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N-nitrosocompounds (NOCs): As chemo-hazards in muscle foods

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ABSTRACT

Nitrite and/or nitrate have been widely used in muscle food processing as a main curing agent, though it can produce several kinds of carcinogenic N-nitrosocompounds (NOCs) reacting with amine components of muscle protein. N-nitrosodimethylamine (NDMA), N- nitrosodiethlamine (NDEA), N-nitrosopyrrolidine (NPYR) and N-nitrosopipperidine (NPIP) are major nitrosamines commonly occur in cured meat products. Additionally, N-Nitrosothiazolidine (NTHZ) and N-nitrosodibenzylamine (NDBzA) could form in smoked and rubber contaminated meat products respectively. The formation of NOCs in muscle foods greatly depend on several factors such as nitrite concentration, processing conditions, ingredients as well as environmental, ecological and biological factors. Excess dietary nitrite intake through muscle food is one of the major sources of endogenous nitrosamines formation in human stomach, which could pose several cancer risks especially gastric and esophageal. However, NOC inhibitors such as organic acids, vitamins, phenolic compounds, nitrate reducing bacteria and Ō-irradiation have been found effective inhibitors to NOCs. This review mainly focuses on the occurrence of NOCs, mechanism, influencing factors, deleterious effects and feasible inhibitors to NOCs in muscle food products.

Key words: Nitrite; Muscle food; N-nitrosocompound (NOC); Cancer risk; NOC inhibitor

INTRODUCTION

Nitrate and /or nitrite have been used for centuries to preserve meat. By the late 1800s, however, the value of saltpeter in meat curing was recognized, and its use in meat was recommended which led to evolve the traditional dry-curing technique in meat industries (NAS, 1982). By virtue of its strong curing effects i.e. formation of cured meat color, flavor, antimicrobial action especially to *Clostridium botulinum* and antioxidant properties, its use in muscle foods processing is still popular in different parts of the world. In contrast, nitrite could produce different nitrosocompounds reacting with amino compounds in muscle foods, which could produce cancer in many animal species (NAS, 1981).Therefore, the use of nitrite and/or nitrate in muscle foods have been regulated and monitored by the US Department of Agriculture (USDA) since the 1900s (NAS, 1981).

N-nitrosamine and N-nitrosamide are two major categories of N-nitrosocompounds based on their chemical structures (Hotchkiss *et al.*,1992).The nitrosamides are direct acting mutagens/ carcinogens but nitrosamines require metabolic activation to exhibit mutagenicity, which is caused by hydroxylation at the free - carbon atom during metabolic bioactivation process (Walker 1990).That is why, the dietary urea and aromatic amines could pose as great a risk as the intake of preformed NDMA in the diet. Nevertheless, carcinogenic potency risk posed by other amines such as primary and secondary were negligibly small (Shephard *et al.*, 1987). N-nitrosodimethylamine (NDMA) and N nitrosodiethlamine (NDEA) are commonly found nitrosamines in foodstuffs, while other NOCs such as N-nitrosopyrrolidine (NPYR), N-nitroso-pipperidine (NPIP), N-Nitrosothiazolidine (NTHZ), N-nitrosodibenzylamine (NDBzA), N-nitrosodiisobutylamine (NDIBA), N-nitrosodibutylamine (NDBA) and N-nitrosomethyl urea (NMU) have been reported in different foodstuffs including meat products, alcoholic beverages (beers), dairy products, fermented vegetable (radish kimchi) and fish or sea food products (Iquierdo-Pulido 1996, Deng *et al.*, 1998, Ku *et al.*, 1999, Lee *et al.*, 2003). These NOCs can be formed either inside stomach after ingestion of nitrites through diets or may directly come through ingestion of food already containing it, thus causing several toxicological implications to human (NAS 1981, Walker 1990).

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2. Mechanisms

The amine compounds such as primary, secondary, tertiary amines and certain primary or secondary amino acids as well as quaternary ammonium salts would be a precursor to form N-nitrosocompounds (Fiddler *et al., 1972a,* Shephard *et al,* 1987).

