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Assessment of Microbiological Qualities and Iodine Contents of Some Brands of Domestic Salt Available in South-eastern Nigeria

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Abstract

Domestic salt is a food condiment, thus, a veritable tool for food contamination. Salt iodisation provides iodine an essential micronutrient and a key component of the thyroid hormones that regulate vital metabolic activities in the body. Insufficiency of iodine results in iodine deficiency disorders (IDD) which affect the functions of vital organs and systems of the body, leading to damaging effects particularly in pregnancy and early childhood, causing miscarriages, stillbirth, mental retardation, and increased infant mortality. Thirty samples of domestic salt made up of ten each of three popular brands in Nigerian markets were obtained from retail stores and evaluated for microbial qualities and iodine contents. Standard microbiological methods and iodometric titration method were employed for sample analysis. Bacillus and fungal species were isolated after sample pre-enrichment and within counts (<10 to 10^1cfu/g) significantly ($p \leq 0.05$) lower than standard specifications. These isolates were however, not confirmed to be either of authochthonous or allochthonous origin or hallophiles. The iodine contents for the three brands were 21.05 ± 0.42, 41.78 ± 0.67 and 29.70 ± 0.27 (SSC). All the salt samples analyzed except for SSC had iodine content significantly ($p \leq 0.05$) different from the standard > 30 ppm iodine at distributor and retail levels and > 50 ppm iodine at port of entry and salt factory level. This could indicate none compliance to standard specifications. There is need for effective legislation and strict monitoring of food fortification at all levels to ensure compliance to standards and adherence to good manufacturing practices/hazard analysis and quality control precepts which are imperative to effective food fortification programmes.

Keywords: Food fortification, Iodine content, salt iodisation, microbial qualities, GMP, HACCP

1.0 Introduction

The Food Fortification Initiative (FFI, 2017) defined fortification as adding vitamins and minerals to foods to prevent nutritional deficiencies. The nutrients regularly used in fortification prevent diseases, strengthen immune systems, and improve productivity and cognitive development. WHO/FAO (2006) opined that food fortification is the deliberate increase of the content of essential micro-nutrients (vitamins and minerals, including trace elements) in a food so as to improve the nutritional quality of the food supply and provide a public health benefit with minimal risk to health.

Micronutrients are used for the fortification because they are obtained in minute quantity from our normal meals. Nutrients generally used for fortification of food products include vitamin A, vitamin C, calcium, iron, vitamin D, vitamin E, vitamin K, thiamine, vitamin B (B_1, B_2, B_6, and B_{12}), foliate, iodine, zinc, copper and manganese (Allen *et al*., 2003; FFI, 2017). Many foods, especially, the staple foods that are used as vehicles for fortification include: the whole wheat flour, maize flour, rice, sugar, salt, vegetable oils, dairy products and margarines (FAO, 1995; FFI, 2017).

Nigeria mandates the fortification of wheat and maize flour with vitamin A and iron, vegetable oil, and sugar with vitamin A. In addition, salt iodisation has been mandatory since 1993 (GAIN, 2014). Increasing the availability of fortified food in Nigeria was observed to be a critical pillar for the country's food and nutrition security plans. Scaling up the availability and consumption of fortified foods in Nigeria would contribute to the achievement of public health goals and a number of Sustainable Development Goals (SDGs), reduce the incidence of spina bifida in unborn children, anaemia among women of reproductive age and enhance cognitive development within the first 1000 days of life (GAIN, 2016).

Iodine is an essential micronutrient required by the body. It is an essential component of the thyroid hormones which affect the functions of vital organs and systems of the body particularly the brain, heart, liver, kidney and muscle. Inadequate production of the thyroid hormones results in a range of disorders collectively known as iodine deficiency disorders (IDD). These disorders include mental retardation, growth impairment, nervous system defects, goiter, reproductive disorders, and physical sluggishness, lower energy and productivity amongst other. The major damaging effects of IDD are in pregnancy and in early childhood resulting in miscarriage, stillbirth, cretinism, and increased infant mortality (Mannar and Dunn, 1995, Nwankwo *et al*. 2016).

Nigeria achieved over 95 percent level of salt-iodisation from from 1998 to 2004 (NDHS 2008), however, since Universal Salt Iodisation (USI) certification in 2005 and the formal recognition of the efforts of the Nigerian government in 2007 at the global Micronutrient Forum, financial support for the program to combat iodine deficiency disorders (IDD) decreased on the part of the government and donors (NDHS 2008). The usual monitoring of iodised salt at the factory, wholesale, retail and household levels became irregular and sporadic. USI regulations were hardly enforced, thus without the needed quality control, manufacturers and marketers also became complacent and failed to comply with the mandatory iodisation standard. This resulted in significant decline in the household consumption of adequately iodised salt, from 98 % in 2003 to 52 % in 2008 (NDHS, 2008). Sablah *et al*. (2013) observed that the impact of food fortification occurs among populations with expected access to adequate quantities of sufficiently fortified foods to meet targeted nutritional needs.

