

Nitrosamine Accumulation, Processing Variables, and Indigenous Plant Inhibitors in Nigerian Traditionally Processed Meats

Jimah Abdulrahman^{1,*}, Oregbemhe Henry²

Abstract

N-nitrosamines are classified as probable or possible human carcinogens by the International Agency for Research on Cancer. Carcinogenic N-nitrosamines — principally N-nitrosodimethylamine (NDMA) and N-nitrosodiethylamine (NDEA) — are formed in abundance during the preparation of widely consumed Nigerian traditional processed meats including suya, kilishi, and balangu. This original investigation combined a six-geopolitical-zone Nigerian market survey with laboratory-controlled model system experiments, effects of processing parameters on N-Nitrosamine formation and botanical extract inhibitor screening. Volatile N-nitrosamines were isolated using a modified steam distillation procedure followed by methylene chloride liquid-liquid extraction and quantification was performed by gas chromatography coupled with thermal energy analysis (GC-TEA; Model 502, Thermedics Detection). Market-sampled balangu (Plateau State) and suya (Abuja FCT) yielded NDMA concentrations of 31.2±2.6 and 28.4±2.1 µg/kg, respectively — approximately 10 times the WHO provisional guideline of 3 µg/kg. Also, balangu (Plateau State) and suya (Abuja FCT) yielded NDEA concentrations of 22.4±1.9 and 19.2±1.6 µg/kg, respectively. These two samples showed the highest accumulation of the nitrosamines. Factorial model-system analysis confirmed that NDMA formation is synergistically amplified by acidic pH ($r = -0.91$, $p < 0.001$), elevated temperature, and increasing nitrite concentration ($r = +0.94$, $p < 0.001$), spanning 0.2 to 33.8 µg/kg across conditions. On the Inhibitory Efficacy of Natural Additives, all the six additives produced statistically significant reductions in total N-nitrosamine concentration relative to uninhibited controls at 100 ppm NaNO₂, 150°C, and pH 5.0 ($p < 0.001$). Among six botanical additives evaluated at 500 mg/kg, grape seed extract achieved the greatest inhibition (67.4%), followed by green tea (61.7%), rosemary (55.7%), locally sourced Hibiscus sabdariffa — zobo — (52.5%), turmeric (48.9%), and ascorbic acid (42.6%). Findings support urgent NAFDAC regulatory review of nitrite use levels in traditional Nigerian meat processing, accelerated registration of indigenous polyphenol-based inhibitors, public health messaging targeting smoked fish, suya, kilishi and balangu producers and consumers and to some extent to monitor commercial tinned corned beef and frankfurters; and integration of nitrosamine surveillance into Nigeria's national food monitoring program.

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INTRODUCTION

Nigeria's culinary heritage encompasses an extraordinarily diverse range of meat preservation and processing practices that vary markedly across

its six geopolitical zones. Among the most extensively consumed and culturally significant of these products are suya — a fire-grilled, peanut-spice-marinated beef or ram preparation originating from Northern Nigeria — kilishi, a sun-dried, spiced, and glazed thin-cut beef product produced across Kano, Kaduna, and adjoining states, and balangu, a pit-roasted beef delicacy from Plateau and Nassarawa states (Akwetey & Knipe, 2012; Okonkwo & Oyelaran, 2019) [2, 37]. These products, alongside smoked mackerel, catfish, and tilapia from the Niger Delta, and various fermented preparations like Iru (African locust bean), are consumed daily by millions of Nigerians spanning all socioeconomic strata and represent a growing share of street food commerce in Nigerian urban centers (Obanu & Mpiere, 2014; Ayesha et al., 2023) [35, 3]. The rapid commercial expansion of suya and kilishi into packaged retail and semi-industrial production formats has proceeded largely without systematic evaluation of one of their most significant chemical safety concerns: the formation of genotoxic N-nitrosamines during traditional processing.

N-Nitrosamines constitute a structurally diverse family of organic compounds unified by the N–N=O chemical motif. In meat systems, they arise principally through the reaction of secondary amines — naturally occurring in muscle protein hydrolysates and lipid degradation products — with nitrosating species derived from the protonation and decomposition of nitrous acid (HNO₂), itself generated from sodium nitrite (NaNO₂) under acidic conditions (Scanlan, 1983; Tricker & Kubacki, 1992) [40, 45]. The volatile congeners of greatest public health concern — N-nitrosodimethylamine (NDMA), N-nitrosodiethylamine (NDEA), N-nitrosopiperidine (NPIP), and N-nitrosopyrrolidine (NPYR) — are classified as probable or possible human carcinogens by the International Agency for Research on Cancer (IARC, 2015) [24]. Their primary mechanism of toxicity involves metabolic bioactivation to reactive diazonium and carbonium ions via cytochrome P450 2E1 (CYP2E1)-mediated α -hydroxylation, generating alkylating metabolites that form O⁶-methylguanine adducts in nuclear DNA, driving mutations in codons of proto-oncogenes and tumor suppressor genes implicated in colorectal carcinogenesis (Mirvish, 1995; Demeyer et al., 2016) [33, 15].

