [3-4]. Although nitrate is not overly toxic to the human body, it changes to toxic nitroso compounds that include nitrite, nitric oxide, and nitrosamine in the natural condition of the stomach [4-5]. Nitroso compounds have several harmful effects on human health; including the formation of methemoglobin, adrenal cortex hyperplasia, and gastric neoplasia [6]. Nitrate is quickly absorbed by the stomach and the beginning of the small intestine in humans. About 5% of nitrate in water and food are transferred to nitrite in the saliva that ultimately leads to the production of nitrosamines. About 80% of nitrate in foods are ingested through consumption of fruits, vegetables, and processed meats [7]. At least 35% of cancers are related to the consumption of food products [8]. The presence of nitrite and nitrate in food increase the risk of stomach and colon cancers (Gastrointestinal cancer) in adults and methemoglobinemia in children [9]. The risk of methemoglobinemia in children and those who are

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suffering from gastrointestinal diseases due to the lower pH in stomach is heavy because of the conversion of nitrate to nitrite. Methemoglobin reductase enzyme is produced in adults and is converted to oxyhemoglobin [6]. Delivering oxygen to tissues is impaired by the formation of methemoglobin [10].

According to the regulations of the European Union Commission and WHO, acceptable daily intake (ADI) of nitrate is from 0 to 7.3 mg/kg of body weight [11]. Nitrate concentration of fruits and vegetables was more than ADI concentration. Fruits such as strawberries and grapes have nitrate more than 100 mg/kg and 17 mg/kg, respectively [11].

There are different varieties of apples with the scientific name of *Malus domestica* or *Pirus malus* from the Rosaceae family. Canned apples are produced from suitable and ripe fruits after separation, cleaning, sorting, washing, peeling, removing seeds, cutting, and filling in the containers. Then the hot sugar syrup is added at a temperature of about 98 °C in order to finally cap and sterilize it in boiling water for a certain period of time before rapidly cooling to temperature of 40-45 °C [12]. Apple contents in 100 gr of apple are about 84% water, 0.3% pulp, 14% carbohydrates, ascorbic acid, and a small amount is minerals. The apple causes disposal of cholesterol due to various acids and especially potassium salts [13].

Pear is another fruit available in the food industry. Contents of 100 gr of pear are about 83% water, 0.3% pulp, 15% carbohydrates and small amount is vitamins. Pear is a laxative due to the presence of pectin and has anti-cancer properties [14].

Lack of diversity in the number of production companies producing canned in the market, there is a short window of production opportunity that often leads to undesired losses of product. The main products of the apples and pears canned in large scales include juice concentrate, puree, juice, vinegar, dried fruit, sauce, canned apples and pears, pie and chips [13]. Regarding the above-mentioned subjects, canned fruits and vegetables contain nitrate; therefore, they can be the source of nitrate in the human body.

The aim of this study was to measure the effect of calcium hydrogen phosphate ($CaHPO_4$) in reduction of nitrate in canned apples and pears.

MATERIALS AND METHODS

Potassium hexacyanoferrate, N-(1-Naphthyl) ethylenediamine hydrochloride, cadmium sulfate ($3CdSO_4.8H2O$), zinc sulfate ($ZnSO_4.7H_2O$), sulfanilamide, hydrochloric acid, sodium tetraborate ($NA_2B_4O_7.10H_2O$), glacial acetic acid, *N*-(1-naphthyl)-ethylene diammonium dichloride reagent ($C_{12}H_{16}C_{l2}N_2.CH_3OH$), sodium nitrate, and saturated borax solution were obtained from Merck (Darmstadt, Germany).

