

## Research Article



## Determination of Nitrate and Nitrite Contents in Pepper (*Capsicum*) and their Derived Products in Syrian Market

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### ABSTRACT

In this study, quantification of both nitrate and nitrite in 120 samples included 70 of green pepper (bell and chili) samples collected randomly from the wholesale market for fruits and vegetables in addition to 50 samples of pepper derived products, included pepper paste, pepper sauce, and dried grinded pepper. Nitrate and nitrite were estimated spectrophotometrically, nitrate was determined after reduction to nitrite with cadmium column. Results showed that the averages amount of nitrate in pepper bell and chili were 54.09 – 38.39 mg/kg respectively, while it was 9.08 mg/kg in pepper paste, the amount of nitrate in pepper sauce was 21.08 mg/kg. The study also showed that the content of nitrate in dried grinded pepper was 655.71 mg/kg. The nitrite averages for pepper fruits (bell and chili), pepper paste, and sauce were very low, under 1mg/kg. The nitrite average for dried grinded pepper was 5.29 mg/kg.

**Keywords:** Nitrate, Nitrite, pepper, pepper derived products, cadmium reduction.

### INTRODUCTION

Because of that the nitrate and nitrite ions have a detrimental adverse effect on human health; increasing interest in the coming years to the content of these two anions in vegetables, continuous monitoring of their content, and trying to decrease their accumulation, has become too persisting. This also applies to the different vegetables derived products. Generally nitrate considered less toxic than nitrite, but transformation of nitrate to nitrite in both saliva and digestive system to nitrite, increases the undesirable effects of these two anions.<sup>1,2</sup>

The toxic effects of nitrite manifested by the possibility of interaction with secondary, tertiary amines, and amides in the stomach, forming N-nitroso compounds which have a carcinogenic potentiality.<sup>1-4</sup> Furthermore, excessive intake of nitrate and nitrite in the diet may cause toxic effects associated with the formation of methaemoglobinaemia (Blue Baby Syndrome) which produces by oxidation of haemoglobin by nitrite. Infants are more susceptible to this syndrome.<sup>1,2,5</sup>

Pepper fruit among the vegetables consumed in largest quantities and containing nitrate with less than 200 mg/kg-1 fresh weight.<sup>3</sup>

Pepper or capsicum is a genus of *Solanaceae* L. family. Its species are native to the Americas and it has been a part of the human diet since about 7500.<sup>6</sup>

Pepper fruit the edible part of pepper – also called pepper, considered as a key element in many cuisines, vegetables food, spices, and even in pharmaceutical and pesticide industries.<sup>6,7</sup>

Peppers varieties can be classified to bell pepper or sweet pepper and chili pepper with piquant (spicy) test due to

the presence of capsaicin (methyl vanillyl nonenamide), especially in the white pith around the seeds.<sup>8</sup>

The accumulation of nitrate and nitrite in vegetables varies according to the harvesting period, the temperature that it is grown at, the sunlight exposure, and the level of natural nitrogen in the soil. Also, whatever the vegetables are, the main role in the nitrate accumulation is the high application of nitrogen fertilizers, from the farmer point of view reducing nitrogen applications, is likely to obtain lower yields. In addition to the problem of irrigation with water contaminated with domestic effluent containing high nitrate concentration.<sup>9,10</sup>

The Syrian green pepper production quantities in 2010 were 76908 tonnes.<sup>11</sup>

Syrians often consume, fresh green pepper next to many other foods, as well as, it constitute one of the major ingredients in salads and cooking, The red pepper is often used in three forms, as, a paste (genitive in many cases with a small amount of olive oil, or other vegetables oil), sauce (which may contain a thickening agent and vinegar) and a grinding red pepper form (paprika) especially the pungent (hot) ones which is very famous in the northern region of the country.

Respecting to chronic effect of nitrate and nitrite, the IARC (2010), stated that, the JECFA (FAO/WHO expert committee on food additives) and SCF (The European Commission's, Scientific Committee on Food) set an ADI (Acceptable Daily Intake) for nitrate ion as 0 - 3.7 mg/kg body weight per day (equivalent to 219 mg/day for a 60 kg person), and the ADI for nitrite as 0 - 0.06 mg/kg of body weight per day (equivalent to 3.6 mg/day for a 60 kg person).<sup>12</sup>



The national academy of sciences (NAS) stated that, vegetables provide 87% of nitrate in a normal diet, while other products including fresh and cured meat, and dairy, and provide the remainder.<sup>13</sup>

Due to the nutritional value and the increasing role of pepper (capsicum) in modern nutrition, this research will focus on the quantitative determination of nitrate and nitrite contents in green pepper fruit and their derived products including red pepper paste, red pepper sauce and dried grinded pepper available in Syrian market.

