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Organochlorine pesticide residues in buffalo meat, liver, kidney, milk, and kariesh cheese

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ABSTRACT

High-quality animal protein, vitamins B1, B6, and B12, as well as minerals like zinc, selenium, and iron, can all be found in buffalo meat and milk. Organochlorine pesticides (OCPs) have been widely utilized for many years because of their long duration of action, low cost, and toxicity against a variety of pests. Despite being outlawed everywhere, OCPs are still being used illegally. One of the main responsibilities of the food safety and public health sectors is to guarantee the safety and wholesomeness of such food products before they are made available to the public. In order to determine the residual OCP contents in retailed buffalo meat, liver, kidney, milk, and kariesh cheese, this study was conducted. The obtained results in the current investigation revealed the detection of OCPs at 75%, 40%, 25%, 60%, and 80% in the examined buffalo raw milk, kariesh cheese, meat, kidneys, and liver. Different OCPs were detected at variable concentrations; however, such concentrations were within the established permissible limits in Egypt. In conclusion, OCPs are still in use in Egyptian agricultural activities and residual concentrations can be detected in the meat, offal, and dairies. Therefore, it is highly advised to continuously check for OCP residues in foods with both animal and plant origins. Additionally, it is strongly advised that farmers become more aware of the negative consequences of OCPs.

Introduction

Recent years have seen a global expansion in the rearing of buffalos, particularly in Egypt and Central Asian nations. According to Cockrill (1981), and Al-Humam *et al*. (2021) buffalo meat is a good source of high-quality animal protein, vitamins B1, B6, and B12, as well as minerals including zinc, selenium, and iron. Because of their distinct flavor, low cost, and high nutritional content, buffalo edible offal (such as masseter muscles, liver, and kidney) is regarded as an ethnic and popular dish in many regions of the world, particularly in the Middle East (Tang *et al*., 2020).

Buffalo milk and other dairy products such as Kariesh cheese are among Egypt's most significant sources of animal protein and minerals (Preiato, 2000; Stadnik and Kska, 2015; Raslan *et al*., 2018). In addition, because xenobiotics are released into milk and then make their way into people's bodies when contaminated milk is consumed, milk is recognized as a bioindicator for animal exposure to xenobiotics (Thompson and Darwish, 2019).

Throughout their lives, buffaloes are exposed to a variety of xenobiotics, such as mycotoxins, heavy metals, antibiotics, and pesticides. Such pollutants can enter the human body by consumption of tainted milk and other edible tissues, which have a number of detrimental effects on the animal (Giantin *et al*. 2008; Darwish *et al*. 2010).

Because of their prolonged duration of action, low cost, and toxicity against a variety of pests, organochlorine pesticides (OCPs) have been widely used for several decades (Pirsaheb *et al*., 2015; Darwish and Thompson, 2023). Aldrin, Dieldrin, and Dichlorodiphenyltrichloroethane (DDT) were three OCPs that saw widespread use in agriculture throughout the 1950s. DDT's toxicity and widespread biomagnification were quickly recognized in other species. Originally, it was thought that DDT was only detrimental to insects. Animals collect lipophilic chlorine residues from OCPs, and top-of-the-food-chain species exhibit biomagnification. Since its adoption in May 2001 (UNEP, 2002), the Stockholm Convention on Persistent Organic Pollutants (POPs) has 179 signatories. Aldrin, chlordane, DDT, dieldrin, endrin, heptachlor, hexachlorobenzene (HCB), mirex, and toxaphene were the first 12 POP compounds that this convention outlawed. While some OCPs have been restricted from use in agriculture, others are still applied as pesticides in many nations. DDT is still being used in many African nations in accordance with a valid exemption for the control of disease vectors. In this situation, chemicals are sprayed inside residences (indoor residual spraying, IRS) or applied to bed nets (insecticide-treated nets). There may be chemical stockpiles or obsolete materials stored in some locations under potentially hazardous conditions. These can only be found out by checking samples of the surrounding environment for contamination (Thompson *et al*., 2017, 2018).