Apparatus

a. Filter papers, nitrate, and nitrite free, 20 µm pore size (Wattman No. 1)
b. Double monochromator UV-visible spectrophotometer Model UV-1601 with quartz cells (1 cm) for measuring absorbance at 538 nm (PG Instruments Ltd. UK)
c. Potentiometer (Inolab, Weilheim, Germany) for pH determination

Sampling

Five common brands of canned apples and pears were selected from different companies of Iran and 240 samples were collected from Tehran market to analyze nitrate content in 2015 in Pharmaceutical Sciences Research Center, Pharmaceutical Sciences Branch, Islamic Azad University, Tehran, Iran. After opening each can, fruits were ground in a food blender with stainless steel cutters to make a completely homogenized sample. Aliquots of samples were refrigerated at (4-6 ºC) before analysis.

Methods

Nitrate content using spectroscopic method and pH with a potentiometer were also analyzed in canned products treated by $CaHPO_4$ (0.01%, 0.02%, 0.03%. 0.05%, 0.07%, 0.1%). The effect of time treatment by the most efficient concentration of $CaHPO_4$ up to 90 min (time 30, 60, and 90 min) was also considered in this study.

Nitrate Determination

Nitrate content was measured according to the Association of Analytical Communities (AOAC, 2002) official spectroscopic method (993.03). 3.0 g of homogenized canned fruits with 150 mL of hot water was extracted and sample extracts (10 mL) were mixed with 2 mL potassium hexacyanoferrate to precipitate protein and after 15 min were filtered on filter paper. Cadmium was added to reduce nitrate to nitrite. Then 5 mL sulfanilamide and 3 mL hydrochloric acid was added and placed for 5 min in the dark. Followed by 1 mL N-(1-Naphthyl) ethylenediamine hydrochloride reagent was added and left for 15 min for color development and create a red complex. The absorbance of the sample was measured colorimetrically at 538 nm using double monochromator UV-visible spectrophotometer (PG Instruments Ltd. UK) with 1 cm quartz cells against blank solution. Nitrate concentration was evaluated using calibration curve of standard solution of 0, 10, 20, 30, 50 and 100 µg $NaNO_3$, and mg $NaNO_3$ contents have been calculated as follows:

mg $NaNO_3$/ kg test portion = b ×100/ m

Where b: sodium nitrate from standard curve (µg) and m: weight of test portion homogenate (g)

Nitrate value of samples was expressed as mg nitrate per kg on a fresh weight basis (mg NO_3/kg FW).

PH Determination

The pH of samples was measured by potentiometer (Inolab, Weilheim, Germany) after calibration using standard buffers pH 6.0 and 9.0. The pH was recorded after the pH meter provided final reading.

Sensory Evaluation

Sensory evaluation was performed for flavor, appearance, texture, and overall acceptance by 15 panelists using 5-point hedonic scales were one represented "extremely dislike" and five indicated "extremely like". Samples were given a three-digit code and sensory analysis was carried out at room temperature. Between each sample, testing the panelists rinsed their mouths with pure, room temperature water [15-17].

Statistical Analysis

Values were expressed as mean (mg/kg FW) ± standard deviation (SD). Statistical analysis was performed by One-Way ANOVA and for comparison between before and after treatment using $CaHPO_4$, paired sample t-test was applied by SPSS 22.0 software (SPSS Inc, IBM, Chicago, IL). A probability value of (P<0.05) was considered statistically significant. All experiments were performed in quintuplicate.

RESULTS

Chemical Analysis

The mean value for nitrate content of canned apple was 233.24±24.90 mg/kg, while the nitrate concentration in canned pear was 195.11±20.32 mg/kg before being treated by $CaHPO_4$ (Figure 1, 2). Nitrate content of canned apple and pear in brand 3 was the highest one and the lowest amount of nitrate was determined in brand 2 in both canned products (P<0.05) (Table 1). Nitrate level in canned apples were significantly greater (P<0.05) than fresh fruit (106.36 ± 8.752 mg/kg) as well as nitrate in canned pears were significantly higher than fresh pears (100.73±8.55 mg/kg) (P<0.05) (Table 1).

