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Removal of Nitrate and Nitrite from Tomato Derived Products by Lemon Juice

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In recent years, many different parts of the world have been facing the problem of nitrate and nitrite contamination in food products, essentially due to the potential reduction of nitrate to nitrite, which is known to cause adverse effects on human and animal health. Therefore a simple, practical and economic process to reduce nitrate and nitrite concentrations in tomato processed products was the major objective of this research. In this study measuring nitrite and nitrate contents of tomato derived products samples commercially available in Iran market was conducted and then by adding different amount of fresh lemon juice the remained nitrate was determined. 280 samples tomato products during 5 consecutive months of 2014 - 2015 were taken and analyzed. In order to conduct a comparison between the content of nitrate and nitrite in the samples studied, dry matter content was determined according to the association of office analytical chemists (AOAC, 2000). A fifty gram sample of the prepared tomato was blended with 50ml deionized 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 tomato samples were determined by spectrophotometric methods at a wavelength of 538 nm, and nitrate concentration was determined after reducing nitrate to nitrite by using cadmium column. The fresh lemon juice in this research show a significant removal effect on the nitrate and nitrite contents in tomato samples tested (pd"0.001). The highest level of nitrate was found in ketchup by range of: 15.01-167.54 and mean content of 65.73±0.007 (mg/kg FW ±SE) which was treated by 0.02%w/w of lemon juice and removing 93% nitrate content in this product. Natural and fresh Lemon juice in other tomato processed treated also removed nitrite contents significantly (pd"0.003).

Key words: Removing nitrate, Tomato processed products, Lemon juice.

In recent years, an increasing remarkable 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. Nitrates and nitrites seem to be among the chemicals that may cause pollution. Many studies have shown these compounds can threaten the environment and living health[1]. However, the largest loading of nitrate and nitrite originates from anthropogenic sources, 2 which mostly result from excessive application of nitrate-based chemical fertilizers in agricultural activities[2-10] and from many industrial processes[9,11,12]. Nitrates and nitrites may accumulate in plants tissues and

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are very dangerous substances for human health, leading health disturbances (methemoglobinemia). Some epidemiological studies linking intake of nitrate and nitrite with gastric cancer in humans indicated a positive correlation[13]. Nitrite (NO2) and nitrate (NO3) are soluble in water and are composed of nitrogen and oxygen atoms. When N-NH3 or other forms of nitrogen present in water are aerated, they produce nitrate. The presence of nitrate and nitrite in food is related to vegetable consumption[8]. The concentration and amount of nitrates levels in plants will vary depending on the type of vegetable, the temperature that it is grown at, the sunlight exposure, soil moisture levels and the level of natural nitrogen in the soil[13]. The Scientific Committee for Food reviewed the toxicological effects of nitrate and nitrite and established an Acceptable Daily Intake (ADI) of 0-3.7 mg/kg b.w. for nitrate in 1990[14] retained the ADI in 1995 and derived an ADI of 0-0.06 mg/kg for nitrite[15]. The JECFA completed its most recent review in 2002 and reconfirmed an ADI of 0-3.7 mg/kg b.w. for nitrate and set an ADI of 0-0.07 mg/kg b.w. for nitrite[16-18]. The high concentrations of nitrate in water causes a phenomenon known as "Eutrophication", which means an excessive growth of the algae in water which consumes the oxygen gas dissolved in water causing fish death[19-22] For these reasons, removal of nitrate and nitrite from water is a necessity. To limit the risk to human health from nitrate and nitrite in drinking water, the World Health Organization (WHO) sat a maximum acceptable concentration to be 50 mg NO3 - /L and 3 mg NO2 - /L[23] Vegetables form the major daily intake source of nitrates in humans supplying about 70-90 % of the total Intake. This intake depends on the type of vegetables consumed, nitrate vegetable levels and the amount of vegetables actually consumed. When large amounts of vegetables are consumed, which have accumulated high amounts of nitrates, a need thus arises for the systematic control of the dietary intake of nitrogenous compounds[26].

