



Event of malondialdehyde and N-nitrosamines and their heralds in some Nigerian solidified yogurts, frozen yogurt, meat and fish species

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Abstract

The malondialdehyde (a deleterious by-product of lipid peroxidation) and N-nitrosamines and their precursors (nitrate and nitrite) content of seven (7) and three (3) brands of Nigerian yogurts and ice-creams respectively as well as that of some meat and fish samples were determined using chemical methods. Malondialdehyde and nitrite were estimated spectrophotometrically, nitrate after reduction to nitrite with cadmium column and N-nitrosamines after decomposition to nitrite using U.V irradiation. Malonaldehyde was detected in all the samples of both ice-creams and yogurts and in some meat and fish samples with mean concentration of 1.79 ± 0.13 - 9.11 ± 2.67 $\mu\text{g/ml}$ in the food drinks and 0.51 ± 0.01 - 1.68 ± 0.03 $\mu\text{g/g}$ and N.D - 12.95 ± 1.07 $\mu\text{g/g}$ in fish and meat samples respectively. All the brands of ice-creams and yogurts (except Brand B) analyzed contained detectable amounts of nitrite. The mean values in $\mu\text{g/ml}$ ranged 0.06 ± 0.01 - 0.2 ± 0.01 $\mu\text{g/ml}$ for ice creams and 0.07 ± 0.01 - 0.04 ± 0.02 $\mu\text{g/ml}$ for yogurts. Measurable concentrations of nitrate (0.64 ± 0.04 - 4.91 ± 0.51 $\mu\text{g/ml}$) and N-nitrosamines were also found in some of these food drinks. The toxicological implications of these findings are discussed.

Keywords: Malondialdehyde, nitrate, nitrite, n-nitrosamines, ice creams, yogurts, fish, meat.

INTRODUCTION

Dietary and environmental chemicals such as N- nitro-samines and their precursors (nitrate and nitrite) and malondialdehyde (or malonaldehyde) involved in the etiology of cancer and other related disease conditions are part of the challenges still facing the world today. The occurrence of these chemical carcinogens in our foods and drinks including fish, meat and food drinks as well as "fresh" super market products is well established (Havery and Fazio, 1985; Ostersahl, 1988; Bidlack et al., 1972; Kuusi et al., 1975; Braddock and Petrus, 1971).

There are relevant lipid peroxidation products which include malondialdehyde (MDA) such that increased level of these products can be used to assay the extent of damage incurred by lipid peroxidation (Ichimoto et al., 1995). Interest in the possible significance of malondialdehyde (a by- product of lipid peroxidation) in human health has been stimulated by reports that it is mutagenic (Mukai and Goldstern, 1976) and carcinogenic (Sharmberger et al., 1974). Malondialdehyde can react with deoxy-

guanosine in DNA resulting in the formation cyclic pyrimidopurinone N^1N^2 malondialdehyde-de-oxyguanosine (M_1dG) adduct. The adduct has the potential to cause mutations that may lead to liver car-cinogenesis (Rajinder et al., 2001).

On the other hand, nitrate and nitrite are known to be precursors of toxic and carcinogenic N-nitrosamines (Bas-sir and Maduagwu, 1978) and has been reported to induce cancer in experimental animals (Sen and Baddon, 1997; Mirvish, 1995). Also some urinary bladder and stomach has been associated with N-nitrosamines (Mirvish, 1995) Nitrate and nitrite is present in foods naturally or may be present as a result of use of fertilizers on crops or from their uses as preservatives. Their presence in relatively high concentrations in fruit juices marketed for consumption in Nigeria has been reported (Okafor and] Ogbonna, 2003; Okafor and Nwogbo, 2005). There have been concerns over the presence of nitrate and nitrite in

foods as they can be metabolized to potentially carcinogenic N-nitroso compounds. For this reason, Reports on the Scientific Committee for Food (1997) considered the implications for human health of nitrate and nitrite in foods and has set Acceptable Daily Intake (ADI) for these compounds to be 0.06 mgKg^{-1} body weight for nitrite and 3.7 mgKg^{-1} body weight for nitrate (Reports on Scientific Committee for Food, 1997).