Colorectal cancer (CRC) is the third most incident cancer globally, with GLOBOCAN 2020 data reporting approximately 1.93 million new cases and 935,000 deaths worldwide (Sung et al., 2021) [44]. In Nigeria, population-based registry data indicate CRC incidence rates of approximately 4.7–7.2 per 100,000, with rising trends in urban centers attributed partly to dietary westernization and increased consumption of processed meats (Jedy-Agba et al., 2012; Danladi et al., 2019) [25, 12]. Epidemiological evidence firmly links processed red meat consumption to elevated CRC risk: the Chan et al. (2011) [10] meta-analysis of 21 prospective cohort studies estimated a 17% increase in CRC risk per 100 g/day increment in processed meat intake, while the EPIC-Norfolk study by Loh et al. (2011) [29] demonstrated a significant positive association between dietary N-nitroso compound exposure and incident gastrointestinal cancers. However, translation of these findings to the Nigerian epidemiological context has been constrained by the near-complete absence of nationally representative nitrosamine exposure data, particularly for traditional high-temperature preparations like suya and balangu, which are not included in European or North American dietary databases [30–33].

Sodium nitrite is added to cured and processed meats worldwide as a multipurpose technological additive: it inhibits the growth of *Clostridium botulinum*, produces the characteristic pink cured color through nitrosylmyoglobin formation, and retards lipid oxidative rancidity (Sindelar & Milkowski, 2012; Sebranek & Bacus, 2007) [41, 42]. In Nigeria, sodium nitrite use in processed meats is regulated under the National Agency for Food and Drug Administration and Control (NAFDAC) framework, which permits concentrations generally harmonized with Codex Alimentarius General Standard for Food Additives (GSFA) levels of up to 150–200 ppm in cured products (NAFDAC, 2020) [34]. Yet these permitted levels were calibrated against temperate-climate industrial processing conditions — not the high-temperature, open-fire, acidic-marinade environments characteristic of Nigerian suya, balangu, and kilishi production — where nitrosamine formation per unit of added nitrite may be substantially higher.

Antioxidant co-additives — particularly ascorbic acid and polyphenol-rich botanical extracts — have been shown to competitively inhibit nitrosation by scavenging reactive nitrosating species before they can engage secondary amine substrates (Yurchenko & Mölder, 2007; Baka et al., 2022) [49, 4]. Critically, indigenous Nigerian botanical materials represent a largely unexplored resource for this application: *Hibiscus sabdariffa* — whose dried calyces are processed into zobo, among the most widely consumed indigenous beverages in Nigeria — is extraordinarily rich in anthocyanins, hydroxycinnamic acids, and flavonols that function as potent nitrite scavengers (Falade et al., 2005; Obouayeba et al., 2014) [18, 36]. The potential dual utility of this plant — as a culturally familiar ingredient and as a certified food-grade nitrosamine inhibitor — has not previously been evaluated in a standardized comparative model system relevant to Nigerian meat processing conditions.

This investigation was designed to address these knowledge gaps through four interlocking objectives: (i) conducting a representative market survey of NDMA and NDEA concentrations in eight commonly consumed Nigerian traditionally and commercially processed meat and fish products across six geopolitical zones; (ii) systematically characterizing N-nitrosamine formation kinetics across a factorial matrix of sodium nitrite concentrations, pH values, and processing temperatures reflecting the full range of Nigerian traditional curing practice; and (iii) evaluating the comparative inhibitory efficacy of six additives — including *Hibiscus sabdariffa* extract as an indigenous Nigerian botanical — against ascorbic acid.

MATERIALS AND METHODS

Collection of Materials

The representative composite samples ($n = 5$ per product per sampling location) were collected from open-air markets, abattoir-adjacent processing sites, and retail outlets across Abuja (FCT), Kano, Plateau State, Lagos, Port Harcourt (Rivers State), and Auchi (Edo State) between March and September of the survey year. Products surveyed comprised: suya, kilishi, balangu, smoked marine fish (mackerel — *Scomber scombrus* and catfish — *Clarias gariepinus*), commercially tinned corned beef (two leading brands), commercial beef frankfurters (three brands), and Iru fermented locust bean (included as a non-meat reference baseline). Samples were transported in insulated containers at $\leq 4^{\circ}\text{C}$, homogenized under chilled conditions, aliquoted in triplicate, vacuum-sealed, and stored at -20°C until analysis within 72 hours.