In Iran the harvest of greenhouse products including cucumber and tomato is done traditionally and by hand in most of provinces such as Kerman and Khuzestan. More than half of the vegetable's area under cultivation in the country of Iran is in seven provinces of Khuzestan, Fars, Hormozgan, Kerman, Hamedan, Zanjan, Golestan[20,24,25]. Among them, Khuzestan has 71077 hectares and 12.73% of the total cultivated area and the first rank devote to it for produced amount and cultivated area .Greenhouse products are harvested gradually and in several harvests. The number of harvesting these products is different in terms of weather conditions in various seasons. The main buyers of the product include provincial buyers, wholesalers and shop owners[27]. The main goal of this study was removing nitrite and nitrate contents of tomato derived products (fruit juice, ketchup, pizza and tomato sauce) in samples commercially available in Iran market.

Sampling Method

A descriptive – analytical and cross-sectional study was conducted for determination nitrate and nitrite in fruit juice, ketchup, pizza and tomato sauce products commercially available in Iran market.

280 samples of 4 varieties of tomato processed products during 5 consecutive months of 2014 and 2015 collected from recognized market in Tehran. Sampling was replicated twice within each month at intervals of two weeks. To evaluate variability of nitrate and nitrite content within sub-samples, five sub-samples (1400 sub-samples) on the whole were analyzed separately by two ways: fresh weight and dried weight methods. In order to be sure about the accuracy of the results 150 samples were analyzed for nitrate and nitrite content by ion chromatography and colorimetric method too. A half of each sub-sample was taken for nitrate determination and another half was used for moisture measurement. Moisture content was determined by the difference between weights before and after heating at 60 – 700 c for 48 hr. 128 tomato processed derived samples (ketchup, pizza sauce, tomato juice and tomato paste samples were analyzed at the same time too.

Quantitative Determination

Quantitative determination of nitrate

For nitrate analysis, sub-samples were mixed with a food processor. Fifty to seventy grams of sub-sample were weighed and placed into a mixer. 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 [21-24]. 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[25-27]. 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.

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 0.2, 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[22].

Treating Samples

After determining nitrate content in all samples, the effect of time on absorption was studied. Samples which have high level of nitrite solution (higher than permissible level or higher than 40 mg/L) were added to fresh lemon juice sample (by different concentration in range of 0.005-0.10 %w/w) at pH 4 - 5.5. The mixture was shaken at 30°C. Aliquots (0.5 mL each) of the clear solution were pipetted out at different time intervals until equilibrium was reached after 24 h.

Statistical Analysis

Values were expressed as the mean (g/kg) ± standard deviation (SD). Nitrate concentration differences on the basis of the type of products were determined by student t-test. Nitrate content changes after treating by lemon juice 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 mean content of nitrate and nitrite in untreated processed tomato products and comparing them after treating by fresh lemon juice (0.02% w/w) were illustrated in figures 1 and 2 respectively.

The results concerning nitrate contents in tomato derived products are shown in figure 1 reveals that nitrate level in tomato products after treating by lemon juice decreased significantly and p value was lower than 0.003. For tomato juice , the nitrate level ranges : 2.01-16.0 with an average of 6.77 ±0.002(mg/kg FW ±SE) . The highest level of nitrate was found in ketchup by range of: 15.01-167.54 and mean content of 65.73±0.007 (mg/kg FW ±SE) which after mixing by

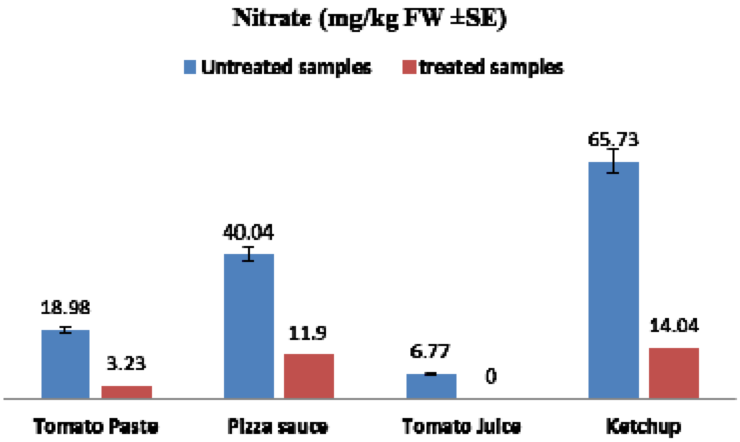

Fig. 1. Remaing Nitrate content (mg/kg FW ±SE) in Tomato processed products after treating by lemon Juice (0.01% w/w)

lemon juice decreased to 14.04±0.04 (mg/kg FW ±SE) . This concentration is significantly higher that other tomato products. The results of nitrite in untreated studied samples shows different trend as in tomato juice the highest level has been found by the range of 0.09 - 0.67 and the mean of 0.28±0.001 (mg/kg FW ±SE) while in ketchup and pizza sauce the lowest nitrite content was found (0.10±0.001 and 0.11±0.001 mg/kg FW ±SE) respectively and all of them after treating by lemon juice were reduced significantly (p d"0.03) . It is clear that the result of our study shows a variation in the nitrate and nitrite levels in the different processed samples.