OCPs can enter the body in several ways, such as through the skin, ingestion of contaminated food or water, or inhalation of polluted air (Mahmoud *et al*., 2013; 2016; Thompson *et al*., 2018). OCP-contaminated foods of animal origin are likely to be a major source of pesticide exposure in humans (Hassal, 1990). Breast milk can carry maternal transfer to neonates, or it can pass the placenta to the fetus. Such OCPs may have a few harmful impacts on a person's health (Sallam and Morshdy, 2008; Morshdy *et al*., 2018). According to their environment and position in the food chain, living animals' amounts of these substances differ (Zhou *et*

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al., 2007).

Assuring the safety and wholesomeness of such food products before they are made available to the public is a key responsibility of the food safety and public health sectors. Therefore, this study was undertaken to examine the OCPs' residual contents in the retailed buffalo meat, liver, kidney, milk, and kariesh cheese.

Materials and methods

Collection of samples

A total of 100 samples of buffalo meat, liver, kidneys, raw milk, and kariesh cheese were randomly collected from market stalls in Zagazig City, Sharkia province, Egypt. Each sample weighs 100 g and is packaged separately in polyethylene bags. The samples were brought into the laboratory at Food Control Department, Faculty of Veterinary Medicine, Zagazig University, Egypt in a chilled container. At the Agricultural Research Centre in Dokki, Giza, Egypt, organochlorine pesticides were extracted and quantified.

Chemicals

Standard OCPs from Sigma-Aldrich (Germany) were used, including pp-DDT, pp-DDD, pp-DDE, HCH, HCH, heptachlor, heptachlor epoxide, aldrin, endrin, chlordane, methoxychlor, and HCB. From Merck (Darmstadt, Germany), petroleum ether, diethyl ether, n-hexane, acetonitrile, anhydrous sodium sulfate, and methylene chloride were purchased. Silica (Silica Co., USA) supplied florisil (PR Grade, 60-100 mesh). All solvents were of HPLC grade or the highest purity available. After being active for 24 hours at 130°C, florisil was cooled to room temperature.

Samples' extraction and preparation

In three separate extraction stages lasting two minutes each, each individual sample (50 g) was mixed and homogenized with 150 g of anhydrous sodium sulfate. Then the homogenates were well-mixed with petroleum ether (150, 100, and 100 ml, respectively) and anhydrous sodium sulfate (100 g), as previously mentioned (Mahmoud *et al*., 2013). Anhydrous sodium sulfate helps to dissolve the material by removing water. Following each extraction, samples were filtered with a vacuum pump. On a rotary evaporator, the solvent was heated to 40°C and evaporated until dry.

Partitioning of the extracts

The Association of Official Analytical Chemists' procedure for partitioning the extracted samples was followed (AOAC, 1999). In order to partition the sample, 500 ml of n-hexane and an equal amount of acetonitrile were first mixed in a separating funnel before each solvent was separated and used separately. The extracted material was put into a 100-ml separating funnel with a solution of 80 ml n-hexane and 20 ml acetonitrile, followed by two minutes of vigorous shaking. Acetonitrile was collected in a flask after being separated into two solvent layers and being passed through anhydrous sodium sulfate to remove any moisture. The partitioning procedure described above was carried out three times with the addition of 20 ml more acetonitrile to the n-hexane. In the end, acetonitrile was evaporated on a rotary evaporator to a volume of less than 10 ml to be used for the cleanup of florisil, while n-hexane was discarded.

Cleanup of the extracts and measurement of OCPs concentrations

To clean up the extracted samples and remove any of the remaining fat, the extract was placed into a glass chromatographic column that contained 20 g of activated florisil (60–100 mesh) covered with anhydrous sodium sulfate. The extracted sample was then placed on the constructed column after being washed with 50 cc petroleum ether. 200 ml of an eluent (10% anhydrous diethyl ether + 90% petroleum ether) was used to elute the column, and then 100 ml of another eluent (1% acetonitrile + 29% n-hexane + 70% methylene chloride) was used for the second elution. The eluent that had been recovered was concentrated using a rotary evaporator before being dissolved in hexane to a volume of 10 ml. In order to prepare each extract for examination using electron capture gas chromatography, an aliquot of each extract was transferred to 2-ml injection vials.