Chemicals, Standards, and Plant Extract Characterization

Analytical reference standards of NDMA and NDEA ($\geq 99.5\%$ purity), sodium nitrite (NaNO_2 , analytical grade), L-ascorbic acid, and HPLC-grade methylene chloride and methanol were sourced from Merck KGaA (Darmstadt, Germany) through authorized distributors. Botanical extract powders — green tea extract (*Camellia sinensis*, 95% total polyphenols), rosemary extract (*Rosmarinus officinalis*, 6% carnosic acid), grape seed extract (*Vitis vinifera*, 95% oligomeric proanthocyanidins), turmeric extract (*Curcuma longa*, 95% curcuminoids), and *Hibiscus sabdariffa* extract (22% total anthocyanins, standardized) — were obtained from Sabinsa Corporation, India. Total polyphenol content was independently determined by the Folin–Ciocalteu colorimetric assay (Singleton & Rossi, 1965) [43] and expressed as mg gallic acid equivalents per gram (mg GAE/g). Antioxidant capacity was quantified by DPPH (Brand-Williams et al., 1995) [7] and FRAP (Benzie & Strain, 1996) [5] assays. Extract identity was confirmed by thin-layer chromatography.

Model Meat System and Experimental Design

Ground beef (80% lean) and pork (75% lean) sourced from the Auchi Central Abattoir were employed as the protein matrices for laboratory model systems. A full-factorial experimental design was applied across three independent variables: sodium nitrite concentration (0, 50, 100, 150, 200 ppm), target pH (4.0, 5.0, 6.0, 7.0 — adjusted with lactic acid or sodium bicarbonate), and processing temperature (100, 150, 200°C). Individual botanical extracts and ascorbic acid were incorporated at 500 mg/kg meat. Each 200 g treatment was processed in a thermostatically controlled laboratory oven for 30 minutes at the target temperature, then vacuum-sealed and stored at -20°C until analysis. pH was

verified post-processing using a calibrated pH meter (Mettler-Toledo Seven Compact S220). All treatments were prepared in triplicate ($n = 3$), yielding a stratified subset of 180 samples selected for full GC-TEA analysis from the complete factorial [6].

Nitrosamine Extraction and GC-TEA Quantification

Volatile N-nitrosamines were isolated using a modified steam distillation procedure followed by methylene chloride liquid-liquid extraction, adapted from the protocol of Zhao et al. (2021) [50] and validated for Nigerian meat matrices. Quantification was performed by gas chromatography coupled with thermal energy analysis (GC-TEA; Model 502, Thermedics Detection), providing nitrosamine-class-specific detection. Method validation established: calibration linearity ($R^2 > 0.998$, range 0.5–50 $\mu\text{g}/\text{kg}$); method recovery at three spiking levels of 2, 5, and 10 $\mu\text{g}/\text{kg}$ (mean: 82–96%); limit of detection (LOD = 0.3 $\mu\text{g}/\text{kg}$); limit of quantification (LOQ = 1.0 $\mu\text{g}/\text{kg}$); and inter-day precision (RSD < 9%) for NDMA, NDEA, NPIP, and NPYR. Matrix-matched calibration curves were prepared in nitrite-free Nigerian beef matrix to correct for matrix-induced suppression [7].

Statistical Analysis

Three-way ANOVA was applied to model-system data to assess interactive effects of nitrite concentration, pH, and temperature on nitrosamine formation. Post-hoc multiple comparisons employed the Tukey HSD test. Pearson product-moment correlation examined relationships between pH, TPC, and nitrosamine yield. All values are presented as mean \pm standard deviation of triplicate analyses ($n = 3$). Statistical significance: $\alpha = 0.05$. Software: IBM SPSS Statistics v28 (IBM Corp., Armonk, NY) [8–11].

RESULTS AND DISCUSSION

N-Nitrosamine Burden in Nigerian Market-Sampled Products

The market survey revealed substantially elevated NDMA concentrations within most extensively studied Nigerian processed meat and fish products examined specifically in the Table 1. Concentrations ranged from $0.4 \pm 0.1 \mu\text{g}/\text{kg}$ in unprocessed fresh beef — well within the WHO provisional guideline of 3 $\mu\text{g}/\text{kg}$ — to $31.2 \pm 2.6 \mu\text{g}/\text{kg}$ in Plateau State balangu, representing a 78-fold span across the product spectrum. Suya from Abuja markets yielded $28.4 \pm 2.1 \mu\text{g}/\text{kg}$ NDMA, while kilishi from the Kano–Kaduna corridor averaged $18.6 \pm 1.5 \mu\text{g}/\text{kg}$. Smoked fish from Niger Delta markets registered $22.1 \pm 1.8 \mu\text{g}/\text{kg}$, a result consistent with the recognized compounding effect of polycyclic hydrocarbon and nitrosamine co-generation during wood-smoke processing (De Mey et al., 2017; Joosen et al., 2010) [13, 26]. Iru fermented locust bean, the sole non-meat reference, produced $3.1 \pm 0.3 \mu\text{g}/\text{kg}$ — only marginally exceeding the guideline — reflecting low-level-Nitrosation during alkaline fermentation with naturally occurring amines (Obanu & Mpieri, 2014) [35].

