International Journal of Chemical and Biochemical Sciences
(ISSN 2226-9614)

Journal Home page:www.iscientific.org/Journal.html

Nutritive value of evaporated milk and assessment of potential contribution to nutritional health of consumers in Owerri, Imo State, Nigeria

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Abstract

Milk is an acclaimed nutrient dense food readily available for human consumption. The wide range of consumption and usage of evaporated milk makes it a worthwhile food to study, for quality assurance. Three evaporated milk brands referred as PM, TCM and HM, sold in markets in Owerri, Nigeria were evaluated for their nutritive value using atomic absorption spectrophotometer and gas chromatograph-electron capture detector. The proximate composition showed appreciable amount of moisture (69%), ash (2.29%), protein (19%), carbohydrates (9%), fibre (0.05%) and fats (3.91%) capable of health-promoting activities. The evaporated milk brands showed appreciable content of vitamin A (1.75±0.24 µg/kg), vitamin D (12.05±1.70µg/kg), vitamin E (5.64±0.39µg/kg), vitamin C (6.37±0.56µg/kg), thiamin (B1), and riboflavin (B2). Also, appreciable amounts of Zn, Fe, Ca, Mg and P were recorded. Toxic heavy metal content were; cadmium (0.37 ±0.03µg/kg) lead (Pb- 0.05 ± 0.004µg/kg), arsenic (0.002 ± 0.0002µg/kg), while mercury was not detected in any of the three brands. Varying concentrations of organochlorine pesticides; dichlorobiphenyl, DDVP, t-nonachlor, isopropylamine, HCB, endosulfan, g-chlordane, biphenyl, aldrin and orgnophosphate pesticides; profenofos and glyphosate were detected, with total residue concentration of 0.1742, 0.3445 and 0.4889 mg/kg for HM, PM and TCM evaporated milk brands respectively. These results shows these evaporated milk brands have nutritional constituent that can fulfill the recommended dietary allowance per day, the concentrations of toxic heavy metals and residual pesticides were generally below the safe limits and are unlikely to be hazardous. The data generated will also contribute to the existing knowledge in the area of nutrition and functional foods studies.

Keywords: Evaporated milk, aldrin, glyphosate, Proximate composition, Minerals, Vitamins, Pesticides, Heavy metals.

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1. Introduction

Milk and milk products are important nutritional substances with essential components for all age groups, meeting the nutritional needs of adults and children better than any single food [1]. Evaporated milk is a commonly available food in Nigeria and represents an essential source of nutrients for many Nigerian families. Evaporated milk is an ingredient used in bakery and confectionary industries. The combination of milk nutrient density and nutrient bioavailability makes the market cost very low and affordable. Consequently, increasing the proportion of milk and dairy products in daily diet is a promising approach to meeting nutrient needs. This approach has been beneficial for B vitamins needs [2]. Milk supplies proteins of high biological value, which include caseinogen, lactalbumin and lactglobulin. Milk proteins are also good source of antioxidative peptides and containing beneficial amount of bioactive peptides. The antioxidant capacity of milk peptides encompasses the preventive role to

various metabolic disorders such as cancer, diabetes, and cardiovascular diseases (CVD) [3]. Milk offers easily digestible fats, milk sugar, calcium, phosphorus, vitamin A and certain B-vitamins in sufficient amounts. Current research trends show that milk and milk products, if consumed as part of a healthy eating pattern are neutral or even have a positive effect on cardiovascular health [4-10]. In Nigeria, many dairy companies operate in partnership or wholly owned by multinational companies. Most of these companies' industrial processes involve reconstitution of imported milk powder into different dairy products. These production processes may affect the quality of milk. Also, some evaporated milk brands in Nigeria claim to have nutritional constituent that fulfills the recommended dietary allowance per day as specified by nutritionists. Recommended Dietary Allowances (RDAs) are the levels of intake of essential nutrients that, on the basis of scientific knowledge, are judged by the Food and Nutrition Board to be adequate to meet the known nutrient needs of practically all healthy persons.Milk consumption is recommended by many nutritional guidelines for meeting daily requirements for macro- and microminerals, animal proteins and B-vitamins. Diets of Nigerians are majorly plant-based, and this leaves some deficiency of nutrient supply especially for children as they need to get a balanced diet at every mealtime. The Nigeria Demographic and Health Survey 2018 revealed that stunting amongst children (>5 years of age) in Nigeria is at 37% and has remained the largest burden of malnutrition, with many states in the North recording prevalence above 40%. It is important to note that during formative years of life in children, deficiencies in nutrients may cause permanent developmental damage. Milk can serve as a valuable addition to Nigerians (especially children) daily diet because it can provide substantial amounts of daily-required (essential) nutrients.

However, studies have associated milk as a potential source of residues of different pesticides and toxic heavy metals [11,12]. Heavy metals are metals in their standard state, presents specific gravity (density) of more than 5 g/cm^3 [13]. These toxic heavy metals may contaminant milk from source (Cow) as a result of exposure of lactating cow to environmental contaminants in feed stuffs and water [14,15]. Raw milk may be contaminated during processing and packaging [16,17].Furthermore, milk can as well be contaminated by residues of different pesticides such as organochlorines (OC) and organophosphorous (OP). Residues of organochlorines pesticides found in milk are mainly from fodder and soil, while residues of organophosphorous pesticides are linked with ingestion of insecticide used for parasite control on the animal [18]. Organochlorine pesticides implicated in chronic poisoning are involved in various pathological processes [19]. Pesticides exposure have been independently or in synergism with modifiable risk factor associated with hemopoetic cancers, cancers of prostate, pancreas, liver and other body system [20].

The content of essential nutrients in milk makes it an excellent nutritional source for children and adult but the introduction of contaminants reduces and/or can cause changes that reduce milk quality. This brings to the front burner the need to regularly evaluate the nutritional and chemical composition of milk and dairy product for the assessment of the quality which may be lost at any possible contamination through feed stuffs and water, processing and packaging, adulteration or by environment. There are greater possibilities of feed stuff and water contamination by free/open cattle grazing practiced in Nigeria. There are many claims of nutritive value of milk by producers and marketers of brands of evaporated milk sold to the Nigerian public. Whereas nutrients and food health advocates give out warning signals concerning consumption of unwholesome and/or adulterated evaporated milk brands in Nigerian markets. It is against this background, the study was conceived to evaluate, the nutritive value and possible chemical contaminants in selected brands of evaporated milk, and to assess the potential contribution to nutritional health of consumers in Owerri, Imo State, Nigeria.

2. Materials and methods

2.1. Collection of samples

A total of fifteen samples were used. Five samples each, of the three popular brands of unsweetened evaporated milk (Table 1) consumed in Imo State South-eastern Nigeria were purchased from various outlets within the Federal Polytechnic Nekede, Owerri Imo State Nigeria. The samples were transported to the laboratory for analyses.

2.2. Determination of Proximate Composition

Proximate composition of each brand sample was determined by standard methods. The percentage moisture, ash, crude fat, crude protein, fibre and carbohydrate were determined in milk samples as described by the Association of Official Analytical Chemists (AOAC) [21]. *Briefly*; to obtain the *moisture content*, samples of evaporated milk were oven dried at 105 ^oC in moisture dish until constant weight was obtained. *For ash content*, the samples obtained from moisture content were subjected to ashing in a muffle furnace at 550 ^oC. *Crude fat* was determined by the exhaustive extraction in petroleum ether, 2.0 g of each evaporated milk sample using Soxhlet extraction apparatus. *Crude protein* of the evaporated milk brands was determined by digesting 1.0 g of the milk sample in a digestion tube containing catalyst salts (Two Kjeltabs Cu 3.5Fg). Concentrated 20 ml of H_2SO_4 was added and mildly shaken for proper mixing. After digestion, the samples were allowed to cool at room temperature and distillation was carried out. Furthermore, the distillate was titrated with 0.025 N H_2SO_4. Finally, the volume of acid used in the titration was recorded. Also, a blank was prepared without milk sample.