Domestic salt is an essential condiment often added to food at any stage before consumption. The use of this condiment as 'ready-to-eat-food' when no further heat treatment or processing is required before consumption makes salt a veritable tool for introducing microbial contaminants into food. The aim of this work therefore is to evaluate the microbial qualities of some popular brands of domestic salt in Nigeria, determine the

level of iodine fortificants in these brands of salt products, and assess the relationship between the fortificant iodine and the microbial load.

2.0 Materials and Method

2.1 Sample Collection

Thirty domestic salt samples made up of ten each of products from companies A, B, C were purchased from supermarkets and retail stores in Onitsha in Anambra state, Owerri in Imo state and Aba in Abia state. The three states are in southeast of Nigeria. Onitsha is located in 6°09'60.00" N 6°46'59.99" E. Owerri is in 5°29'1.07" N 7°01'59.70" E while Aba is situated at 5°06'23.69" N 7°22'0.01" E. All samples purchased were within expiry date and there were no observable physical damage to the polyethylene packs. All the samples collected were adequately labeled as SSA (samples from company A), SSB (samples from company B) and SSC (samples from company C). They were aseptically conveyed in sterile specimen containers to the Department of Biological Science laboratory, Covenant University, Ota, Ogun state, Nigeria for microbiological and chemical analyses.

2.2 Determination of Microbial Load in Salt Samples

Salt samples were mixed thoroughly and triplicate ten grams of each sample was homogenized in 90 ml sterile nutrient broth as diluents. The sample homogenates were further serially diluted in tenfold to 10^6 using nutrient broth as diluents. Aliquot 0.1 ml of appropriate dilutions of the sample homogenates were inoculated onto triplicate plates of nutrient agar (Oxoid, England) for total aerobic plate count (TAPC) and isolation of contaminating heterotrophic organisms. MacConkey agar (Biolab, Hungary), eosin methylene blue (EMB) agar and broth (HiMedia, India) were inoculated for coliform count, isolation and coliform tests. Potato dextrose agar (PDA) and mannitol salt agar (MSA) (both from Biolab, Hungary) were inoculated for count and isolation of fungi and Staphylococci respectively. The PDA plates were incubated for 3-5 days at room temperature 28±3°C while all other plates were incubated at 37°C for 24 to 48 h. Sample homogenates in nutrient broth were also incubated for 24 h at 37°C for resuscitation (pre-enrichment) and subsequently inoculated on all the media as described above. At the end of incubation period, colonies were counted using the digital colony counter (Gallenkamp, England) and mean total microbial counts was expressed as colony forming units per gram (cfu/g) of sample.

Colonial morphology and other cultural characteristics was observed and recorded and pure cultures of microbial isolates were obtained by repeated sub-culturing on appropriate media. Preliminary identification of bacterial isolates was based on cultural, morphological and basic biochemical characteristics; Gram staining, catalase activity, indole, methyl red, Voges proskaur test, motility, citrate utilization, urease production, oxidase, starch hydrolysis, gelatin liquefaction, coagulase and fermentation of sugars. Further identification of bacterial isolates was based on standard bacteriological procedures (Jolt *et al*., 1994) and employing the Biomerieux® sa API system. Confirmation for coliform organisms was based on presumptive, confirmatory and completed tests following the description of Speck (1976) and Oranusi *et al*., (2013). Fungal isolates were identified based on cultural and morphological characteristics, pigmentation on media, and microscopic characteristics, sporulation, mycelia arrangement, and sugar assimilation tests and with reference to standard identification key and atlas (Tsuneo, 2010).

2.3 Determination of Iodine content of Salt Samples by Iodometric Titration

The salt iodine content was determined by standard iodometric titration method and estimated in mg/kg (ppm) following the description of EuSalt Sodium Chloride Analytical Standard (2005) and Ashwini *et al.* (2013). Fifty (50) grams of salt was dissolved in water and made up to 250 ml in a volumetric flask. Aliquot 1ml of 1mol/l tetraoxosulphate-6- acid (H_2SO_4) was added to liberate free iodine from the salt sample. Excess potassium iodide (KI) (5 ml of 100 g/l) was added to solubilize the free iodine, which is quite insoluble in pure water under normal conditions. The free iodine was titrated with 0.0025 mol/l sodium thiosulphate. Starch was added as an external indicator, it produce blue colour on reaction with the free iodine. When it is added near the end of the titration when only a trace amount of free iodine is left, the loss of the blue colour (end point) indicates that no free iodine is left in the reaction with sodium thiosulphate. The amount of sodium thiosulphate which is used is proportional to the amount of free iodine which is liberated from the salt.

