Study and Characterization of nitrate and nitrite content in canned tomato paste on Al Zawia City market - Libyan

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الملخص:

أجريت هذه الدراسة لقياس محتويات النترات والنتريت في معجون الطماطم المعلب الذي يعد عنصراً أساسياً في الوجبة الغذائية، ولقد تم جمع 57 عينة عشوائية من معجون الطماطم المعلب المستورد لعدد 19 منتج، والمتوفرة تجارياً في الأسواق المحلية بمدينة الزاوية – ليبيا، خلال مايو 2023 لتقييم محتوى النترات والنتريت، وتم تحديد تركيز النتريت بالطرق الطيفية عند الطول الموجي 538 نانومتر، وتم تحديد تركيز النترات بعد اختزالها إلى نتريت بواسطة الكادميوم.

أظهرت النتائج، أن تركيز النترات في عينات الطماطم تتراوح ما بين – 2.50±0.04 102.00±2.33 مجم/كجم، بمتوسط 26.83 مجم/كجم، وكان أعلى تركيز في معجون الطماطم (N) 102±2.33 مجم/كجم.

أما بالنسبة لتركيز النتريت تراوح بين 0.18±0.03 – 10.00±0.43 مجم/كجم، بمتوسط 3.32 مجم/كجم، وكان أعلى تركيز في العينة (L) 10±0.43 مجم/كجم.

وفقاً للنتائج يعتبر تركيز النتريت اقل بكثير من النترات، وكان متوسط تركيز النترات في جميع العينات ضمن الحدود المقبولة دولياً ولم تتجاوز المستوى الذي حددته منظمة الصحة

العالمية، أما بالنسبة لتركيز النتريت كان مرتفع نسبياً، حيث وجد أن 68.42% من العينات تحتوي على أعلى من الحد المسموح به. وبما أن النتريت قد يكون لها عامل خطر على الصحة، فيجب مراقبة المنتج بشكل صحيح أثناء الزراعة وخلال عملية التعليب والتخزين.

Abstract:

This study was conducted to measure the contents of nitrates and nitrites in canned tomato paste, which is an essential component of the diet 57 random samples were collected from imported canned tomato paste from 19 products, which are commercially available in the local markets in the Zawia city – Libya during May 2023 to evaluate the content of nitrates and nitrites. The concentration of nitrite was determined by Spectroscopic methods at wavelength of 538 nm, and the concentration nitrate was determined after it was reduced to nitrite by cadmium.

The results showed that the concentration of nitrates in the tomato samples ranged between 2.50±0.04 - 102.00±2.33 mg/kg, with an average of 26.83 mg/kg, and the highest concentration in the samples (N) was 102 ±2.33mg/kg. as for the concentration of the nitrite, it ranged between 0. 18±0.03 - 10.00± 0.43 with an average of 3.32mg/kg, and highest concentration in tomato paste samples (L)was 10 ± 0.43.

According to the results, the concentration of nitrite is considered much lower than that of nitrate. The average concentration of nitrate in all samples was within internationally accepted limits and did not exceed the level set by the World Health Organization. As for the concentration of nitrite ,it was relatively high, as it was found that 68.42% of the samples contained higher than the permissible limit. Since nitrites may be a health risk factor, the product must be properly monitored during cultivation and during the canning and storage process.