In this present study, a survey was made of the concentrations of malondialdehyde (MDA) and n-nitrosamines precursors (nitrate and nitrite) in some Nigerian ice creams, yogurts, as well as in some fish and meat samples. This study has become necessary following the high consumption rate of both yogurts and ice creams among Nigerian School Children and the concern that these deleterious compounds may be in high concentrations in these products. The way and manner of selling fish and meat could result in significant lipid peroxidation in them. In most cases, meat and fish are sold displayed in open air after slaughter or purchase from cold room.

MATERIALS AND METHODS

Ice creams and yogurts

Samples of seven (7) and three (3) brands of yogurts and ice-creams respectively commonly marketed and consumed in Nigeria were purchased from supermarkets and fast food joints and used in this study. The brands were designated A, B, C, D, E, F, and G for yogurts and X, Y, Z for ice creams. The brands were selected based on their wide distributions in most cities of Nigeria. The samples were analyzed immediately they are brought to the laboratory. The samples were centrifuged or filtered and further clarified using animal charcoal.

Meat and fish samples

Different meat types and species of fish (processed and unprocessed) were purchased from city markets and cold rooms in Aba and Umuahia in Nigeria in the forms being sold to the public. The meat types include beef, goat meat, turkey and chicken that were fresh, frozen or exposed to the air after 6 – 8 h before analysis. Different species of processed and unprocessed fish were also analyzed fresh, frozen and after exposure to air for 6 – 8 h.

Malondialdehyde (MDA) determination

There are several works on assessing the lipid peroxidation index using the indicative level of MDA in plants (Obi and Umeh, 2003; Ichimoto et al., 1995). Malondialdehyde was determined by the modified thiobarbituric acid (TBA) method of Guitteridge and Wilkin (1982). 2 ml of clarified samples was added into a clean test-tube containing 3 ml of glacial acetic acid followed by addition of 3 ml of 1% thiobarbituric acid in 2% NaOH. The mixture was placed in boiling water for 15 min, allowed to cool and the absorbance of the pink colored product was read at 532 nm.

Nitrate, nitrite and N-nitrosamine determination

Nitrite was determined by spectrophotometric method as described by Follet and Ratcliff (1963). Nitrate was determined after reduction to nitrite using cadmium column (Follet and Ratcliff, 1963) while N-

nitrosamines were determined after decomposition to nitrite by U.V irradiation. For color formation, nitrite was reacted with sulphanilic acid and N (1-naphthyl) ethylenediamine hydrochloride. And the purple color developed after 20 min read spectrophotometrically at 520 nm.

Standard curves for malondialdehyde and nitrite were constructed using malondialdehyde derivative 1, 1, 3, 3-tetramethoxy propane (Aldrich Chemical Company) that hydrolyzes under acidic conditions to form free dialdehyde and sodium nitrite respectively.

Statistical analysis

The data represent the mean \pm standard deviation for n number of samples. The mean values of the various brands were compared using the Students't - test for comparing means and the significant level was set at 0.05

RESULTS

The results of the analyses are summarized in Table 1 for ice-creams and yogurts and in Figures 1 and 2 for fish and meat respectively. From Table 1, only one brand did not contain detectable amounts of nitrite (NO_2^-), two did not contain nitrate (NO_3^-) and five did not contain N-nitrosamines, while malonaldehyde was detected in the samples of all the brands. There were marked variations in the concentrations of these chemical carcinogens in these food drinks as shown in Table 1. The highest concentration of MDA was found in ice-cream (brand Z) and the least in brand Y with mean concentrations of 9.11 ± 2.67 and $1.79 \pm 0.13 \mu\text{g/ml}$ respectively.

The mean concentrations of MDA in the yogurts ranged $3.00 \pm 0.44 - 6.05 \pm 0.73 \mu\text{g/ml}$ with the highest occurring in brand F. All the brands of ice creams and yogurts contained more than $1 \mu\text{g/ml}$ of MDA. Handling of products with fatty substances could result in induction of lipid peroxidation and generation of malondialdehyde. On the other hand all the samples has less than $1 \mu\text{g/ml}$ of NO_2^- and only two brands (A and C) contained nitrate level above $2.00 \mu\text{g/ml}$.