By analyzing samples with an electron capture gas chromatograph (Hewlett Packard GC Model 6890) fitted with a Ni63-electron capture detector, organochlorine residues were identified. The HP- 5MS capillary column was used in the GC under the following conditions: carrier gas: N2 at a flow rate of 4 ml/min; injector and detector temperatures were 230°C and 300°C, respectively; 30m length X 0.32mm internal diameter, X 0.25m film thickness. the gas chromatography oven temperature program was started at 150°C for 5 min, raised to 170°C (at a rate of 5°C/min) and held for 10 min, then raised to 220°C (at a rate of 10°C/min) and held for 20 min (with a total run time of 44 min); the injection volume was 1, ll, and the flow rates of nitrogen make-up gas were 20 ml/min. Residual concentrations of the tested OCPs were determined based on the calibrated standard curves and presented as ng/g.

Statistical analysis

All data are presented as means ± SE, and each measurement was done twice. JMP statistical software; SAS Institute Inc., Cary, NC) was used to determine statistical significance using the comparison of means approach (the Tukey-Kramer HSD test), where $p < 0.05$ is considered significant.

Results and Discussion

Organochlorine pesticides have been banned in Egypt since the 1980s, however, due to their persistent nature, they can be detected in animal tissues, water, soil, and agricultural crops (Mahmoud *et al*., 2013; 2016; Darwish and Thompson, 2023). Living animals are exposed to OCPs via ingestion of contaminated animal feed and water, or via inhalation of contaminated air. The residues of the OCPs can find their way into the human body via ingestion of contaminated animal tissues, milk, and other dairy products.

The obtained results in the current investigation revealed the detection of OCPs at 75%, 40%, 25%, 60%, and 80% in the examined buffalo raw milk, kariesh cheese, meat, kidneys, and liver (Fig. 1). Such results go in agreement with previous reports such as Mahmoud *et al*. (2013) who detected OCPs in the buffalo's tongue, kidneys, and liver at variable rates, and Raslan *et al*. (2018) who detected OCPs in the raw milk of cattle (50% positive samples), buffalo (75% positive samples, and goats (75% positive samples). Globally, Heck *et al*. (2007) detected OCPs in 100% of the examined milk samples retailed in in Rio Grande do Sul, Brazil.

The obtained results in Fig 2 showed that ΣDDTs were significantly highest in the tested raw milk (114±12.4 ng/g ww), and liver (109.7±7.8 ng/g ww), followed by kidneys (65.8±3.6 ng/g ww), kariesh cheese (17.3±0.9 ng/g ww), and meat (5.6±0.6 ng/g ww), respectively. Different DDT isomers were detected including pp-DDT, pp-DDD, and pp-DDE in the positive samples. These results indicate that DDTs are still illegally in use in Egypt. Similarly, Mahmoud *et al*. (2013) detected different DDTs in the edible offal of the buffalo. Besides, Sallam and Morshdy (2008) detected DDTs in the carcasses of cattle, camel, and sheep. Regarding dairy samples, Darko and Acquaah (2008) found DDTs in milk, yoghurt, and cheese sold in Ghana at quantities ranging from 0.01 to 119 ng/g ww, which is in agreement with the findings of this study. Contrary to expectations, greater DDT concentrations (1230 and 874.4 ng/g ww) were found

in samples of goat and calf milk taken from Ethiopian markets (Deti *et al.*, 2014). In contrast, DDTs were not found in the buffalo milk samples taken from Assuit City in Egypt by Shaker and Elsharkawy (2015).

