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Odinga T
Department of Biochemistry, Faculty of Science, Rivers State University, Nigeria

Uzuanu CK
Department of Biochemistry, Faculty of Science, Rivers State University, Nigeria

Gabriel-Brisibe CU
Department of Medical Biochemistry, Faculty of Basic Medical Sciences, College of Medical Science, Rivers State University, Nigeria

Austin-Asomeji I
1. Department of Community Medicine, Faculty of Clinical Sciences, College of Medical Sciences, Rivers State University, Nigeria
2. Department of Community Medicine, Rivers State University Teaching Hospital, Nigeria

Bademosi A
1. Department of Community Medicine, Faculty of Clinical Sciences, College of Medical Sciences, Rivers State University, Nigeria
2. Department of Community Medicine, Rivers State University Teaching Hospital, Nigeria

Corresponding Author:
Odinga T
Department of Biochemistry, Faculty of Science, Rivers State University, Nigeria

Study on the concentration of tertiary butyl hydroquinone in selected brands of instant noodles without their spices in Port Harcourt, Nigeria

Odinga T, Uzuanu CK, Gabriel-Brisibe CU, Austin-Asomeji I and Bademosi A

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Abstract
Background/Aim: The use of synthetic antioxidants to maintain products condition to consumer's end is broadly used in food industries. Tertiary butyl hydroquinone (TBHQ) is a synthetic aromatic organic compound that is commonly used in food to serve as an antioxidant and preservative. This study evaluated the concentration of TBHQ in four (4) widely sold brand instant noodles and consumed in Port Harcourt, Nigeria; W, X, Y, and Z which represented Indomie, Tummy-Tummy, Supreme and Masters Noodles respectively.
Methodology: This study employed the use of High-Performance Liquid Chromatography (HPLC) for the analysis of the noodles samples. The results obtained were compared with FAO/WHO maximum level of TBHQ in such foods (200mg/kg). The noodles alone were analyzed for TBHQ without their spices.
Results: The results obtained revealed that Y-Noodles had the highest TBHQ concentration (23.6841µg/10g) while W-noodles had the least concentration (0.4789 µg/10g) of TBHQ. Z-Noodles had (22.2315 µg/10g) and X-Noodles had (10.9924 µg/10g). All concentrations of TBHQ in all brands of noodles evaluated were below the maximum level of FAO/WHO.
Conclusion: This study suggests that caution in frequent consumption of noodles is imperative, and a continuous evaluation of TBHQ in noodles is recommended as an increase in the concentration of TBHQ can be cytotoxic and deleterious to the consumers. This study thus ensures the safety of processed food consumed associated with the high rate of noodles consumption.

Keywords: Tertiary butylhydroquinone, antioxidant, instant noodles, port-harcourt

Introduction
The use of synthetic Antioxidants in the food industries has found wide application. This is due to the potency of the antioxidants to retain the products original state to the end consumers. In synthetic state, antioxidants are used in food during processing, storage and distribution of the food until they get to the consumers because of their ability to avert lipid oxidation [1]. Oxidation of lipids in food results in deterioration of the quality of the product, especially the flavor and odor. A combination of scavenging free radicals, chelating pro oxidative metals, quenching singlet oxygen and photosensitizers, and inactivating lipoxygenase are implied by antioxidants to slow down the oxidation rates of foods [2].
Noodles are among the most available, affordable and convenient foods in the market and consumed among people of all socioeconomic status including both urban and rural areas of the country [3]. It is believed that Asian noodles originated from China as early as 5000 BC [4, 5]. Instant noodles are consumed in more than 80 countries and have become internationally recognized food.
World Instant Noodles Association reported that Nigeria was the eleventh largest consumer of instant noodles in the World in 2019 [6].
Noodles with sodium stearoyl lactylate (SSL) had a better quality product such as; improved tensile strength, cutting force and elasticity, whereas the addition of lecithin, MG or SFAE had a negative impact on the strength of extruded noodles [7]. Synthetic Antioxidants are widely used to prevent rancidity in food and of all studied, TBHQ revealed to have the best antioxidant activity [4].

Tert-butylhydroquinone (TBHQ) also known as 2-(1, 1-dimethylethyl)-1, 4-benzenediol (NIST Chemistry web book) is a synthetic phenolic antioxidant used in frying process with highly unsaturated vegetable oils. TBHQ has been reported as the most effective synthetic phenolic antioxidant used for stabilizing edible oils, because of its diphenolic structure and heat-stable properties, it also showed excellent synergism with other antioxidants [8, 9].

