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Determination of Histamine and Heavy Metal Concentrations in Tomato Pastes and Fresh Tomato (*Solanum lycopersicum*) in Iran

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During the last decade, the increasing demand of food safety has stimulated research regarding the risk associated with consumption of foodstuffs contaminated by contaminates especially heavy metals. Tomatoes and their products provide an essential source of vitamin C, potassium, and antioxidants. Iran is the 7th biggest producer after Egypt and Italy with 4,826,396 tons according to the data of "Food and Agriculture Organization of The United Nations". Regarding to the high consumption of tomato paste in this study, the levels of lead, cadmium, copper, zinc, tin and iron in fresh tomato and in tomato canned paste as well as the histamine content of tomato paste are reported. Due to this descriptive study the effect of processing method; samples were studied in 2 different conditions: fresh and canned paste forms. Fresh Tomato samples were collected during the spring 2014 from five regions of Fars and Kermanshah province farmlands. A total of 480 symptomatic samples were collected from 24 fields located in Fars and Kermanshah Provinces. Meanwhile 400 canned tomato pastes from 12 popular brands in 3 consequences months were purchased. All canned paste samples had the same date of producing. Samples were randomly purchased for analysis and analyzed according to standardized international protocols by wet digestion method. Histamine content was Determined by Capillary Electrophoresis while Lead, Cadmium , Zinc, Copper, Tin and Iron contents in fresh and canned paste tomato samples were determined by Atomic absorption spectrometer. According to variance analyses of data, Tin and most of the heavy metals in canned samples were significantly affected by storage time. The results showed that, the highest concentration in canned food samples was for tin, 357.88±14.33 and lowest 26.54± 5.73 (mg/kg DW). According to variance analyses of data, heavy metal concentration and histamine in canned food samples were significantly affected by company factory. There was a positive correlation between the storage time and heavy metal contents especially tin, zinc and iron and they were varied significantly ($p < 0.003$) and after 6 and 12 months of storage the heavy metal contents were much higher in the same brand in comparison by the newer samples.

Key words: Food Safety, Tomato, Canned Tomato Paste, Histamine, Heavy Metals.

Tomatoes are unique fruit vegetables composed of varied types of tissues that play a critical role in the perception of texture[1]. There are known different varieties of tomato, round, oval, "cherry", but all have the same nutritional characteristics, being an important source of: - potassium, phosphorus, magnesium, iron, so necessary to the normal activity of nerves and muscles[2]. The tomato (Solanum lycopersicum, syn. Lycopersicon lycopersicum & Lycopersicon esculentum) is an herbaceous plant. It is perennial, that usually grown outdoors in temperate climates as an annual plant[3]. Tomatoes and their products provide an essential source of vitamin C, potassium, and antioxidants (primarily lycopene). Lycopene,

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present at high concentrations in tomatoes and tomato products, has attracted considerable attention because of epidemiological evidence that suggests this compound may provide protection against cancer and other degenerative diseases[4] . Iran is the 7th biggest producer after Egypt and Italy with 4,826,396 tons according to the data of "Food and Agriculture Organization of The United Nations" [5]. One of the most important industries in the agricultural sector of Iran is tomato processing industry[6]. The agricultural department of Fars Province has played an important role in the production and occupancy areas of the food industry of the country, moreover a great part of the Gross National Product. This section could provide the raw materials for industry, thus stimulating the section[7]. Two-thirds of the total world production of tomatoes is processed, and application of thermal preservation treatments significantly affects product firmness, viscosity, and consistency[8]. However, heavy metals are natural components of the Earth's crust that main rout enter to the human body is through Food and intake of heavy metal-contaminated fruit or vegetables may pose a risk to the human health[9-16]. On the other hand food packaging can retard product deterioration, retain the beneficial effects of processing, extend shelf-life, and maintain or increase the quality and safety of food. Undoing so, packaging provides protection from 3 major classes of external influences: chemical, biological, and physical[17]. The presence of heavy metals in the food chain and genotypical differences in the critical toxicity levels of heavy metals in plants has been often reported[1]. The most common heavy metal contaminants are Cd, Cu, Pb and Zn, and for canned food there is a great concern about tin . Although tin is not a toxic element, there are studies reporting gastrointestinal perturbations when it is present in concentrations above 200 mg/kg[19, 20,21]. Moreover, when the contamination reaches at this level the organoleptic properties of the food can be seriously affected. Tinplate is widely used in food industry as a robust form of packaging, allowing minimization of headspace oxygen and sterilization of foodstuff within the hermetically sealed can, giving a long, safe, ambient shelf life with no or minimal use of preservatives[21].