Key words: tomato paste - Nitrate - Nitrite - concentration

Study and Characterization of nitrate and nitrite

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Introduction:-

Nitrate (NO_3^-) and nitrite (NO_2^-)are polyatomic ion that are naturally preset in plant food and vegetables and are also used as additives in processed food. The main sources of exposure to external nitrates are vegetables and drinking water (1). The concentration of nitrates in vegetables can vary greatly, Sometimes reaching 3-4 g/kg of fresh weight, and these levels can have potential health implications (2) mainly due to the potential reduced of nitrate into nitrite, which is known to cause harmful effects on human and animal health (3). Therefore quality control of plant products must be strengthened. Tomatoes are a vegetable that plays a major role in the human diet, and its products are a major source of Vitamin C, Potassium, and the antioxidants, and attracted great attention due to evidence indicating that this compound may provide protection against cancer. And other diseases (4)degenerative disease, and in systems mammals Nitrate is converted to nitrite by the action of bacterial enzyme, and nitrite then reacts with Amines, Amides and amine acids to form compounds N-nitroso(5) some of them cause cancer, and this increases the risk of stomach and esophageal cancer. Some studies reported evidence of positive relationship between to high nitrate consumption and risk of infection (6)

Currently the concentration of nitrates in vegetables exceeds the permissible limits globally(7) this due to the spraying of pesticides and the rate of use of manufactured fertilizers. This has led to setting limits at the permissible levels based on various standards, including those related to the environment and the amount of nutrients present in the soil (8). Others are related to the plant, such as variety, type, maturity, and temperature.

The importance study:

Nitrates are considered one of the most important pollutants that accumulate mainly due to the excessive use of nitrogen fertilizers, in

addition to the use of these compounds, such as potassium or sodium nitrate salt, to improve the quality of food and protect against pollution and chemical changes that occur. There it is necessary to study pollution in these products that have a negative impact on the consumer.

Objectives of the study:

This study aims to measure the amount of nitrate and nitrite contents of number of imported canned tomato paste samples commercially available in the Libyan market in comparison with international standard specifications, as well as to evaluate the health risks to humans when consuming these products.

Material and the static method:-

Materials:

Disodium tetra borate ($Na_2B_4O_710H_2O$), potassium hexacyano ferrate II [$K_4Fe(CN)_6.3H_2O$], zinc acetate [$Zn(CH_3COO)_2.2H_2O$], sulfanilamide ($C_6H_8N_2O_2S$),N-(1-naphthyl)ethylene diamine dihydrochloride ($C_{10}H_7NHCH_2CH_2NH_2.2HCl$), hydrochloric acid(HCl), zinc, cadmium sulfate octahydrate ($CdSO_4.8H_2O$), ammoniacal buffer solution (P H=9.6), sodium nitrite ($NaNO_2$).

*ammoniacal buffer solution is used to establish and maintain ion activity within narrow range. It is used to establish hydrogen ion activity for calibration of pH meter, in the analytical procedures, it is also used to maintain the stability of various dosage forms.

The method used:

The Molecular absorption method is the selected and use way to determine Nitrate and Nitrite content due to their deformation, speed and accuracy, and high sensitivity, and the device used in this study. DR 3900 spectrophotometer .

The practical experiences of this research were conducted in the laboratory of Al Sadim Company, laboratory Technology in June 2023.

Samples collection:

Fifty seven random samples were collected from nineteen different brand products of canned tomato paste to present the Nitrate and Nitrite content in these samples, and they were coded with the following codes (A to S)where three samples were collected from each product so that it met most of the types available in the local markets, as shown in the table (1).

Table (1)Shows the samples of canned tomato paste used in this study

N^0	Samples Cod	Number of replicates
1	A	3
2	B	3
3	C	3
4	D	3
5	E	3
6	F	3
7	G	3
8	H	3
9	I	3
10	J	3
11	K	3
12	L	3
13	M	3
14	N	3
15	O	3
16	P	3
17	Q	3
18	R	3
19	S	3

Molecular absorption Spectrometry Method:

Method:

- Determination of Nitrite Extraction was done using hot water, and proteins were precipitated using filtering the Zinc acetate and Potassium hexacyano Ferrate II solution of N-(1-

naphthyl)ethylene diamine dihydrochloride and the precipitate, adding Sulfanil amide chloride to the filtrate, and measuring the Spectrophotometer of the red complex obtained in the presence of Nitrite at wave length of 538nm.