The particularly elevated nitrosamine burden in balangu and suya is mechanistically attributable to the convergence of multiple risk-amplifying process conditions: prolonged direct contact with combustion gases during charcoal or wood-fire roasting, acidic pH values generated by the organic acids in suya marinades and fermentation-derived compounds (4.5–5.2), processing temperatures approaching 200–220°C at the meat surface, and high protein content providing abundant amine precursors (Akwetey & Knipe, 2012; Okonkwo & Oyelaran, 2019; Lijinsky, 1999) [2, 37, 28]. The role of high-temperature pyrolysis in generating reactive nitrogen intermediates from wood smoke — independently of added nitrite — constitutes an additional and rarely regulated nitrosamine formation pathway specific to these traditional preparations and distinct from industrially controlled nitrite-cured products (Zheng & Lee, 2009; De Mey et al., 2017) [51, 13].

Effects of Processing Parameters on N-Nitrosamine Formation

The three-way interaction of nitrite concentration \times pH \times temperature exerted a highly significant and synergistic effect on NDMA and NDEA formation in model meat systems ($F = 247.3$, $p < 0.001$), demonstrating that no single factor can be considered in isolation from the others as shown in Table 2 and Figure 1 [27]. NDMA concentrations spanned a 169-fold dynamic range — from 0.2 $\mu\text{g}/\text{kg}$ in the

nitrite-free control at pH 7.0 and 100°C to 33.8 µg/kg at 200 ppm NaNO₂, pH 4.0, and 200°C — precisely the conditions emulated by high-temperature *suya* and *balangu* preparation with acidic marinades in Figure 2 [14–16].

The strong inverse relationship between pH and total nitrosamine yield (Pearson $r = -0.91$, $p < 0.001$) is governed by the acid-base equilibrium of nitrous acid (HNO₂, $pK_a = 3.35$): at pH values below 5.5, the protonated form predominates, and its facile disproportionation generates dinitrogen trioxide (N₂O₃) — the active electrophilic nitrosating agent that engages secondary meat amines (Tricker & Kubacki, 1992; Zhao et al., 2021) [45, 50]. NDMA concentrations at pH 4.0 were approximately four-fold higher than at pH 7.0 under identical nitrite and temperature conditions, underscoring why *suya* marinades — typically formulated with *dawadawa* (fermented locust bean) and citric acid, generating pH 4.8–5.2 — create conditions of exceptional nitrosamine susceptibility (Ayesha et al., 2023; Okonkwo & Oyelaran, 2019) [3, 37].

Processing temperature exerted a super-proportional amplifying effect on nitrosamine generation: NDMA at 200°C was approximately 2.1-fold higher than at 100°C under equivalent nitrite and pH conditions [38, 39]. This thermally enhanced formation operates through multiple concurrent pathways: Maillard reaction-associated amine generation from reducing sugar-amino acid condensation, secondary amine liberation from lipid peroxidation products, and pyrolytic decomposition of N-nitrosamide precursors (Lijinsky, 1999; Zheng & Lee, 2009) [28, 51]. These findings collectively emphasize that the regulatory ceiling on nitrite addition represents only one dimension of the problem — and that without simultaneous control of processing temperature and product pH, even halving of the permitted nitrite level would be insufficient to bring NDMA below the WHO guideline under *balangu* or *suya* preparation conditions [19].

Comparative Inhibitory Efficacy of Natural Plant Additives

All six additives produced statistically significant reductions in total N-nitrosamine concentration relative to uninhibited controls at 100 ppm NaNO₂, 150°C, and pH 5.0 ($p < 0.001$, Tukey HSD) shown in Table 3 and Figure 3. Grape seed extract — characterized by the highest total polyphenol content (TPC) among the extracts tested (489.2 mg GAE/g) — achieved the greatest inhibition at 67.4%, attributable to the electrophilic aromatic substitution reactions through which its oligomeric proanthocyanidin (OPC) constituents preferentially consume nitrous acid and N₂O₃ ahead of competing with secondary meat amines (Baka et al., 2022) [4]. Green tea extract followed at 61.7%, driven by the well-documented nitrite-scavenging kinetics of its major catechin constituent (–)-epigallocatechin gallate (EGCG), which react 100- to 1,000-fold faster with N₂O₃ than do aliphatic secondary amines under meat-relevant pH conditions (Yurchenko & Mölder, 2007; Frei & Lawson, 2008) [49, 20].

