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Evaluation of lead and cobalt residues in locally processed yoghurt from native dairy cattle raised under traditional system of management

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Abstract

Heavy metals are partitioned into two different groups of essential (non-toxic) and non-essential (toxic) elements. The non-toxic heavy metals are of essential health and nutritional benefits to animals and humans. The clinical consequences of non-essential heavy metals if ingested even in low doses are grave. This study was the concluding part of our previous works on the detection of drug residues and bacterial contaminations of locally processed yoghurt sold for human consumption in Jigawa State of Northern Nigeria. In this present study, we attempted to evaluate the presence of Lead (Pb) and Cobalt (Co) heavy metal residues in samples of locally processed yoghurt hawked for human consumption in selected parts of Jigawa State, Nigeria. About 10 mL each of four samples of locally processed yoghurt samples were randomly collected from each of five collection centers/points in the morning from large containers into sterilized collection tubes and cooled and transported in iced pack box for laboratory analyses. The evaluation of heavy metal residues was performed using high performance liquid chromatography (HPLC)-based assay. We presented from our results that sample of locally processed yoghurt collected from Kiri had the highest Cobalt (Co) residue (75.18 mg/kg) followed by Majiyawa (65.84 mg/kg) both of which were from Ringim dairy collection center while Dakayyawa, Andaza and Balbadu dairy collection centers had Co residue-free yoghurt. Majority of the locally processed yoghurt samples collected from the study area had elevated Pb contamination levels which were far higher than those collected from Rumbawa (0.00 mg/kg), Dakayyawa (0.00 mg/kg), Kyambo (0.02 mg/kg), Dangyati (0.02 mg/kg) and Andaza (0.02 mg/kg) dairy collection centers/points. Our results suggested that Pb contamination levels detected in the study area is clinically unsafe and unhygienic for human consumption and it is of grave consequences to public health. Therefore, urgent action is needed to be taken by appropriate agency of government to address this health threat to the consumers' safety of dairy products in the study area.

Keywords: Yoghurt, Dairy Cattle, Milk, Milk products, Heavy metals

Une Évaluation des résidus de plomb et de cobalt dans les yaourts transformés localement provenant de bovins laitiers indigènes élevés dans le cadre d'un système de gestion traditionnel

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R**ésumé**

Les métaux lourds sont répartis en deux groupes différents d'éléments essentiels (non

toxiques) et non essentiels (toxiques). Les métaux lourds non toxiques présentent des avantages essentiels pour la santé et la nutrition des animaux et des humains. Les conséquences cliniques des métaux lourds non essentiels en cas d'ingestion même à dosesfaibles sont graves. Cette étude était la conclusion de nos travaux antérieurs sur la détection des résidus de médicaments et des contaminations bactériennes de yaourts transformés localement vendus pour la consommation humaine dans l'État de Jigawa au nord du Nigéria. Dans l'étude actuelle, nous avons tenté d'évaluer la présence de résidus de métaux lourds de plomb (Pb) et de cobalt (Co) dans des échantillons de yaourt transformé localement destiné à la consommation humaine dans certaines régions de l'État de Jigawa, au Nigéria. Environ 10 ml de chacun des quatre échantillons d'échantillons de yaourt transformés localement ont été prélevés au hasard dans chacun des cinq centres / points de collecte le matin à partir de grands conteneurs dans des tubes de collecte stérilisés et refroidis et transportés dans une boîte d'emballage glacée pour les analyses de laboratoire. L'évaluation des résidus de métaux lourds a été réalisée en utilisant un test basé sur la chromatographie liquide à haute performance (HPLC). Nous avons présenté à partir de nos résultats que l'échantillon de yaourt transformé localement recueilli à Kiri contenait le résidu de cobalt (Co) le plus élevé (75,18 mg / kg) suivi de Majiyawa (65,84 mg / kg), tous deux provenant du centre de collecte laitière de Ringim tandis que Dakayyawa, Andaza et les centres de collecte des produits laitiers de Balbadu disposaient de yaourt sans résidu de Co. La majorité des échantillons de yaourt transformés localement prélevés dans la zone d'étude présentaient des niveaux de contamination élevés au plomb qui étaient bien supérieurs à ceux recueillis à Rumbawa (0,00 mg / kg), Dakayyawa (0,00 mg / kg), Kyambo (0,02 mg / kg), Dangyati Centres / points de collecte laitière (0,02 mg / kg) et Andaza (0,02 mg / kg). Nos résultats suggèrent que les niveaux de contamination au plomb détectés dans la zone d'étude sont cliniquement dangereux et insalubres pour la consommation humaine et ont de graves conséquences pour la santé publique. Par conséquent, des mesures urgentes doivent être prises par l'agence gouvernementale appropriée pour faire face à cette menace pour la santé vis-à-vis la sécurité des consommateurs des produits laitiers dans la zone d'étude.