The percentage of protein content was calculated accordingly.

$$\% \text{ Nitrogen} = \frac{0.014 \times VD \times N \times 100 \times TV}{\text{Weight of sample} \times AD}$$

% Protein =% N x F

Where, VD = Volume of digest, N = Normality of acid, TV = Titre value, AD = Aliquot of digest, F = Conversion factor for nitrogen to protein (6.25).

Carbohydrate content obtained by calculation based on difference:

Carbohydrate =100% - (% moisture + % ash + % fat +% Fibre + % crude protein).

2.3. Determination of Vitamins

B-complex vitamins (thiamine, and riboflavin) were determined by methods described by Kirk and Sawyer. [22]. Briefly**;** One gram of sample was dissolved with 100ml of deionized water in a conical flask**.** This was shaken thoroughly and heated for 5 minutes and allowed to cool and filtered. The filtrate was poured into cuvette and their respective wavelength (B1 = 261nm and B2 = 242nm) for the vitamins set to read the absorbance using spectrophotometer. Vitamins A, E, D and C contents of the evaporated milk brands were determined using the methods described by Kirk and Sawyer. [22].

2.4. Determination of Heavy Metals, Macro- and Microminerals Content of Evaporated Milk

A portion, 2 ml of milk sample was digested [23]in 20 ml of acid mixture (650 ml Conc. HNO_3; 80 ml Perchloric acid; 20ml Conc. H_2SO_4). The setup was heated until a clear digest was obtained and this was diluted with distilled water to the 100 ml mark of a standard flask. The resulting digest was used to determine the concentration of Magnesium, potassium, sodium, calcium, phosphorus, zinc, iron, cadmium, arsenic, lead and mercury using Agilent FS240AA Atomic Absorption Spectrophotometer according to the method of APHA [24] (American Public Health Association). The samples were aspirated into the oxidising air-acetylene flame (at a sensitivity of 1% absorption).A series of standard metal solutions in the optimum concentration range were prepared by diluting the single stock element solutions with water containing 1.5 ml concentrated nitric acid/litre. A calibration blank was prepared using all the reagents except for the metal stock solutions. Calibration curve for each metal was prepared by plotting the absorbance of standards versus their concentrations.

2.5. Determination of Pesticide Residues by Gas Chromatography (GC) Analysis

Ten grams of the homogenized milk sample was mixed with 60g of anhydrous sodium sulphate in agate mortar to absorb moisture. The homogenate was delivered into a 500ml beaker and extraction was carried out with 300ml of n – hexane for 24h. The crude extract evaporated to dryness at 40^{o}C using a rotary vacuum evaporator. One milliliter (1 ml) of the filtered residue was dissolved in 50ml of chloroform. Pesticide residues were extracted by n-hexane, benzene and methanol as described by AOAC [21] and Pesticide analytical manual [25]. Aliquots of 1-2 µl of extracts of pesticide residues were injected into a Buck 530 gas chromatograph equipped with an on – column, automatic injector, Electron capture detector, HP 88 capillary column (100m x 0.25µm film thickness) CA, USA. The Buck 530 gas chromatograph was run under the following instrument conditions: Detector Temperature A: 280^{o}C, Column temperature 210^{o}C, Injector temperature 250^{o}C, and Integrator chart speed: 2cm/min. The oven temperature of the GC was set to 180^{o}C and was allowed to warm up. While warming, the following temperature condition was set thus: Initial oven temperature was 120 ^{o}C, held for 4 min and ramp for 10 min to final temperature of 180 ^{o}C. The injection and detector temperatures were 180^{o}C and 300^{o}C, respectively. Ten microliter (10µl) of accu standard was injected in the chromatography and the retention time compared with retention time of standard. Concentrations of pesticide residues in the brands of evaporated milk studied were calculated.

2.6. Statistical Analysis

Data obtained were subjected to analysis of variance (ANOVA) and least of significant difference (LSD) at $P<0.05$. Data were analyzed using SPSS 20.0 software and results were presented as mean values ± standard deviation (SD).

3. Results and Discussions

'Evaporated milk' is the commercial name for sterilized unsweetened condensed milk, i.e. fresh cows' milk from which a considerable portion of the water has been removed. The composition of evaporated milk is regulated by the Codex Alimentarius and by the legislation of individual countries, such as NAFDAC in Nigeria. In this study, fifteen samples, consisting, three commercial brands of evaporated milk were purchased to determine the proximate composition, micro- and macrominerals and vitamins composition. The result of proximate is presented in Table 2. The moisture content of 67.47± 0.68 to 69.94±1.39 %, for evaporated milk studied agrees with other studies [26,27], and that presented by Okoye and Ulasi. [28] for evaporated milk sold in Nsukka, Enugu State, Nigeria. Crude fat content of the milk brands which ranged from 2.87 ± 0.15 to 3.91 ± 0.79 % were significantly low compared to 6.5 to 7.5% reported by others [27,29]. Milk fat is a very complex natural fat, presenting about 400 different types of fatty acids [30]. Milk contains small amounts of *trans*-fats, such as vaccenic acid and conjugated linoleic acid (CLA) with considerable health effects [31]. Conjugated linoleic acid (CLA) is a family of positional and geometric isomers of linoleic acid present in the milk and meat of ruminants. CLA is associated to health-promoting activities, such as the capacity to inhibit various types of cancer, hypertension, atherosclerosis and diabetes and improve immune function and body composition. Protein content of the three brand which varied from 16.20±0.45 to 19.40 ± 0.59% were significantly higher compared to previous reports [26,27,32]. Milk provides proteins of high-quality supplying all nine essential amino acids in appropriate proportions [33]. The high protein content observed in the milk brands studied indicate great potential to confer nutritional benefits to consumers, especially boosting the immune function in this period of covid-19 pandemic. Milk proteins are of high nutritive and medicinal value with potential ingredients of health-promoting functional foods. Milk proteins have comparably same composition as the egg protein, except lower values of methionine and cystine. It is important to note that milk proteins indicate 95% of crude protein, however the other 5% represent free amino acids, small peptides and non-protein nitrogen. As an important source of essential amino acids, milk protein can suppress protein catabolism and provides substrates for gluconeogenesis [34,35]. Other protein constituents of milk include immunoglobulins, serum proteins, milk fat globule proteins, transferrin, lactoferrin, β2-microglobulin, several enzymes, peptides and proteolytic products [36,37]. Furthermore, milk

proteins (intact proteins or its derivatives) are attributed to the anticarcinogenic activities, antihypertensive properties, immune system modulation, and other metabolic features [38]. Furthermore, these evaporated milks showed significantly high content of Ash (1.89± 0.17 to 2.29 ±1.07 %), than reported previously [26,27,32]. This result suggests that PM milk with the highest ash content can act as a better source of macro- and microminerals compared to other milk brands. These minerals play essential functions for good health. Dietary minerals are essential for biological processes, such as cofactors for biochemical processes, for normal metabolism, growth and development, as constituents of complexes with various proteins, lipids, carbohydrates and low molecular weight ligands such as citrate and amino acids [39]. The three brands of evaporated milk showed significantly very low fiber compared to other solids, however, PM milk with 0.10 ± 0.02 % showed the highest fiber content. Carbohydrate content of evaporated milk brands ranged from 5.44± 2.27 to 9.03 ± 2.00 % and was within the values reported in other studies [26,40,41].Milk contains appreciable amount of carbohydrate that is predominately lactose with trace amounts of monosaccharides and oligosaccharides. Lactose plays significant role in the intestinal uptake of minerals such as calcium, magnesium and phosphorus, and in the use of vitamin D [42]. This quality makes the consumption of milk very important for the management of immune-compromised and dehydrated patients where electrolytes intake is a major management tool.

