

Original Article**Nitrate Reduction in Canned Apples and Pears Using Calcium Hydrogen Phosphate (CaHPO₄)***Parisa Ziarati*^{*1}, *Estatira Sepehr*², *Saeed Heidari*³, *Maryam Moslehisad*⁴

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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 CaHPO₄ 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 CaHPO₄ did not influence sensory attributes of canned apples while overall acceptance of canned pears decreased only in 0.1% CaHPO₄ ($P<0.05$). The most efficient time for treating by CaHPO₄ was 90 min, but the most practical one is recommended 30 min. Addition of CaHPO₄ did not change pH of canned samples.

Conclusion: Application of CaHPO₄ 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

1. PhD of Chemistry, Young Researchers & Elite Club, Pharmaceutical Sciences Branch, Islamic Azad University, Tehran, Iran.

2. PhD of Cellular and Molecular Medicine, Division of Biochemical Toxicology, National Center for Toxicological Research, U.S. Food and Drug Administration, Jefferson, AR, USA.

3. MSc of Food Sciences & Technology, Nutrition and Food Sciences Research Center, Pharmaceutical Sciences Branch, Islamic Azad University, Tehran, Iran.

4. Department of Food Science and Technology, Safadasht Branch, Islamic Azad University, Tehran, Iran.

*Corresponding Author: E-Mail: ziarati.p@iaups.ac.ir

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 ($3\text{CdSO}_4 \cdot 8\text{H}_2\text{O}$), zinc sulfate ($\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$), sulfanilamide, hydrochloric acid, sodium tetraborate ($\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$), glacial acetic acid, N-(1-naphthyl)-ethylene diammonium dichloride reagent ($\text{C}_{12}\text{H}_{16}\text{C}_{12}\text{N}_2 \cdot \text{CH}_3\text{OH}$), sodium nitrate, and saturated borax solution were obtained from Merck (Darmstadt, Germany).

Apparatus

- Filter papers, nitrate, and nitrite free, 20 μm pore size (Wattman No. 1)
- Double monochromator UV-visible spectrophotometer Model UV-1601 with quartz cells (1 cm) for measuring absorbance at 538 nm (PG Instruments Ltd. UK)
- 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 °C) 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 $\mu\text{g NaNO}_3$, and mg NaNO_3 contents have been calculated as follows:

$$\text{mg NaNO}_3/\text{kg test portion} = b \times 100 / m$$

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 $\text{NO}_3/\text{kg FW}$).

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) \pm 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% CaHPO_4 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 ($P \geq 0.05$) (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 ($P \geq 0.05$). 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 \pm 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 \pm 0.56 ^b	130.021 \pm 1.03 ^b	131.489 \pm 0.98 ^{ab}	135.223 \pm 2.33 ^{ab}	141.242 \pm 1.84 ^{ab}	151.889 \pm 2.26 ^{ab}	226.514 \pm 3.11 ^b	A1
108.321 \pm 0.45 ^a	110.207 \pm 1.29 ^a	118.798 \pm 0.78 ^a	121.568 \pm 1.83 ^a	126.020 \pm 1.23 ^a	136.12 \pm 2.01 ^a	195.899 \pm 2.89 ^a	A2
148.908 \pm 0.34 ^c	150.733 \pm 1.44 ^c	156.710 \pm 0.67 ^c	163.121 \pm 1.29 ^c	172.423 \pm 1.56 ^c	186.561 \pm 3.07 ^c	280.191 \pm 5.02 ^d	A3
138.206 \pm 0.38 ^{bc}	140.218 \pm 0.67 ^{bc}	143.552 \pm 0.88 ^{bc}	146.872 \pm 1.22 ^{bc}	152.408 \pm 1.04 ^b	164.712 \pm 1.87 ^b	245.956 \pm 4.18 ^c	A4
121.032 \pm 0.36 ^{ab}	124.092 \pm 0.43 ^{ab}	128.183 \pm 1.06 ^{ab}	131.250 \pm 1.00 ^{ab}	136.655 \pm 0.96 ^{ab}	146.060 \pm 1.02 ^{ab}	217.635 \pm 3.44 ^b	A5
117.444 \pm 0.13 ^a	120.110 \pm 0.28 ^a	123.549 \pm 0.78 ^a	125.888 \pm 1.11 ^a	131.045 \pm 1.66 ^a	137.535 \pm 2.09 ^{ab}	204.078 \pm 3.89 ^c	P1
115.218 \pm 0.65 ^a	117.652 \pm 0.78 ^a	119.825 \pm 0.66 ^a	120.556 \pm 1.59 ^a	124.320 \pm 1.56 ^a	130.520 \pm 1.88 ^a	154.286 \pm 2.16 ^a	P2
124.213 \pm 0.76 ^a	126.872 \pm 0.91 ^a	130.777 \pm 0.29 ^a	134.523 \pm 1.67 ^a	141.820 \pm 1.06 ^{ab}	150.200 \pm 1.76 ^{ab}	220.616 \pm 1.45 ^c	P3
116.782 \pm 0.88 ^a	118.111 \pm 0.24 ^a	121.115 \pm 0.14 ^a	122.295 \pm 1.45 ^a	126.123 \pm 1.23 ^a	130.718 \pm 1.23 ^a	185.152 \pm 1.01 ^b	P4
118.442 \pm 0.72 ^a	121.056 \pm 0.76 ^a	125.776 \pm 0.16 ^a	129.897 \pm 1.23 ^a	136.795 \pm 1.11 ^{ab}	144.100 \pm 1.43 ^{ab}	211.431 \pm 2.86 ^c	P5