- Determination of Nitrate, Extraction of part from the test with hot water, precipitating the proteins were precipitated using filtering the Zinc acetate and Potassium hexacyano Ferrate II by adding a solution of it, filtering the precipitate, then reducing the nitrates to nitrites with metallic cadmium, and after that, N-naphthyl)ethylene diamine dihydrochloride and Sulfanil amide chloride are added. To the filtrate, the spectrum of the red complex obtained in the presence of nitrite was measured at a wavelength of 538nm.

Devices and tools used:

All glassware is washed and rinsed well with distilled water to ensure that it is free of nitrites and nitrates. 50, 200ml volumetric flask, 200ml beakers, selected filter paper, 1,2,3,5,10 ml volumetric pipettes, 25ml conical flask. Water bath, Balance stir bar spectrometer suitable for measurements at wavelength of 538nm and equipped with cells 1 cm thick DR 3900 spectrophometer.

The work method:

First determination of nitrite content.

- Preparation of sample solution and extraction to prepare the filtrate.

Weigh 5 g of the homogenized test sample, transfer the quantitatively weighed sample to one of the beakers, add 5ml of disodium tetra borate solution and about 100ml of hot distilled water 70-80^{0}C, then heat the beaker for 15 minutes in a water bath while shaking from time to time, then successively add 2ml of potassium hexacyano ferrate(II) solution and 2 ml of Zinc acetate solution, shaking after each addition. Transfer quantitatively to a 200ml

volumetric flask, then supplement to the mark with rise water, and stir after then through fluted filter paper and, if necessary, repeat the filtration until a clear liquid is obtained.

Preparation the test solution for measuring nitrite (NO_2^-)

Transfer with a pipette a portion of the filtrate at least 10ml to the volumetric flask with capacity of 50ml, dilute to about 30ml with distill water, and add by pipette 5ml of Sulfanilamide solution, then add 3ml of hydrochloric acid solution and stir. Leave the solution at room temperature away from light. Add 1ml of solution N-(1-naphthyl) ethylene diamine di hydrochloride and stir be carefully and leave the solution 3 minutes in the room temperature away from light. Fill the volumetric flask with distilled water to the mark, stir during 15 minutes, measure the absorbance of the solution using a Spectrometer. Subtract the blank test value and read the mass of nitrite from the calibration graph.

Blank test:

Perform a blank test in the same way but replace the test sample with 10ml of distilled water.

Prepare the calibration curve and prepare the standard calibration chart. Prepare a series of 50ml standard volumetric flask. Place in each flask 0,0.5, 1, 2, 2.5, 3ml of standard sodium nitrite solution, and 30,29.5, 29, 28, 27.5, 27 ml of distilled water respectively. Then follow the same procedures for preparing the test solution for measurement. Draw a graph showing the masses of nitrite in micrograms in titration solutions as dotted letters, and the corresponding values of absorbance measured by the spectrometer as coordinates.

Calculations:

Nitrite content is expressed in milligrams of nitrite ion(NO_2^-) per Kilogram of product.

$$m_1 \times 200/v_1 \times m_0$$

where : m_0: is the mass of the sample in grams.

m_1: is the mass of nitrite ion(NO_2^-) in micrograms present in the portion of filtrate taken(v_1) read from the calibration graph.

V_1:The value in milliliters of the portion of filtrate taken for measurement.

Second: determination of nitrite content

- Preparation of sample solution and extraction to prepare the filtration : to prepare the test sample and extraction to prepare using the same method to nitrite prepare sample.
- Transfer using a pipette a portion of the filtrate 10ml to a 25ml conical flask, which contains about 2 grams of cadmium and 5 ml of buffer solution. Previously , the flask was closed and shaken on the mechanical stirrer for 5 minutes .then filter it through filter paper and collect the filtrate in standard 50ml volumetric flask. Rinse the filter paper several times with small amount s of water, then the collect the rinse water into standard beaker. Replenish with distilled water to the mark, shake, and continue to determine total nitrite in the same way the previous method using 10ml of test solution. Subtract the blank test value and read the total mass of nitrite from the calibration diagram.
- Blank test: perform a blank test in the same way, replacing the test part with 10ml of water.