Mineral content of the selected brands of evaporated milk is presented in Table 3. The result showed concentrations of micro-minerals in evaporated milk ranged thus; Zn (1.04±0.08 - 1.22±0.09 mg/kg); Fe (0.07 ± 0.01 - 0.17± 0.02 mg/kg). These values for Zn and Fe were less than 8.12-14.55 mg/kg and 0.45- 2.3 mg/kg reported earlier for Zn and Fe respectively [28] for evaporated milk. The values obtained in this study are within the range of 0.27 - 18.77 mg/kg for Zn but less than 3.63 - 42.84 mg/kg for Fe as reported by Eleboudy et al. [43]. Holland et al. [44] reported a permissible limit of 9.00 mg/kg for Zn and 2.6 mg/kg for Fe in evaporated milk. The amount of Zn and Fe in the milk brands sampled indicates them as good sources. Adequate Zn intake is important in growth and development, immunological processes, reproductive immunity, neurological function and reproduction [45,46]. Zinc containing enzymes are vital in macronutrient metabolism and cell replication [47], while Fe is a constituent of heme, cytochromes, electron transport, and involved in activation of some enzymes. Zn and Fe are important co-factors of enzymes and are involved in numerous physiological functions. Deficiencies of these metals cause metabolic derangements, disturbances and pathological conditions [45]. Despite the essential status for health of these metals, acute and/or chronic exposure to levels above permissible limit may be hazardous. The macro-minerals such as Mg varies as 1.75± 0.06 - 1.89 ± 0.09 mg/kg and are higher than values reported in other studies [41,48]. Calcium (3.09±0.08- 4.170 ± 0.05 mg/kg) was within the range reported in other studies [41,48], but higher than 1.165-1.335 mg/kg reported by Asubiojo *et al*. [26]. Phosphorus (8.06±0.06- 9.38 ± 0.31 mg/kg) values were also appreciable. Calcium, magnesium and phosphorus supplied by dairy products are needed for bone and teeth formation and protein synthesis respectively [49]. Adequate consumption of milk and milk products will positively affect supply of calcium, which, is important in metabolic processes such as skeletal calcium retention for peak bone mass in early adulthood [50], and in the prevention of bone loss and osteoporotic fractures in elderly people [49,50,51]. However, low milk intake may have negative health implications [52]. Milk as a functional food shows that if removed from diet can lead to inadequate intake of calcium leading to osteoporosis. This is important for women over the age of 50 and the elderly, who have high calcium needs.
Potassium (K) and sodium (Na) of evaporated milk brands ranged from 5.70±0.15 to 17.06±0.72 mg/kg and 4.93±0.08 - 12.67 ± 0.29 mg/kg respectively, Olagunju et al. [53] reported, 15.287±5.54 to 17.988±4.08 mg/kg for K and 2.680±0.85 to 3.0081±0.70 mg/kg for Na. Calcium, sodium and potassium are important in membrane permeability, muscle contraction, transmission of nerve impulses and in neuromuscular excitability. Calcium is vital in enzyme (adenosine triphosphatase (ATPase), succinic dehydrogenase, lipase activation. Sodium participates in the absorptive processes of monosaccharides, amino acids, pyrimidines, and bile salts. Potassium is involved in glycogenesis and phosphate group transfer from ATP to pyruvic acid. Magnesium is important constituent of bones, teeth, and enzyme cofactor (kinases). Phosphorus content varied from 8.06±0.06 to 9.38 ± 0.31 mg/kg, and within the value (9.4 mg/kg) reported by Górska-Warsewicz et al. [52], but lower than 23.3mg/kg and 20.3 mg/kg reported Dairy UK. [54] and Diet/Fitness Today. [55] respectively. These indicate that the milk brands studied requires further fortification. Phophorus functions in the synthesis of ATP, phospholipids and phosphoproteins. Recent study showed that, milk and milk products can supply one- quarter of required phosphorus [52]. Results of previous studies indicated that milk and milk products can provide 12.3% to 32 % of phosphorus needs [56,57].

Vitamin contents of the selected brands of evaporated milk are presented in Table 4.Vitamins are involved in metabolism as co-factors, synthesis of heamoglobin and antioxidants. Vitamins are involved in metabolism and use of carbohydrates, protein, and fat. The evaporated milk brands studies showed appreciable content of fat soluble vitamins A, D, E and water soluble vitamins ascorbic acid (vitamin C), thiamin (vitamin B1), and riboflavin (vitamin B2). Vitamin A and D varied from 0.67 ± 0.10 to 1.75 ± 0.24 mg/kg and 7.30 ± 1.04 to 12.05± 1.70 mg/kg respectively, and are within the range of 0.28 to 0.61mg/kg for vitamin A and 10.0 to 13.0 mg for vitamin D reported by USDA [58] for cow milk. However, vitamin E content (3.41 ± 0.45 to 5.64± 0.39 mg/kg) of the milk brands was higher than 0.1 to 0.6 mg/kg reported by USDA [58]. Vitamin A content of the three brands of evaporated milk can contribute to daily vitamin A needs. Vitamin A is required for differentiation of epithelial structures, reproduction, vision, immune system function, stimulates thyroid gland to produce thyroid stimulating hormone (TSH) for thyroxin (T4) and triidothyronine (T3) synthesis [59]. Deficiency of vitamin A can lead to night blindness, visual impairment and growth disturbance [60]. Vitamin C ranged from 5.34± 0.34 to 6.37 ± 0.56 mg/kg. Vitamin B1 and B2 content varied non-significantly as 1.19 ± 0.10 to 1.22± 0.07 mg/kgand 1.18 ± 0.05 to 1.19± 0.02 mg/kg respectively. The

vitamin C and B1 contents of the milk brands were higher than 0.0 to 2.0 mg/kg reported by USDA [58] for cow milk. However, vitamin B2 content was within the rage of 1.30 to 1.80 mg/kg reported by USDA [58] for cow milk. Vitamin B1 (Thiamine) biological active form is thiamine pyrophosphate (TPP), acts as coenzyme to pyruvate dehydrogenase complex necessary for the conversion of pyruvate to acetyl CoA and as coenzyme to α-ketoglutarate dehydrogenase complex that converts α-ketoglutarate to succinyl CoA [61]. Similarly, vitamin B2 (riboflavin) is required for metabolic energy synthesis and as a factor in co-enzymes such as flavin mononucleotide (*FMN*) and flavin adenine dinucleotide (*FAD*) serving as hydrogen transport systems [62,63]. B-vitamins of milk especially from cow have excellent bioavailability. Low intake of B-vitamins has been shown to increase the incidence of chronic diseases, neurological impairment, and several types of cancer [2]. Vitamin C content of the three brands of evaporated milk varied significantly. Vitamin C is an excellent antioxidant and free radical scavenging nutrient, which protects cells from oxidative damage [64]. The RDA of vitamin C is 60mg/day for a male adult [65]. Therefore, adequate and judicious intake of these milk brands will provide the required daily intake.