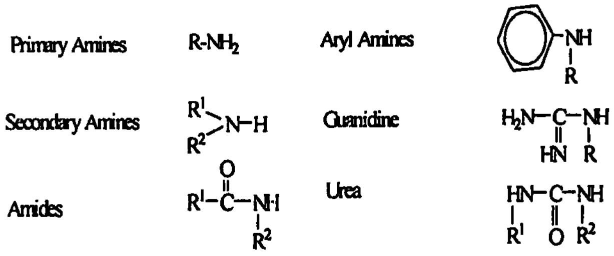

Fig 1- Names and general formula for main amines precursors to NOCs.
Source: Shephard *et al.*, (1987)

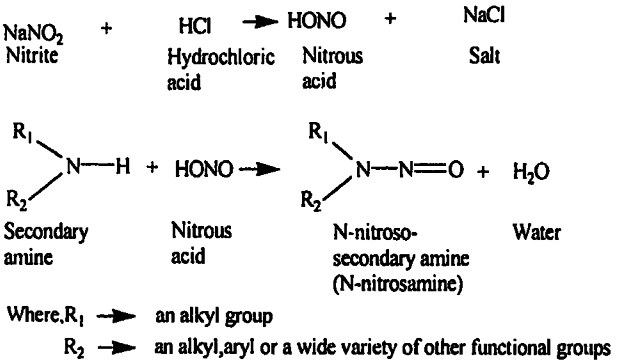

Fig 2- Mechanism of nitrosamine formation in muscle foods

N-nitrosamines are formed by the reaction between secondary amines and nitrous acid from sodium nitrite in acidic condition optimally at around pH 3.5 (Fig 2). However, the rate of nitrosation of secondary amine is greatly influenced by its basicity (Walters, 1985). In a similar way, N-nitrosamides are formed by reaction of nitrous acid with the corresponding amides (Crosby,1983). Since, the rate of formation of N-nitrosamine is proportional to the square of nitrite concentration (Walters,1985), a good correlation between initial nitrite concentrations and formation of nitrosamines in final products was observed by Sen *et al.*,(1974).

3. Occurrence of NOC in muscle foods

N-nitrosodimethylamine (NDMA) is a most commonly reported nitrosamine in muscle foods. Song and Hu (1988) reported the occurrence of NDMA and NDEA up to 7.4ppb and 3ppb in Beijing roast ducks respectively. Besides, other nitrosamines such as NPYR and NPIP have also been reported in muscle foods available in different countries (Table 1).

4. Human exposure to NOC

Human may expose to N-nitrosocompounds by several ways, though exogenous and endogenous exposures are more common. Muscle foods are one of the major sources for different nitrosamines (Table 1).The data from survey reports for different countries showed that the muscle foods had significant contribution to daily intake of NOCs (Table 2).

Since, endogenous nitrosation may occur at the sites other than stomach such as oral cavity, small intestine, bladder and caecum (Gangolli and Phillips,1985) the metabolism and pharmacokinetics of nitrate and nitrite may vary among individuals due to differences in physiology, age group, and general health condition. In person with normal health, the gastric acidity is enough to convert nitrate into nitrite mainly by micro flora in the saliva (NAS, 1981).Therefore, the factors such as gastric pH, gastric nitrite concentration, and in vivo nitrate reductase activity, dietary nitrate, ascorbic acid, and amine/amide precursor ingested may influence the amount of NOCs formed and hence, cancer risk (Hotchkiss *et al.* 1992). Furthermore, the significant positive correlation between the intragastric pH values (pH 4 to 7.5) and total volatile N-nitrosamine content was reported but it was not found at low pH range (pH 1-3) (Dallinga *et al.*, 1998).More recently, Krul *et al.*, (2004) have reported that NDMA was formed when nitrite and dimethylamine or codfish and/or codfish like fish species were introduced into in vitro gastrointestinal model system.

Another possible source of human exposure to N-nitrosocompounds is biogenic amines e.g. putrescine and cadaverine which, can react with nitrite to form carcinogenic nitrosamines i.e. N-nitrosopyrrolidine and N-nitrosopiperidine respectively (Warthesen *et al.*, 1975).