According with WHO legislation[31] all the analyzed fresh tomatoes and derived products present nitrate levels lower than those imposed by legislation. In conclusion, they meet the safe limits for toxic elements specified by the food standards and are safe to be marketed and consummated.

The nitrite contents of tomato have been investigated in several countries. For instance, in China, the study of the center for food safety in Hong Kong in 2012 shows average concentration of 0.5 mg/kg in Hong Kong[35]. In 2008 in Iran; Ardabil Province, the concentration ranges between ND – 0.76 mg/kg[36]. In Romania, the study of Simion et al, in 2008 shows nitrite concentration less than 1 mg/kg[37] .In Turkey, nitrite average concentration was 0.36 mg/kgin 2007[38]. In Syria in 2013, the amounts of nitrate were within satisfactory limits and even lower than those recorded in numbers of international studies[39]. Despite the fact that presence of nitrate and nitrite in tomato vegetable fruit are in low concentration (<200 mg kg"1 FW) [40], it can be suggested that the consumption of these products does not pose a health risk for the consumers as the values obtained are below the FAO/WHO limits, but the tomato and its derived products is not alone source of dietary nitrate and nitrite. Therefore, certain group of consumer such as vegetarians, young children and elderly with cardiovascular problems and kidney deficiency who may intake these food items for long term should be extra cautions as they are more susceptible to toxicities.

CONCLUSION

The accumulation of nitrates in crops and vegetables depends on many factors such as genetic, agricultural (e.g. type of soil, the dose and chemical forms of nitrogen, availability of other nutrients, herbicide application etc.) and some environmental factors such as air humidity, soil water content and photoperiodicity. Further differences in nitrate accumulation may arise from the time of harvest, vegetation season and storage time[41,42,43]. Due to nitrates and nitrites are among the major chemical indicatives monitored by different organizations that are concerned about environment pollution and safety of food products, surveillance of these chemical levels in food products especially most consumed products such as tomato derived samples is recommended. According with this, the objective of the present study was to determine whether the nitrate and nitrite content present in tomatoes' derived products might be sufficient to cause the associated health problems and to establish if

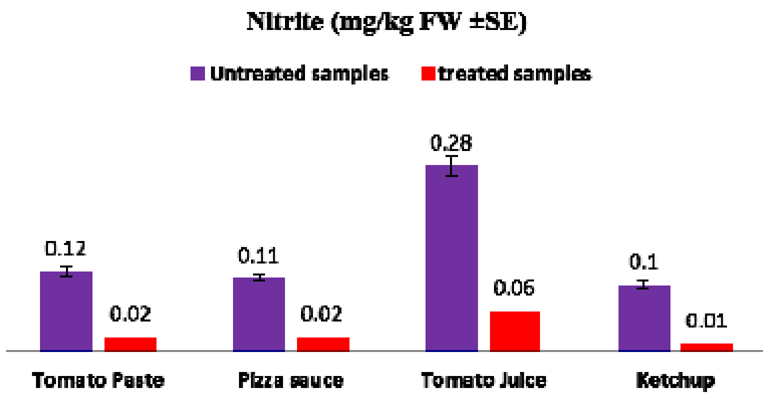

Fig. 2. Remaing Nitrite content (mg/kg FW ±SE) in Tomato processed products after treating by lemon Juice (0.01% w/w)

these vegetables are safe for human health. 1400 tomato derived samples were analyzed and the results revealed that nitrate in ketchup and nitrite content in tomato juice were much higher that maximum permissible and nitrite content. Treating these processed products by fresh lemon juice in comparison by untreated samples showed significant differences and lead to safe limits set by Iranian legislation and the products are safe from this point of view. Also low concentration of nitrate in final products and mix with lemon juice result to very healthy and optimal conditions. Our result indicate that people who use tomato sauce and ketchup and other tomato derived product can eat them safely if add some fresh lemon juice by them. Further investigation for clear the mechanism of juice compounds and its effect on other products is recommended.

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