The results of the toxic heavy metals shows that Mercury was not detected, but the concentrations of lead (Pb) in the evaporated milk samples ranged from 0.02 – 0.05 mg/kg. This Pb level is slightly outside the safe limits values of 0.02 to 0.03 mg/kg for Pb in milk as recommended by the Egyptian standard [66] and German Federal Health Agency [67] respectively. The Pb levels obtained in this study were higher than 0.001 – 0.003 mg/kg reported by Okoye and Ulasi [28] for powdered and evaporated milk sold in Nsukka, Enugu State, Nigeria. However, this amount was lower than Pb level of 0.55 mg/kg in milk reported by Zamir and Hussain [68] in Quetta (Pakistan) and 1.17 ± 0.13 mg/kg reported by Eleboudy *et al.* [43] in Alexandria, Egypt. Furthermore, the Pb level observed in this study was within required 0.02 mg/kg for Pb in milk according to the Turkish Food Codex Regulation for Contaminants [69]. Cadmium (Cd) (0.02 - 0.37 mg/kg) and Arsenic (As) (0.00 - 0.002 mg/kg) concentration obtained for the evaporated milk samples studied were below, 0.01 to 1.0 mg/kg for Cd and 0.1 to 1.0 mg/kg for Arsenic, allowed in milk as reported by the Turkish Food Codex Regulation for Contaminants [70]. According to the Turkish Food Codex Regulation for Contaminants, the limit values of the elements likely to be present in foods are given as 0.1-1 mg/kg for As, 0.01-1 mg/kg for Cd, and 0.02 mg/kg for Pb in milk [70]. Eleboudy *et al.* [43] reported 0.07 ± 0.01 mg/kg for Cd in evaporated milk and it is within range for Cd in this study but above 0.02 mg/kg for Cd recommended by the Codex Alimentarius Commission [71]. Based on the report of WHO [72] setting the permissible total weekly intake (PTWI) of Pb for man at 0.05 mg/kg body weight and 3.0 mg/person. The concentrations of Pb and Cd measured in this study which was generally lower than recommended standards are unlikely to be hazardous to the consumer of these products. However, the presence of these toxic heavy metals (Pb and Cd) calls for attention, because even at low concentrations, toxic heavy metals may trigger metabolic derangement and disorders leading to adverse health issues including but not limited to weakness, heart failure, cancer, liver and kidneys damage [73].Chronic Pb ingestion causes a disease called plumbism [74]. Similarly, exposure to heavy toxic heavy metals, affects intercellular messenger protein kinase C, and limits smooth muscle contraction [75]. A synergy of lead, cadmium, arsenic and mercury can affect male reproductive functions including sperm counts [76], motility and morphology [77] and spermatogenesis [78]. The good news here is that, due to appreciable and available amount of iron, calcium, and zinc reported for evaporated milk (Table 3), the tendency for uptake of Pb by milk consumers will be minimal. This is so because Pb absorption is indicated to be enhanced by iron, calcium, and zinc deficiencies [74,79].

Table 5 presents the toxic heavy metal content of selected brands of evaporated milk sold in Owerri, Imo State, Nigeria. Food intake present about 7% cadmium absorbed in the gastrointestinal system is taken up with food. Cadmium exposure adversely affects the liver, placenta, kidneys, lungs, brain, and bones by directly and indirectly promoting oxidative stress, depleting glutathione and altering sulfhydryl homeostasis [75,80,81]. Cadmium is an inhibitor of antioxidant enzymes [82,83], thereby increasing oxidative damage to cellular membrane and loss of membrane-bound enzymes such as ATPases [84]. The presence of cadmium in the milk can be attributed to the type and quality of cow feed stuff, because cadmium is present in insecticides, fungicides, sludge, and fertilizers used in agriculture [85].In Nigeria, open grazing of cattle is practiced, though in serious legislative and administrative contentions and logjams because of farmers and herders clashes. This open/free grazing of cattle in Nigeria predisposes the cow, an important source of raw milk to high levels of contaminants in the environment.

Table 1: Characteristic of evaporated milk samples

SAMPLE 1	SAMPLE 2	SAMPLE 3
TCM	PM	HM
1. PD: 02/2021	PD: 03/2021	PD: 26/01/2021
BB: 02/ 2022	BB: 03/ 2022	BB: 25/07/ 2021
Batch No. 2	Batch No. 1	Batch No. C 12
2. PD: 12/2020	PD: 10/2020	PD: 21/01/2020
BB: 12/ 2021	BB: 10/ 2021	BB: 20/07/ 2021
Batch No. 1	Batch No. 2	Batch No. A26
3. PD: 09/2020	PD: 02/2021	PD: 19/12/2020
BB: 09/ 2021	BB: 02/ 2022	BB: 18/06/ 2021
Batch No. 3	Batch No. 2	Batch No. 3
4. PD: 07/2020	PD: 09/2020	PD: 16/03/2021
BB: 07/ 2021	BB: 09/ 2021	BB: 15/09/ 2021
Batch No. 1	Batch No. 3	Batch No. A43
5. PD: 03/2021	PD: 02/2021	PD: 28/01/2021
BB: 03/ 2022	BB: 02/2022	BB: 27/07/ 2021
Batch No. 3	Batch No. 3	Batch No. C26

Key: Production Date (PD), Best Before (BB) date and Batch number

Table 2: Proximate Composition of Selected Brands of Evaporated Milk Sold in Owerri, Imo State, Nigeria

	Moisture	% Crude Fats	% Crude Protein	% Ash	% Crude Fibre	% Carbohydrate
TCM milk	69.94±1.39^{a}	2.87±0.15^{a}	16.20±0.45^{a}	1.89±0.17^{a}	0.05±0.01^{a}	9.03±2.00^{b}
PM milk	67.47±0.68^{a}	2.98±0.08^{a}	18.30±1.01^{a}	2.29±1.07^{c}	0.10±0.02^{b}	8.86±1.04^{c}
HM milk	69.07±0.93^{a}	3.91±0.79^{b}	19.40±0.59^{b}	2.14±0.83^{b}	0.05±0.02^{a}	5.44±2.27^{a}

Proximate composition (%) of three brands of evaporated milk consumed in Owerri. Columns represent mean ± standard deviation of quintuplet determinations. Values bearing different superscripts per column are significantly ($p<0.05$) different.

Table 3: Mineral Content of Selected Brands of Evaporated Milk Sold in Owerri, Imo State, Nigeria (mg/kg)

	Zinc	Iron	Magnesium	Calcium	Phosphorus	Potassium	Sodium
TCM Milk	1.22±0.09^{a}	0.17±0.02^{c}	1.75±0.06^{a}	3.67±0.07^{b}	9.38±0.31^{b}	6.70±0.10^{b}	6.04±0.13^{b}
PM Milk	1.04±0.08^{a}	0.14±0.02^{b}	1.88±0.12^{a}	3.09±0.08^{a}	8.99±0.13^{b}	17.06±0.72^{c}	12.67±0.29^{c}
HM Milk	1.12±0.09^{a}	0.07±0.01^{a}	1.89±0.09^{a}	4.17±0.05^{c}	8.06±0.06^{a}	5.70±0.15^{a}	4.93±0.08^{a}

Mineral composition (mg/kg) of three brands of evaporated milk consumed in Owerri. Columns represent mean ± standard deviation of quintuplet determinations. Values bearing different superscripts per column are significantly ($p<0.05$) different

Table 4: Vitamin Content of Selected Brands of Evaporated Milk Sold in Owerri, Imo State, Nigeria (mg/kg)

	Vitamin A	Vitamin D	Vitamin E	Vitamin C	Vitamin B1	Vitamin B2
TCM Milk	1.75±0.24^{b}	7.30±1.04^{a}	5.64±0.39^{b}	6.37±0.56^{b}	1.22±0.07^{a}	1.19±0.02^{a}
PM Milk	0.94±0.07^{a}	12.05±1.70^{b}	5.01±0.12^{b}	5.34±0.34^{a}	1.21±0.13^{a}	1.18±0.02^{a}
HM Milk	0.67±0.10^{a}	9.41±0.56^{a}	3.41±0.45^{a}	5.84±0.15^{ab}	1.19±0.10^{a}	1.18±0.05^{a}

Vitamin composition (mg/kg) of three brands of evaporated milk consumed in Owerri. Columns represent mean ± standard deviation of quintuplet determinations. Values bearing different superscripts per column are significantly ($p<0.05$) different.

