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Effect of Plant Powders as Natural Nitrate Source on Reduction of Nitrosamine Compounds in Beef Burgers

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ABSTRACT

Recent epidemiological studies have shown a positive association between cancer incidence and high intake of processed meats**.** N-nitrosamines (NAs) in these products have been suggested as one potential causative factor. Most NAs are classified as probable human carcinogens. Nitrite, a curing agent of meat products and is a precursor of carcinogenic, so one of the main research topics in meat industry is the reduction of chemical contamination with nitrite. This research aimed to evaluate the effect of replacement of artificial nitrite by tomato, red beet and celery powders (at levels of 1.5 and 3%) as natural sources of nitrates into beef burger formula on residual nitrite and NAs concentrations. The results showed that the tested plant powders were much decreased the residual nitrite content in beef burger samples during the frozen storage periods by different rates depending on the type and level of powder added. Where, tomato powder was the most effective in reducing these contents, followed by red beet powder. Also, the results indicated that the compounds of NDMA, NDEA, NPYR, NPIP, NDBA, NDPA and NSAR were detected in all tested samples and ranged from 0.30 to 10.0µg/kg. While, the other nitrosamine compounds such as NPRO, NMOR, NMEA, NMA and NHPRO were not detected. In general, beef burger samples containing plant powders were lower in the total nitrosamine content (0.84 to 3.66 µg/kg) compared with the sample prepared with 150 ppm sodium nitrite (10.0 µg/kg). The most abundant nitrosamine compounds in the burger samples were NDMA (0.17 - 2.71 µg/kg), NDEA (0.05 - 1.78 µg/kg) and NPYR (0.08 - 2.11µg/kg). It could be concluded that the nitrite (precursor of NAs) can be avoid by replace it with natural nitrate/nitrite sources.

Key words: Beef burger, tomato, celery, red beet, residual nitrite, nitrosamines

Introduction

Several epidemiologic studies show strong associations between consumption of processed red meat and many risks (Herrmann *et al.,* 2015) such as pancreatic cancer (Larsson and Wolk, 2012), colorectal cancer (IARC, 2015), cardiovascular diseases and other causes of death (Rohrmann *et al.,* 2013). Among the compounds responsible for these risks in processed meats are N-nitroso compounds and polycyclic aromatic hydrocarbons (Khambete and Kumar, 2014). So, IARC (2015) classified processed meat as carcinogenic to humans (group 1) based on sufficient evidence in humans, that the consumption of processed meat causes colorectal cancer. It's concluded that each 50 gram portion of processed meat eaten daily increases the risk of colorectal cancer by 18%.

Nitrite is one of the additives, which is widely used currently in different meat products along with salt, sugar, ascorbate and polyphosphate (Goswami *et al.,* 2014). It has been used in mainly to maintain their microbial quality, flavor, color and to prevent lipid oxidation (Alahakoon *et al.,* 2015). Numerous studies have questioned the toxicological risks that consumption of nitrates and nitrites pose to human health (Bryan and van Grinsven 2013). The main concern is the reaction of the nitrosating species such as NO^+ and N_2O_3 (formed from residual nitrite) with primary and secondary amines to form N-nitrosamines.

N-nitrosamines are amongst the most potent and versatile carcinogens (Bhangare *et al.,* 2015), which may exhibit potential genotoxicity and increase the risk of gastric and colorectal cancer (Oostindjer *et al.,* 2014). These harmful substances which have been detected in meat products are widely present in meat products and considered to be carcinogens and mutagenic (Zhu *et al.,* 2015). IARC (2010) classified N-nitrosodimethylamine (NDMA) and N-nitrosodiethylamine (NDEA) as most carcinogenic to humans. While N-nitrosodi-n-butylamine (NDBA), N-nitrosopiperidine (NPIP) and N-nitrosopyrrolidine (NPYR) as possibly carcinogenic to humans. It causes conversion of hemoglobin to methmyoglobin which lacks oxygen transporting ability (Bryan and van Grinsven, 2013).