The $CaHPO_4$ in all applied concentrations (0.01% to 0.1%) caused a decrease in the nitrate levels of canned apples and pears (P<0.05) (Figure 1, 2). The reduction of nitrate level in canned pears using $CaHPO_4$ in the range of 0.01% to 0.1% was determined (P<0.05) (Figure 2). The concentration of 0.1% of $CaHPO_4$ showed the greatest effect on the reduction of nitrate content (P<0.05) both in canned apples and pears (Figure 1, 2).

Adding 0.1% CaHPO4 could reduce about 30% of nitrate in apples and 35% in pears (Figure 3).

Treatment times of $CaHPO_4$ were tested as 30, 60, and 90 min (Figure 4). The most effective time to reduce nitrate content was determined at 90 min (P<0.05).

The variation of pH in canned apples and pears after 30, 60, and 90 min treated by $CaHPO_4$ indicated that there was not significantly different in pH after 60 min treatment (P≥0.05) (Figure 5).

Sensory Analysis

A sensory evaluation was carried out on canned apples and pears after treatment by different concentrations of $CaHPO_4$ (Table 2). In canned apples, concentration of $CaHPO_4$ did not influence overall sensory attributes of canned fruits (P≥0.05). Sensory evaluation of canned pears revealed that the overall acceptance in samples contained less than 0.05% $CaHPO_4$, similar to that of the untreated product, while a significant decrease in overall acceptance score was observed for treatment by 0.1% $CaHPO_4$ (P<0.05). This may suggest that 0.05% $CaHPO_4$ did not affect sensory attributes and no off-flavor was produced in both types of canned products. The percentage of panelists who selected the product for purchasing was also considered in the questionnaire; more than 60% of panelists selected the canned apples and pears for purchasing (Table 2).

Table 1: Mean value ±SD of nitrate content (mg kg^{-1} Fresh weight) after addition of different percentages of $CaHPO_4$.

0.1%	0.07%	0.05%	0.03%	0.02%	0.01%	Not-treated by$CaHPO_4$	Brand	
127.556±0.56^{b}	130.021±1.03^{b}	131.489±0.98^{ab}	135.223±2.33^{ab}	141.242±1.84^{ab}	151.889±2.26^{ab}	226.514±3.11^{b}	A1	Canned Apple
108.321±0.45^{a}	110.207±1.29^{a}	118.798±0.78^{a}	121.568±1.83^{a}	126.020±1.23^{a}	136.12±2.01^{a}	195.899±2.89^{a}	A2	
148.908±0.34^{c}	150.733±1.44^{c}	156.710±0.67^{c}	163.121±1.29^{c}	172.423±1.56^{c}	186.561±3.07^{c}	280.191±5.02^{d}	A3	
138.206±0.38^{bc}	140.218±0.67^{bc}	143.552±0.88^{bc}	146.872±1.22^{bc}	152.408±1.04^{b}	164.712±1.87^{b}	245.956±4.18^{c}	A4	
121.032±0.36^{ab}	124.092±0.43^{ab}	128.183±1.06^{ab}	131.250±1.00^{ab}	136.655±0.96^{ab}	146.060±1.02^{ab}	217.635±3.44^{b}	A5	
117.444±0.13^{a}	120.110±0.28^{a}	123.549±0.78^{a}	125.888±1.11^{a}	131.045±1.66^{a}	137.535±2.09^{ab}	204.078±3.89^{c}	P1	Canned Pears
115.218±0.65^{a}	117.652±0.78^{a}	119.825±0.66^{a}	120.556±1.59^{a}	124.320±1.56^{a}	130.520±1.88^{a}	154.286±2.16^{a}	P2	
124.213±0.76^{a}	126.872±0.91^{a}	130.777±0.29^{a}	134.523±1.67^{a}	141.820±1.06^{ab}	150.200±1.76^{ab}	220.616±1.45^{c}	P3	
116.782±0.88^{a}	118.111±0.24^{a}	121.115±0.14^{a}	122.295±1.45^{a}	126.123±1.23^{a}	130.718±1.23^{a}	185.152±1.01^{b}	P4	
118.442±0.72^{a}	121.056±0.76^{a}	125.776±0.16^{a}	129.897±1.23^{a}	136.795±1.11^{ab}	144.100±1.43^{ab}	211.431±2.86^{c}	P5	

Mean values with different small letters are different for the same percentage of $CaHPO_4$ in canned apples and pears ($P<0.05$).