Some tin will dissolve into the food content as a result of the use of tinplate for food and beverage packaging, especially when plain uncoated internal surfaces are used. The Provisional Tolerable Weekly Intake for tin is 14 mg/kg body weight[22-25] and recommended maximum permissible levels of tin in food are typically 250 mg/kg (200 mg/kg UK)[23] for solid foods and 150 mg/kg for Beverages[25].Biogenic amines such as histamine are natural components of food and feed that can affect quality and physiology[26,27]. There are many foods that contain histamine or cause the body to release histamine when ingested. Histamine intolerance results from a disequilibrium of accumulated histamine and the capacity for histamine degradation. The main enzyme for metabolism of ingested histamine is diamine oxidase (DAO)[28-34]. An impaired histamine degradation based on a reduced DAO activity and the resulting excess of histamine may cause numerous symptoms mimicking an allergic reaction.

Nevertheless, limited information on histamine and heavy metal levels of canned tomato paste marketed in Iran has been reported. Regarding to the high consumption of tomato paste in this study, the levels of lead, cadmium, copper, zinc, tin and iron in fresh tomato and in tomato canned paste as well as the histamine content of tomato paste are reported.

MATERIAL AND METHODS

Study Area

In Iran tomatoes could be cultivated on open farms in the whole of a year because of very different climate exist from north to south of country in the same season, such as, on a winter day in some Northern cities the temperature is by day –5°C and by night –20°C, meantime, in southern cities is +8°C to +25°C. But the winter crop in south of Iran is not suitable to produce paste and peeled tomato because of its poor color, so 95% are consumed fresh, but in summer crop because of ideal climate for tomato cultivation: fully and enough sunny days and large difference between day and night-time temperature tomatoes are produced in good color which results good color quality paste[35]. Tomatoes are cultivated in many parts of Iran, but processing tomatoes are mainly cultivated in 5 areas where the factories mostly are located : Fars province in south 35%, Khorasan

Pr. in north east 20%, Azarbaijan Pr. in north west 18%, Tehran Pr. in North 12%, and Golestan Pr. in north 6% and the rest 9% is cultivated in other provinces.

Fars Province has got a great deal of potentialities in this area of production, being one of the greatest producers of the horticultural products, form the total number of Fars Province gardens, 22 percent is fertile. The acreage totaled 63,391 acres and the rest being 283,623 acres fertile [5]. Agriculture is of great importance in Fars[6]. The other area of tomato and tomato paste production is Kermanshah province. Kermanshah province, with its diverse climate, soil and water resources enjoys higher potential capability for agricultural development[36].

Major cities and towns in Kermanshah Province: Kermanshah; Eslamabad-e Gharb; Paveh; Harsin; Kangavar; Sonqor; Javanrood; Salas-e-babajani; Ravansar ; Dalahoo ; Gilan-Gharb ; Sahneh ; Qasr-e Shirin ; Sarpol-e-Zahab. The province's capital is Kermanshah (WikiMiniAtlas
34°182 N 47°42 Eÿþ / ÿþ34.300°N 47.067°Eÿþ / 34.300; 47.067), located in the middle of the western part of Iran. The population of the city is 822,921. The city is built on the slopes of Mt. Sefid Kooh and extended toward south during last two decades. The built up areas run alongside Sarab River and Valley [37,38,39]. City's elevation average about 1350 meters above sea level. It is the trade center of rich agricultural region that produces grain, rice, vegetable, fruits, and oilseeds.

Sample Collection and Preparation

Due to this descriptive study the effect of processing method; samples were studied in 2 different conditions: fresh and canned paste forms. Fresh Tomato samples were collected during the spring 2014 from five regions of Fars and Kermanshah province farmlands. A total of 480 symptomatic samples were collected from 24 fields located in Fars and Kermanshah Provinces. Samples were taken by hands protected with vinyl gloves and carefully packed in polyethylene bags. The samples were collected during the period when tomato is usually picked. Each sample of tomato fruits were manually collected and kept at +4 °c. Meanwhile 400 canned tomato pastes from 12 popular brands in 3 consequences months were purchased. All canned paste samples had the same date of producing. Samples were randomly purchased for analysis and analyzed according to standardized international protocols by wet digestion method[40,41]. To ensure the uniform distribution of metals in the sample, all materials were milled in a micro-hammer cutter and sieved through a 1.5-mm sieve. Dried and milled samples were powdered and kept in clean polyethylene bottles[42, 43]. All necessary precautions were taken to avoid any possible contamination of the sample as per the AOAC guidelines.