Mots clés : Yaourt, Bovins laitiers, Lait, Produits laitiers, Métaux lourds

Introduction

Yoghurt and other dairy products are of animal by-products, they supply man with readily available protein both in rural and urban areas where hawking of dairy products through informal channel are source of income (Onasanya *et al.*, 2019a). Locally processed yoghurt is easily available because it is relatively cheap and affordable in Nigeria especially in communities with local cattle herders (Onasanya *et al.,* 2019a; FAO, 2013). In Africa, Milk and Milk products have been reported to have been contaminated with elevated levels of heavy metals contaminants (Meshref *et al.*, 2014). Milk and dairy products are staple food which form a vital part of human protein source both in rural and urban areas. They are very important sources of essential nutrients for daily improvement of human health (Dikko *et al.,* 2010). Milk and dairy products are critical for poverty alleviation among millions of people especially in rural communities (FAO, 2013) especially in Africa. However, hazardous chemicals and contaminants in form of toxic heavy metals are found in milk and other dairy products, some of which constitute a wide range of public health challenges when consumed by unsuspecting members of the general public (Licata *et al.,* 2004). According to Meshref *et al.* (2014), heavy metals contaminants in milk and dairy products

can be partitioned into essential elements for example; Iron, Copper, Zinc etc. but at low doses and non- essential or toxic ones e.g. Lead, Cadmium, Ascenic among others (Meshref *et al.*, 2014). These essentials elements are capable of causing cascades of nutritional and/or health disorders and imbalance when ingested in disproportionate doses. The presence of toxic and non-essential heavy metals even in low doses in milk and dairy products upon consumptions has grave health consequences (Meshref *et al.*, 2014).

According to WHO (2006), among the most prominent sources of exposure of man and human to heavy metals are through the food and water supply. Many heavy metals for instance, Lead, Cadmium Copper, Cobalt, etc. are genotoxic to both human and mammalian species and are capable of causing deleterious mutation which subsequently changes genetic information of functional protein products. In 2006, World Health Organisation (WHO) reported that heavy metals e.g Cobalt, Lead, Cadmium etc. are released into the atmosphere and are deposited in both soil and water which is usually absorbed as sediments or particles and this can be consumed by animals either from water or feed concentrate. Similarly, dairy animals ingest heavy metals while grazing on the pasture planted on heavy metals polluted soil, when fed on contaminated concentrate feeds and when served with water from heavy metals contaminated water bodies during grazing (Meshref *et al.*, 2014) and this will subsequently find its way into the blood stream and finally into the milk of dairy animals and other by-products. Milk and other products from these contaminated sources are thus consumed by unsuspecting humans who may subsequently experience health related issues. For instance, heavy metal pollution and fumes from industries, emissions from heavy duty and earth moving automobiles, all of this got soil and water bodies polluted and finally find their way into the food chain and got it contaminated **(**Bilandzic *et al.,* 2011; WHO, 2007**)**. And this subsequently have grave implication on the health status of human and animals within the entire habitat. Many heavy metals are emitted into the atmosphere via automobiles, heavy duty machines, earth moving vehicles and other industrial activities and finally into humans via respirations**.** Therefore, this study attempts to evaluate the presence of Pb and Co heavy metals in locally processed yoghurt collected from selected dairy collection points from across the study area with a view to hypothesizing its clinical implications towards providing baseline information necessary for policy formulation for the improvement of public health

Materials and methods

Collection of yoghurt samples and sampling procedure

About 10 mL each of four samples of locally processed yoghurt samples were randomly collected from each of five dairy collection centers/points in the morning from large containers into sterilized collection tubes and were cooled and transported in iced pack box and stored in the refrigerator for further analyses. Pb and Co heavy metals were evaluated in the study.