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Since consumer demand for natural meat products has increased due to the concerns of health risk of synthetic additives, the meat industry is currently focusing on the development of nitrite alternatives. Many studies review the potential alternatives to replace nitrite salts that are used completely or partially in the manufacturing of meat products (Alahakoon *et al.,* 2015). Therefore there is need to find alternative natural plant material which provides alternative color, antioxidant and antimicrobial activities since they are non-carcinogenic and reliable hence can substitute or reduce the amount of nitrates and nitrites with minimal or no quality compromise with respect to sensory attributes and shelf-life. The plant ingredients may be used as a natural nitrate/nitrite source. They are chosen for their ability to supply nitrate, but nitrate concentrations vary widely among types of plant parts from 1.0 to 10000 ppm (Correia *et al.,* 2010).

Hernandez-Ramirez *et al.* (2009) reported that the N-nitrosamines formation inhibited by some antioxidants, such as polyphenols and vitamins (C and E). Also, Li *et al.* (2013) found that plant polyphenols decreased total N-nitrosamine formation in dry-cured sausage. Polyphenols are widespread compounds in fruits and vegetables such as apple, tomato, celery, red beet, etc. (Thilakarathna and Rupasinghe, 2013).

Red beet contains high amounts of biologically active substances including betalains and inorganic nitrate (Oksuz *et al.,* 2015), vitamins (A, B and C), fiber and natural dyes. Also, it's rich in polyphenol compounds, which have antioxidant properties (Kavalcova *et al.,* 2015). Beta-cyanin can decrease LDL cholesterol oxidation and is effective on cardiovascular diseases (Wootton-Beard and Ryan, 2011).

Tomatoes are considered healthy foods. It contains other active compounds such as neoxanthin, lutein, α-cryptoxanthin, α-carotene, β-carotene, cyclolycopene and β-carotene 5, 6-epoxide. These components provide synergistic effect against various threats (Perveen *et al.,* 2015). Epidemiological studies have proven the bioactive role and potential disease prevention property of carotenoids and their consumption has been associated with reduced risk of degenerative diseases. Lycopene is reported to have a variety of pharmacological and nutritional effects in animals and humans, on one hand and promising health benefits as antioxidant on the other (Srivastava and Srivastava, 2015).

Celery is a flavour tasty vegetable much appreciated for its nutritive and therapeutic values. It has been used in traditional medicine primarily: tonic, aphrodisiac, antipyretic, antiscorbutic, antiseptic, aperitif, carminative, depurative, emmenagogue, blood regenerator, hypotensive, oxytocic, vermifuge and as a diuretic. Celery is also used for the treatment of high-blood pressure, cirrhosis, hepatitis, cancer, diabetes hyper-glycemia and regulates heart function (Sowbhagya, 2014). The phytochemicals investigation of celery have shown the presence of polyphenols, flavonoids, steroids, tannins, saponins and terpenoids including biomolecules which have hepatoprotective, cytotoxic, estrogenic, antiestrogenic and antioxidant properties (Pandey *et al.,* 2012). The chemical constituents in celery showed the analgesic, anti-inflammatory and antimicrobial effect (Shad *et al.,* 2011 and Din *et al.,* 2015).

Only a few countries have specific regulations for the N-nitrosamines content in food. In the United States the maximum permitted amount of volatile nitrosamines is 10µg/kg in cured meat products (USDA, 2011).

The current study mainly aimed to produce safe meat products free or may as possible, from nitrosamines components, by evaluate the effect of using some dietary plant powders into beef burger formula as natural source of nitrite and nitrate which they responsible on the formation of nitrosamine compounds during grill of beef burger patties. The quality stability of beef burger patties treated by such high nitrate plants was also studied after frozen storage at -18±2°C for 4 months.

Materials and Methods

Materials:

Beef meat and fat:

Imported frozen beef meat and fat tissues (sheep tail) were purchased from the private sector shop in the local market, Nasr City, Cairo, Egypt.

Plant materials:

The red beet roots (*Beta vulgaris*), tomato fruits (*Lycopersicon esculentum*) and celery (*Apium graveolens)* leaves and stalk were purchased from the local market in Cairo, Egypt.

Other ingredients:

Soy flour was purchased from the Food Technology Institute Agriculture Research Center- Giza, Egypt. Sodium chloride, sodium triphosphate and sodium ascorbate were obtained from El-Gomhoria for Chemicals Co., Cairo- Egypt. Egg, fresh onion and spices were purchased from local market at Nasr city, Cairo, Egypt.

