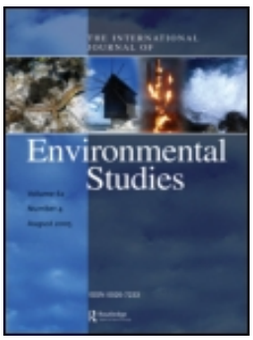

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Organochlorine pesticide residues in skin, flesh and whole carrots (*Daucus carota*) from markets around Lake Victoria basin, Uganda

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Residual concentrations of organochlorine pesticides in vegetables cause concern because of their adverse health effects. Pesticides have been applied in agricultural production and vector control in Uganda. Vegetables may absorb high residual levels of cyclodienes necessitating regular monitoring. Carrots are commonly consumed in Uganda as raw salads or components of different dishes. A gas chromatograph with electron capture detector was used to quantify organochlorine pesticides. Pesticide residues were confirmed by gas chromatography with a mass spectrometer. Trace amounts of 4,4′-dichlorodiphenyltrichloroethane (DDT), 2,4′-DDT, 2,4′-dichlorodiphenylchloroethane (DDE), α- endosulphan, β-endosulphan, α-lindane, γ-lindane and dieldrin were detected in carrots. Levels of organochlorine residues in carrots were below the maximum residue limits considered safe for human consumption by Codex Alimentarius and the European Union Commission.

Keywords: Organochlorine pesticides; Carrots; Lake Victoria basin

Introduction

Lake Victoria basin is characterized by fertile soils and a favourable climate, suitable for commercial farming. The favourable climate also promotes easy multiplication and survival of crop pests. As a result, farmers use fertilizers and pesticides to increase productivity and ensure high quality of produce [1]. Recent efforts to diversify crops for the export market have also contributed to substantial use of agrochemicals [2]. Between 1950 and 1980, various synthetic pesticides were used in farming and public health in Uganda. Among the organochlorines, dichlorodiphenyltrichloroethane (DDT) was applied in substantial volume to control mosquitoes that spread malaria [1]. In spite of the benefits of pesticide use in boosting agricultural production and controlling vectors, it has resulted in contamination of the environment [3]. Discharge of untreated industrial and anthropogenic effluents has in addition increased the contamination of Lake Victoria basin [4]. This necessitates monitoring of contaminants in water, fish and vegetables to protect consumers.

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Carrots are commonly consumed as raw salads or in cooked form as components of different dishes. Mature carrots contain high amounts of β-carotene, a vitamin A precursor, and an antioxidant known to have anti-carcinogenic and anti-aging properties. They are also a rich source of various micronutrients including potassium, manganese, sodium, fluoride, phosphorus, iron, zinc, copper, selenium, calcium, thiamine and riboflavin [5]. Despite this rich nutritional profile, carrots are known to pick up cyclodienes from the contaminated soil [6]. Levels of organic pesticides in fresh vegetables are, however, shown to diminish after washing, peeling, blanching and cooking [7–9]. The extent to which processing reduces levels of pesticides residue varies with the types of vegetable and pesticide [10]. Limited information is, meanwhile, available on the pesticide residue status of vegetables consumed in Uganda. Our team studied levels of organochlorine pesticide residues in carrots from markets around Lake Victoria basin (Uganda). This study provides information on pesticide residues in skin, flesh and whole carrots.

Materials and methods

Sampling

Carrots were collected from four main markets located within Lake Victoria basin. Two hundred samples of mature fresh carrots were obtained from Nakasero and Nakawa markets found in the central region of Kampala district. In Mukono district, 200 pieces of carrots were collected from Seeta and Mukono markets. A total of 100 pieces of carrots were sampled from each of the four markets. Samples were wrapped in aluminium foils and placed in cool boxes containing ice packs. Carrots were stored in a refrigerator maintained at 0 °C and extracted within a period of two days.

Sample preparation

Fresh whole carrots (*ca*. 600 g), peeled carrots (*ca*. 600 g) and carrot skins (*ca*. 400 g) obtained by scrapping were separately homogenized using a Warring blender (Patterson Scientific, Blender 800E, USA) at high speed for five min. Samples (25 g) for each carrot portion were dehydrated by mixing with anhydrous sodium sulphate (50 g) in a glass beaker. Dehydrated carrots were dissolved in a mixture of acetone (50 ml) and that comprising ethyl acetate and cyclohexane (1:1 v/v) (50 ml). Extracts were filtered over anhydrous sodium sulphate through glass wool conditioned with acetone. The filtrate was further filtered over sodium sulphate through Glass Microfibre Filters (GF/C, Whatman 47 mm, England), florisil (PR grade 60–100 mesh) and washed further using a mixture of cyclohexane and ethyl acetate (1:1 v/v). The supernatant was transferred to a 100 ml flask and concentrated to dryness using a rotary evaporator (Laborota 4000 Heidolph, Germany). The residue was reconstituted with hexane (2 ml) and kept for clean-up.