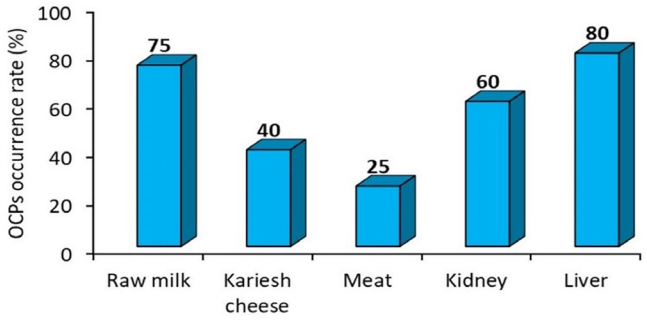

Fig. 1. Detection rates (%) of OCPs in the examined buffalo raw milk, kariesh cheese, meat, kidneys, and liver (n=20/each).

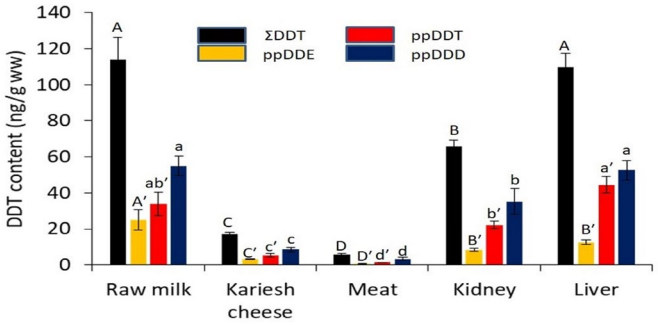

Fig. 2. DDTs concentrations (ng/g ww) in the examined buffalo raw milk, kariesh cheese, meat, kidneys, and liver. Values represent means ± SE, and columns for each tested parameter carrying different characters are statistically different at $p < 0.05$.

The results recorded in Fig. 3 showed the residual concentrations (ng/g ww) of Hexachlorocyclohexanes (HCHs) in the examined samples. The average total HCHs in the examined samples were 140.6±4.6, 48.6±2.4, 5.0±0.2, 97.8±7.6, and 134.0±11.5 in the examined raw milk, kariesh cheese, meat, kidneys, and liver, respectively. α-HCH and lindane (ɣ-HCH), the most active and stable HCH isomer, were also detected at variable rates, where the meat had the lowest concentrations while the liver and raw milk had the highest concentrations. The recorded HCH concentrations were comparable to that recorded in the buffalo offal retailed in Egypt (Mahmoud *et al.*, 2013), raw milk in Egypt (Raslan *et al.*, 2018), raw milk in Mexico (Waliszewski *et al.*, 2003), and raw and pasteurized milk in Uganda (Kampire *et al.*, 2011).

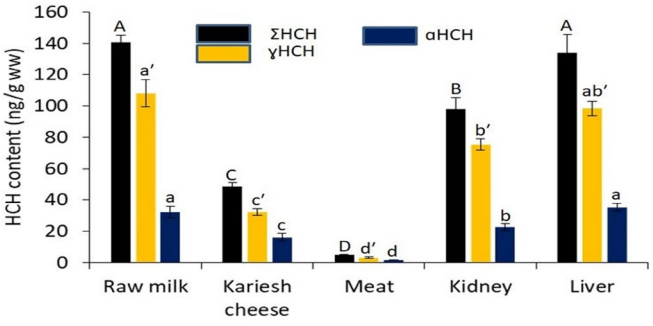

Fig. 3. HCHs concentrations (ng/g ww) in the examined buffalo raw milk, kariesh cheese, meat, kidneys, and liver. Values represent means ± SE, and columns for each tested parameter carrying different characters are statistically different at $p < 0.05$.

Heptachlors either heptachlor or its epoxide were detected in all positive samples for OCPs contamination. The liver had the highest residues followed by raw milk, kidneys, kariesh cheese, and meat, respectively (Fig. 4). Likely, heptachlors were detected in the liver, kidney, and tongue of Egyptian buffalo, and cattle (Mahmoud *et al.*, 2013; 2016), and in raw buffalo and cattle milk in Egypt (Raslan *et al.*, 2018) at relatively similar concentrations. However, higher concentrations were detected in the raw milk sampled in India (John *et al.*, 2001).