Chemicals:

The standards of N-nitrosodiethylamine (NDEA), N-nitrosodimethylamine (NDMA), N-nitrosopyrrolidine (NPYR), N-nitrosopiperidine (NPIP), N-nitrosodibutylamine (NDBA), N-nitrosomethylethylamine (NMEA), N-

nitrosomorpholine (NMOR), N-nitrosoproline (NPRO), N-nitrosodi-n-propylamine (NDPA), N-nitrosomethylaniline (NMA), N-nitrosohydroxyproline (NHPRO), N-nitrososarcosine (NSAR), Ammonium hydroxide, acetonitrile, anhydrous sodium sulfate, sodium chloride, ethanol, methanol, octane, dichloromethane, silica gel, sodium hydroxide, *N*-(1-naphthyl)-ethylenediamine-2-HCl, sulfanilamide, acetic acid, sodium nitrite, sodium nitrate, Ag_2SO_4, sulphuric acid 98%, phenol and Na_2CO_3 were purchased from Sigma-Aldrich (USA).

Methods:

Experimental Treatments:

A- Preparation of red beet roots, tomato and celery powders:

Red beet roots, tomato and celery powders prepared according to the methods described by El-Gharably and Ashoush (2011), Nicholas (2012) and Kooti *et al.* (2015), respectively. The plant powders were sieved using a 60 mesh, packed in polyethylene bags and stored at -18°C until used.

B- Preparation of Beef burger:

The control beef burger patties were prepared according to the formula present in Table (1) as described by Oroszvari *et al.* (2005). The other burger formulations were prepared by adding sodium nitrite (at 150 ppm) and others by partial replacement of burger formula with red beet, tomato and celery powders (at 1.5 and 3%) and also with their mixtures (at 3%). All formulations were aerobically packaged in a foam plate, wrapped with polyethylene film and stored in a freezer at -18±2°C for 4 months. The samples were periodically taken for analysis every month.

Table 1: The beef burger control formula prepared without sodium nitrite or partial replacement by tested plant powders

Ingredients	Formulation (%)
Minced beef meat and fat	62.0
Soybean (rehydrated)	12.0
Fresh ground onion	7.0
Whole eggs	7.0
Ice water	10.0
Sodium chloride	1.37
Spices mixture	0.50
Sodium triphosphate	0.10
Sodium ascorbate	0.03

Analytical Methods:

Determination of nitrite:

Residual nitrite level of tested plant powders and beef burger samples were determined (as mg $NaNo_2$/kg sample) by a spectrophotometer (model: CT2200-s/n: RE1310004-Germany) at 540 nm according to the method described in A.O.A.C. (2000).

Determination of nitrate:

Nitrate content of tested plant powders and beef burger samples were determined by a spectrophotometer (model: CT2200-s/n: RE1310004-Germany) at 407 nm according to the method described by Tanaka *et al.* (1982).

Determination of nitrosamine:

Extraction of N-nitrosamines was performed according to the method described by Al-kaseem *et al.* (2013). Approximately 6 g of grilled burger sample was placed in the Pyrex tube into which 10 mL of sodium hydroxide 1N was poured. The tube was capped tightly and autoclaved at 121˚C for 10 min. After being allowed to stand at room temperature, the autoclaved solution was transferred to 50 mL separatory funnel. The tubes was rinsed twice with 5 mL of ethanol and then 10 mL of dichloromethane, and the rinsing solutions and 10 mL of 10% aqueous sodium chloride were combined with the original extract in the separatory funnel. After being shaken, the dichloromethane layer was collected and the water layer was re-extracted with 10 ml of dichloromethane.

The dichloromethane extracts were combined, dried over anhydrous sodium sulfate and concentrated to approximately 0.5 mL using rotary evaporator concentrator (EVF-530-010K-GallenKamp) and nitrogen gas flow. The concentrate was loaded into a silica gel column (30 cm × 1.5 cm). The column was eluted with 10 ml of dichloromethane. After the addition of 100 µL of octane (to prevent exsiccation of the solvent), the elute was concentrated to 1 mL using rotary evaporator concentrator and nitrogen gas then extracting 3 mL methanol. This

was repeated three times. The combined methanol extracts were concentrated to about 100 µL under a nitrogen stream and analyzed for nitrosamines by HPLC in Agriculture Research Center, Giza, Egypt.

Statistical Analysis:

The obtained results were analyzed using analysis of variance (ANOVA). The least significant difference (LSD) at a significance of probability 5 % to evaluation different beef burger samples as reported by Snedecor and Cochran (1994).