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

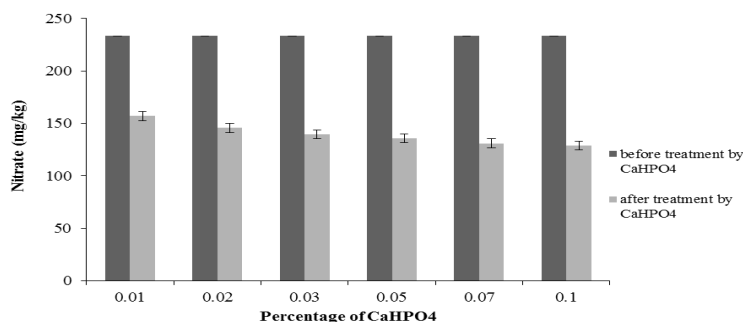


Figure 1. Nitrate content of canned apples before and after treatment by CaHPO_4 .

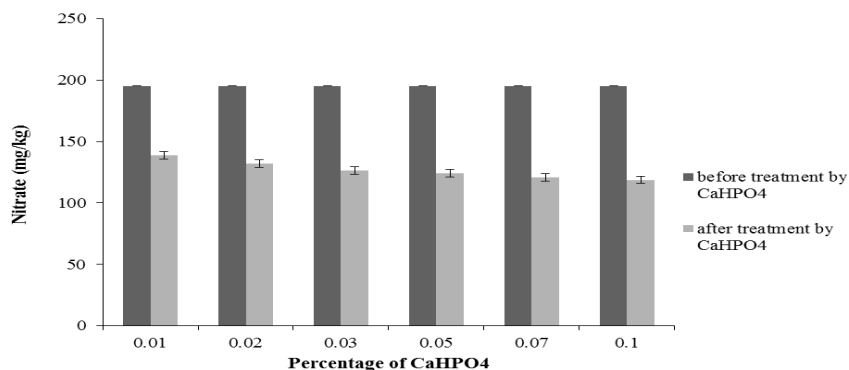


Figure 2. Nitrate content of canned pears before and after treatment by CaHPO_4 .

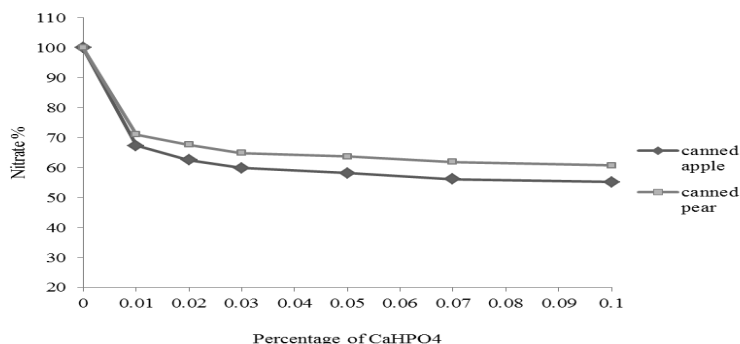


Figure 3. Removal yields of nitrate content of canned apples and pears using CaHPO_4 .