The health risk as well as benefits of TBHQ in food products has been reported, thus, the strict restriction of its usage in food products. TBHQ showed a non-typical mode of cell death and proved cytotoxic toward human monocytic leukemia cells, caused apoptosis and significantly promoted DNA damage [10]. Along with sodium nitrite, it promoted forestomach carcinogenesis [9]. Due to the strict regulations on TBHQ, there is need to develop effective and convenient analytical methods for analytically monitoring the concentration of TBHQ in food products [11]. Vision disturbances have been reported when humans consume TBHQ, causes liver enlargement, neurotoxic effects, convulsions, and paralysis in laboratory animals [12]. The European food safety authority (EFSA) and the united states food and drug administration (FDA), as well as the Joint FAO/WHO Expert Committee on food additives (JECFA) have evaluated TBHQ and determined that it is safe to consume at the concentration allowed in food been 200mg/kg [13].

It is in view of these assertions that this study was conducted to evaluate the concentrations of TBHQ in selected brands of Instant Noodles sold and consumed in Port Harcourt, Rivers State in Nigeria, West Africa

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Fig 1: Instant Noodles

Materials and Methods

Materials and Reagents

Noodles, blender, weighing balance, magnetic stirrer, methanol, filter paper, centrifuge and high-performance liquid chromatography (HPLC). Noodles samples sold in Port Harcourt were purchased from grocery shops and labeled W-Noodles (Indomie), X-Noodles (Tummy Tummy), Y-Noodles (Supreme), and Z-Noodles (Masters). Methanol and 2-(1,1-dimethylethyl)-1, 4-benzenediol (analytical grade and were purchased). Water was purified using distillatory system twice to meet the requirement of HPLC grade water.

Instrumentation

High performance liquid chromatography DAD detector was used to analyze the amount of TBHQ in the extracted sample using N200 chromatography software. HPLC was performed on Hangzhou LC-8518 with a low-pressure gradient and solvent delivery LC-8518 pump with a high-pressure switching valve, a high-sensitivity LC-8518 Diode Array Detector. A micro syringe for sample injection. The column size was 150 x 4.6mm, Sample volume of 40 micro liter was injected. DAD detector was used, with Methanol/Water 60:40 as the mobile phase, at Wavelength 210nm. The Column temperature was 40°C and run time was 8 minutes.

Preparation of Standard Solution

Stock solution of the reference compound was made by weighing 0.001g of reference standard into a test tube, the standard was dissolved with 10ml of absolute methanol. Each of the standard was then agitated for 10 minutes using vortex mixer and filtered using micron filter into the sample bottle.

Sample Preparation and Extraction

Samples were ground, sieved (70 mesh), weighed (5g), soaked with absolute methanol and stirred at 200rmp for 2hrs. Samples were then centrifuged at 400rmp for 15mins. The supernatant was decanted, filtered using 0.45 µm filter membrane, degassed, and injected to HPLC using a micro syringe [14].

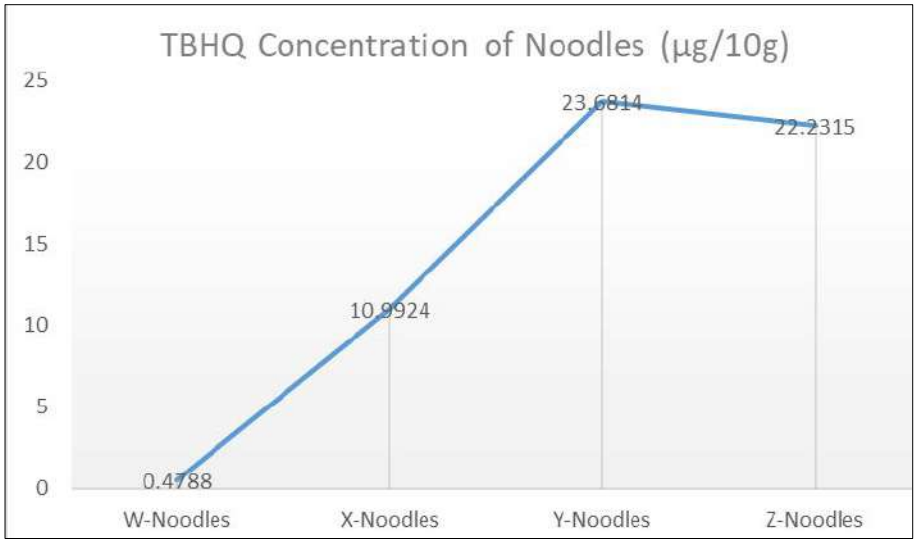
Results

Table 1: Concentration of THQ in Instant Noodles.