Calculation:

Iodine, mg/kg (ppm) = titration volume (ml) × 21.15 × Normality of sodium thiosulphate × 1000 / weight of salt sample weight (gram). (1)

2.4 Statistical Analysis

Data were expressed as mean ± standard error of the samples. The mean microbial counts and mean fortificants levels were compared using analysis of variance (ANOVA). The least significant difference (LSD) was used to separate variance means. A probability value $p \leq 0.05$ was considered significant in all cases. Also the relationship between microbial counts and the fortificants were determined using Pearson's correlation method. Correlation was considered significant at the 0.05 level (2-tails).

3.0 Results

Bacillus and fugal species were scantily isolated after resuscitation that enhanced the chances of microbial survival. Tables 1 and 2 shows the mean microbial counts (total aerobic plate count, total fungal count, total staphylococcal count, and total coliform count) from the salt samples before and after resuscitation. Samples of brands SSA and SSC had TAPC to the level of 10^1cfu/g sample. TFC were <10 while staphylococci and coliforms were not detected. The counts obtained were significantly ($p \leq 0.05$) lower when compared to the International Commission on Microbiological Specifications for Foods (ICSMF), 1996 general microbiological reference criteria for foods requiring further cooking at temperature above 70°C (>70°C) and reference sampling plans for spices, condiments, and gums.

Table 3 shows the mean values of iodine contents for samples SSA, SSB, and SSC respectively. In comparison to the Government approved level, the iodine content of SSA was significantly ($p \leq 0.05$) lower while the iodine content of SSB was significantly ($p \leq 0.05$) higher. There is no significant ($p \geq 0.05$) variation between iodine content of SSC and Government approved level. The results showed iodine contents/levels of the salts was negatively correlated ($r = -0.815$, $p \leq 0.05$) with microbial loads/counts (TAPC and TFC).

Table 1: Mean (±SEM) total microbial count (cfu/g) before resuscitation

Parameter	SSA	SSB	SSC	ICMF, 1996 standard
TAPC	3.0 ± 0.00	-	4.0 ± 0.00	$5 \times 10^5 \pm 0.00$
TFC	2.0 ± 0.00	-	2.0 ± 0.00	$1 \times 10^2 \pm 0.00$
TSC	-	-	-	$1 \times 10^4 \pm 0.00$
TCC	-	-	-	-

Key: TAPC = Total Aerobic Plate Count, TFC = Total Fungal Count, TSC = Total Staphylococci Count, TCC= Total Coliform Count, SSA = Salt sample company A, SSB = Salt sample company B, SSC = Salt sample company C, SEM = Standard Error of Mean

Table 2: Mean (±SEM) total microbial count (cfu/g) after resuscitation

Parameter	SSA	SSB	SSC	ICMF, 1996 standard
TAPC	$1.1\times10^1 \pm 0.00$	<10	$1.0 \times10^1 \pm 0.00$	$5 \times 10^5 \pm 0.00$
TFC	<10	<10	<10	$1 \times 10^2 \pm 0.00$
TSC	-	-	-	$1 \times 10^4 \pm 0.00$
TCC	-	-	-	-

Key: TAPC = Total Aerobic Plate Count, TFC = Total Fungal Count, TSC = Total Staphylococci Count, TCC= Total Coliform Count, SSA = Salt sample company A, SSB = Salt sample company B, SSC = Salt sample company C, SEM = Standard Error of Mean

Table 3: Mean (±SEM) iodine contents (ppm) of the samples

Fortificant	SSA	SSB	SSC	GAL
Iodine	21.05 ± 0.42*	41.78 ± 0.67*	29.70 ± 0.27	> 50 ppm iodine at port of entry and salt factory level > 30 ppm iodine at distributor and retail levels > 15 ppm iodine at household level (UNICEF, 2006).

Key: *Values differ significantly ($p < 0.05$) from the GAL
SSA = Salt sample (company A), SSB = Salt sample (company B), SSC = Salt sample (company C), GAL = Government approved level, SEM = Standard error of mean

4.0 Discussion

The result of this work shows that bacillus and fungal species were isolated from the samples. The near absence of viable microbial cells in the samples prior to resuscitation, and the scanty counts of few cells after resuscitation point to the fact that the domestic salts have good antimicrobial activities. (Bautista *et al*., 2008., Albarracin *et al*., 2010). This is corroborated by the negative correlation ($r = -0.815$, $p \leq 0.05$) of iodine contents of the salts with microbial counts. Hallophiles are known to thrive in salt concentrations (Hedi *et al*. 2009, Casamayor *et al.,* 2013, and Yang, *et al*. (2016). The few bacterial and fungal cells obtained after resuscitation could have existed as spores or could be Hallophiles.