Calculations:

Nitrite content is expressed in milligrams of nitrite ion(NO_2^-) per Kilogram of product.

$$m_1 \times 200/v_1 \times m_0$$

where : m_0: is the mass of the sample in grams.

m_1: is the mass of nitrite ion(NO_2^-) in micrograms present in the portion of filtrate taken(v_1) read from the calibration graph.

V_1:The value in milliliters of the portion of filtrate taken for measurement.

Second: determination of nitrate content (NO_3^-)

- Preparation of sample solution and extraction to prepare the filtration : to prepare the test sample and extraction to prepare using the same method to nitrite prepare sample.

Transfer using a pipette a portion of the filtrate 10ml to a 25ml conical flask, which contains about 2 grams of cadmium and 5 ml of buffer solution. Previously , the flask was closed and shaken on the mechanical stirrer for 5 minutes .then filter it through filter paper and collect the filtrate in standard 50ml volumetric flask. Rinse the filter paper several times with small amount s of water, then the collect the rinse water into standard beaker. Replenish with distilled water to the mark, shake, and continue to determine total nitrite in the same way the previous method using 10ml of test solution. Subtract the blank test value and read the total mass of nitrite from the calibration graph.

Calculation:

Nitrate content is expressed in milligrams of nitrate ion (NO_3^-) per kilogram of the product in the following format.

$1.348(m_2 \times 1000/ V_3 \times V_2 \times m_0 – m_1 \times 200/ V_1 \times m_0)$

Where:

m_2 is the total mass of nitrite (NO_2^-), present in the volume (V_2) of the solution test taken for spectrophotometry , read from calibration graph.

V_2 is the volume of test solution in milliliters taken for spectrophotometric measurement.

V_3 it is the volume of the fraction of filtrate taken to prepare the test solution in milliliters.

m_0 is the mass of the sample in grams.

m_1 is the mass of nitrite in micrograms present in the portion of the leachate taken (V_1) ,read from calibration graph.

V_1it is the volume in milliliters of portion of filtrate taken to prepare the test solution.

1.348 is the ratio between the relative molecular masses of the nitrate ion (NO_3^-) and the nitrite ion (NO_2^-).

Analysis and discussion:

Table (2)shows the concentration of nitrate and nitrite in tomato paste samples by mg/kg

Samples Cod	Nitrate concentration (NO_3^-) mg/kg	Nitrite concentration (NO_2^-) mg/kg
A	**44.00±2.12**	**0.80±0.06**
B	**3.00±0.02**	**2.73±0.11**
C	**45.00±1.20**	**6.50±0.91**
D	**30.77±1.10**	**3.08±0.65**
E	**57.27±1.11**	**0.27±0.02**
F	**39.00±1.23**	**5.40±0.10**
G	**25.00±1.10**	**7.75±0.43**
H	**34.29±0.91**	**4.07±0.02**
I	**42.50±1.31**	**2.25±0.06**
J	**16.36±0.88**	**7.64±0.22**
K	**18.46±1.07**	**2.77±0.05**
L	**11.00±0.12**	**10.00±0.43**
M	**6.43±0.67**	**2.57±0.04**
N	**102.00±2.33**	**5.10±0.10**
O	**27.86±1.07**	**0.21±1.01**
P	**21.82±1.21**	**1.09±0.04**
Q	**15.00±.98**	**0.30±0.04**
R	**3.53±0.12**	**0.18±0.03**
S	**2.50±0.04**	**0.50±0.02**

*Values are the mean of three replicates ± standard deviation.