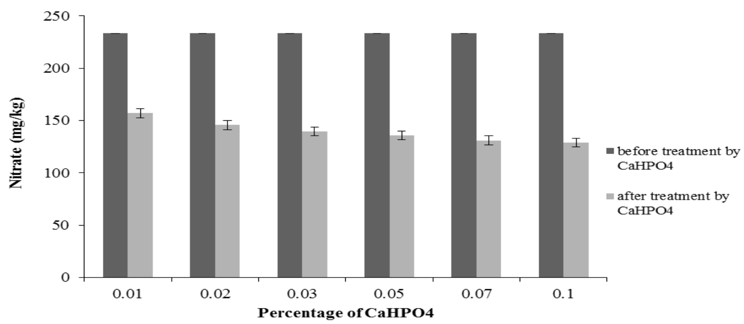

Figure 1. Nitrate content of canned apples before and after treatment by CaHPO4.

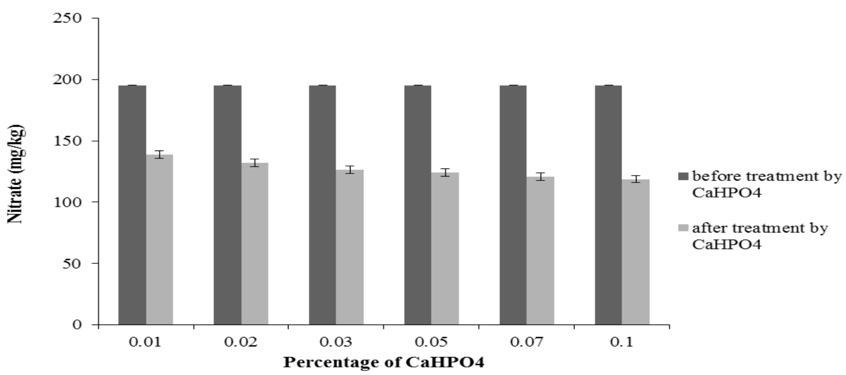

Figure 2. Nitrate content of canned pears before and after treatment by CaHPO4.

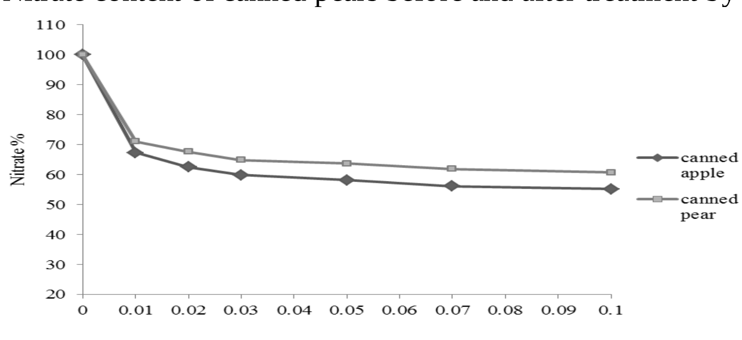

Figure 3. Removal yields of nitrate content of canned apples and pears using $CaHPO_4$.