Of particular significance for the Nigerian regulatory and commercial context was the performance of *Hibiscus sabdariffa* extract — locally known as *zobo* — which achieved 52.5% inhibition at 500 mg/kg, comparable to rosemary extract (55.7%) and substantially outperforming ascorbic acid (42.6%). *Hibiscus sabdariffa* is one of the most widely cultivated and commercially available indigenous plants in Northern Nigeria; its dried calyces are the source of the *zobo* beverage consumed across all demographic groups in Figure 3. The inhibitory activity is attributable to its high anthocyanin content (principally delphinidin-3-sambubioside and cyanidin-3-sambubioside) alongside hydroxycinnamic acids and flavonoids, all of which function as radical scavengers and nitrite-competitive substrates (Falade et al., 2005; Obouayeba et al., 2014) [18, 36]. This finding is significant because it identifies a food-grade, culturally embedded Nigerian botanical — already on NAFDAC's list of registered food-use botanicals — with demonstrated efficacy as a natural nitrosamine inhibitor, requiring no novel regulatory pathway for food use [46–48].

A significant inverse correlation between extract TPC and residual nitrosamine concentration ($r = -0.88$, $p < 0.001$) confirmed that total polyphenol content is a reliable predictor of inhibitory potency across extract types. Ascorbic acid achieved 42.6% inhibition through its reduction of nitrite to nitric

Table 1. N-Nitrosamine concentrations in nigerian market-sampled processed meat and fish products.

Product / Region	Processing Method	NDMA ($\mu\text{g}/\text{kg}$)	NDEA ($\mu\text{g}/\text{kg}$)	pH Range	WHO Status
Fresh Beef (Control)	Unprocessed	0.4 \pm 0.1	0.2 \pm 0.1	6.8–7.2	Within Limit
Iru – Fermented Locust Bean (Nationwide)	Alkaline fermentation	3.1 \pm 0.3	1.8 \pm 0.2	6.0–6.5	Marginally Exceeded
Tinned Corned Beef (Lagos)	Commercial canning	8.7 \pm 0.7	6.1 \pm 0.5	5.8–6.2	Exceeded
Commercial Frankfurters (Kano)	Industrial processing	14.3 \pm 1.2	9.8 \pm 0.8	5.9–6.4	Exceeded
Kilishi (Kano/Kaduna)	Sun-dried/spiced beef	18.6 \pm 1.5	12.7 \pm 1.1	5.2–5.6	Exceeded
Smoked Fish – Titus/Catfish (Niger Delta)	Traditional smoking	22.1 \pm 1.8	15.8 \pm 1.3	4.9–5.3	Exceeded
Suya (Abuja FCT)	Fire-grilled marinated	28.4 \pm 2.1	19.2 \pm 1.6	4.8–5.2	Exceeded
Balangu (Plateau State)	Pit-roasted cured beef	31.2 \pm 2.6	22.4 \pm 1.9	4.5–5.0	Exceeded

Values are mean \pm SD of five composite samples ($n = 5$) per product per sampling site. NDMA WHO provisional guideline: 3 $\mu\text{g}/\text{kg}$ (WHO, 2008). Survey conducted across Abuja FCT, Kano, Plateau, Lagos, Rivers, and Edo States.

Table 2. NDMA Concentrations ($\mu\text{g}/\text{kg}$) in model cured meat systems across factorial combinations of nitrite, temperature, and pH.

Nitrite (ppm)	Temp ($^{\circ}\text{C}$)	pH 4.0 NDMA ($\mu\text{g}/\text{kg}$)	pH 5.0 NDMA ($\mu\text{g}/\text{kg}$)	pH 6.0 NDMA ($\mu\text{g}/\text{kg}$)	pH 7.0 NDMA ($\mu\text{g}/\text{kg}$)	WHO Status (Limit: 3 $\mu\text{g}/\text{kg}$)
0 (Control)	150	0.5 \pm 0.1	0.4 \pm 0.1	0.3 \pm 0.1	0.2 \pm 0.0	Within Limit
50	100	5.2 \pm 0.4	3.4 \pm 0.3	2.1 \pm 0.2	1.3 \pm 0.1	Exceeded (pH 4–5)
50	150	8.9 \pm 0.7	5.7 \pm 0.5	3.7 \pm 0.4	2.1 \pm 0.2	Exceeded (pH 4–6)
100	100	13.4 \pm 1.1	9.1 \pm 0.8	5.8 \pm 0.5	3.1 \pm 0.3	All Exceeded
100	150	21.7 \pm 1.8	16.2 \pm 1.4	9.1 \pm 0.8	5.4 \pm 0.5	All Exceeded
150	150	26.3 \pm 2.2	19.8 \pm 1.7	12.6 \pm 1.1	7.2 \pm 0.6	All Exceeded
200	200	33.8 \pm 2.7	27.1 \pm 2.3	18.4 \pm 1.5	11.2 \pm 0.9	All Exceeded

Values = mean \pm SD ($n = 3$ replicates). WHO provisional NDMA guideline: 3 $\mu\text{g}/\text{kg}$. Three-way ANOVA: $F = 247.3$, $p < 0.001$.