5. Cancer risk, toxicity and safety

NAS (1981) has recommended that most of the N-nitrosocompounds(tested over 300) have been found carcinogenic in one or more species of animals, mutagenic in microbial and mammalian test systems, and some were teratogenic in laboratory animals. Therefore, NOCs are likely to be carcinogenic to human and have been found to be associated with the etiology to different cancer in vivo particularly esophageal, gastric, nasopharyngeal, urinary bladder, liver, colon and brain cancer (NAS 1981, Mirvish 1995, De Stefani *et al.*, 1999, Waddell 2003). NDEA could pose esophageal tumor on rats depending on the dose concentrations; however, the threshold concentration of NDEA for carcinogenicity has been reported as 10^{17} molecules/ kg/ day in rats (Waddell, 2003).In US west coast study, Preston-Martin *et al.*, (1996) reported that an increasing risk of childhood brain tumor was associated with the daily intake of average nitrite from cured meat and frequency of such meat consumption by their mothers during pregnancy. They also postulated that the presence of sodium nitrite and alkyl amide may lead to the endogenous formation of genotoxic and carcinogenic nitrosamines and other N-nitrosocompounds. Mirvish (1995) has also reviewed the association between the consumption of Hotdog (frankfurters) and childhood leukemia and brain cancers which could be induced by the dietary exposure of nitrite to children or of pregnant mothers.

A case-control study among the patients afflicted with cancer of upper aerodigestive tract (UADC) concluded that the cured salted meat was associated with an increased risk of 60% for esophageal cancer in Uruguay (De Stefani *et al.*, 1999).Likewise, positive correlation between gastric cancer and dietary nitrate and/or nitrite concentration has been found, caused by the formation of N-nitrosomethyl urea (NMU) in vivo (Deng *et al.*, 1998). NMU could be formed chemically from nitrite and methyl urea both in vitro(pH 1-3) and in the stomach of rats and

Table 1- Occurrence of N-nitrosamines in muscle foods (μg/kg) in different countries

COUNTRY	NDMA	NDEA	NPYR	NPIP	REFERENCES
China[a]	n.d.to2.3	n.d.to1.2	n.d. to 0.2	No data	Song and Hu 1988
W.Germany[b]	0.22 to 1.01	No data	n.d. to 0.10	n.d.to 0.25	Tricker *et al.* 1991
Japan[c]	n.d.to 6.3	n.d.to 3.9	n.d.	n.d.to29.5	Yamamoto *et al.* 1984
USSR	18.3(Max.)	20.5(Max.)	No data	No data	Tutelyan *et al.* 1990
Thailand[d]	0.2 to 2.1	No data	n.d. to 3.4	n.d. to1.8	Mitacek *et al.* 1999
UK[e]	<2 to 8	No data	<2 to 19	No data	Massey *et al.* 1991
UK[f]	<2 to 5	No data	<2 to 9	No data	Massey *et al.* 1991
Sweden[g]	0.3	No data	0.1	0.04	Österdahl and Alriksson 1990
Sweden[h]	1.2	No data	4.5	0.2	Österdahl and Alriksson 1990
USA[i]	4.0/5.3	No data	17.0/32.0	0.7/1.5	Vecchio *et al.* 1986

Nitrosamines are in range of mean value detected in different kinds of meat products available in Chinese market; [b]Mean content of nitrosamines in different meats and meat products in West Germny; [c]N-nitrosamines in Japanese salami; [d]Mean concentration of nitrosamines in range detected in different type of pork and beef meat products available in Thailand during 1988 to 1996; [e]Data reported in smoked bacon; [f]Data reported in unsmoked bacon; [g,h] Data from microwave cooked and pan cooked Swedish bacons respectively; [i]Average data from rasher(edible fried bacon part)/fried out fat, N-Nitrosothiazolidine (NTHZ) was also detected in average of 8.9/3.4(μg/kg) in the same samples.