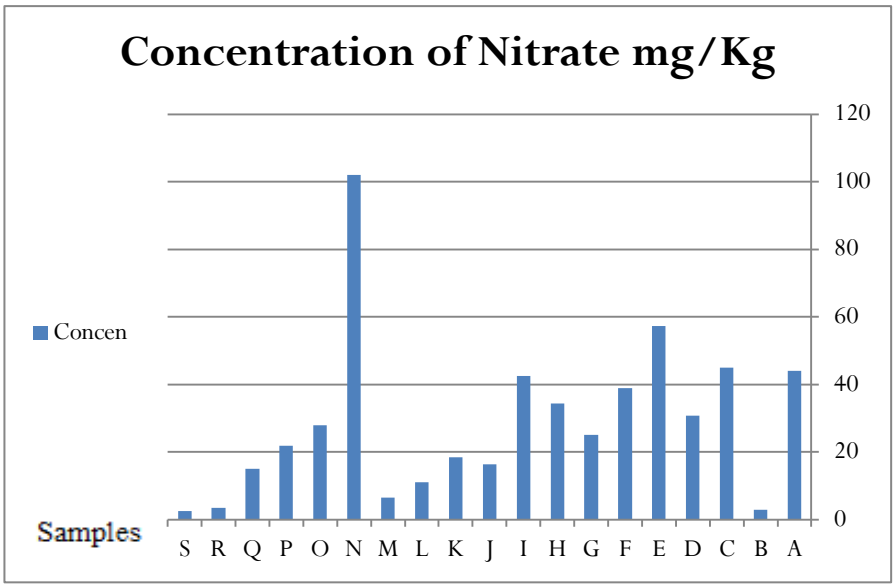

Figure (1)shows the Nitrate Concentration mg/Kg content in the samples

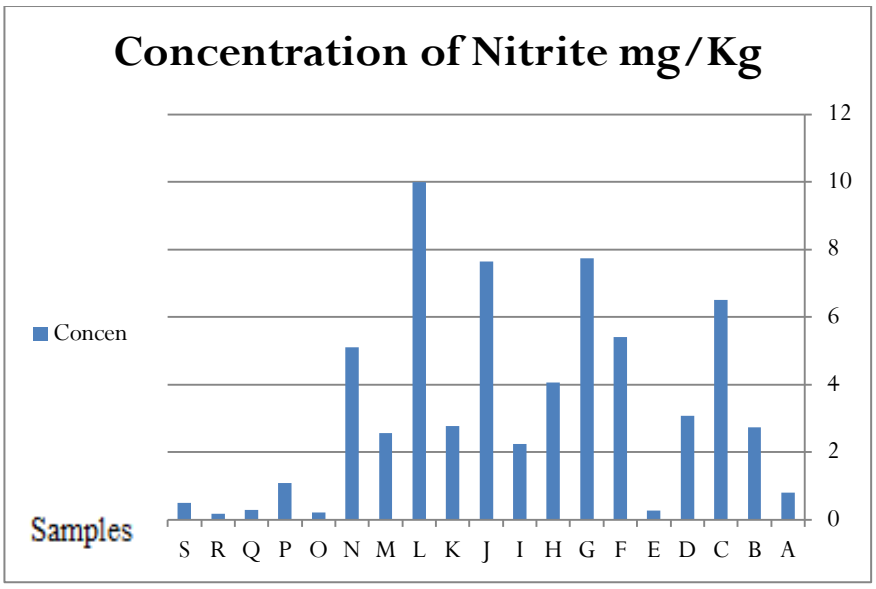

Figure (2)shows the Nitrite Concentration (mg/Kg) content in the samples

The presence of nitrates and nitrites in agricultural products has generated a lot of interest in recent years.

Many factors affect the level of these compounds in plants, such as agricultural practices, herbicide use and environmental and climatic condition.

Chemical and physical processes during canning and strong agricultural products are among the factors that affect the presence of nitrates and nitrites in agricultural products. affect the level of nitrates.

The World Health Organization also set the maximum levels of nitrates in the food products at 300mg/Kg(9). since nitrates and nitrites may be present for various reasons in food of plant origin and accumulate in the plant tissues, and they are substance dangerous to human health, their concentrations were determined .