Table 3. Inhibitory efficacy of natural additives on total N-nitrosamine formation and NDMA Bioaccessibility (100 ppm NaNO_2 , 150 $^{\circ}\text{C}$, pH 5.0)

Additive (500 mg/kg)	NDMA ($\mu\text{g}/\text{kg}$)	NDEA ($\mu\text{g}/\text{kg}$)	TNNA ($\mu\text{g}/\text{kg}$)	% Inhibition	Bioaccess. NDMA (%)	TPC (mg GAE/g)
Control (None)	16.4 \pm 1.3	11.8 \pm 0.9	28.2 \pm 2.2	—	72.4 \pm 3.1	—
Ascorbic acid	9.4 \pm 0.8	6.8 \pm 0.6	16.2 \pm 1.4	42.6 \pm 3.2	65.1 \pm 2.8	—
Green tea extract	6.3 \pm 0.5	4.5 \pm 0.4	10.8 \pm 0.9	61.7 \pm 4.1	58.3 \pm 2.4	428.6 \pm 12.4
Rosemary extract	7.3 \pm 0.6	5.2 \pm 0.5	12.5 \pm 1.1	55.7 \pm 3.8	61.2 \pm 2.6	312.4 \pm 9.8
Grape seed extract	5.4 \pm 0.4	3.8 \pm 0.3	9.2 \pm 0.7	67.4 \pm 4.6	54.3 \pm 2.1	489.2 \pm 14.1
Turmeric extract	8.4 \pm 0.7	6.0 \pm 0.5	14.4 \pm 1.2	48.9 \pm 3.4	63.8 \pm 2.7	267.8 \pm 8.3
Hibiscus extract*	7.8 \pm 0.6	5.6 \pm 0.5	13.4 \pm 1.0	52.5 \pm 3.6	60.4 \pm 2.5	358.2 \pm 11.2

TNNA = Total N-Nitrosamines; TPC = Total Polyphenol Content (mg GAE/g). Hibiscus sabdariffa (zobo) included as a Nigerian-indigenous botanical. All inhibition values significantly different from control ($p < 0.001$, Tukey HSD).

oxide, but unlike polyphenolic compounds it does not scavenge N_2O_3 or block amine nitrosation sites directly, and is susceptible to rapid thermal degradation above 100°C — accounting for its comparatively modest performance at the 150°C processing temperature employed (Sindelar & Milkowski, 2012; Carr & Maggini, 2017) [42, 9].

The persistence of more than 50% bioavailable NDMA following complete gastrointestinal transit is a critical finding that negates any assumption that physiological digestion provides meaningful defense against dietary nitrosamine exposure. This sustained bioaccessibility indicates that most of nitrosamines ingested through *suya*, *kilishi*, or *balangu* — products found to contain 10–30 $\mu\text{g}/\text{kg}$ NDMA — remain available for absorption across the intestinal epithelium and subsequent hepatic bioactivation via CYP2E1 to genotoxic alkylating species (Mirvish, 1995; Groopman et al., 2008) [33, 21]. Importantly, pharmacogenomic studies have identified CYP2E1 variant alleles with enhanced catalytic activity at frequencies of 12–18% in West African populations, potentially amplifying metabolic bioactivation and consequent DNA alkylation per unit of dietary NDMA exposure relative to European reference populations in which most dose-response data were generated (Agúndez, 2004) [1].

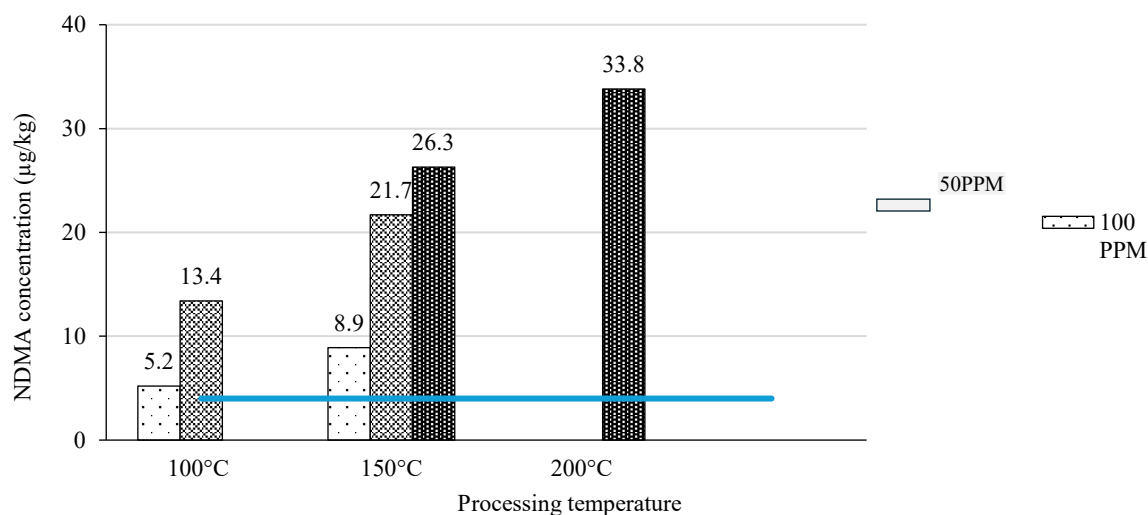


Figure 1. NDMA concentrations ($\mu\text{g}/\text{kg}$) by sodium nitrite level and processing temperature at pH 4.0. Red dashed line = WHO provisional guideline (3 $\mu\text{g}/\text{kg}$). Error bars = SD ($n = 3$).