Histamine Determination by Capillary Electrophoresis

All chemicals were of analytical reagent grade. Deionized water was used throughout. The following compounds were used: 0.1 mol/l HCl; 1.0 mol/l NaOH; 1.2 mol/l H_3PO_4 (dilute 12.2 ml of 85% acid in a 100ml flask); 1% solution of orthophtalate aldehyde; 1 mg/ml histamine solution (dissolve 167.4 mg of histamine hydrochloride and add 0.1 mol/l HCl in 100ml flask). The capillary electrophoresis instrument was programmed to run a voltage gra-dient of 5–30 kV over 13 min, with replenishment of the sodium citrate (20 mM, pH 2.5) buffer after every injection. The following rinses were used after each sample: water 1 min, aqueous NaOH (0.1M for paste extracts, 0.5M for fruit ex-tracts) 1 min, water 1 min, running buffer 1 min. Detection was at 212 nm and the operating temperature was set to 35°C. Samples and standards were injected hydrodynamically (50 mbar for 10 s)[44, 45].

Quantitative determination of Heavy Metals

For heavy metal analyses approximately 20.0 g of each sample (fresh tomato and canned paste tomato) accurately weighed and digested in accordance with U.S. Analar grade nitric acid, hydrogen peroxide (about 30%) , Sulfuric Acid (about 98%) and concentrated per chloric acid(37%) [3:1:2:1] were used for the digestion. Application of concentrated HNO_3 along with thirty percent hydrogen peroxide H_2O_2 (Merck) for mineralization of samples to the complete digestion of samples[11,46] following Environmental Protection Agency (EPA) Method 3052 was done.

All glassware and plastic containers used were washed with liquid soap, rinsed with water, soaked in 10% volume/volume nitric acid at least overnight, and rinsed abundantly in deionized water and dried in such a manner to ensure that any contamination does not occur. Five-point calibration curves (five standards and one blank) were constructed for each analyte. The calibration curve correlation coefficient was examined to ensure an $r^2 \geq 0.998$ before the start of the sample analysis. The digested samples were diluted with 10% HNO3 and brought up to 50 mL and analyzed by a graphite furnace atomic absorption spectrophotometry, (GFAAS). The measurements were performed using a PerkinElmer PinAAcle 900T atomic absorption (AA) spectrophotometer and using at least five standard solutions for each metal. All necessary precautions were taken to avoid any possible contamination of the sample as per the AOAC guidelines[10,11,39,46] certified standard reference material (Alpha – Line, Chem Tech Analytical, England) was used to ensure accuracy, and the analytical values were within the range of certified values. All recoveries of the metals studied were greater than 95%.

Statistical Method

State differences on the basis of the states: fresh tomato and canned paste samples were determined by student t-test. The changes were calculated by one way Anova and for analysis of the role of multiple factors univariate analysis was used by SPSS 17.Probability values of <0.05 were considered significant.

RESULTS AND DISCUSSION

Results were determined as mean ± SD of dry weight from three replicates in each test. The samples were analyzed by wet digestion method and standardized international protocols were followed for the preparation of material and analysis of heavy metals contents and analyzed by Atomic Absorption Spectrophotometer in Research Laboratory in Pharmaceutical Sciences Branch, Islamic Azad University. Results obtained using AAS technique for determination of heavy metals in canned tomato paste and in fresh tomato grown in farmlands. The mean values of Cd, Cu, Fe, Sn, Pb, Zn and Histamine concentrations in canned paste tomato samples studied are given in Table 1. In table 1, obtained results show that, the highest concentration in canned food samples was for tin, 357.88±14.33 and lowest 26.54± 5.73 (mg/kg DW). According to variance analyses of data, heavy metal concentration and histamine in canned food samples were significantly affected by company factory.

There was a positive correlation between the food industries machinery manufactured and Food processing and packaging machinery to the food industry and production lines as the new industry founded by new machinery mostly has less heavy metal contents in canned samples ($p \leq 0.05$). There was a positive correlation between the storage time and heavy metal contents especially tin, zinc and iron and they were varied significantly ($p \leq 0.003$) and after 6 and 12 months of storage the heavy metal contents were much higher in the same brand in comparison by the newer samples. The heavy metal contents in comparison by maximum limit set by WHO were indicated in figure 2.