Table 5: Toxic Heavy metal Content of Selected Brands of Evaporated Milk Sold in Owerri, Imo State, Nigeria

	Lead	Cadmium	Arsenic	Mercury
TCM Milk	0.05±0.004^{b}	0.37±0.03^{b}	0	ND
PM Milk	0.03±0.001^{b}	0.05±0.01^{a}	0.002	ND
HM Milk	0.02±0.003^{a}	0.02±0.01^{a}	0	ND
Range	0.02- 0.05	0.02- 0.37	0 - 0.002	ND

ND = Not detected

Table 6: Summary of Residual Pesticides in Evaporated Milk Brands Sold in Owerri, Imo State, Nigeria

Sample A Component	Retention	Area	Height	External (mg/kg) A
Isopropylamine	0.283	4135.003	167.246	0.1909
HCB	5.353	4319.453	161.35	0.097
g-chlordane	18.516	7898.905	199.251	0.1823
Biphenyl	29.28	4753.193	177.528	0.0187
Total				0.4889
Sample B Component	**Retention**	**Area**	**Height**	**External (mg/kg)**
DichloroBiphenyl	3.086	15.639	0.226	0.000
DDVP	34.716	30.894	0.467	0.0006
Isopropylamine	0.026	3278.459	0.462	0.1513
HCB	6.903	18.074	0.239	0.0004
g-chlordane	18.19	5352.544	420.034	0.1236
Heptachlor	37.32	12.153	0.389	0.000
Biphenyl	29.963	3620.852	279.777	0.0143
Aldrin	10.260	2954.141	219.934	0.0543
Total				0.3445
Sample C Component	**Retention**	**Area**	**Height**	**External (mg/kg)**
DichloroBiphnyl	3.630	3161.531	26.358	0.0099
Isopropylamine	0.866	43.598	0.777	0.002
Endosulfan	8/983	20.493	0.028	0.0005
t-nonachlor	41.94	3723.89	29.485	0.0685
Glyphosate	19.94	4508.064	35.60	0.0933
DDT	28.72	3818.805	29.686	0.000
Total				0.1742

The toxic heavy metals and pesticides (being lipophilic) accumulate in mammary organs serving as a major route for human exposure. Furthermore, non-compliance in the application of industrial tools and equipment with standard for dairy products manufacturing are significant in the exposure of milk to heavy metal contamination. Therefore, the near absence of arsenic (present in only one of the brand) and mercury not detected in any of the brands studied indicate good laboratory practice by the manufacturers of these milk brands. Results of residues of pesticides assessed in the evaporated milk brands showed varying concentrations of Organochlorine pesticides (Table 6) such as: Lindane, DichloroBiphenyl, DDT, DDVP, t-nonachlor, Isopropylamine, HCB, Endosulfan, g-chlordane, p'p'-DDD, Carbofuran, Dichlorvos, Heptachlor, Biphenyl, and Aldrin; and orgnophosphates pesticides which include; Profenofos and Glyphosate. Summary of total residue concentration of pesticide in the three brands of evaporated milk studied were 0.1742 mg/kg, 0,3445 mg/kg and 0.4889 mg/kg for HM milk, PM milk and TCM milk respectively. The lipophilic nature of organochlorine pesticide residues enhances their concentration in milk fat, thereby making organochlorine pesticides metabolism highly restricted [86]. The organochlorine pesticide residue, g-chlordane at concentrations of 0.1236 to 0.1823 mg/kg obtained in this study was less than 0.0232 mg/kg reported by Maitre *et al*., [87], but above 0.022 mg/kg reported by Abou-Arab *et al.* [88] and exceeds the recommended tolerance levels of 0.002 mg/kg by FAO/WHO [89]. HCB residues (0.0004 - 0.097 mg/kg) were within the range for Ultra heat-treated milk reported by Abou-Arab *et al.*[88] and for pasteurized milk (0.0068 mg/kg) by Maitre *et al*. [87]. Also, PM evaporated milk brand was contaminated by aldrin at a concentration of 0.0543 mg/kg and this is within the value (0.0048 mg/kg) reported by Abou-Arab *et al.* [88]. The concentration of aldrin residues (0.0543 mg/kg) was higher than the aldrin value (0.0388 mg/kg) reported by Maitre *et al*., [87] (1994) in pasteurized milk analyzed in Argentina and above 0.006 mg/kg tolerance levels recommended for aldrin by FAO/WHO [89]. Studies have shown that pasteurization and ultra heat treatment (UHT) of milk degrades and/or eliminates residues of pesticides in milk. Rachev *et al*. [90] and Abou-Arab *et al.* [91], reported heat treatment as an efficient degradation process of residual pesticides in milk.

It is important to state that, of all organophosphorus pesticides measured (glyphosate, malathion, profenofos) only one evaporated milk brand presented, glyphosate at 0.0933 mg/kg. These results could be attributed to ease of conversion of organophosphorus pesticides to other compounds in exposed cow and subsequent elimination of the chemicals in urine and feces [92]. Heat treatment during pasteurization may also lead to their degradation. It is interesting to state that DDT was not detected in any of the evaporated milk brands sold in Owerri Nigeria. However, previous report indicated that 3.83 mg/kg DDT was detected in raw milk in Nigeria [93]. The presence of organochlorine pesticides in milk above its maximum residue limits (MRL) is viewed with serious concern. Pesticides levels obtained in this study showed limited pesticide contamination and, in most cases, not detected. This may be attributed firstly to the state of the raw milk used and good industrial practice which included efficient heat treatment.

4. Conclusions

This study revealed that the three brands of evaporated milk are nutrient-dense foods capable of supplying energy and significant amounts of protein and micronutrients. The concentrations of toxic heavy metals and pesticides residues were mostly below the safe limits adopted from international food standards. By these results, evaporated milk sold in Owerri can be said to be relatively safe with respect to toxic heavy metals and pesticide content. These findings contribute to the existing knowledge in the area of nutrition and functional food research. Furthermore, it establishes the role of milk and dairy products in providing essential and nonessential nutrients consumers, despite the below average intake by Nigerians. Also, this study has shown the need for continuous and regular monitoring of these foods to confirm food safety in order to eliminate any potential risk to human health. These findings may be relevant in developing dietary guidelines on the quantity of milk consumption so as to increase the population size consuming milk and to achieve better nutritional and health status of the population.

Author's disclosure statement: We wish to confirm that no competing financial or non-financial interests exist between the Authors and any other people or institution.

Competing Interests: The authors declare that there are no conflicts of interest.

Author's Contributions*:* All authors contributed to this study and the writing of the paper.

References

[1] D.K. Sharmaand and D.V. Joshi (1992). Bacteriological Quality of Milk and Milk Products with Special Reference to *Salmonella* and its Public Health Significance. Journal of Science & Technology.22: 100 –103.

[2] G. Benoît and C.L. Girard (2017). Chapter 15 - B Vitamins in Cow Milk: Their Relevance to Human Health, Editor(s): Ronald Ross Watson, Robert J. Collier, Victor R. Preedy, Dairy in Human Health and Disease Across the Lifespan Academic Press 211-224.