The results replated to the nitrate and nitrite contents are shown in table (2) and range between 2.500.04 – 202.002.33 mg/Kg, with an average of 26.83 mg/Kg. these values did not exceed the maximum permissible limit according to the World Health Organization. The highest content of nitrates was found in the samples of quality tomatoes(N)102.002.23mg/Kg, and the lowest content was found in the samples (S) 2.50±0.04mg/Kg shown in figure (1) as for the nitrite content in the same of tomatoes, it ranged between– 0.18±0.03 - 10.00±0.43 -mg/Kg, with an average of 3.322g/Kg, and the highest level of nitrite in samples (L) 10.00±0.43mg/Kg, and the lowest level in the samples (R)was 0.18±0.03mg/Kg, shown in the figure (2). The level nitrite must be less than one mg/Kg (10). Thus we note that the concentration of nitrite was relatively high in the samples, it was found that 68.42% of the samples contained higher than one mg/Kg of nitrite but did not exceed 10 mg/Kg.

The nitrite content is generally lower than that of nitrate, and nitrate can be converted to nitrite through Enzymatic reactions or microorganism activity. These reaction take place without control and

therefore, can these reactions occur in food or in the human digestive system, and they occur in the intestines of adults when this happens in the stomach or duodenum of children where nitrite is easily absorbed, therefore nitrates are removed the toxic to infants. Although nitrates are not considered dangerous, they should be always keep in mind that its toxicity may be due to the presence of nitrite. When comparing tomatoes which represent 47.37% of the samples size and are the most abundant in the Libyan market, the nitrate level ranged between– 3.00±0.02 - 57.27±1.11 mg/Kg, with an average of 35.65mg/Kg, and the highest level of nitrate was found. Nitrate tomato sample (E)was 7.75±0.43 mg/Kg, and the lowest level in the samples tomato (B)was 3.00±0.02 mg/Kg. as for the nitrite concentration in these same samples, it range between 0. 27±0.02 - 7.75±0.43mg/Kg with an average of 3.65mg/Kg. the highest level of nitrite was 7.75±0.43 mg/Kg in the tomato samples (G), and the lowest level was 0.27±0.02 mg/Kg samples(E).

As for the samples tomato (M-N-O-P-Q-R) which represent 31.58% the number of samples which are also available in the level of nitrates in the Libyan market ranged between– 3.53±0.12 - 102.00±2.33 mg/Kg. with an average of 29.44mg/Kg. the highest level of nitrates was found in quality tomato samples (N) 102.00±2.33 mg/Kg, and the lowest level in the sample (R) was 3.53±0.12mg/Kg, while the nitrite content in these same samples ranged between 0.18±0.03 - 5.10±0.10 –mg/Kg, with an average of 1.58mg/Kg. the highest level of nitrite was detected in quality samples 5.10±0.10mg/Kg, and the lowest level in the samples (R) 0.18±0.03 mg/Kg.

The tomato samples(J - K) represented 10.52% of the percentage of samples studied, and the average nitrate content in these samples was 17.42 mg/Kg, where the sample (K)contained the highest level of nitrates, 18.46±1.07 mg/Kg, while the lowest level of nitrates was

found. In the sample (J) it was 16.36±0.88 mg/Kg. as for the average content of nitrite in the same samples, it was 5.20mg/Kg. and the highest contain was found in the sample (J) 7.64±0.22 mg/Kg, and the lowest contain was found in the sample (K) 7.64±0.22mg/Kg.