The tin content in mostly canned samples were lower than maximum level set by WHO and only 8% of samples has tin content above this limitation which all of them had acidic pH (pHd" 4.1). In figure 3, obtained results show that, all mean tin and histamine concentrations in tomato canned paste were lower than maximum levels set by WHO in canned food. The sources of Cd in industrial activities are mining, ore dressing, and smelting of nonferrous metals, Cd compound

Table 1. Concentrations of histamine and heavy metals (mg/kg ± SD) in canned Tomato paste samples. Data represent the mean of three replicates

	Minimum	Maximum	Mean
Histamine	3.3± 0.6	211.2± 15.4	36.73± 4.8
Lead	0.011± 0.009	0.5678±0.12	0.0932±0.11
cadmium	0.0032±0.002	0.0876±0.09	0.0443±0.054
Tin	26.54± 5.73	357.88±14.33	120.011±42.77
Zinc	0.1111±0.056	38.7106±5.78	17.3112±1.42
Copper	2.444±0.92	10.9932±2.37	5.7761±0.98
Iron	0.0150±0.005	18.2222±3.50	7.0529±1.03

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

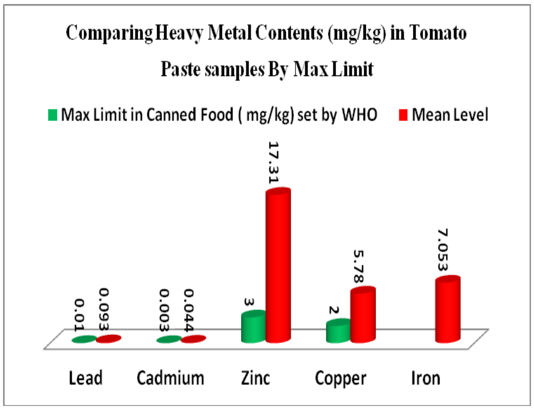

Fig. 2. The mean level of Pb, Cd, Zn , Cu and Fe content (mg/kg DW) in tomato canned paste and comparing these concentrations by maximum limit in canned Food set by WHO[47,48].

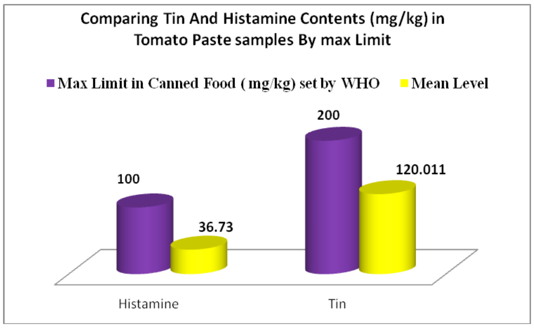

Fig. 3. The mean level of Histamine and Sn content (mg/kg DW) in tomato canned paste and comparing these concentrations by maximum limit in canned Food set by WHO and AOAC[41,42]

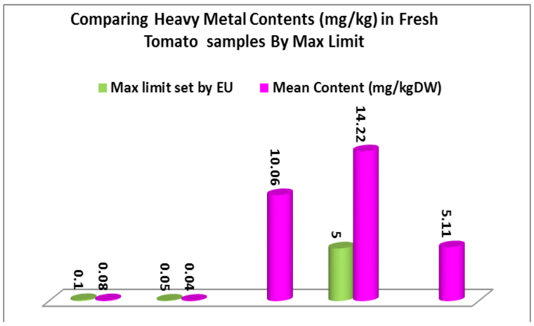

Fig. 4. The mean level of Pb, Cd, Zn , Cu and Fe content (mg/kg DW) in fresh tomato samples and comparing these concentrations by maximum limit in vegetables set by European Commission[51]

production, and battery manufacturing industry and electroplating[49,50].

According to EU regulation[51] , maximum allowed content of some heavy metals per kilo of fresh vegetables and in comparison by heavy metal contents in fresh tomato samples picked up from Kermanshah , Fars ,Khorasan and Tehran farmlands at the same time of studying canned tomato paste samples, results are showed in Fig. 4.

CONCLUSION

To estimate dietary exposure to a substance migrating from the food packaging material, information is needed on the nature of the packaging material, migration data, packaging usage factors and food consumption migration testing using food stimulants is the normal procedure for checking compliance of a food packaging material against specific migration limits (SMLs). The identities of the migrants should be well known although it may be difficult to analyze them. Therefore, the use of mathematical modeling to predict migration, which can reduce the amount of tests must be undertaken and would be introduced into legislation. Based on above issues, the following can be concluded that monitoring of presence of heavy metals in soil and in other fresh vegetables and crops is really important. Adding pesticide and protection of vegetables and crops has to be performed properly.

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