Laboratory analyses of heavy metals using high performance liquid chromatography-based instrumentation

The HPLC column packing material was AxxiChrom Octyl (Cole Scientific Inc., Moorpark, Calofornia), with a particulate size of 5 mm. HPLC analyses was performed using Shimadzu HPLC machine (Schimadzu Coporation, Kyoto, Japan). For mobile phase, HPLC separation of Pb, and Co residues were carried out at a 1:1.5:5 mixture of methanolacetonitrile (0.01 M), aqueous oxalic acid solution and oxalic acid

was modified with ammonia solution of 2.0 pH according to procedure earlier described by Oka and Uno (1984) with the flow rate of 0.8 mL/min. (Karno and Sean, 2003).

Regarding the standard Stocks for Pb and Co elements, the standard stock solutions for mobile phase were formulated by precision weighing of 20 mg each of Pb and Co reference analogues using digital scale and immediately dissolving them each in 10 mL of methanol (Onasanya *et al*., 2019b; Oka and Uno, 1984). The standard stock solutions of the Pb and Co heavy metals were subsequently stored in refrigerator for further analyses. Thereafter, the working stocks were subsequently prepared from the previously constituted stock solutions by diluting it with appropriate volume of methanol to make total concentrations range of 0.0092–1.1 mg/mL. The residues of heavy metals were successfully optimised to identify and separate these heavy metal residues and other organic compounds in a biological matrix of locally processed yoghurt sample, by spiking 0.2 mg/mL each of heavy metal analogues with 10 mL of locally processed yoghurt (Onasanya *et al*., 2019b; Oka and Uno, 1984). The locally processed yoghurt samples were later acidified with 1 mL of TFA and centrifuged for 15 minute using 0.2 µm syringe filter and subsequently injected directly into the supernatant liquid which was subsequently drawn in the HPLC system for appropriate identification and separation (Onasanya *et al*., 2019b; Oka and Uno, 1984). Evaluation of the specific rotation was accomplished using the peak height method as previously reported by (Rice *et al.,* 1989). Regarding the separation of heavy metals analogue (Pb and Co) and other organic compound, HPLC-UV detection system was performed out according to earlier published works of Onasanya *et al.* (2019b) and Oka and Uno (1984). The UV detector of the Schinadzu HPLC was visualised in millivolts (Onasanya *et al*., 2019b). All chemical reagents used for this study were specific for HPLC instrumentation. Pb and Co analogues were obtained from reputable laboratories. Other chemical reagents for example, acetonitrile, methanol, oxalic acid, trifluoroacetic acid (TFA), and aqueous ammonia were obtained from Thermo Fisher Scientific (Waltham, Massachusetts, United States of America).

Results

The contamination levels of Co residues detected in samples of locally processed yoghurt collected from the Ringim dairy collection center/point showed that locally processed yoghurt from Kiri had the highest Co residue (75.18 mg/kg) followed by Majiyawa (65.84 mg/kg) while Dakayyawa, Andaza and Balbadu had Co residue-free yoghurt (Table 1). Regarding the contamination of Pb in the locally processed Yoghurt from the study area, Table 2 shows that Surko had Yoghurt with the highest Pb contamination level of 218.06 mg/kg followed by Sule Tankarkar (72.02 mg/kg) while Rumbawa and Dakayyawa had Pb-free yoghurt.