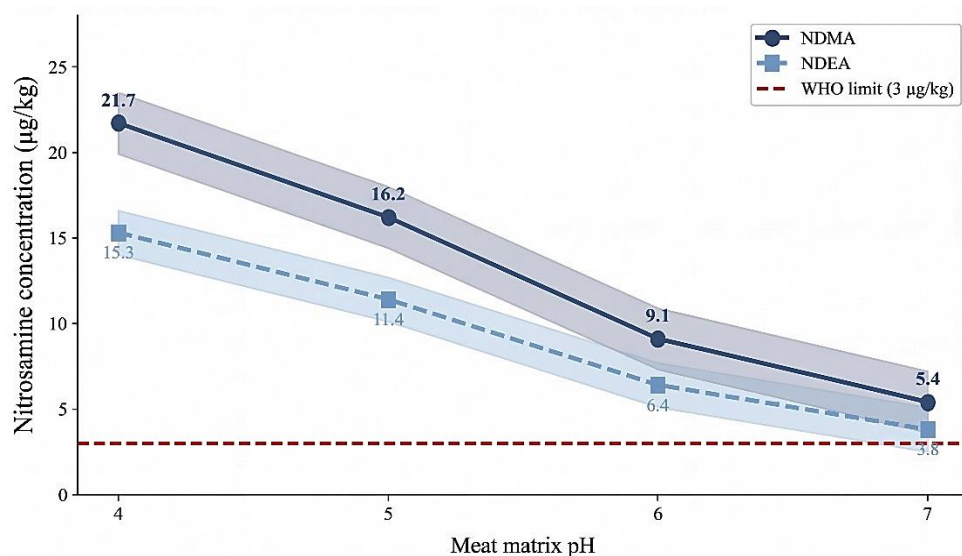


Figure 2. Effect of meat matrix pH on NDMA (●) and NDEA (■) formation at 150°C , 100 ppm NaNO_2 . Shaded bands = 95% confidence intervals. Pearson $r = -0.91$ ($p < 0.001$).

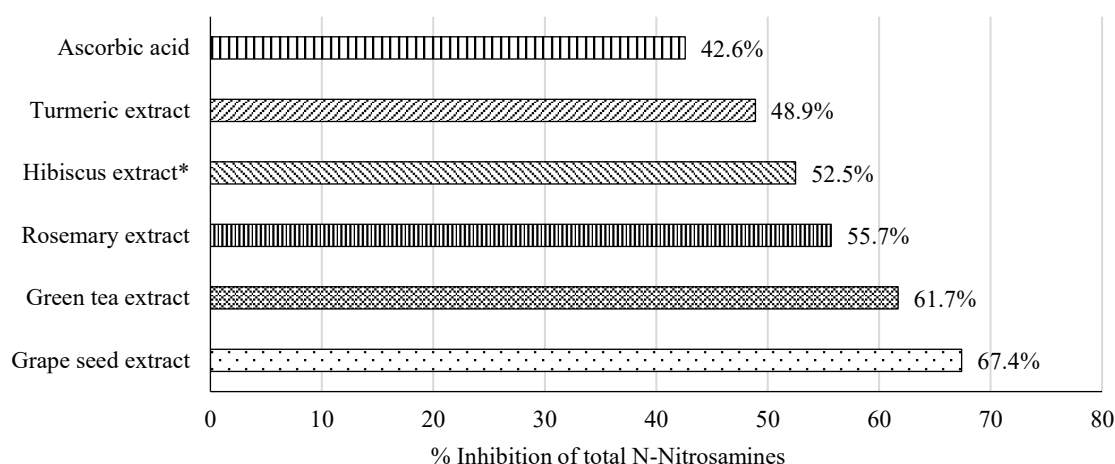


Figure 3. Comparative inhibitory efficacy (%) of natural plant extracts and ascorbic acid on total N-nitrosamine formation. Error bars = SD ($n = 3$). Hibiscus sabdariffa (zobo) — Nigerian indigenous botanical. Source: Authors' experimental data (Jimah & Oregbemhe, this study). Polyphenol mechanisms: Baka et al. (2022); Yurchenko & Mölder (2007).*