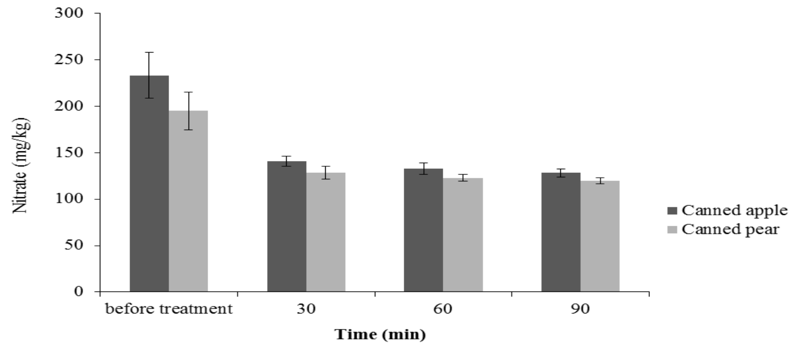

Figure 4. Nitrate content of canned apples and pears after 30, 60, and 90 min of treatment by $CaHPO_4$.

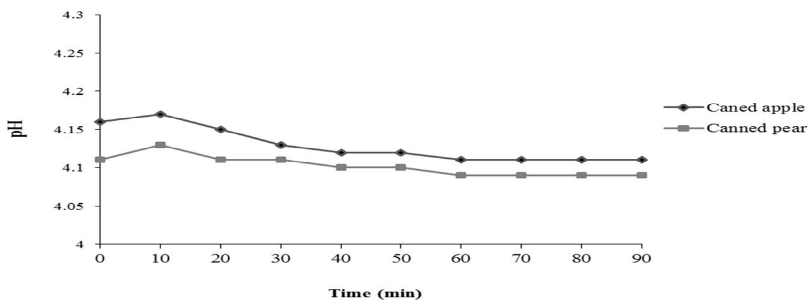

Figure 5. Variation of pH of canned apples and pears after 30, 60, and 90 min of treatment by $CaHPO_4$.

Table 2. Mean value ±SD of overall acceptance scores of sensory evaluation.

0.1%	0.05%	0.03%	0.01%	Not-treated by $CaHPO_4$		
3.96±0.34[a]	4.01±0.25[a]	4.11±0.19[a]	4.18±0.20[a]	4.28±0.14[a]	Overall acceptance	Canned Apples
66.66%	80%	80%	93.33%	93.33%	Consumer acceptance	
3.79±0.27[b]	3.98±0.26[ab]	4.06±0.19[ab]	4.12±0.15[ab]	4.17±0.90[a]	Overall acceptance	Canned Pears
60%	73.33%	80%	93.33%	93.33%	Consumer acceptance	

DISCUSSION

In the food industry, determination of nitrate content in fruits, vegetables, and their derived products has been considered by different organizations for food safety management [18]. In Germany, the maximum allowed limit of nitrate in fruits is determined as following: 108 mg/kg for apples, 11 mg/kg for pears, 31 mg/kg for peaches, 34 mg/kg for grapes and in France, the maximum nitrate contents of pears is 35 mg/kg and 24 mg/kg in cherries and apples are allowed [18-19].

Nitrate accumulation in fruit depends on three major factors, including the type and dose of fertilizer and herbicide application, physiologically active substances, and the type of soil and environmental conditions, such as air humidity [20-21]. Nitrate content in fresh fruit is significantly (P<0.05) lower than canned products; this may be explained by the effect of water for washing fruits during processing. The mean value of nitrate in canned apples were significantly (P<0.05) higher compared to the canned pears; this may be related to the type of fruits and its texture and composition. Acceptable Daily Intake (ADI) for nitrate content based on the European Commission (EC)'s Scientific Committee for Food (SCF) is recommended as 3.65 mg/kg body weight (equivalent to 219 mg/day for a person weighing 60 kg). Whereas, the Joint Expert Committee of the Food and Agriculture (JECFA) Organization of the United Nations/WHO established the ADI as 0–3.7 mg per kg body weight (the maximum amount is equivalent to 222 mg per day for a person weighing 60 kg) [17-22]. However, high-level consumption of canned fruits may exceed the ADI approximately two-fold. The accumulation of nitrates in fruits and its derived products can create health defects. Therefore, finding methods for reduction of nitrate content has been observed to decrease associated health problems and increase food safety. An application of $CaHPO_4$ for reduction of nitrate was considered in the present study. Calcium hydrogen phosphate ($CaHPO_4$) as generally regarded as Safe (GRAS) food additive was used in the present study. 0.5% phosphate level did not appear to have adverse physiological effects in humans. The addition of CaHPO4 to canned products can cause a decrease of nitrate content due to the following process: [22]