.d.- not detected

guinea pigs in vivo (Yamamoto *et al.*, 1987).Therefore, urea and aromatic amines are most potent risk factors for gastric cancer due to endogenous nitrosation as compared to any other nitrogenous compounds where nitrosourea acts as a base analogue to produce DNA lesion, while aromatic amines produces diazo compounds which can react efficiently with cell components (Shephard *et al.*, 1987).However its lethality is directly proportional to the degree of methylation of tissue DNA and protein caused by itself. The transformation of N-nitrosamines through hydroxylation reactions can form active carcinogens such as carbonium ions and diazonium salts which ultimately may induce tumor formation (Crosby 1983, Hotchkiss 1992, Nayak *et al.*,1998).

Nayak *et al.*, (1998) reviewed the acute LD_{50} for several species of animals in the ranges from 25 to 41mg per kg body weight for NDMA and 216mg per kg body weight for NDEA. N-nitrosocompounds are toxic at very high doses and death caused by irreversible liver damage (cirrhosis) (NAS, 1981).

The mandatory standard for N-nitrosamines in muscle foods have not clearly been established yet, though the US Food and Drug Administration (FDA) has established its mandatory limit for bacon as below 5g/kg (Warner *et al.*,1983).Therefore, N-nitrosamines below 5 µ g/kg in muscle foods can be considered as safe to consume.

6. Analytical technique

To determine N-nitrosamines in food products, many kinds of analytical techniques have been developed such as, thin layer chromatography (TLC), gas-liquid chromatography (GLC), UV absorption spectroscopy, gas chromatographic-thermal energy analyzer (GC-TEA), gas chromatographic mass spectrometry (GC-MS), combined HPLC-TEA (Tutelyan *et al.*,1990, Nayak *et al.*, 1998, Lijinsky 1999). However, gas chromatographic-thermal energy analyzer method is most popular and can detect up to 0.1-0.4ppb with validity for 14 different kinds of nitrosamines in cured meat products (Warner 1983).

Firstly, nitrosamines are extracted by different ways such as a mineral oil vacuum distillation(Warner *et al.*, 1983),a solid phase extraction (Pensabene and Fiddler,1994),or steam distillation combined with extraction and concentration(Tutelyan *et al.*, 1990) then finally, determined using a gas chromatographic-thermal energy analyzer detector .Confirmation of nitrosamine peaks is made by GC-MS.

Table 2- N-nitrosamines intake (µg/person/day) in different countries published survey reports

Country	Year	NOC	Sources of N-nitrosamine	Reference
France	1987-1992	0.19	NDMA,12.5% from meat and meat products	Biaudet *et al.* 1994
West Germany (For men)	1989-1990	0.3	Cooked meat and fish products	Tricker *et al.* 1991
West Germany (For women)	1989-1990	0.2	Cooked meat and fish products	Tricker *et al.* 1991
Japan	1982	0.5	88%from fish products, ~11%from Ham and sausages	Yamamoto *et al.* 1984
Netherlands[a]	1984 -1985	= 0.1	Duplicate portions of 24-hours diets.	Ellen *et al.* 1990
UK[b]	1978	0.53	81%from cured meats	Gough *et al.* 1978
USA	1979	0.17	NPYR, bacon	NAS 1981
USA	1984	0.076	NPYR, Bacon	Vecchio *et al.* 1986

[a] 24 hour dietary duplicate portion technique used in the Netherlands on 110 adult volunteers.
[b] cured meats contributes 0.43µ g /person/day

7. Factors influencing NOCs formation in muscle foods

7.1 Environmental and ecological factors

Rywotycki (2003a and 2003b) reported that different concentrations of nitrosamines in raw meat, which greatly depended on different factors like environmental, mode of nutrition, animal species, breeding, seasons etc.The mean content of dimethylonitrosamine (DMNA) in the range of 6.02 to 9.11 µg /kg and diethylonitrosamine-DENA in the range of 6.35to 8.83µg/kg of meat were reported in different wild animals (Rywotycki 2003a).The nitrosamines concentration was found at lowest level in winter season and highest level in autumn. Likewise, pork and beef from non-ecologically bred meats showed the highest level of nitrosamine; while ram, goat and veal meats which were ecologically bred had lowest (Rywotycki 2003b).This fact was also supported by the findings of Shahidi *et al*., (1995) who have reported higher level of NDMA (0.37-0.52µg/kg) produced in chicken salami prepared either with seal meat or seal protein hydrolysate than in only chicken meat (0.33 µg/kg) salami as control.