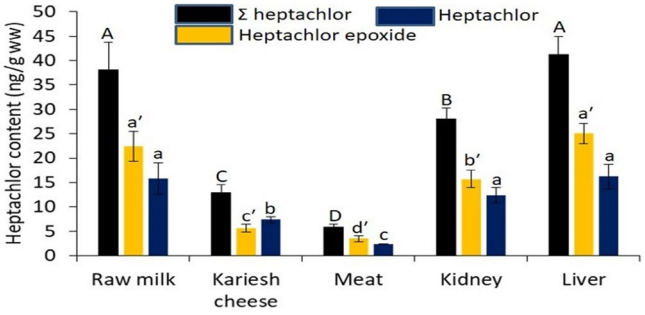

Fig. 4. Heptachlors concentrations (ng/g ww) in the examined buffalo raw milk, kariesh cheese, meat, kidneys, and liver. Values represent means ± SE, and columns for each tested parameter carrying different characters are statistically different at $p < 0.05$.

The results presented in Fig. 5 demonstrated the detected concentrations of aldrins in the examined samples. Similar to other OCPs, the liver, and raw milk had the highest residual contents of aldrins, followed by kidneys, kariesh cheese, and raw meat, respectively. The recorded concentrations of aldrins were in agreement with other reports focusing on edible offal in Egypt (Mahmoud *et al.*, 2013; 2016), raw milk in Egypt (Shaker and Elsharkawy, 2015; Raslan *et al.*, 2018), and cheese in Ghana (Darko and Acquaah, 2008).

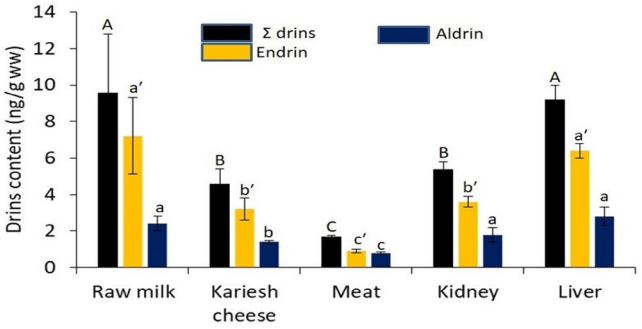

Fig. 5. Drins concentrations (ng/g ww) in the examined buffalo raw milk, kariesh cheese, meat, kidneys, and liver. Values represent means ± SE, and columns for each tested parameter carrying different characters are statistically different at $p < 0.05$.

The obtained results in Fig. 6 revealed the detection of chlordane, HCB, and methoxychlor in the examined samples at variable rates which go in correspondence with the findings of Mahmoud *et al.* (2013), and Raslan *et al.* (2018).

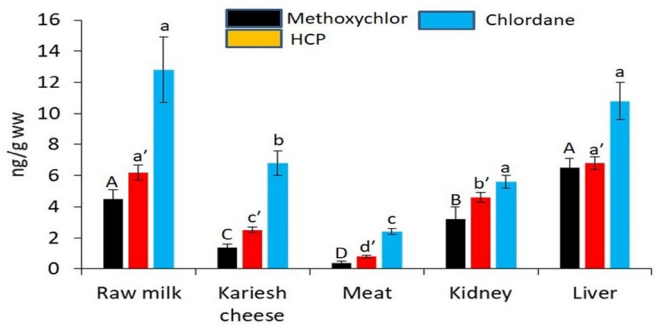

Fig. 6. Chlordane, HCB, and methoxychlor concentrations (ng/g ww) in the examined buffalo raw milk, kariesh cheese, meat, kidneys, and liver. Values represent means ± SE, and columns for each tested parameter carrying different characters are statistically different at $p < 0.05$.