Results and Discussion

Nitrate and nitrite content in the tested plant powders

The nitrite content of tested plant powders was illustrated in Figure (1). It could be observed that differences were found between the tested plant powders. The highest content of nitrite was noticed in red beet powder (12.0 ppm) followed by celery powder (10.82 ppm). These results are in line with the findings of studies by Shah *et al.* (2013) and Raczuk *et al.* (2014) they found that, the nitrite contents of red beet and celery powders were from 2.50 to 15.0 ppm and 10.94 ppm, respectively. While, tomato powder exhibited of the lowest content (1.6 ppm) for nitrite. A similar concentration of nitrites has been found by study of Zamrik (2013) who mentioned that nitrite content was ranged from zero to 27.16 mg/kg in tomatoes.

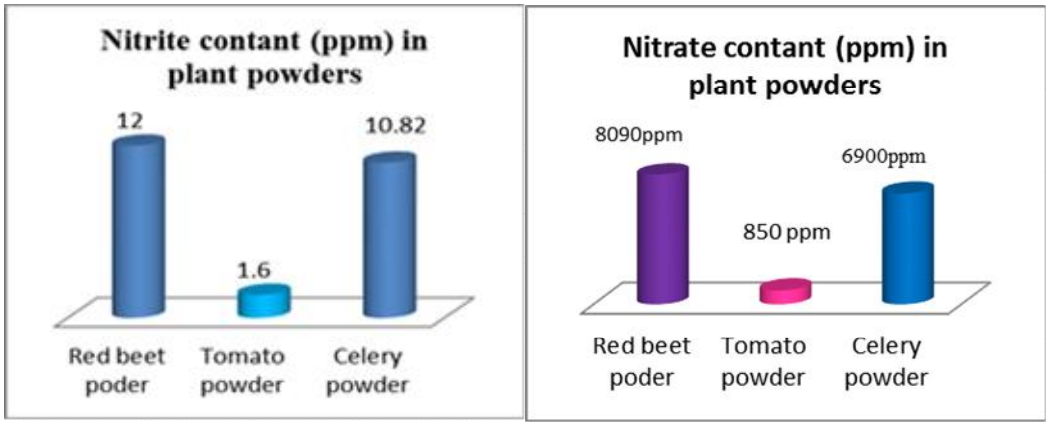

Fig. 1: Nitrite and nitrate concentrations (ppm) of different plant powders.

With regard to nitrate content, it was varied between 850 to 8090 ppm in tested plant powders (Fig 1). These results are in agreement with the findings of a study by Correia *et al.* (2010) they found that the nitrate content in vegetables ranged from 1.0 to 10000 ppm. The highest content of nitrate (8090 ppm) was recorded in red beet powder followed by celery powder (6900 ppm). The present results of nitrate in tested plant powders are in agreement with those reported by Keeton *et al.* (2009) and Raczuk *et al.* (2014), they found that the nitrate contents of beet roots and celery powders were from 480 - 2000 ppm and 391 to 2052 ppm on fresh weight, respectively. While, the lowest content of nitrate was found in tomato powder (850 ppm). These results are go in line with the results obtained by Zamrik (2013) who found that tomato had an average of 11.55−1078.9 mg/kg dry weight of nitrate.

Effect of the different additives on residual nitrite content in beef burgers

Residual nitrite content in the beef burger samples containing sodium nitrite at (150 ppm) or red beet, tomato and celery powders at levels (1.5 and 3%) during frozen storage at -18±2˚C up to fourth month was presented in Table (2). From this Table, it could be noticed that there were significant differences ($P<0.05$) in residual nitrite content between all beef burger samples as affected by different additives at zero time or along frozen storage periods. The differences in residual nitrite content might be due to the type of curing process (nature or synthetic cured and uncured). The obtained results illustrated that residual nitrite content of all tested samples ranged from 10.60 ppm (for control sample) to 82.05 ppm (for sample prepared with 150 ppm sodium nitrite) at the beginning of storage. As noticed by Zsarnoczay (2011), prepared sausages without added nitrite contains nitrite, may be from meat itself and other ingredients used in processing. Also, he indicated that the sodium nitrite content in meat products was significantly decreased starting immediately after addition. Generally, the obtained results are in agreement with those reported by Zahran and Kassem (2011) who found that the raw dry-cured sausages exhibited level of nitrite from 10.45 to 100.36 ppm.