7.2 Processing time and temperature

The formation of nitrosamines was strongly affected by the frying time and temperature (Österdahl and Alriksson, 1990). Furthermore, they observed microwave cooked bacon contained significantly ($p<0.01$) lower level of volatile N-nitrosamines than bacon fried in pan. This fact was supported by the findings of Shahidi *et al*., (1995), where frying caused highest content 0.79µg/kg of NDMA in chicken salami compared to boiled or micro-waved products that contained upto 0.55and 0.65 µg/kg NDMA respectively. In parallel, indirect heating could produce less amount of NDMA in seafood products (Lee *et al*., 2003).Furthermore, increase in processing time and nitrite concentration both increased the levels of NDMA concentration in frankfurters (Fiddler *et al*.,1972b).

N- Nitrosopyrrolidine (NPYR) is another important NOC, which is frequently reported in fried bacon. Pensabene *et al*., (1974) found no NPYR produced in bacon when fried at 99°C, while maximum level (17µg/kg) was formed at 204°C. Moreover, higher concentration was deposited on the exterior portion of bacon. Nevertheless, integral relationship was found between slice thickness and frying time to form NPYR during frying of bacon (Theiler *et al*., 1981a). Fiddler *et al*., (1974) have described the possible pathways to form nitrosopyrrolidine (NPYR) in fried bacon, either by the action of nitrite on collagen itself or its pyrolytic decomposition during heating; where adipose tissue served as precursor for formation of NPYR.

7.3 Smoking

Although, Kimoto *et al*., (1982) isolated and identified the N- Nitrosothiazolidine (NTHZ) in fried bacon very clearly, the main cause of formation of NTHZ in bacon was smoking process especially wood smoke which was found by Pensabene and Fiddler (1983).Cysteamine was reported as most likely precursor of NTHZ formation in bacon (Pensabene and Fiddler 1985).Likewise, Ikins *et al*.,(1988) have reported that the presence of formaldehyde and acetaldehyde in the liquid smoke could produce N-Nitrosothiazolidine-4-carboxylic acid (NTCA) and Methylnitrosothiazolidine-4-carbocylic acid (MNTCA) in vitro or vivo, which would be the precursors of NTHZ.Similarly, Sen *et al*.,(1986) also showed a positive correlation between the formation of NTHZ from the NTCA and the frying time and temperature.

7.4 Nitrate and/or nitrite concentration

Since ingoing nitrite concentration in muscle foods is a main source of N-nitrosocompounds lowering the nitrite concentration can significantly reduce the level of such NOCs in muscle foods. According to Robach *et al*.,(1980), the reduction of ingoing levels of nitrite from 120ppm to 80ppm or 40ppm along with 0.26% potassium sorbate significantly reduced the levels of NPYR in the fried bacon. Likewise, a highly significant reduction in NDBzA (31%) in ham products prepared from lower concentration of nitrite (100ppm) compared to products prepared from higher concentration (200ppm) was reported by Fiddler *et al*.,(1996).Similarly, a good correlation between the level of NPYR and ingoing concentrations of nitrite in fried bacon was observed by Sen *et al*.,(1974).Nevertheless, occurrence of NDMA in frankfurter failed to show such correlation (Fiddler *et al.*, 1972b).

7.5 Processing and Packaging materials

N-nitrosodi-n-butylamine, N-nitrosodibenzylamine (NDBzA), N-nitrosodiisobutylamine (NDIBA) and N-nitrosodibutylamine (NDBA) are another important group of NOCs, which can be formed when meat and meat products come in contact with rubber or rubber containing materials (Sen *et al.*, 1987, Petersen 1993, Fiddler *et al.*, 1998).The main amine precursor in rubber is dithiocarbamate, which can react with nitrite in meats to produce NDEA and NDBA (Sen *et al.*, 1987).