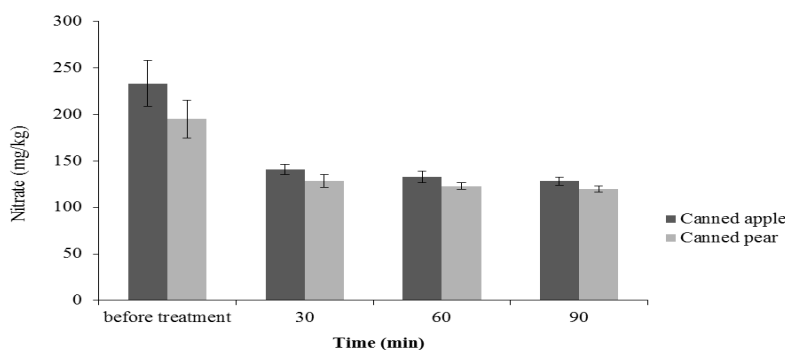


Figure 4. Nitrate content of canned apples and pears after 30, 60, and 90 min of treatment by CaHPO_4 .

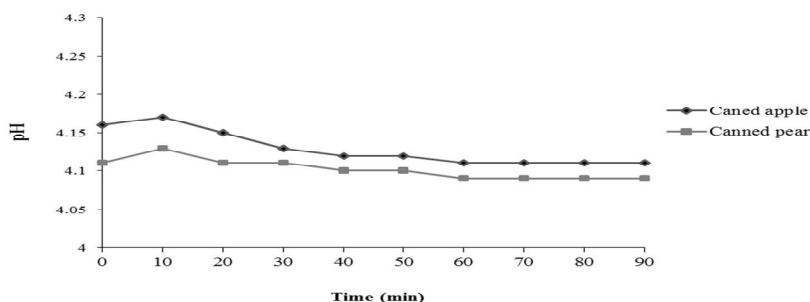


Figure 5. Variation of pH of canned apples and pears after 30, 60, and 90 min of treatment by CaHPO_4 .

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

0.1%	0.05%	0.03%	0.01%	Not-treated by CaHPO_4	
3.96 \pm 0.34 ^a	4.01 \pm 0.25 ^a	4.11 \pm 0.19 ^a	4.18 \pm 0.20 ^a	4.28 \pm 0.14 ^a	Overall acceptance
66.66%	80%	80%	93.33%	93.33%	Consumer acceptance
3.79 \pm 0.27 ^b	3.98 \pm 0.26 ^{ab}	4.06 \pm 0.19 ^{ab}	4.12 \pm 0.15 ^{ab}	4.17 \pm 0.90 ^a	Overall acceptance
60%	73.33%	80%	93.33%	93.33%	Consumer acceptance

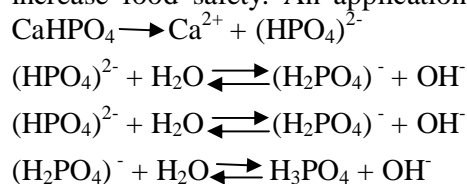
DISCUSSION

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].

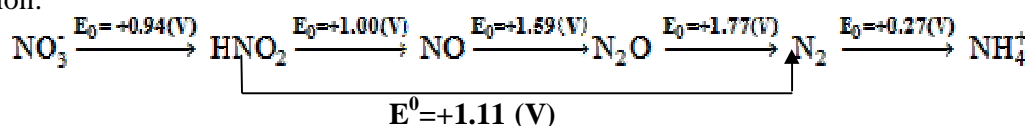
Nitrate accumulation in fruit depends on three major factors, including the type and dose of fertilizer and herbicide application, physiologically active substances, and the type of soil and environmental conditions, such as air

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 CaHPO₄



In acidic condition:



Based on the above-mentioned formula, this process removes nitrate in the form of nitrogen (N₂) gas from the canned fruit. CaHPO₄ 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. CaHPO₄ 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 CaHPO₄ 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 CaHPO₄ (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 CaHPO₄ was 0.1%. However, the result of current study showed that increasing the concentration of CaHPO₄ to cause a decrease in panelists desires to purchase the product.

The application of 0.1% CaHPO₄ in canned apples did not change sensory evaluation, while in canned pears no changes in overall acceptance scores were detected in 0.05% of CaHPO₄. Time

for reduction of nitrate was considered in the present study. Calcium hydrogen phosphate (CaHPO₄) 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]

of treatment is another important factor in terms of the efficiency of CaHPO₄ 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 CaHPO₄ 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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