$$CaHPO_4 \rightarrow Ca^{2+} + (HPO_4)^{2-}$$

$$(HPO_4)^{2-} + H_2O \rightleftarrows (H_2PO_4)^- + OH^-$$

$$(HPO_4)^{2-} + H_2O \rightleftarrows (H_2PO_4)^- + OH^-$$

$$(H_2PO_4)^- + H_2O \rightleftarrows H_3PO_4 + OH^-$$

In acidic condition:

$$NO_3^- \xrightarrow{E_0=+0.94(V)} HNO_2 \xrightarrow{E_0=+1.00(V)} NO \xrightarrow{E_0=+1.59(V)} N_2O \xrightarrow{E_0=+1.77(V)} N_2 \xrightarrow{E_0=+0.27(V)} NH_4^+$$

$$\underbrace{HNO_2 \longrightarrow N_2}_{E^0=+1.11\ (V)}$$

Based on the above-mentioned formula, this process removes nitrate in the form of nitrogen (N2) gas from the canned fruit. $CaHPO_4$ can be applied for the removal of nitrate in canned products.

Besides, higher levels of phosphate often create chemical, physical, and sensory defects in food products. $CaHPO_4$ is used in powder form as a food additive in cereal products, chewing gums, and in food processing to act as humectants, acidity regulator, flour treatment agent, anti-caking agent, firming agent, stabilizer, thickener, and emulsifier [23-25]. In food industry, optimum time is short time processing; 60 min for treatment by $CaHPO_4$ has been suggested in the production of canned fruit [21]. Consequently, reduction of nitrate by CaHPO4 in minimum time has been considered in this study.

CONCLUSION

Treating canned apples and pears by $CaHPO_4$ (0.01% to 0.1%) in comparison with untreated canned products revealed a significant reduction in nitrate content ($P<0.05$). The most efficient concentration of $CaHPO_4$ was 0.1%. However, the result of current study showed that increasing the concentration of $CaHPO_4$ to cause a decrease in panelists desires to purchase the product.

The application of 0.1% $CaHPO_4$ in canned apples did not change sensory evaluation, while in canned pears no changes in overall acceptance scores were detected in 0.05% of $CaHPO_4$. Time of treatment is another important factor in terms of the efficiency of $CaHPO_4$ in order to reduce nitrate content. The most effective time was obtained at 90 min, but due to the preference of short time processing in the food industry, 30 min for treatment by $CaHPO_4$ is recommended. No variation in pH of canned products was detected for 60 min of treatment.

A new and safe method suggested for a reduction of nitrate content of canned apples and pears using CaHPO4. Further investigation for treatment of other types of canned products is recommended.

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REFERENCES

1. Ward MH. Too much of a good thing? Nitrate from nitrogen fertilizers and cancer. Rev Environ Health 2009;24: 357-63.
2. Zhao X , Chen L, Zhang H. Nitrate and ammonia contaminations in drinking water and the affecting factors in Hailun, northeast China. J Environ Health 2013 ;75(7):28-34.
3. Lundberg JO, Weitzberg E, Cole JA, Benjamin N. Nitrate, bacteria and human health. Nature Rev Microbiol 2004; 2: 593-602.
4. Bednar C, Kies C. Nitrate and vitamin C from fruits and vegetables: Impact of intake variations

on nitrate and nitrite excretions of humans. Plant Foods Hum Nutr 2004*;* 45:71-80.