A survey of boneless hams processed in elastic rubber nettings showed over 100 ppb NDBzA on the surface of bacon (Fiddler *et al.*, 1998) but in Danish cured meat products the occurrence of NDBA was found not more than 0.05ppm (Petersen, 1993).The another factor was netting contact time with meat, which could increase the NDBzA formation in ham products so immediate removal of such netting after processing was suggested (Fiddler *et al.*, 1996). Additionally, the use of natural casing and dry smoking also increased the formation of N-nitrosoproline (NPRO) (precursor of NPYR) as high as 353mg/kg than in use of synthetic casing and liquid smoking (Hamburg and Hamburg, 1992).

7.6 Ingredients

Different kinds of food additives are used in muscle foods either as flavor enhancers or preservatives. However Sen *et al.*, (1973) reported the occurrence of NPYR (up to 105ppb) and NPIP (up to 60ppb) in sausages, which were mainly derived from the reaction of curing mixture contained amine components of black pepper and /or paprika with the nitrite in cured meats. This finding has also been confirmed by Song and Hu (1988) in Chinese sausages. Likewise, Yamamoto *et al.*, (1984) also detected NPIP up to 29.5ng/g in Japanese salami, which were originated by spices and pepper used in formulation.

8. NOC Inhibitors

Since the findings that nitrite could produce NOCs caused by several health implications, had greatly concerned public attention to nitrite cured meats and meat products. That is why the USDA and FDA were involved in this area to assess the public health effects of dietary nitrate and/or nitrite as well as the feasible alternatives to nitrite (NAS 1981). Even though, no single substitute to nitrite has been found, some feasible alternatives to nitrite is summarized in Table 3.

Recently, ã- irradiation has been reported as NOC inhibitor for meat and meat product (Ahn *et al.*, 2002a and 2002b, Jo *et al.*, 2003). In aqueous solvent systems such as water and dichloromethane, the NDMA and NPYR could completely decompose at 5kGy dose of ƒ-irradiation (Ahn *et al.*, 2002b), however at least 20kGy irradiation dose required to reduce the NDMA and NPYR level significantly in either aerobic or vacume packaged sausages stored at 4°C for 4weeks (Ahn *et al.*, 2002b), while only 10kGy ƒ- irradiation was required getting the same effect for CO_2 packaged pork sausages in same storage condition (Jo *et al.*, 2003).

Since the lower pH in muscle products dissipates residual nitrite content, both factors can reduce potential nitrosamine formation (Bacus 1984). Therefore use of microbes in muscle foods processing especially, *Lactobacilli* strains would have beneficial effect for this purpose (Bacus 1984; Xu and Verstraete 2001).Furthermore, strains of *Lactobacillus fermentum* (JCM1173 and IFO3956) were reported in the formation of nitrite cured pigment without addition of nitrite in model or smoked fermented sausages. Therefore, the use of *Lactobacilli* in meat processing seems beneficial to replace nitrite as well as biopreservative action (Møller *et al.*,2003).Likewise, the cooked cured-meat pigment prepared from the reaction of beef red blood cells with a nitrosating agents through a hemin intermediate has already been reported by Shahidi *et al.*, (1985).This kind of pigment can be used in nitrite free curing system, which exhibited no risk of NOC formation in cured meat and fish products (Shahidi and Pegg 1990 and 1994).

9. Conclusions

Although, it has been claimed that significant reduction (appx.80%) of residual nitrite from 1970s in meat and meat products is mainly due to lowered ingoing nitrite concentration, extensive use of nitrosamine inhibitors and technological advancement, the nitrosamine problem is not resolved yet (Cassens, 1997). Direct use of nitrite and /or nitrate in cured meats and meat products is a common source of endogenous nitrosation to form NOCs especially NDMA, however environmental and occupational contamination with NOCs are also considerable factors for human exposure to NOCs. Likewise, some fish species like codfish or codfish like fish species have also been reported to form NDMA in vitro gastrointestinal model, which could pose several toxic and mutagenic affect to humans (Krul, 2004).From the evidence of available data N-nitrosamines have been found to be associated with the etiology of several cancer, e.g. gastric, esophageal, nasopharyngeal, bladder, liver, colon and brain cancer in human and several animal species.