[3] K, Naresh, R. Midathala, T. Jayanti and R.S. Hari (2017) Chapter 24 - Milk Proteins: Precursors of Antioxidative Peptides and Their Health Benefits, Editor(s): Ronald Ross Watson, Robert J. Collier, Victor R. Preedy, Dairy in Human Health and Disease Across the Lifespan. Academic Press 313-323.

[4] C.M. Skeaff and J. Miller (2009). Dietary Fat and Coronary Heart Disease: Summary of Evidence from Prospective Cohort and Randomised Controlled Trials. Annals of Nutrition & Metabolism.55:173–201. doi: 10.1159/000229002.

[5] J.P. Drouin-Chartier, D. Brassard, M. Tessier-Grenier, M.È. CôtéJA,Labonté, S. Desroches, P. Couture and B. Lamarche (2016). Systematic Review of the Association Between Dairy Product Consumption and Risk of Cardiovascular-Related Clinical Outcomes. Advances in Nutrition. 7:1026–40.

[6] R. Lordan and I. Zabetakis (2017). Invited Review: The Anti-Inflammatory Properties of Dairy Lipids. Journal of Dairy Science. 100:4197–4212. doi: 10.3168/jds.2016-12224.

[7] K. Lee and W. Cho (2017). The Consumption of Dairy Products is Associated with Reduced Risks of Obesity and Metabolic Syndrome in Korean Women but not in Men. Nutrients. 9:630. doi: 10.3390/nu9060630.

[8] M. Dehghan, A. Mente, X. Zhang, S. Swaminathan, W. Li, V. Mohan, R. Iqbal, R. Kumar, E. Wentzel-Viljoen and A.Rosengren et al (2017). Associations of Fats and Carbohydrate Intake with Cardiovascular Disease and Mortality in 18 Countries from Five Continents (pure): A Prospective Cohort Study. Lancet. 390:2050–2062. doi: 10.1016/S0140-6736(17)32252-3

[9] R. Lordan, A. Tsoupras, B. Mitra and I. Zabetakis (2018). Dairy Fats and Cardiovascular Disease: Do We Really Need to be Concerned?. Foods. 7(3):29. doi:10.3390/foods7030029

[10] A. Giosuè, I. Calabrese, M. Vitale, G. Riccardi and O. Vaccaro (2022). Consumption of Dairy Foods and Cardiovascular Disease: A Systematic Review. Nutrients. 14, 831. https://doi.org/10.3390/nu14040831

[11] S.M.Waliszewski, V.T.Pardio, K.N.Waliszewski, J.M.Chantiri, A.A.Aguirre, R.M. Infanzon and J. Rivera (1997).Organochlorine Pesticide Residues in Cow's Milk and Butter in Mexico. Science of The Total Environment. 208, 127-132.

[12] A. Abdulkhaliq, K.M. Swaileh, R.M. Hussein and M. Matani (2012). Levels of Metals (Cd, Pb, Cu and Fe) in Cow's Milk, Dairy Products and Hen's Eggs from the West Bank, Palestine. International Food Research Journal. 19 (3): 1089-1094

[13] A.F. Holleman and E. Wiberg (2001).Inorganic Chemistry, San Diego Academic Press.

[14] M. Carl (1991). Heavy Metals and other Trace Elements. Monograph on Residues and Contaminants in Milk and Milk Products. Special Issue. 9101: 112-119.

[15] I.A. Okada, A.M. Sakuma, F.D. Maio, S. Dovidauskas and O. Zenebon (1997). Evaluation of Lead and Cadmium Levels in Milk due to Environmental Contamination in the Paraiba Valley Region of Southeastern Brazil. Journal of Public Health. 31(2): 140-143

[16] A. Oskarsson, I.P. Hallen and J. Sundberg (1995). Exposure to Toxic Elements via Breast Milk. Analyst. 120: 765- 770.

[17] M.M. El-Batanouni and G. Abo El-Ata (1996). Metals in Food. Proceedings of Food Born Contamination and Egyptian's Health Conference. 11-25. Mansoura, Egypt.

[18] S.M. Waliszewski, V.T. Pardio, K.N. Waliszewski, J.M. Chantiri, A.A. Aguirre, R.M. Infanzon and J. Rivera (1997). Organochlorine Pesticide Residues in Cow's Milk and Butter in Mexico. Science of The Total Environment.208, 127-132.

[19] E. Sitarska, W. Klucinski, A. Winnicka and J. Ludwicki (1991). Residues of Organochlorine Pesticides in Milk Gland Secretions of Cows in Prenatal Period. Bulletin of Environmental Contamination and Toxicology. 47: 817-821.

[20] K. Jaga and C. Dharmani (2005). The Epidemiology of Pesticide Exposure and Cancer: A review. Rev Environ Health. 20(1): 15-38.

[21] AOAC (1990). Official Methods of Analysis of the Association of Analytical Chemists, Washington D.C.

[22] R.S. Kirk and Sowyer (1991). Pearsons Food Composition and Analysis (4th Edition). Macmillan Publisher Company United Kingdom.

[23] W.J. Adrian (1973). A Comparison of a Wet Pressure Digestion Method with other Commonly used Wet and Dry-Ashing Methods. Analyst. 98(1164): 213-216ISSN/ISBN: 0003-2654 doi: 10.1039/an9739800213

[24] APHA (1995).Standard Methods for the Examination of Water and WastewaterAmerican Public Health Association, Washington USA.

[25] Pesticide Analytical Manual (1991). Pesticide Analytical Manual, Vol. 1, Multiresidue Method, Food and Feed. U.S. Department of Health and Human Services. Public Health Service, Food and Drug Administration.

[26] O.I. Asubiojo, F.M. Adebiyi, A.A. Adenipekun and J.G. Ayenimo (2007). Proximate Analysis and Determination of Some Physicochemical Characteristics of Evaporated Milks. Ife J Sci. 9(1): 109-113.

[27] N. Faiza, O. Mariam, A. Amal, E. Ali, Y. Manal, A. Amira and Y. Kholoud (2017). Comparison of the Nutritive Values of Different Types of Evaporated Milk Available in Local Marketing in Benghazi City, Libya. Sch J App Med Sci. 5(6B):2188-2197

[28] C.O.B. Okoye and C.U. Ulasi (2009).Levels of Essential and Toxic Metals in Milk and Baked Products. Bio-Research. 7(1): 474 – 476

[29] V. Sikand, P.S. Tong and Walker (2010). Heat Stability of Reconstituted, Protein-Standardized Skim Milk Powders. J Dairy Sci. 93(12):5561-71.

[30] H.L. Månsson (2008). Fatty Acids in Bovine Milk Fat. Food & Nutrition Research. 52, 10.3402/fnr.v52i0.1821. https://doi.org/10.3402/fnr.v52i0.1821

[31] K. Koba and T. Yanagita (2014). Health Benefits of Conjugated Linoleic Acid (CLA). Obesity Research & Clinical Practice. 8:e525–32. doi: 10.1016/j.orcp.2013.10.001

[32] A.A.K. Abou-Arab, M.A. AbouDonia and A. Enb (2008). Chemical Composition, Metals Content and Pesticide Residues in Raw, Pasteurized and UHT Milk and Their Dietary Intake. Journal of the Egyptian Society of Toxicology. 39: 111-121

[33] G.D. Miller, J.K. Jarvis and L.D. McBean (2007). Handbook of Dairy Foods and Nutrition. 3rd ed. Boca Raton: CRC press, Taylor & Francis group. 1–55

[34] P.C. Pereira (2014). Milk Nutritional Composition and its Role in Human Health. Nutrition. 30: 619–627.