5. Ward MH, DeKok T M, Levallois P, Brender J, Gulis G, Nolan B T, VanDerslice J Workgroup report: Drinking-water nitrate and health-recent findings and research needs. Environ Health Perspect 2005; 1607-14.
6. Authority E. Nitrate in vegetables: scientific opinion of the panel on contaminants in the food chain. The EFSA 2008*;* 689: 1-79.
7. Choi BC. N-nitroso compounds and human cancer: a molecular epidemiologic approach. Am J Epidemiol 1985;121:737-43.
8. Kennedy D. Leafy vegetables and nitrates in nitrate and nitrite in food and water. Harwood Publishers, London. 2003.
9. Hord NG, Tang Y, Bryan NS. Food sources of nitrates and nitrites: the physiologic context for potential health benefits. Am J Clin Nutr 2009; 90: 1-10.
10. Santamaria P. Nitrate in vegetables: toxicity, content, intake and EC regulation. J Sci Food Agr 2006; 86: 10-7.
11. Ziarati P, Mohammad-Makki FM. Removal of Nitrate and Nitrite from Tomato Derived Products by Lemon Juice. BBRA 2015; 12(2):767-72 ·
12. Schuddeboom L. Nitrates and nitrites in foodstuffs. Council of Europe 1993; 23: 385-90.
13. Tabatabaee J, Nazari-e-Deljo M, Rostami R, Azarmi F, Fazilat F, Pahnaei S. Nitrate concentration evaluation of leafy, fruit bearing and tuberous vegetables in Tabriz. In: Proceeding of 4th Conference of Farming Sciences. 2005; 11-3.
14. Kirovska-Cigulevska O. Determination of nitrates in food products. Balikesir Üniversitesi Fen Bilimleri En-stitüsü Dergisi 2002;4:70-3.
15. Šušin J, Kmecl V, Gregorčič A. A survey of nitrate and nitrite content of fruit and vegetables grown in Slovenia during 1996–2002. Food Addit Contam 2006; 23(4): 385-390.
16. Ameh BA, Gernah DI, Obioha O, Ekuli G K. Production, Quality Evaluation and Sensory Acceptability of Mixed Fruit Juice from Pawpaw and Lime. Food Nutr Sci 2015; 6: 532-3.
17. Chambers B, Chambers E, Bowers JA, Craig JA. Sensory detection and population thresholds for sodium tripolyphosphate in cooked ground turkey patties. J Food Sci 1991; 56: 200-9.
18. AOAC. Official Methods of Analysis, 17th ed. Arlington, VA: AOAC.2002.
19. Susin J, Kmecl V, Gregorcic A. A survey of nitrate and nitrite content of fruit and vegetables grown in Slovenia during 1996-2002. Food Addit Contam 2006 ; 23(4):385-90.
20. Anjana SU, Iqbal M. Nitrate accumulation in plants, factors affecting the process, and human health implications. A review. Agron Sustain Dev 2007; 27: 45-57.
21. Tahmasi A, Ziarati P. Histamnie and Chemical Composition of Canned and Frozen Green Pea (Pisum sativum). OJC 2015; 9(2):739-49.
22. Tas AC. Monetite (CaHPO4) synthesis in ethanol at room temperature. J Am Ceram Soc 2009; 92: 2907-12.
23. Mohammadi S, Ziarati P. Nitrate and Nitrite Content in Commercially-available Fruit Juice Packaged Products. JCPR 2016; 8(6):335-41.
24. Maga, J. A., Types of food additives, in Food Additive Toxicology, Maga, J. A. and Tu, A. T., Eds., Marcel Dekker Inc., New York, 1995, 1.
25. Sundaralingam T, Gnanavelrajah N. Phytoremediation potential of selected plants for Nitrate and Phosphorus from ground water. Int J Phytoremediation 2014; 16: 275-84.