Interestingly, all detected OCPs concentrations were below the established maximum permissible limits (MPL) of OCPs in Egypt, HCHs

(1000 ng/g), DDTs (5000 ng/g), drins (600 ng/g), HCB (200 ng/g), and chlordane (200 ng/g) (EOS, 1992). However, OCPs are incriminated in several adverse health effects including teratogenesis, impaired growth, infertility, and cancer risk (Thompson and Darwish (2019).

Conclusion

The obtained results of the current study revealed that OCPs are still illegally in use in Egypt as they were detected in the buffalos' edible offal and dairy products. Despite being below the MPL of OCPs in Egypt, continuous monitoring for OCPs residues in foods of animal and plant origins is highly recommended. Besides, increasing the awareness of the farmers of the adverse effects of the OCPs is also highly suggested.

Conflict of interest

The authors declare that they have no conflict of interest.

References

Al-Humam, N.A., Reda, L., Mohamed, R.E., El-Ghareeb, W.R., Darwish, W.S., Ibrahim, N.A., 2021. Prevalence and antibiogram of *Listeria monocytogenes* in retailed buffalo raw meat and mince with a protection trial using nisin, and gingerol. Buffalo Bulletin 40, 47-57.

AOAC (Association of Official Agricultural Chemists), 1999. International, "Official methods of analysis of AOAC International," P. Cunnif, Ed., Association of Official Agricultural Chemists (AOAC) International, Gaithersburg, Md, USA, 16th edition, 1999.

Cockrill, W.R., 1981. The water buffalo: a review. Br. Vet. J. 137, 8-16.

Darko, G., Acquaah, S.O., 2008. Levels of organochlorine pesticide residues in dairy products in Kumasi, Ghana. Chemosphere 71, 294-298.

Darwish, W.S., Ikenaka, Y., El-Ghareeb, W.R., Ishizuka, M., 2010. High expression of the mRNA of cytochrome P450 and phase II enzymes in the lung and kidney tissues of cattle. Animal 4, 2023-2029.

Darwish, W.S., Thompson, L.A., 2023. Soil, water, and air: potential contributions of inorganic and organic chemicals. In Present Knowledge in Food Safety. Academic Press. pp. 26-43.

Deti, H., Hymete, A., Bekhit, A.A., Mohamed, A.M.I., Bekhit, A.E.D.A., 2014. Persistent organochlorine pesticide residues in cow and goat milk collected from different regions of Ethiopia. Chemosphere 106, 70-74.

EOS (Egyptian Organization for Standardization), 1992. Maximum limits for pesticide residues in meat. Paper number 2078. Egyptian Organization for Standardization and Quality Control, Cairo, Egypt.

Giantin, M., Carletti, M., Capolongo, F., Pegolo, S., Lopparelli, R., Gusson, F., Nebbia, C., Cantiello, M., Martin, P., Pineau, T., Dacasto, M., 2008. Effect of breed upon cytochromes P450 and phase II enzyme expression in cattle liver. Drug Metab. Dispos. 36, 885-893.

Hassal, K.A., 1990. Biochemistry and uses of pesticides. Macmillan Press Ltd., Basingstoke.

Heck, M.C., dos Santos, J.S., Junior, S.B., Costabeber, I., Emanuelli, T., 2007. Estimation of children exposure to organochlorine compounds through milk in Rio Grande do Sul, Brazil. Food Chem. 102, 288-294.

John, P.J., Bakore, N., Bhatnagar, P., 2001. Assessment of organochlorine pesticide residue levels in dairy milk and buffalo milk from Jaipur City, Rajasthan, India. Environ. Inter. 26, 231-236.

Kampire, E., Kiremire, B.T., Nyanzi, S.A., Kishimba, M., 2011. Organochlorine pesticide in fresh and pasteurized cow's milk from Kampala markets. Chemosphere 84, 923-927.