Also, from the same Table it could be noticed that residual nitrite content of beef burgers containing tested plant powders obviously increased ($P<0.05$) with increasing powder levels compared with control beef burger

sample. Hence burger sample contain sodium nitrite (at 150 ppm) had the highest residual nitrite content (82.05 ppm) followed by burger sample contain 3% mixture of powders (25.52 ppm). While, control sample (uncured) showed the lowest residual nitrite content among all other beef burger samples (10.60 ppm).

On the other hand, a gradual decrease (P<0.05) in sodium nitrite content of all beef burger samples was observed during frozen storage up to 4 months. Where reached this content ranged from 0.25 to 6.35 ppm at the end of frozen storage period. These results are in agreement with those obtained by Ahn *et al.* (2002); Fernandez-Lopez *et al.* (2007) and Miller *et al.* (2015) they reported that the nitrite level declines further during frozen storage for cured burgers and sausages.

Table 2: Residual nitrite content (ppm) of different beef burger treatments as affected by different additives and their percentages during frozen storage at - 18±2°C up to 4 months

Storage period (month)	Control	Treatments						
		$NaNo_2$ (150ppm)	Red beet		Tomato		Celery 1.5%	Mix* 3%
			1.5%	3%	1.5%	3%		
0	10.60^{Ga}	82.05^{Aa}	19.79^{Da}	25.50^{Ba}	11.73^{Fa}	12.30^{Ea}	21.81^{Ca}	25.52^{Ba}
1	4.23^{Eb}	52.08^{Ab}	16.42^{Bb}	16.50^{Bb}	7.14^{Db}	10.86^{Cb}	16.57^{Bb}	16.44^{Bb}
2	0.95^{Gc}	24.55^{Ac}	11.68^{Cc}	12.30^{Bc}	5.17^{Fc}	7.63^{Ec}	9.12^{Dc}	11.33^{Cc}
3	0.50^{Ed}	14.50^{Ad}	9.87^{Bd}	8.25^{Cd}	1.03^{Ed}	2.98^{Dd}	8.48^{Cd}	8.23^{Cd}
4	0.25^{Ge}	4.25^{Ce}	1.50^{Ee}	3.20^{De}	0.32^{Ge}	0.87^{Fe}	6.35^{Ae}	5.22^{Be}

Mean values in the same row (as a capital letter) or column (as a small letter) with the same letter are not significantly differences
Mix consists of red beet, tomato and celery with equal weights (1:1:1).*

Based on Egyptian standard specification (ESS) for residual nitrite in meat products, all examined beef burger treatments had acceptable level for residual nitrite (10.60 to 82.05 ppm). Where ESS stated that 100 ppm is the maximum permissible limit for residual nitrite in cured meat products and didn't differentiate between cooked, raw meat products, salted and dry products in their residual nitrite (ESS, 2005).

Effect of the different additives on sodium nitrate content in beef burgers

Sodium nitrate content of different beef burger treatments as affected by the type of additives and their percentages during frozen storage at -18±2˚C up to the fourth month was presented in Table (3). From statistical analysis of these data, it could be observed that, there were significant differences (P<0.05) in sodium nitrate content between all beef burger treatments at either a zero time or along frozen storage periods. The obtained data illustrated that the nitrate content of all beef burger samples ranged between 30.25 ppm and 205.10 ppm at the beginning of storage.

Also, from the same Table it could be noticed that sodium nitrate content of beef burgers containing tested plant powders obviously increased (P<0.05) with increasing powder levels as compared with control beef burger sample. Beef burger treatment which prepared with 3% red beet powder had the highest nitrate content (205.10 ppm) followed by beef burger prepared with 3% mixture of powders (200 ppm). This might be due to the high content of nitrate in red beet (8090 ppm) when compared with other plant powders as shown in Fig (1) similar results were obtained by Oksuz *et al.* (2015). As reported by Sarhan *et al.* (2014) mentioned that red beet had higher nitrate content (1500 mg/kg). While, control sample (uncured) showed the lowest sodium nitrate content among all other samples (30.25 ppm) at zero time.