All the fresh fish analyzed contained measurable levels of MDA (Figure 1). The highest levels were measured when fresh fish samples were exposed to air for 6 – 8 h, while dried fish samples contained the lowest levels of this compound. All the dried fish samples contained $< 1 \mu\text{g/g}$ while the smoked ones contained $0.98 - 1.55 \mu\text{g/g}$ and the fresh ones $0.5 - 1.54 \mu\text{g/g}$. Among the meat types, frozen chicken contained the highest concentration of MDA ($12.95 \mu\text{g/g}$), followed by fresh turkey and chicken after exposure to air for 6 – 8 h ($5.75 \mu\text{g/g}$) and ($5.56 \mu\text{g/g}$) respectively. Fresh beef contained non-detectable amounts of MDA even after exposure to air for 6 – 8 h (Figure 2).

The different concentrations of this relevant lipid peroxidation product (MDA) in these analyzed samples indicate the different degrees of their deterioration (rancidity). These variations in concentration may be a consequence of differences in the methods of handling, storage condi-

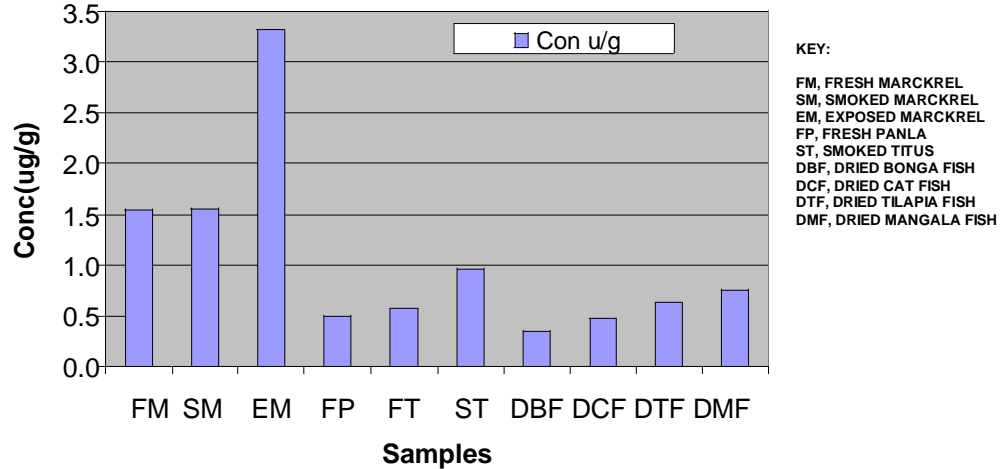


Figure 1. Concentration of malondialdehyde in samples of some Nigerian fish species.

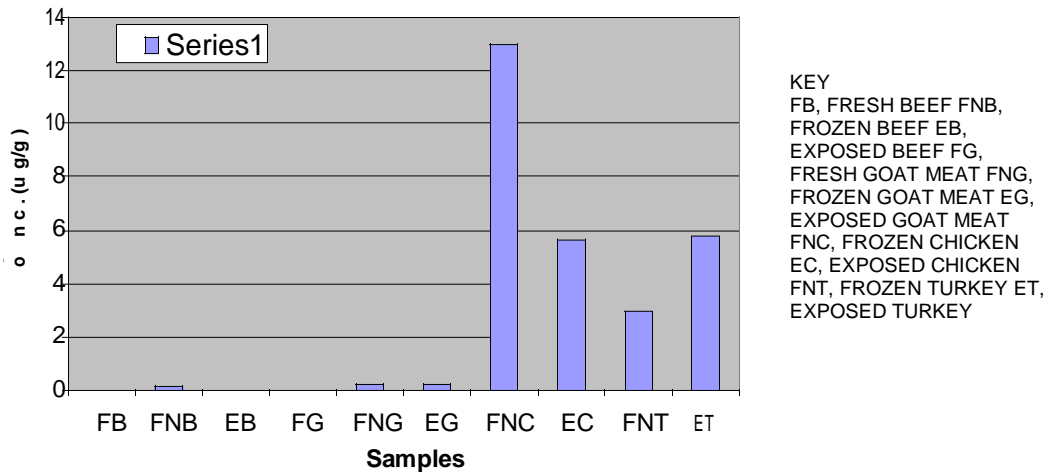


Figure 2. Concentration of malondialdehyde in some Nigerian meats.