## MATERIALS AND METHODS

### Samples Collection

#### Raw material

A total of seventy green pepper (35 bell pepper and 35 chili pepper) samples were collected from the wholesale fruit and vegetable markets in Damascus - Syria covering the period from July to the end of October, 2012. Samples were transported to the laboratory in cool box, after purchase. Prior to analyses, pepper samples were washed with distilled water for air pollutants removal, and placed on absorbent paper towel (nitrite –nitrate free) then, stalk and core were removed and a sample of 60 g (bulk sample) was produced from the parts fit for consumption.

#### Pepper derived products

All samples were obtained randomly from Damascus local and open markets in Syria, with twenty samples of red pepper paste, nine samples of red pepper sauce, and twenty grinding red pepper. All samples were kept at +4°C before starting the analysis.

### Method of Analysis

#### Determining of the dry matter

Dry matter contents of all samples were determined by oven-drying at 105°C until a constant weight was obtained.<sup>14</sup>

#### Determining of nitrate and nitrite

The extraction and determination procedures were based on ISO 6635.<sup>15</sup> Initial preparation of the sample, passed through six stages, beginning from cutting, blending, weighing, dissolution in warm water, precipitation of the fat and proteins, ending with filtration.

The nitrate in a portion of filtrate was reduced to nitrite by means of metallic cadmium in glass column. For the determination of nitrate in the reducing form (nitrite) and nitrite, sulphanilamide and N-1-naphtyl-ethylenediamide were used as colouring developing reagents. Measurement of colour intensity was made by Spectronic Genesys 2 spectrophotometer at a wavelength of 538 nm, and compared with standard nitrite solutions. The method was continuously tested by standard addition of nitrate and nitrite. Recoveries have been found to be between 98 and 102%. The limit of detection was 0.34 ppm for nitrate and 0.05 ppm for nitrite. Analyses were run in duplicate. All reagents were of analytical grade quality.

#### Statistics

(PASW Statistics 18) one way ANOVA – Duncan test were conducted for statistical comparisons.

## RESULTS AND DISCUSSION

In this study, dry matter, nitrate and nitrite were determined in all samples with a total of hundred twenty samples of green pepper fruit (bell and chili) and its derived products available in Syrian market, including red pepper paste, red pepper sauce and red dried ground pepper.

#### The dry matter content

In order to conduct a comparison between the content of nitrate and nitrite in the studied samples, dry matter content was determined according to the association of office analytical chemists (AOAC, 2000).<sup>15</sup> Results for dry matter content (%) are presented in table 1.

**Table 1:** Dry matter content (%) in pepper and derived products

Sample	Number of samples	Dry matter content %			
		Minimum	Maximum	Mean	Standard Deviation
Pepper Bell	35	4.61	8.79	6.98	0.96
Pepper Chili	35	5.93	20.31	9.96	3.87
Pepper Paste	20	12.19	36.47	28.34	9.09
Pepper Sauce	10	7.49	13.47	12.09	1.76
Dried grinded pepper	20	87.94	93.43	90.67	1.68

Regarding to the previous table, for bell and chili pepper and by manipulating the results statistically, it can be noticed that, the differences between the values were statistically significant at  $p=0.05$ , and this return the retention of water for bell pepper more than that for chili.

For the pepper derived products, the ascending orders of content of solid material were as follows: pepper sauce (12.09%) < pepper paste (28.34%) < dried ground pepper (90.67%).

## The Nitrate and Nitrite content

### The Nitrate content

Results concerning nitrate contents in pepper and its derived products are represented in table 2 and table 3 respectively.

Regarding to the fresh weight basis, the nitrate concentration (mg/kg) in analyzed green bell peppers ranges between 0.56 – 238.80 mg/kg with an average of 54.09 mg/kg while for chili pepper ranges between 5.65 – 201.15 mg/kg with an average of 38.39, statistically, the differences between the values were insignificant at  $p=0.05$ .

Comparing the results obtained with other global researches results regarding the fresh weight basis, revealed, that the existing amounts of nitrate was within satisfactory limits and even lower than those recorded in numbers of international studies.

For instance, in China, the study of the centre for food safety in Hong Kong (2012), shows averages

concentration of 77 mg/kg for bell pepper and 33 mg/kg in chili pepper, while the study of Feng et al (2006) in Beijing, shows averages concentration of 218 mg/kg for bell pepper and 203 mg/kg in chili pepper.<sup>16</sup> In European Countries the averages of nitrate were 108 mg/kg for bell pepper and 67 mg/kg for chili pepper.<sup>1</sup> In Romania, The study of Terbe (2006) represent concentration of nitrate ranged between 21 – 276 mg/kg.<sup>7</sup>

For the derived pepper products, by arranging the results for averages of nitrate content mg/kg on the basis of fresh weight in the ascending order, the results showed the following: pepper paste (9.08) < pepper sauce (21.05) < dried ground pepper (655.71).