On the other hand, a gradual decrease (P<0.05) in sodium nitrate content of all beef burger samples was observed during frozen storage up to 4 months, where reached this content ranged from 0.39 to 64.15 ppm at the end of frozen storage period. These results are in agreement with those obtained by Ahn *et al*. (2002) and Honikel (2008) they found that the sodium nitrate of all beef burger sample decreased with extension of storage time. This due to apart of nitrate may be transformed to nitrite during manufacturing in presence of bacteria (Zsarnoczay, 2011).

Table 3: Sodium nitrate content (ppm) of different beef burger treatments affected by different additives and their percentage during frozen storage at - 18±2°C up to 4 months.

Storage period (month)	Control	Treatments						
		$NaNo_2$ (150ppm)	Red beet		Tomato		Celery 1.5%	*Mix 3%
			1.5%	3%	1.5%	3%		
0	30.25^{Ga}	32.10^{Ga}	106.75^{Da}	205.10^{Aa}	49.50^{Fa}	65.00^{Ea}	115.00^{Ca}	200.00^{Ba}
1	18.50^{Fb}	20.70^{Fb}	85.25^{Cb}	177.00^{Ab}	35.40^{Eb}	52.70^{Db}	87.80^{Cb}	163.00^{Bb}
2	10.63^{Hc}	13.97^{Gc}	59.12^{Dc}	135.65^{Ac}	18.95^{Fc}	33.96^{Ec}	62.98^{Cc}	118.60^{Bc}
3	2.56^{Hd}	8.26^{Fd}	36.36^{Dd}	107.88^{Ad}	6.87^{Gd}	20.55^{Ed}	41.35^{Cd}	95.19^{Bd}
4	0.39^{He}	2.85^{Fe}	25.14^{De}	53.75^{Be}	1.96^{Ge}	9.09^{Ee}	29.28^{Ce}	64.15^{Ae}

Mean values in the same row (as a capital letter) or column (as a small letter) with the same letter are not significantly differences
Mix consists of red beet, tomato and celery with equal weights (1:1:1).*

Effect of different additives on nitrosamine contents in beef burgers:

The compounds of NDMA, NDEA, NPYR, NPIP, NDBA, NPRO, NMOR, NMEA, NDPA, NMA, NHPRO and NSAR in grilled beef burger samples were determined. Data presented in Table (4) show the influence of sodium nitrite and tested plant powders on formation of total and carcinogenic nitrosamines in grilled beef burgers. Results showed that, NDMA, NDEA, NPYR, NPIP, NDBA, NDPA and NSAR were detected in tested samples. On the other hand, other N-nitrosamine compounds (NMEA, NHPRO, NMOR, NPRO and NMA) were not found or were under the level of detection. These results are consistent with those studies of Sannino and Bolzoni (2013) and Herrmann *et al.* (2015).

The most abundant nitrosamine compounds in the tested beef burger samples were NDMA, NDEA as probably carcinogenic compounds (ranged from 0.22 to 4.49µg/kg) and NPYR as possibly carcinogenic compound, (ranged from 0.08 to 2.11µg/kg). These results support earlier studies showing that compounds of NDMA, NDEA and NPYR are commonly found in meat products (Yurchenko and Molder, 2007 and Ozel *et al.,* 2010). Also, Campillo *et al.* (2011) found NDMA in meat products with concentrations ranged from 1.7 to 5.7µg/kg. Second abundant of nitrosamine compounds in beef burger treatments were NPIP (ranged from 0.16 to 1.61µg/kg), NDPA (ranged from 0.22 to 0.82µg/kg) and NDBA (ranged from 0.11 to 0.76 µg/kg), it is worth mentioning that these compounds not detected in each of control sample and beef burger treatments prepared with red beetroots and tomato powders at 1.5%. Similar results were obtained by Scheeren *et al.* (2015), who found that the most abundant nitrosamine compounds in cooked sausage and bologna were NDPA and NPIP, which ranged from 1.75 to 34.75µg/kg and from 4.12 to 4.26µg/kg, respectively.