SAMPLES	TBHQ Concentration (µg/10g)	TBHQ Concentration (mg/kg)	FAO/WHO Maximum level (mg/kg)
W-Noodles	0.4789	0.04189	200
X-Noodles	10.9924	1.09924	200
Y-Noodles	23.6841	2.36841	200
Z-Noodles	22.2315	2.22315	200

Footnote: W-Noodles (Indomie), X-Noodles (Tummy Tummy), Y-Noodles (Supreme), and Z-Noodles (Masters).

The result showed that W-noodles had the least concentration of TBHQ while Y-Noodles had the highest concentration of TBHQ. The concentrations were all below the FAO/WHO maximum level in food.



Footnote: W-Noodles (Indomie), X-Noodles (Tummy Tummy), Y-Noodles (Supreme), and Z-Noodles (Masters).

Fig 2: TBHQ concentrations in Instant Noodles sold in Port Harcourt

Discussion

This study evaluated the concentration of TBHQ in common Instant Noodles sold and consumed in Port Harcourt, Nigeria. TBHQ has been reported to be used as a preservative and antioxidant in food products to protect against Oxidative deterioration or rancidity, thus extending the storage stability in the food product.

The result as shown in Table 1 and Figure 2, revealed that TBHQ had the highest concentration of 23.6841µg/10g in Y noodles with brand name; Supreme noodles. The least concentration (0.4789 µg/10g) of TBHQ was observed in W noodles with brand name Indomie Noodles. X noodles (Tummy Tummy) had 10.9924 µg/10g) while Z noodles (Masters Noodles) had a concentration of 22.2315 µg/10g of TBHQ. A higher concentration of synthetic phenolic antioxidants than normal in food products when consumed has been reported to be associated with carcinogenicity, cytotoxicity, oxidative stress induction and endocrine-disrupting effects [15]. The concentrations of TBHQ in all tested noodles were below the FAO/WHO maximum level, thus, healthy for consumption. A study showed a beneficial potency of TBHQ to protect L6 myoblasts against the toxicity induced by sodium palmitate due to a synergistic activation of different signaling pathways such as Nrf2 and NF-κB [16]. However, via the process of bioaccumulation of xenobiotics in the body, a low concentration of a substance can undergo bioaccumulation if consistently consumed to become cytotoxic and can fragment the DNA [17]. Consumption of TBHQ in high/ long term doses (1-4 grams) has been associated with various disorders such as; DNA damage, affect estrogen levels in women, hyperactivity in children, asthma, dermatitis, nausea, vomiting, tinnitus (ringing in the ears) etc. In also lead to development of cancerous precursors in the stomach [18]. TBHQ caused a concentration-dependent increase in CYP1A1 at the mRNA and activity levels in human HepG2 cells, thus can directly induce cyp1a1 gene expression in an AHR-dependent manner and may represent a novel mechanism by which TBHQ promotes carcinogenicity [19].

The Joint FAO/WHO Expert Committee on Food Additives (JEFSA), and the World Health Organization (WHO) concluded that dogs are most sensitive to TBHQ, so should have an accepted daily intake (ADI) of 0–0.7 mg/kg body weight (BW) [20]. Also, since human exposure to TBHQ depends largely on consumed fats containing TBHQ, Children and infants should have Acceptable Daily Intake of TBHQ higher than adults (1.3mg/kgBW) because Children take more fats due to their energetic requirements [20].

The average intake of TBHQ as established by Codex General Standard for food additives varies;

USA (90%), Australia and New Zealand (180%) of the ADI. It has been reported that the best estimate of the national average intake of TBHQ for the USA ranges from 50% while China (100%) [21, 22]. The use of TBHQ as a food additive are legalized thus: Australia (200 mg/kg), Brazil (200 mg/kg), China (200 mg/kg), the USA (200 mg/kg) and Iran (120 mg/kg). Several studies have observed that the concentration of TBHQ in some foods were all below the permissible range; (corn oil: 115 mg/kg [23], Brazil nut crude oil: 82.37 mg/kg [24], butter/margarine: 153.1–180.3 mg/kg and snacks: 160.7–180.7 mg/kg [25]). This present study also reported the TBHQ concentration of selected Instant Noodles sold in Port Harcourt, Nigeria to be below the permissible limit.

Conclusion

In conclusion, TBHQ had its concentration in Instant Noodles tested as negligible, however, caution should be employed in its intake. This is because, via the process of bioaccumulation in the body tissues, it can become deleterious, toxic and possibly cause disorders and diseases when ingested consistently as is the case in many homes in Port Harcourt, Nigeria.

Conflict of Interest

The Authors in this paper hereby declare no conflict of interest

Role of Authors

The authors collaborated on every part of this paper together

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