[35] M. Zhou, L. Xu, F. Zhao and H. Liu (2021). Regulation of Milk Protein Synthesis by Free and Peptide-Bound Amino Acids in Dairy Cows. Biology. 10(10), 1044; https://doi.org/10.3390/biology10101044

[36] P.F. Fox, T.P. Guinee, T.M. Cogan and P.L.H. McSweeney (2017). Chemistry of Milk Constituents. In Fundamentals of Cheese Science; Springer: Boston, MA, USA. 71–104.

[37] N. Landi, S. Ragucci and A. Di Maro (2021). Amino Acid Composition of Milk from Cow, Sheep and Goat Raised in Ailano and Valle Agricola, Two Localities of 'Alto Casertano' (Campania Region). Foods. 10:2431. https://doi.org/10.3390/ foods10102431

[38] S.H. Davoodi, R. Shahbazi, S. Esmaeili, S. Sohrabvandi, A. Mortazavian, S. Jazayeri and A. Taslimi (2016). Health-Related Aspects of Milk Proteins. Iranian Journal of Pharmaceutical Research. 15(3), 573–591.

[39] C. Sola-Larrańaga and I. Navarro-Blasco (2009). Chemometric Analysis of Minerals and Trace Elements in Raw Cow Milk from the Community of Navarra, Spain. Food Chemistry.112, 189-196.

[40] P.F. Fox (1997).Lactose, Water, Salts and Vitamins Advanced Dairy Chemistry, 2nd Edn, Vol 3, Chapman & Hall, London. 12p.

[41] N.P. Wong (1998). Fundamental of Dairy Chemistry, 3rdEdn,39-75

[42] Y.W. Park, M. Juárez, M. Ramos and G.F.W. Haenlein*(*2007).Physico-chemical Characteristics of Goat and Sheep Milk. Small Ruminant Research. 68(1–2): 88–113.

[43] A.A. Eleboudy, A.A. Amer, H.S. Abo El-Makarem and H.H. Abo Hadour (2016). Heavy Metals Residues in Some Dairy Products. Alexandria Journal of Veterinary Science. 51: 334-346 doi: 10.5455/ajvs.230723.

[44] B. Holland, A.A. Welch, I.D. Unwin, D.H. Buss, A.A. Paul and D.A.T. Southgate (1995). Composition of Foods, 5th Edn. London: Royal Society of Chemistry and Ministry of Agriculture, Fisheries and Food

[45] C.O. Ujowundu, F.N. Kalu, E.C. Nwosunjoku, R.N. Nwaoguikpe, R.I. Okechukwu and K.O. Igwe (2011). Iodine and Inorganic Mineral Contents of some Vegetables, Spices and Grains Consumed in Southeastern Nigeria. African Journal of Biochemistry Research. 5(2):57-64.

[46] A.M. Rangan and S. Samman (2012). Zinc Intake and its Dietary Sources: Results of the 2007 Australian National Children's Nutrition and Physical Activity Survey. Nutrients. 4, 611–624

[47] O.G. Arinola (2008). Essential Trace Elements and Metal Binding Proteins in Nigerian Consumers of Alcoholic Beverages. Pakistan Journal of Nutrition. 7(6): 763-765.

[48] F. Harding (1995). Milk Quality, Chapman and Hall 1st Edn,75-161

[49] R. Rizzoli (2014). Dairy Products, Yogurts, and Bone Health. American Journal Clinical Nutrition. 99:1256S–1262S. doi: 10.3945/ajcn.113.073056.

[50] K. Zhu and R.L. Prince (2012). Calcium and Bone. Clinical Biochemistry. 45:936–942. doi: 10.1016/j.clinbiochem.2012.05.006.

[51] Y.W. Park (2009). Introduction: Overview of Bioactive Components in Milk and Dairy Products. In: Park Y.W., Editor. Bioactive Components in Milk and Dairy Products. Publishers, Wiley-Blackwell; Ames, Iowa: Oxford, UK.. 3–14.

[52] H. Górska-Warsewicz, K.Rejman, W. Laskowski and M. Czeczotko (2019). Milk and Dairy Products and Their Nutritional Contribution to the Average Polish Diet. Nutrients. 11(8):1771. doi: 10.3390/nu11081771.

[53] A. Olagunju, A. Muhammad, S. Aliyu, S.B. Mada, R. Isah, S.A. Abdullahi and Z.O. Audu (2013). Nutritional Values of Powdered Milk Commercially Consumed in West Africa. International Journal of Food Nutrition & Safety. 4(2): 55-61.

[54] U.K. Dairy (2022). The Nutritional Composition of Dairy Products. nutrition@dairyuk.orgAccessed on March 3, 2022, https://milk.co.uk/nutritional-composition-of-dairy/milk/#evaporatedmilklight. Last reviewed: 01/2021

[55] Diet and Fitness Today (2022). Amount of Phosphorus in Milk Accessed on March 3, 2022 http://www.dietandfitnesstoday.com/phosphorus-in-milk.php.

[56] M.J. Tijhuis, J. Ezendam, S. Westenbrink, C. Van-Rossum and L.Temme(2011). Replacement of Meat and Dairy by More Sustainable Protein Sources in The Netherlands Quality of the Diet. National Institute for Public Health and the Environment, Ministry of Health, Welfare and Sport; Bilthoven, Denmark.

[57] C.E. O'Neil, D.R. Keast, V.L. Fulgoni and T.A. Nicklas (2012). Food Sources of Energy and Nutrients Among Adults in the US: NHANES 2003-2006. Nutrients. 4:2097–2120.

[58] United States Dept. of Agriculture (USDA) (2006). Nutrient Database. Version 18. Accessed October 2006. http://www.nal.usda.gov/fnic/foodcomp/Data

[59] C.O. Ujowundu, R.N. Nwaoguikpe, D.C. Belonwu, F.N. Ujowundu and K.O. Igwe (2013). Effects of Roasted Beef Based-Diet on Thyroid Hormones and Vitamins: the Biochemical Roles of *Lycopersicon esculentum* L. and *Allium cepa*. Nigerian Journal of Biochemistry & Molecule Biology. 28(1&2): 59-69. ISSN: 0189-4757/96.

[60] C.O. Ujowundu, L.A. Nwaogu, F.N. Ujowundu and D.C. Belonwu (2013):Effect of Gas Flaring on the Phytochemical and Nutritional Composition of *Treculia africana*and *Vigna subterranean*. British Biotechnology Journal. 3(3): 293-304. ISSN: 2231-2927. doi : 10.9734/BBJ/2013/3033

[61] F.N. Ujowundu, A.I. Ukoha, A.O. Ojiako and R.N. Nwaoguikpe (2015): Nutritional Characterization of *Combretum dolichopentalum* Leaves. Biochemistry & Analytical Biochemistry. 4: 232.doi:10.4172/2161-1009.1000232. ISSN: 2161-1009

[62] C.O. Ujowundu, C.U. Igwe, V.H.A. Enemor, L.A. Nwaogu and O.E. Okafor(2008). Nutritional and

Anti-Nutritive Properties of *Boerhavia diffusa* and *Commelina nudiflora* Leaves. Pakistan Journal of Nutrition. 7(1): 90-92. DOI:https://dx.doi.org/10.3923/pjn.2008.90.92

[63] S. Mosegaard, G. Dipace, P. Bross, J. Carlsen, N. Gregersen and R.K.J Olsen (2020). Riboflavin Deficiency—Implications for General Human Health and Inborn Errors of Metabolism. International Journal of Molecular Sciences. 21(11):3847. https://doi.org/10.3390/ijms21113847

[64] C.O. Ujowundu, L.A. Nwaogu, K.O. Igwe, F.N. Ujowundu and D.C. Belonwu (2013). Evaluation of the Impact of Oil and Gas Pollutants on the Chemical Composition of *Abelmoschus esculentus Moench* and *Pterocarpus mildbraedii Harms.* Journal of Research in Biology. 3(3): 8611-869, ISSN: 2231 – 6280, EISSN: 2231-6299.http://jresearchbiology.com/documents/RA0327.pdf

[65] W.F. Ganong (2003).*Review of Medical Physiology*, 21st edn. Macmillan Hill Companies Inc, New York. 316-318 & 514.