Mahmoud, A.F.A., Darwish, W.S., Morshdy, A.E.M.A., Eldaly, E.A., Ikenaka, Y., Ishizuka, M., 2013. Determination of organochlorine pesticides (OCPs) in the edible offal of Egyptian buffalo. Jpn. J. Vet. Res. 61, S58-S63.

Mahmoud, A.F.A., Ikenaka, Y., Yohannes, Y.B., Darwish, W.S., Eldaly, E.A., Morshdy, A.E.M., Nakayama, S.M., Mizukawa, H., Ishizuka, M., 2016. Distribution and health risk assessment of organochlorine pesticides (OCPs) residue in edible cattle tissues from northeastern part of Egypt: High accumulation level of OCPs in tongue. Chemosphere 144, 1365-1371.

Morshdy, A.E.M.A., Darwish, W.S., Daoud, J.R., Hussein, M.A., Sebak, M.A., 2018. Monitoring of organochlorine pesticide residues in *Oreochromis niloticus* collected from some localities in Egypt. Slov. Vet. Res. 55, 303-311.

Pirsaheb, M., Limoee, M., Namdari, F., Khamutian, R., 2015. Organochlorine pesticides residue in breast milk: a systematic review. Med. J. Iran 29, 228.

Preiato, D., 2020. All you need to know about bison meat. Health Line Nutrition 2020. https://www.healthline.com/nutrition/bison-meat-nutrition.

Raslan, A.A., Elbadry, S., Darwish, W.S., 2018. Estimation and human health risk assessment of organochlorine pesticides in raw milk marketed in Zagazig City, Egypt. J. Toxicol., 2018, 3821797.

Sallam, K.I., Morshedy, A.E.M.A., 2008. Organochlorine pesticide residues in camel, cattle, and sheep carcasses slaughtered in Sharkia Province, Egypt. Food Chem. 108, 154-164.

Shaker, E.M., Elsharkawy, E.E., 2015. Organochlorine and organophosphorus pesticide residues in raw buffalo milk from agroindustrial areas in Assiut, Egypt. Environmental Toxicology and Pharmacology 39, 433-440.

Stadnik, J., Kęska, P., 2015. Meat and fermented meat products as a source of bioactive peptides. Acta Sci. Pol. Technol. Aliment. 14, 181-190.

Tang, H., Darwish, W.S., El-Ghareeb, W.R., Al-Humam, N.A., Chen, L., Zhong, R.M., Xiao, Z.J., Ma, J.K., 2020. Microbial quality and formation of biogenic amines in the meat and edible offal of Camelus dromedaries with a protection trial using gingerol and nisin. Food Sci. Nutr. 8, 2094-2101.

Thompson, L.A., Darwish, W.S., 2019. Environmental Chemical Contaminants in Food: Review of a Global Problem. J. Toxicol. 2019, 2345283.

Thompson, L.A., Darwish, W.S., Ikenaka, Y., Nakayama, S.M., Mizukawa, H., Ishizuka, M., 2017a. Organochlorine pesticide contamination of foods in Africa: incidence and public health significance. J. Vet. Med. Sci. 79, 751-764.

Thompson, L.A., Ikenaka, Y., Yohannes, Y.B., Ichise, T., Ito, G., Bortey-Sam, N., van Vuren, J.J., Wepener, V., Smit, N.J., Darwish, W.S., Nakayama, S.M.M., 2018. Human health risk from consumption of marine fish contaminated with DDT and its metabolites in Maputo Bay, Mozambique. Bull. Environ. Contam. Toxicol. 100, 672-676.

UNEP, 2002. Africa Environment Outlook. Past, present and future perspectives. United Nations Environment Programme, Nairobi.

Waliszewski, S.M., Villalobos-Pietrini, R., Gomez-Arroyo, S., Infanzon, R.M., 2003. Persistent organochlorine pesticide levels in cow's milk samples from tropical regions of Mexico. Food Addit. Contam. 20, 270-275.

Zhou, R., Zhu, L., Kong, Q., 2007. Persistent chlorinated pesticides in fish species from Qiantang River in East China. Chemosphere 68, 838–847.