Table 4: Effect of different additives on the formation of nitrosamine levels (µg/kg) for grilled beef burgers

Nitrosamine Components	Control	Treatments						
		$NaNo_2$ (150ppm)	Red beet		Tomato		Celery	Mix
			1.5%	3%	1.5%	3%	1.5%	3%
*Probably carcinogenic components								
N-nitrosodimethylamine (NDMA)	0.17	2.71	0.56	0.97	0.20	0.36	0.63	0.76
N-nitrosodiethylamine (NDEA)	0.05	1.78	0.43	0.65	0.28	0.31	0.52	0.58
**Possibly carcinogenic components								
N-nitrosopyrrolidine (NPYR)	0.08	2.11	0.67	1.26	0.36	0.51	0.81	0.95
N-nitrosopiperidine (NPIP)	ND	1.61	ND	0.20	ND	0.18	0.31	0.16
N-nitrosodibutylamine (NDBA)	ND	0.76	ND	0.15	ND	0.11	0.30	0.21
Total carcinogenic nitrosamines	0.30	8. 97	1.66	3.23	0.84	1.47	2.57	2.66
N-nitrosomethylethylamine (NMEA)	ND	ND	ND	ND	ND	ND	ND	ND
N-nitrosomorpholine (NMOR)	ND	ND	ND	ND	ND	ND	ND	ND
N-nitrosoproline (NPRO)	ND	ND	ND	ND	ND	ND	ND	ND
N-nitrosodi-n-propylamine (NDPA)	ND	0.82	ND	0.38	ND	0.22	ND	0.26
N-nitrosomethylaniline (NMA)	ND	ND	ND	ND	ND	ND	ND	ND
N-nitrosohydroxyproline (NHPRO)	ND	ND	ND	ND	ND	ND	ND	ND
N-nitrososarcosine (NSAR)	ND	0.21	ND	0.05	ND	0.02	0.11	0.08
Total nitrosamines	0.30	10.00	1.66	3.66	0.84	1.71	2.68	3.00

** Components classified as probably carcinogenic to humans according to IARC (Group 2a),*
*** Components classified as possibly carcinogenic to humans according to IARC (Group 2b) ND: Not detected*
Mix consists of red beet, tomato and celery with equal weights (1:1:1).*

Also, from the same Table, total carcinogenic nitrosamines in grilled beef burger samples ranged from 0.30 for control sample (untreated with any additives) to 8.97µg/kg for beef burger containing $NaNo_2$. Nevertheless, total carcinogenic nitrosamines not-exceeded 10 µg/kg, which is the highest level tolerated by some countries in retail food products (USDA, 2011). These results were similar to those obtained by Herrmann *et al.* (2015), found total carcinogenic nitrosamines of various meat products were ranged from 0.05 to 17.10µg/kg.

Furthermore, compounds of NMEA, NMOR, NPRO, NMA and NHPRO not detected in all tested burger samples, while NDPA and NSAR were detected in burger samples treated with sodium nitrite, powders of red beet and tomato, and mixture at 3% level, in addition to the sample treated with 1.5% celery was recorded 0.11 µg/kg of NSAR.

It is worth mentioning that the beef burger samples containing plant powders were lower in the total nitrosamines content (0.84 to 3.66µg/kg) compared with the sample prepared with 150 ppm sodium nitrite (10.0 µg/kg). Also, total nitrosamines content of beef burger treatments increased with increasing level of plant powder from 1.5 to 3.0% in beef burger formula as compared with control sample. The treatment containing tomato powder was the lowest (1.71µg/kg) in the content of nitrosamines followed by sample containing a mixture of powders (3.0µg/kg) then sample prepared with red beet powder (3.66µg/kg) at 3% level. This is due to presence of antioxidant compounds such as polyphenols in plant powders and inhibitor of nitrosamine formation through reduction of nitrate to nitrogen oxide which will not be able to react with amines to form

nitrosamines (Okafor and Nwogbo 2005). These results are agreement with Li *et al.* (2013) they found that plant polyphenols decreased total nitrosamine formation in dry-cured sausage.

Conclusion

Nitrite is an essential component for meat products but because their negative effects it is recommended to reduce as much is possible the residual nitrite from this products. Higher concentrations of sodium nitrite resulted in higher yields of detected N-nitrosamines in grilled beef burgers. In most cases, the effect of sodium nitrite was more significant. However, formation of N-nitrosamines does not seem problematic when the concentration level of the added sodium nitrite is within the legally prescribed limits (150 mg/kg).

Using of some dietary plant powders into beef burger formula proved to be effective sources of nitrite and nitrate for maintenance of low residual levels of nitrite and nitrate compared with the added chemical sodium nitrite. The decrease in residual nitrite level could prevent nitrosamines formation and hence improve safety and quality of meat products. Therefore, it could be concluded that the nitrite can be avoid by replace it with natural nitrate/nitrite sources.

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