[66] Egyptian Standard. Maximum Levels of Heavy Metal Contaminants in Food (2010). Egyptian Organization for Standardization. No. 7136.

[67] E. Merian (1991). Metals and their Compound in the Environment – Occurrence Analysis and Biological Relevance, VCH, Weiheim.704p.

[68] T. Zamir and A. Hassain (2001). Determination of Lead and Cadmium Level in Powdered Milk in Quetta (Pakistan) by Atomic Absorption Spectrometry. Online Journal of Biological Sciences. 1 [5]: 412 – 413.

[69] A.M.S. Meshref, W.A. Moselhy and NE-HY Hassan (2014). Heavy Metals and Trace Elements levels in Milk and Milk Products. Food Measure. 8:381–388. doi 10.1007/s11694-014-9203-6

[70] M.S. Seğmenoğlu and E. Baydan (2021). Comparison of Heavy Metal Levels of Organic and Conventional Milk and Milk Products in Turkey. Turkish Journal of Agriculture - Food Science and Technology. 9(4): 696-700, doi: https://doi.org/10.24925/turjaf.v9i4.696-700.4007

[71] Codex Alimentarius Commission (2004). Joint FAO/WHO Food Standard Programme Codex Committee on Milk and Milk Products, Auckland, Newzealand.

[72] WHO (1972). World Health Organization. Evaluation of Certain food Additives WHO Tech. Rep. Ser. 505, Geneva.

[73] P. Licata, D. Trombeta, M. Cristiano, F. Giofrè, D. Martino, M. Calò and F. Naccari (2004). Levels of "Toxic" and "Essential" Metal in Samples of Bovine Milk from Various Dairy Farms in Calabria, Italy. Environment International.30: 1-6.

[74] C.O. Ujowundu, C.O. Ajoku, L.A. Nwaogu, D.C. Belonwu and K.O. Igwe (2014).Toxicological Impact of Gas flaring and Other Petroleum Production Activities in the Niger Delta Area of Nigeria. Journal of Advances in Chemistry. 10 (2): 2297-2304. doi.org/10.24297/jac.v10i2.5496

[75] C.O. Ujowundu, K.L. Ihekweazu, C.S. Alisi, F.N. Ujowundu and C.U. Igwe (2014). Procarcinogens: Polycyclic Aromatic Hydrocarbons and Heavy Metal Content in Some Locally Processed Foods in South Eastern Nigeria. Current Journal of Applied Science and Technology. 4(1). 249-260https://doi.org/10.9734/BJAST/2014/4668.

[76] S.E. Chia, C.N. Ong, S.T. Lee and F.H. Tsakok (1992). Blood Concentrations of Lead, Cadmium, Mercury, Zinc and Copper and Human Semen Parameters. Archives of Andrology..29: 177-183.

[77] J.D. Meeker, M.G. Rossano, B. Protas, M.P. Diamond, E. Puscheck, D. Daly, N. Paneth and J.J. Wirth (2008). Cadmium, Lead and other Metals in Relation to Semen Quality: Human Evidence for Molybdenum as a Male Reproductive Toxicant. Environ. Health Perspectives.116: 1473-1479.

[78] S. Telisman, B. Colak, A. Pizent, J. Jurasovic and P. Cvitkovic (2007). Reproductive Toxicity of Low-Level Lead Exposure in Men. Environmental Research.105: 256-266.

[79] M. Slota, M. Wąsik, T. Stoltny, A. Machoń-Grecka, A. Kasperczyk, F. Bellanti, M. Dobrakowski, A. Chwalba, and S. Kasperczyk (2021). Relationship between lead absorption and iron status and its association with oxidative stress markers in lead-exposed workers, Journal of Trace Elements in Medicine and Biology. 68:126841, https://doi.org/10.1016/j.jtemb.2021.12684.

[80] The Agency for Toxic Substances and Disease Registry (ATSDR) (1999). Study on Combined Effects of Organophosphates and Heavy Metals in Bird; Toxicological Profile for Chlorpyrifos.40p.

[81] E.M. Alissa and G.A. Fern (2011).Heavy Metal Poisoning and Cardiovascular Disease. Journal of toxicology. 870125:1-21. https://doi.org/10.1155/2011/870125

[82] S.O. Asagba and G.E. Eriyamremu (2007). Oral Cadmium Exposure Alters Haematological and Liver Function Parameters of Rats Fed a Nigerian-Like Diet. Journal of Nutritional & Environmental Medicine. 16(3–4): 267–274.

[83] S. Chater, T. Douki, A. Favier, M. Sakly and H. Abdelmelek (2009). Changes Inantioxidant Status and Biochemical Parameters after Oral Cadmium Administration in Female Rats. Acta. Biol. Hung. 60(1): 79-88.

[84] M. Galazyn-Sidorczuk, M.M. Brzoska, M. Jurczuk and J. Moniuszk-Jaconiuk (2009). Oxidative Damage to Proteins and DNA in Rats Exposed to Cadmium and or Ethanol. Chem. Biol. Interac. 180(1): 31-38

[85] C.O. Ujowundu, K.I. Ogamanya, F.N. Ujowundu, V.O. Adejoh, C.I. Iheme and K.O. Igwe (2020). Biochemical and Liver Histological Changes in Rats Exposed to Sub-Lethal Dose of Uproot-Pesticide and the Protective Potentials of Nutritional Supplements. Journal of Applied Biology & Biotechnology. 8(04): 26-32

[86] J. Falandysz, B. Wyrzkowska, J. Warzocha, I. Barska, A. Garbacik Wesolowska and P. Szefer (2004). Organochlorine Pesticides and PCBs in Perch *Perca fluviatilis* from the Odra/Oder River Estuary, Baltic Sea. Food Chemistry. 87: 17-23.

[87] M.I. Maitre, P. Sierra, A. Lenardon, S. Enrique and F. Marino (1994). Pesticide Residue Levels in Argentinean pasteurized Milk. Science of The Total Environment. 155: 105-108.

[88] A.A.K. Abou-Arab, M.A. AbouDonia and A. Enb (2008). Chemical Composition, Metals Content and Pesticide Residues in Raw, Pasteurized and UHT Milk and Their Dietary Intake. Journal of Egyptian Society of Toxicology. 39: 111-121.

[89] FAO/WHO (1993). Food Standards Programme. Codex Alimentaries, Pesticide residues in food.

[90] R. Rachev, L. Lotov and D. Sergeeva (1974). Attempts to Reduce Contents of Organochlorine Pesticides in Milk and Milk Products. Dairy Science. 37:197.

[91] A.A.K. Abou-Arab (1999). Effect of Processing and Storage of Dairy Products on Lindane Residues and its Metabolites. Food Chem. 64: 467-473.

[92] IDF (1979). International Dairy Federation Bulletin, Chemical Residues in milk and milk products. I.D.F. Document. 133.

[93] FAO (1986). International Code of Conduct on the Distribution and Use of Pesticide, Food and Agriculture Organization of the United Nations, Rome. 28p.