Table 1: Contamination levels of Cobalt residues detected from samples of locally processed yoghurt collected from the study area

S/N	Dairy Collection centers/points	Source (Villages) of the yoghurt	Concentration residues per yoghurt sample mg/kg	% concentration of Co residue per yoghurt samples relative to other organic impurities
1	Sule tankarkar	Zareku	37.77	33.64
2	Kiyawa	Yar'gaba	5.12	56.60
3	Sule tankarkar	Sule Tankarkar	5.60	7.53
4	Dutse	Jaudi	0.05	0.75
5	Kiyawa	Rumbawa	0.02	0.88
6	Brini Kudu	Surko	0.09	0.06
7	Kiyawa	Shuwarin gabas	1.23	1.08
8	Brini Kudu	Kyambo	0.02	0.88
9	Dutse	Laraba	0.02	0.88
10	Ringim	Kiri	75.18	62.83
11	Ringim	Majiyawa	65.84	94.74
12	Dutse	Dutse	4.41	6.91
13	Sule tankarkar	Dakayyawa	0.00	0.00
14	Sule tankarkar	Dangyati	0.45	4.69
15	Brini Kudu	Balbadu	0.00	0.00
16	Brini Kudu	Bamaina	61.33	67.08
17	Ringim	Barawa	4.82	6.97
18	Dutse	Andaza	0.00	0.00
19	Ringim	Rinial Arewa	0.14	2.40
20	Kiyawa	Fake	4.49	33.53

Table 2. Contamination levels of Lead residues detected from samples of locally processed yoghurt collected from the study area

S/N	Dairy Collection centers/points	Source (Villages) of yoghurt	Concentration of Pb residues per yoghurt sample mg/kg	% concentration of Pb residues per yoghurt sample relative to other organic impurities
1	Sule tankarkar	Zareku	25.19	33.64
2	Kiyawa	Yar'gba	3.94	24.42
3	Sule tankarkar	Sule Tankarkar	72.02	92.47
4	Dutse	Jaudi	0.87	13.30
5	Kiyawa	Rumbawa	0.00	0.00
6	Brini Kudu	Surko	218.06	84.97
7	Kiyawa	Shuwarin gabas	25.47	20.31
8	Brini Kudu	Kyambo	0.02	1.75
9	Dutse	Laraba	0.69	8.67
10	Ringim	Kiri	55.72	37.17
11	Ringim	Majiyawa	1.96	5.26
12	Dutse	Dutse	0.28	2.57
13	Sule tankarkar	Dakayyawa	0.00	0.00
14	Sule tankarkar	Dangyati	0.02	0.23
15	Brini Kudu	Balbadu	0.80	9.00
16	Brini Kudu	Bamaina	1.08	0.82
17	Ringim	Ba'awa	0.29	0.53
18	Dutse	Andaza	0.02	2.24
19	Ringim	Rinial Arewa	0.18	1.73
20	Kiyawa	Fake	5.04	7.69