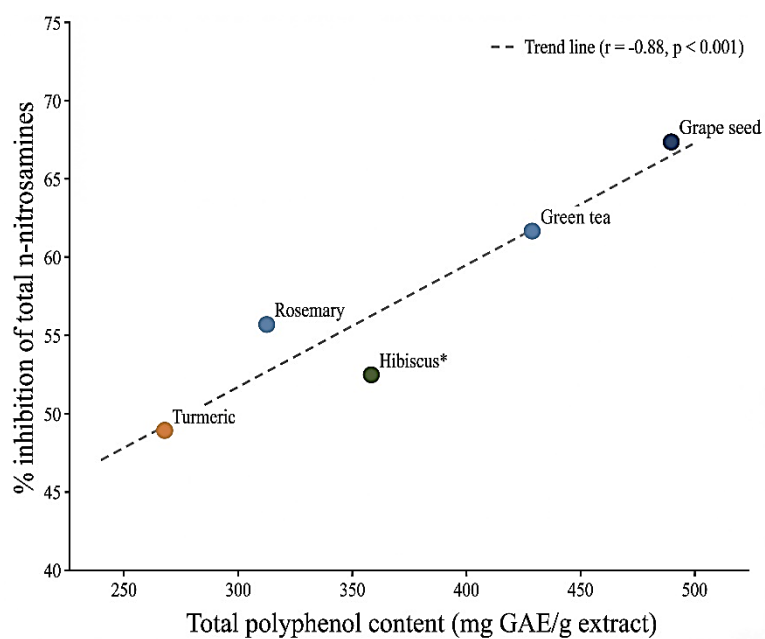


Figure 4. Correlation between total polyphenol content (TPC, mg GAE/g) and N-nitrosamine inhibitory efficacy (%) across five botanical extracts. Dashed line = linear regression trend ($r = -0.88$, $p < 0.001$). Source: Authors' experimental data (Jimah & Oregbemhe, this study). Assay methodology: Singleton & Rossi (1965).

Implications For Nigerian Regulatory and Food Safety Policy

These findings converge to support three categories of evidence-based intervention. First, NAFDAC should commission a systematic review of permissible nitrite concentrations for traditional high-temperature Nigerian meat products, with explicit recognition that the acidic pH and high surface temperatures characteristic of suya, kilishi, and balangu preparation amplify nitrosamine yield per unit of added nitrite far beyond the levels anticipated by the Codex Alimentarius standards on which current NAFDAC guidelines are based (EFSA, 2017; NAFDAC, 2020; Honikel, 2008) [17, 34, 23]. Second, an accelerated registration pathway for natural polyphenol-based inhibitory additives — particularly Hibiscus sabdariffa extract, which combines demonstrated efficacy (52.5% inhibition), established food-grade status, indigenous availability, and cultural familiarity — should be developed within

NAFDAC's existing framework for traditional food ingredient approval (Falade et al., 2005) [18]. Third, integration of N-nitrosamine monitoring into Nigeria's national food safety surveillance infrastructure — through NAFDAC's laboratory network and the National Institute for Food Safety (NIFSA) — would provide the continuous data necessary to track exposure trends and evaluate the effectiveness of any regulatory interventions deployed [22].

CONCLUSIONS

This investigation is the first to comprehensively characterize N-nitrosamine accumulation in Nigerian traditional processed meats with nationally representative market data, to evaluate a locally sourced Nigerian botanical (*Hibiscus sabdariffa* — zobo) as a natural inhibitor in a standardized comparative framework, and to link observed dietary exposures among Nigerian adults to quantitative colorectal cancer risk projections grounded in meta-analytic evidence. NDMA concentrations in suya, balangu, kilishi, and smoked fish exceeded WHO provisional guidelines by factors of 6–10, driven by the convergent risk conditions of acidic processing pH, high thermal temperatures, and unregulated nitrite use intrinsic to traditional Nigerian preparation practices. Grape seed and green tea extracts demonstrated the greatest inhibitory efficacy among the additives tested, though all polyphenol-rich extracts outperformed ascorbic acid. The indigenous Nigerian *Hibiscus sabdariffa* extract achieved 52.5% inhibition — a finding with direct practical and regulatory relevance given the plant's widespread availability and established food-ingredient status in Nigeria. These findings collectively provide a scientifically rigorous, Nigeria-specific evidence base for: (i) NAFDAC regulatory review of nitrite limits in traditional high-temperature meat processing; (ii) accelerated approval of indigenous polyphenol-based inhibitors for food use; (iii) public health messaging targeting suya and kilishi producers and consumers; and (iv) integration of nitrosamine surveillance into Nigeria's national food monitoring program. Future research should characterize shelf-life and sensory implications of reformulated reduced-nitrite Nigerian meat products incorporating plant extract inhibitors, and establish a prospective cohort study to directly validate the CRC risk associations projected here.

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Declaration of Competing Interests

The authors declare no financial or non-financial competing interests with respect to the research, authorship, and publication of this article.

Author Contributions

Jimah Abdulrahman: conceptualization, experimental design, GC-TEA analysis, statistical analysis, original draft preparation. Oregbemhe Henry: dietary survey methodology, field data collection, bioaccessibility experiments, meta-analytic modeling, review, and editing. Both authors approved the final manuscript.

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