To date, there is no single alternative to nitrite and it is unlikely that an acceptable alternative will be discovered in the near future. However nitrite free curing system has already emerged, which comprises the cumulative effects of several nitrite substitutes such as cured meat pigment, a sequesterant and/or antioxidant and an antimicrobial agent (Table 3) (Shahidi and Pegg 1992). Furthermore, /-irradiation would be another most effective tool to decontaminate

Table 3- Feasible N-nitrosocompound inhibitors in muscle foods

Inhibitor	NOC	Inhibitory action	Reference
Sodium chloride (1.5%)	NPYR	Inhibits about 50% compare to no salt added as control	Theiler *et al.* 1981b
[a]Bovine Serum Albumin (BSA) and Trypsinized Casein	NDMA	50% by BSA and 100% by try-psinized casein in vitro.	Kato and Kikugawa 1992
δ-tocopherol coated salts (500mg/kg)	NDMA, NPYR	39-67% for NDMA and 63 -73% for NPYR in bacon storaged at70°F for 0 to3 month.	Skrypec *et al.* 1985
Fruit juice(Kumquat, sw-eet orange, strawberry , kale 15ml of each)	NDMA, NPYR	10.2 to 45%compare to control	Choi, *et al.* 2002
Green Tea and Maelil extract 15ml of each.	NDMA	45.6 to 48.4% compared to control[b]	Choi, *et al.* 2002
Orange juice ,black tea and ascorbic acid	NDMA	about 60% reduction	Krul *et al* 2004
Pasteurization(Pork)	NDMA NDEA	NDMA and NDEA decreased by 36.1% and 35.7% respectively compare to unpasteauraized	Rkywotycki 2002
Vitamin-E and Selenium	ONOO-[c]	reduce or scavenge ONOO-	Chow, and Hong 2002
Sorbic acid	NDMA	In vitro test	Tanaka et al. 1978
Ascorbate	NTCA	90% in vivo	Ikins *et al.* 1988
Sodium ascorbate (550-ppm) or liquid smoke	NTHZ	Significant reduction of NTHZ	Pensabene and Fiddler 1985
Rubber netting threads reformulated with zinc di-isobutyldithio carbamate	NDIBA	>91% reduction compare to control on the basis of mean content.	Fiddler *et al.*1998
Ascorbyl palmitate, Pep-erazine and Propyl-gallate	NPYR	59 to 87%, 90 to 91%and 50 to 97% respectively	Sen *et al.* 1976

[a] In vitro reaction condition: 200mM-dimethylamine and 50mM-nitrite at pH 3 and 37°C

[b] Distilled water used as control

[c] Nitric Oxide (NO) from nitrate or nitrite can react with super oxide to form highly reactive peroxynitrite (ONOO-) which is responsible to adverse cellular effects.

the level of N-nitrosamines in muscle foods, though the consumer acceptance of such irradiated foodstuffs is still a major problem caused by undesired organoleptichanges during Irradiation (Ahn *et al.*, 2002a).

From data available, muscle foods showed its significant contribution to dietary N-nitrosamines. One of the reasons might be lack of legal regulatory and monitory standard to control such problems in foodstuffs. Therefore, the need of hour is to establish mandatory hygienic standards for NOCs in foods and beverages including muscle foods to control nitrosamine problems.

Moreover, it is hoped that collaborative actions of process modification, extensive use of substitutes and/or inhibitors, mandatory hygienic standards and efforts against the indiscriminate use of pesticides, fertilizer and other environmental pollutants (these substances could also accumulate the nitrate and /or nitrite level in foods) could provide hygienic muscle foods hence pose no further potential health risks to its consumers.

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