Bacillus and fungal species are known to be spore bearers and food contaminants (Chukwu *et al*. 2016). Although, all necessary aseptic measures to prevent microbiological contaminants were taken, the study, however, did not investigate to ascertain that the isolated organisms were of authochthonous origin and not just mere contaminants or organisms of allochthonous origin, introduced during sampling and handling, investigation was also not carried out at this level of the work to establish if the isolates were hallophiles. Bacillus species and several other hallophiles have however, been isolated from salt and saline environments (Russel (2000), Van-der-Heide (2002), Hedi *et al*. (2009), Weimer *et al*. (2009), Oren *et al*. (2009), Oren, (2013), Casamayor *et al* (2013), and Yang, *et al*., 2016) The absence of coliforms which are indicator organisms of faecal contamination (Aydin *et al*., 2009) and staphylococci which are normal human flora often implicated in food contamination (Rohilla, 2010) could be attributed to the antimicrobial activity and extreme harsh environmental condition presented by domestic salt.

The government approved levels of iodine in domestic salt is > 50 ppm (mg/kg) iodine at port of entry,and salt factory level, > 30 ppm iodine at distributor and retail levels, and > 15 ppm iodine at household level (UNICEF, 2006). Samples used in this work were from retail stores, thus the iodine levels for samples of brand SSA examined was significantly lower ($p < 0.05$) than the Federal government specification. The low level of iodine fortificant in products SSA could be due to deliberate under fortification by the manufacturers or loss of iodine by oxidation in the course of storage and handling of products. Potassium iodide in salt is known not to be very stable; it can easily be oxidized to molecular iodine and lost by evaporation. Iodine is also easily lost when iodised salt is subjected to conditions such as high moisture content, humidity, aerated environment, sunlight, heat, acid, impurities and damped packaging material ((Mannar and Dunn, 1995; WHO/FAO, 2006) and in the presence of excess water, the iodide may be separated from the salt in the water film (FAO/WHO, 1991, 2006). In Nigeria, salt iodisation is with potassium iodate which is considered to be more stable in storage than iodide (FAO,1995., WHO/FAO, 2006), the samples analyzed in this study are all within expiry date as stipulated by the manufacturers on the packs, they are all packaged in polyethylene bags, and no physical damage was observed on the packs during sampling. Chauhan *et al*. (1992), and Silveira (1993) reported that stability studies of iodized salt using potassium iodate as the fortificant showed no significant loss of iodine on storage in polyethylene bags for up to two years, and the boiling of the salt solutions led to negligible loss of iodine. The plausible reason for the low level of iodine in SSA could be under fortification. Since the result of this study and similar other

reports confirmed the stability of potassium iodate fortificants (Mannar and Dunn, 1995, WHO/FAO,2006).

The level of iodine in samples of salt brand SSB examined was higher than the recommended 30.0 mg/kg sample at distributor and retail levels, although this is lower than the 50 mg iodine/kg salt stipulated for iodisation at production level (Mannar and Dunn, 1995; UNICEF, 2006).This could mean non-compliance to standard specification. Food fortification requires standards to ensure consistency across an industry, and ensure that fortification levels meet international recommendations and micronutrient requirements of the population at safe levels based on evidence. These standards can therefore serve as the basis for quality control and monitoring of industry practices (Sablah, et al., 2013. Standardization is pertinent because as much as adverse effects would be present at very low intakes because of a deficiency condition, which would decrease in severity with an increase in intake, adverse effects would also be present at high intakes because of toxicity, which would increase in severity with an increase in the dose (FAO,1995; Renwick, 2006; Shenkin, 2006). It is important for salt iodisation to be restricted to standard specifications because iodine intakes of up to 1mg (1000/g) per day are tolerated by most people. Nevertheless, evidence abound that an acute, excessive increase in iodine intake can increase the risk of iodine toxicity in susceptible individuals resulting in iodine-induced hyperthyroidism (IIH) and Iodine-induced thyroiditis (WHO/FAO, 2006).

Conclusion

Food condiments including domestic salt could be veritable source of microbial contaminants to food, specifically were such condiment can be added to processed food prior to consumption ie condiments used as ready to eat food (RTF). Micronutrients play a central part in metabolism and in the maintenance of tissue function. An adequate intake therefore is necessary, but provision of excess supplements to people who do not need them may be harmful (Shenkin, 2006). There is need for effective legislation and strict monitoring of food fortification at all levels to ensure compliance to standards and specifications. Adherence to good manufacturing practices and hazard analysis and quality control precepts are imperative to effective food fortification programmes.

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