The samples(S - L) each constitute 5.26% of the samples under study, and the average nitrate content 2.50±0.04 mg/Kg and 11.0±0.12 mg/Kg, respectively. And the average Nitrite in the sample (S) was 0.50±0.02, and the sample (L)equal 10.00±0.43mg/Kg. which represents the highest content among all the samples studied. Thus the results indicate the all the canned samples under study contained nitrate that did not exceed the maximum permissible limits according to the World Health Organization, 300mg/Kg, and these results were similar(11). Soon for a study conducted by those who found that The average nitrate contents in the samples was 18.98±0.003 mg/Kg, which ranged between 1.78 – 98.44mg/Kg. as for the nitrite content, which must be less than 1 mg/Kg (10), the results in this study were 31.58% of the samples containing less than 1 mg/Kg of nitrite, while the rest of the samples which represent 68.42% of these samples, were higher than 1 mg/Kg. but not exceeding 10mg/Kg is considered relatively, and the nitrite content in this study was higher compared to a study conducted by where they found that the average nitrite contents 0.12±0.001mg/Kg which ranged between 0 – 0.22 mg/Kg.

Conclusion:

Molecular absorption spectrometry method was used to determine the nitrate and nitrite content in range of tomato paste products spectrophotometric absorption is a highly efficient method they were used to determine nitrate and nitrite in the samples of the most commonly used and sold canned tomatoes Libyan market. The study showed that the nitrate content in the samples ranged between 2.50±0.04 - 102.00±2.33 mg/Kg. with an average of 26.83mg/Kg. and the highest concentration was found in the sample (N)

102.00±2.33mg/Kg, and the lowest concentration was found in the sample (S) 2.50±0.04mg/Kg. as for the nitrite content in the same study it ranged between 0.18±0.03 - 10.00±0.43mg/Kg. with average of 3.32mg/Kg and the highest concentration was found in the sample (L) 10.00±0.43mg/Kg, while lowest concentration was found in the sample (R) 0.18±0.03mg/Kg.

By comparing the results obtained in this study with the specifications of the World Health Organization, which stipulates that the concentration of nitrates in food products must be not exceed 300mg/Kg,(11, 12) the results of all samples under study contained the amount of nitrates conforms to World Health Organization specifications. As for the concentration of nitrite in the same studied samples, which should be less than 1 mg/Kg, were relatively high, it was found that 68.42% of commercially available samples contained nitrite higher than 1 mg/Kg. but did not exceed 10 mg/Kg.

However. Studies conducted on nitrates cannot be ignored, proving that they also have potential beneficial health effects, such as preventing microbial infections and reducing high blood pressure(13) and cardiovascular disease.

Results:

The results of the study to determine the nitrate and nitrite content in different types samples by molecular absorption spectroscopy showed the following results:

- molecular absorption spectroscopy is a highly efficient method that has been used to determine nitrate and nitrite content in various sample of imported in the Libyan market.
- The nitrate content in the samples ranged between 2.50±0.04 - 102.00±2.33 mg/Kg.
- The nitrite content in the samples ranged between 0.18±0.03 - 10.00±0.43 mg/Kg.

- Sample (N)was given the highest value for nitrate content, 102.00±2.33mg/Kg. while the lowest value for nitrate content was for sample(S) 2.50±0.04mg/Kg.
- Sample (L)was given the highest value for nitrite content, 10.00±.43mg/Kg. while the lowest value for nitrite content was for sample(R) 0.18±0.03 mg/Kg.
- The nitrate content in samples of commercially available did not exceed the maximum permissible limit according to the World Health Organization which stipulates that it should not exceed 300mg/Kg**.**
- Nitrite content in the samples was relatively high, which should be less than 1 mg/Kg, as it was found that 68.42% of the studied sample contained nitrites higher than 1 mg/Kg, but did not exceed 10 mg/Kg.

Recommendations:

Food surveillance should be extensive and carried out regularly by relevant authorities, food business owners and food business operators and owners. Food producers and food business owners must monitor the amount to nitrates and nitrites in the food products in order to reduce the risks these food pose to consumers. Continuous health monitoring of canned tomato paste to ensure that the types of tomatoes available in the market contain nitrates and nitrites that do not exceed the permissible limits. It is necessary to pay attention to and study other sources, such as water and processed meat products. Spreading nutritional knowledge about canned food and choosing the best.

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