Table 1. Concentrations of nitrate, nitrite, N-nitrosamines and malondialdehyde in some Nigerian ice creams and yogurts.

| Samples | Nitrite (µg/ml) | Nitrate (µg/ml) | N-nitrosamine (µg/ml) | Malondialdehyde (MDA) (µg/ml) |
|---------|------------------------|------------------------|------------------------|-------------------------------|
| A | 0.07±0.01 ^a | 4.9±0.51 ^e | ND | 3.90±0.71 ^h |
| B | ND | 0.93±0.02 ^c | 0.13±0.01 [†] | 3.68±0.53 ^h |
| C | 0.21±0.01 ^b | 2.83±0.21 ^d | 0.02±0.00 ^g | 3.58±0.83 ^h |
| D | 0.81±0.03 ^b | 0.40±0.02 ^c | ND | 4.95±0.65 ⁱ |
| E | 0.05±0.01 | 0.78±0.01 ^c | 0.06±0.01 ^g | 4.32±0.37 ^h |
| F | 0.41±0.02 ^b | 0.64±0.04 ^c | 0.12±0.00 [†] | 6.05±0.73 ⁱ |
| G | 0.25±0.02 | 0.91±0.05 ^c | ND | 3.00±0.44 ^h |
| X | 0.06±0.01 ^a | 1.92±0.10 ^a | 0.09±0.02 [†] | 5.16±0.56 ⁱ |
| Y | 0.18±0.02 ^b | ND | ND | 1.79±0.13 ⁱ |
| Z | 0.20±0.01 ^b | ND | ND | 9.11±2.67 ^j |

ND... Non-detectable within the limit of analytical method used. N = 10 where n is the number of samples of each brand analyzed with an average of two determinations. Means with the same superscripts in each column are not statistically significant. A-G: Yogurts samples. X-Z: Ice cream samples.

tions, age and source of the samples.

DISCUSSION

The presence of malondialdehyde (MDA) usually associated with oxidative rancidity (Koning and Silk, 1963), N-nitrosamines and their precursors; nitrate and nitrite in these food drinks, meat and fish types has been demonstrated in this study. The different concentrations of this relevant lipid peroxidation product (that is, MDA) in these products and samples indicate the different degrees of their deterioration (rancidity), since MDA is normally used to assay the extent of damage incurred by lipid peroxidation (Ichimoto et al., 1995; Obi and Umeh, 2003). The presence of MDA in these food drinks, fish and meat is consistent with earlier reports on the occurrence MDA in 'fresh' supermarket products such as orange juice essence (Braddock and Petrus, 1971), meats (Bidlack et al., 1972) and fish (Kuusi et al., 1975).

There were statistically significant differences ($p < 0.05$) in the concentrations of MDA of brand Y (ice-cream) compared to those of brands X and Z (ice-creams) as well as those of yogurts (see Table 1). These difference could be accounted for in part by their storage time. It is important to note that brand Y is a product of a Fast-Food Company found virtually in all the cities of Nigeria and their products don't normally stay for more than 16hrs after production. Samples of the other two brands of ice-creams (X and Z) contained higher concentration of MDA (5.16 ± 0.56 and $9.11 \pm 2.67 \mu\text{g/ml}$ respectively) than the yogurt samples ($3.00 \pm 0.44 - 6.05 \pm 0.73 \mu\text{g/ml}$) although most of the yogurts are stored for longer days under room temperature in supermarkets. These differences in degree of deterioration over time could be attributing partly to the levels of unsaturated fatty acids content of these products. Metabolism of these unsaturated fatty acids leads to generation of MDA, which can be present in high amounts in rancid foods (Yahya et al., 1996). Also handling of products could result in induction of lipid peroxidation and generation of malondialdehyde.

Fresh meat and fish samples yielded lower MDA levels than the frozen samples (Figures 1 and 2) confirming the earlier reports of Kuusi et al. (1975) who reported increases in MDA in Baltic herring during storage at low temperatures.