Returning to table (3), which demonstrates the results of nitrate mg/kg according to dry matter weight. For bell and chili pepper there were no significant differences between the values at  $p=0.05$ .

For pepper sauce, the analyses for nitrite contents showed a level of variation between 59.25 to 363.54 mg/kg.

**Table 2:** Nitrate content ( $\text{NO}_3^{-1}$  mg/kg FW) in pepper and derived products according to fresh weight (FW).

Sample	Number of samples	Nitrate mg/kg FW			
		Minimum	Maximum	Mean	Standard Deviation
Pepper Bell	35	0.56	238.8	54.09	65.15
Pepper Chili	35	5.65	201.15	38.39	42.01
Pepper Paste	20	0.61	29.86	9.08	9.09
Pepper Sauce	10	7.75	45.66	21.05	10.91
Dried grinded pepper	20	147.29	1589.55	655.71	438.12

**Table 3:** Nitrate content ( $\text{NO}_3^{-1}$  mg/kg DM) in pepper and derived products according to dry matter weight (DW).

Sample	Number of samples	Nitrate mg/kg DW			
		Minimum	Maximum	Mean	Standard Deviation
Pepper Bell	35	6.87	3316.67	828.85	985.54
Pepper Chili	35	38.16	2849.15	453.97	574.45
Pepper Paste	20	2.04	116.14	33.16	36.52
Pepper Sauce	10	59.25	363.54	179.57	94.42
Dried grinded pepper	20	163.75	1756.21	722.24	483.14

**Table 4:** Nitrite content ( $\text{NO}_2^{-1}$  mg/kg FW) in pepper and derived products according to fresh weight (FW)

Sample	Number of samples	Nitrite mg/kg FW			
		Minimum	Maximum	Mean	Standard Deviation
Pepper Bell	35	ND	0.86	0.23	0.25
Pepper Chili	35	ND	1.49	0.28	0.30
Pepper Paste	20	0.08	1.21	0.40	0.40
Pepper Sauce	10	0.06	0.47	0.26	0.11
Dried grinded pepper	20	1.65	14.01	5.29	4.05

ND: Not Detected.

**Table 5:** Nitrite content ( $\text{NO}_2^{-1}$  mg/kg DM) in pepper and derived products according to dry matter weight (DW).

Sample	Number of samples	Nitrite mg/kg DW			
		Minimum	Maximum	Mean	Standard Deviation
Pepper Bell	35	ND	12.5	3.4	3.66
Pepper Chili	35	ND	17.02	3.28	3.66
Pepper Paste	20	0.23	6.31	1.58	1.74
Pepper Sauce	10	0.8	3.75	2.13	0.81
Dried grinded pepper	20	1.86	15.55	5.85	4.49

ND: Not Detected.

In respect of the red pepper, in both form, grinded (average 33.16 mg/kg) and paste (average 722.24 mg/kg) (paste may contain a little amount of olive oil or other vegetable oil), there was statistically significant difference between the values, which is mainly due to the steps for preparations of pepper paste, starting from chopping and in most cases, filtration to get rid of larger magnitude of water and electrolytes including nitrates and nitrite.

#### The Nitrite content

Results for nitrite content are represented in table 4 and table 5 respectively.

Regarding to the fresh weight basis, the nitrite levels in bell and chili pepper were under 1mg/kg except one result for chili pepper (1.49 mg/kg). There were undetected nitrite in thirty four percent of bell pepper studied samples, and seventeen percent of chili pepper. The nitrite content in pepper sauces were under 1mg/kg in all samples studied, while the nitrite content in pepper paste was less than 1 mg/kg except two results with 1.18 and 1.21 mg/kg. For dried grinded pepper, the analyses for nitrite contents reveal a level of variation between 1.65 to 14.01 mg/kg.

Returning back to table 5, which represents the results of nitrite mg/kg according to dry matter weight, the differences between the values for bell and chili pepper were statistically insignificant at  $p=0.05$ .

For the derived pepper products, by arranging the results for averages nitrite content mg/kg on the basis of dry matter weight in ascending order, the results showed the following: pepper paste (1.58) < pepper sauce (2.13) < dried grinded pepper (5.85).

Whatever the nitrite content in pepper and its derivatives, the differences of nitrite content may be due to the unstable nature of nitrite in addition to the fact that normally small amounts of nitrite are present in pepper, and the possibility of forming nitrite by the reduction of nitrate if the peppers are stored incorrectly.

#### CONCLUSION

Although the presence of nitrate and nitrite in peppers relatively lies in low category in the classification of edible vegetables according to nitrate content (not exceeding 250 mg/kg for nitrate and less than 1 mg/kg for nitrite),

the increased consumption of fruits pepper as an essential component of a healthy diet, call for the enhanced monitoring and surveillance of the quality of peppers and their derived products in terms of content of these two anions.

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