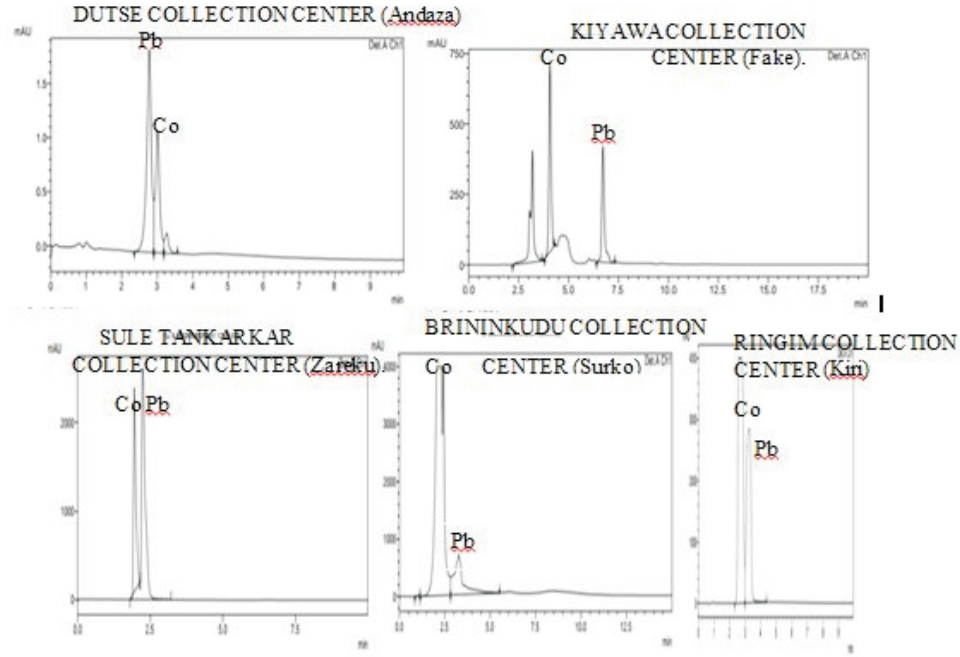

Fig. 1 Chromatogram maps showing contamination levels of some Co and Pb residues detected in the samples of locally processed yoghurt collected from the study area.

Discussions

Elevated levels of toxic heavy metal beyond recommended MRLs have grave consequences of public health and as such action must be taken to minimize their levels in animal meat and dairy products. We presented from our results that sample of locally processed yoghurt collected from Kiri had the highest Co residue (75.18 mg/kg) followed by Majiyawa (65.84 mg/kg) both of which are from Ringim dairy collection center while Dakayyawa, Andaza and Balbadu had Co residue-free yoghurt. The FAO and WHO have yet to determine MRLs for Cobalt in milk and milk products and as such we have no acceptable recommended MRLs to compare our data though Co is non-toxic element which is beneficial to both human and animals. The low Cobalt levels recorded in our study was clinically followed similar trend with previous works in the literature because most of dietary Co ingested by animals is excreted mainly through Urine. Few amounts are also excreted through faeces, sweat and milk (European Agency for Evaluation of Medicinal products, 1998). Co is made available to animals inform of salts namely; Cobalt oxide, Cobalt trioxide, Cobalt carbonate, Cobalt chloride, Cobalt gluconate and Cobalt sulpahte and it is incorporated into diets of animals as a component of Vitamins B_{12} for treating Co deficiency diseases in all food producing species of animals and enhancement of other biological functions (European Agency for Evaluation of Medicinal products, 1998). The non-toxic/essential heavy metals like cobalt if present in disproportionate doses could cause grave clinical consequences on the health of animal species which could manifest in nutritional imbalance and health disorder (Meshref *et al.*, 2014). Most of the yohgurt

samples collected from the study area had Pb contamination levels far higher than the Maximum Residue Limits (MRLs) for Milk and Milk products (0.02 mg/kg) as recommended by Food and Agriculture Organisation and World Health Organisation (2012) except for few yoghurt samples collected from Rumbawa (0.00 mg/kg), Dakayyawa (0.00 mg/kg), Kyambo (0.02 mg/kg), Dangyati (0.02 mg/kg) and Andaza (0.02 mg/kg) which had Pb levels within FAO and WHO (2012) recommended MRLs. The presence of Pb residues in milk and dairy products could be due to environmental sources (atmospheric deposition, automobile exhausts, fumes from automobiles and earth moving vehicles, industrial effluents etc.). However, it is important to note that the levels of Pb residues obtained in our study were very high, since most samples were above the (FAO and WHO (2012) recommended level of 0.02 mg/kg for Milk, yoghurt and other dairy products. Food is the major medium of exposure of Pb to the general public (WHO, 2007). Pb is potentially carcinogenic and is associated with etiology of myriads of diseases in the cardiovascular system including kidneys, nervous system, blood and skeletal system (Zhuang *et al*., 2009) and as such the elevated levels of Pb in dairy products constitute a grave public health risk.

Conclusion and recommendation

The present study gives clinical information on the levels of Pb and Co present in the samples of locally processed yoghurt collected from the study area. Our results suggested that contamination levels of Pb detected in the study area is clinically unsafe and unhygienic for human consumption and it is of grave consequences to public health. Therefore, urgent action needed to be taken by appropriate agency of government to address this threat to the consumers' safety of dairy products in the study area.

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