The samples exposed to air for 6 - 8 h had higher MDA than the fresh ones which is consistent with increased damage by lipid peroxidation as demonstrated by Ichimoto et al. (1995).

Fresh beef contained non-detectable concentration of MDA even after exposure to air for 6 - 8 h, while frozen and exposed chicken and turkey contained higher concentration of this compound (Figure 2). Our result did not agree with the earlier report of Sui and Draper (1978) who reported lower levels of MDA in raw chicken and pork than beef. Non-detection of MDA in the fresh beef in

our result could be explained by the fact that the part analyzed is lean meat and not fat meat. Thus, Nigerian beef could be of health advantage on account of very low content of MDA. Again beef is known to contain a nutrient (carnosine) that is critical to preventing lethal glycation reaction in the body. Glycation is the toxic binding of glucose to the body's protein and is known to alter body protein and renders them non-functional.

Our results from fish analysis (Figure 1) agree with earlier report on the presence of MDA in fish sold in supermarkets (Sui and Draper, 1978). Generally samples exposed to the air for 6 - 8 h had higher concentration of MDA than the dried and smoked ones which indicate increased damage by lipid peroxidation as earlier reported by Ichimoto et al. (1995). From our results fish samples appear more susceptible to lipid peroxidation than the meat samples, although the highest concentration of MDA was recorded in frozen meat.

The health implications of these findings cannot be over emphasized. Although, the Acceptable Daily Intake (ADI) for malondialdehyde has not been set or its concentration that can cause toxicity established, increased levels of lipid peroxidation products have been associated with a variety of chronic diseases in both humans and animal model systems. The potential carcinogenicity of MDA and acetaldehyde (a probable metabolite of malondialdehyde) has been reported in Current Intelligent Bulletin 55 (2005) a publication of National Institute for Occupational Safety and Health (NIOSH). MDA is also known to react readily with several functional groups on molecules including proteins, lipoproteins, and DNA (Yahya et al., 1996) to form a variety of adducts including cross-linked products.

The concentrations of nitrate (NO_3^-) and nitrite (NO_2^-) in these products fall below the WHO (1978) Acceptable Daily Intake (ADIs) which is set at 5.0 and 0.2 mg/Kg body weight. They also fall below the ADIs set European Commission's Scientific Committee for Food (1997); 3.7 mg/kg body weight for nitrate and 0.06 mg/kg body weight for nitrite. The total concentrations of nitrate and nitrite in a total volume pack (500 ml) for the brands with the highest concentrations of these compounds will be 2.46 mg (NO_3^-) and 0.2 mg (NO_2^-). These values are quite below the ADIs. However, these food drinks contribute to the total dietary nitrate and nitrite intakes of many Nigerians along with fruit juices and water (Okafor and Ogbonna 2003; Okafor and Nwogbo, 2005).

Nitrosamines were also detected in some of these products together with nitrate and nitrite. This is in agreement with the reports of some Investigators who have also demonstrated nitrosamine formation in food-nitrite mixture (Saddiqi et al., 1988; Atewodi et al., 1991) However, it is important that that the nature of nitrosamines contained in these drinks be determined using better analytical methods such as thermal energy Analyzer. Also the levels of volatile nitrosamines such as N-nitrosodimethylamine need be quantified. It is only then that

the extent of toxic and carcinogenic potential of the nitrosamines be ascertained.

Studies have shown that children could be more susceptible to the toxic effects of these chemical carcinogens as they have low body weight, immature enzymatic system (especially for xenobiotic metabolism) and low gastric acidity (a good condition for N-nitrosamine formation). Infant illness and death from nitrite induced methemoglobinemia has been reported in places like, Provincial Children Hospital, Krakow, Poland (Lutynski et al., 1996) and South Dakota (Johnson and Kross, 1990).

In this study, we have demonstrated the presence of malondialdehyde, a deleterious by-product of lipid peroxidation, N-nitrosamines and their precursors, nitrate and nitrite in ice creams, yogurts, meats and fish. The results unequivocally show that these products are good sources of toxic and carcinogenic chemicals in some Nigerian dairy and fish products. Thus, there is need to develop plans and practices to reduce the exposure of Nigerians to these compounds to the lowest feasible levels in view of the health implications associated with their consumption.

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