Clean-up of carrot extracts

Extracts were cleaned using Gel Permeation Chromatography (GPC) with chromatographic tube (50 cm $\times$ 1 cm i.d.), with two adapters, six-way valve with a sample injector loop (1 ml) and Teflon tubing. The carrot extract (1 ml) was loaded in the sample loop using a syringe, while the GPC valve was at the loading position and the loop was turned to the injection point. The mixture of cyclohexane and ethyl acetate (1:1 v/v) was used to convey the extract

through the column and also for elution. During the clean-up, the first fraction (18 ml) was discarded because the pesticides were eluted in the volume range 18 to 38 ml. The second fraction (18–38 ml) was collected, evaporated to dryness and the residue dissolved in cyclohexane (2 ml) for gas chromatograph with electron capture detector (GC-ECD) and gas chromatography with a mass spectrometer (GC-MS) analyser [11]. The column was cleaned with cyclohexane/ethyl acetate (1:1 v/v) (25 ml) before injection of the next extract. Extracts were evaporated and reconstituted in cyclohexane ready for analysis of pesticide residues.

Determination of organochlorine pesticide residues

Pesticide residues in carrots were determined using gas chromatography as described by Specht *et al*. [12]. Organochlorine pesticide residues were analysed using Varian (CP-3800, USA) Gas Chromatograph equipped with an Electron Capture Detector (ECD), with a model 1079 injection port (1079, USA). Two columns were fitted, viz., a semi-polar (CP-Sil 19 CB, USA) and a non- polar (CP-Sil 5 CB, USA) fused-silica capillary GC column of 30 m × 0.25 mm i.d., 0.25 mm film thickness (DF). The column temperature was maintained at 90 °C for 1 min, raised at rate of 30°C min^{-1} to 180 °C, then at 4 °C min^{-1} to 260 °C, and held for 16 min. The carrier gas was hydrogen (99.99% purity) with electronic flow control at 1.2 ml min^{-1}. Other GC operating conditions were: 230 °C injector and 300 °C detector temperatures, and 30 ml min^{-1} make-up gas (nitrogen) flow. A Turbochrom (PerkinElmer Corporation, USA) Chromatography work station was used for chromatographic data processing. Analysis was carried out by injecting 1 µl of sample into the GC. Identification and quantification were accomplished by external standards.

An Agilent 6890 N GC-MS, USA version with an HP-5MS-fused silica capillary column 30 m × 0.25 µm × 0.25 mm i.d. was used for confirmation. The mass spectrometer used was equipped with a selective mass detector (Agilent 5975 inert XL Quadrupole, USA). Samples were injected in a splitless mode at an injector temperature of 260 °C. The detector was maintained at 200 °C and the initial column temperature was 90 °C. After 1 min, the temperature was programmed to rise at 30°C min^{-1} to 180 °C. The temperature was further increased at a rate of 4°C min^{-1} to 260 °C and held for 10 min. The injector temperature was 250 °C and the detector was maintained at 250 °C. Helium (99.999%) was used as the carrier gas at a flow rate of 1.0 ml min^{-1}. The GC-MS was operated in a splitless mode with a purge-off of 1 min and the volume of injection was 1 µml for each injection. The mass spectrometer solvent delay time was 3.57 min and the scanned mass range was 50–550 *m*/*z*. The full scan ion monitoring mode was used for the determination of the pesticide residues and identification of the analytes was done using the internal standards. GC-MSD Chemstation Software (G1701, JAS CWA, USA) was used for data processing.

Quality assurance and limits of detection

Quality assurance of the pesticide residue was done by use of blanks, spikes and reference sample and standards. Arithmetic means and standard deviations were calculated from positive quantifiable samples only, and in all cases, differences in means were significant at $p < 0.05$. Limits of detection (LOD) were established based on a signal to baseline (3:1) noise ratio and reported as averages of individual chromatograms [11]. The LOD was obtained for α-endosulphan, β-endosulphan, dieldrin, 4, 4′-DDT, 2, 4′-DDT, 2, 4′-DDE, α-lindane and γ-lindane (table 1). Samples with pesticide values greater than LOD were considered positive.

Table 1. Validation parameters of analytical methodologies for organochlorine in skin and whole carrot.

			Carrot skin			Whole carrot		
Pesticide	Linearity ($\mu g\,kg^{-1}$)	LOD ($\mu g\,kg^{-1}$)	Standards ($\mu g\,kg^{-1}$)	Precision (%)	Recovery (%)	Standards ($\mu g\,kg^{-1}$)	Precision (%)	Recovery (%)
α-endosulphan	0.04–19.5	0.04	0.10	92.4–92.5	92.5 ± 1.9	0.10	95.6–95.6	95.6 ± 0.1
β-endosulphan	0.05–17.0	0.05	0.50	104.3–104.3	104.3 ± 2.2	0.50	101.1–101.3	101.2 ± 1.3
Dieldrin	0.10–20.5	0.10	0.50	92.3–92.4	92.4 ± 1.4	0.50	98.7–98.9	98.9 ± 0.8
α-Lindane	0.09–14.5	0.09	0.50	98.3–98.4	98.4 ± 1.5	0.50	121.2–121.2	121.2 ± 2.1
γ-Lindane	0.08–14.0	0.08	0.50	95.2–95.3	95.2 ± 4.2	0.50	103.9–104.0	104.0 ± 1.4
2, 4′-DDT	0.06–19.5	0.06	1.00	101.8–101.8	101.8 ± 2.6	1.00	96.4–96.5	96.4 ± 0.7
4, 4′-DDT	0.07–19.0	0.07	1.00	97.5–97.6	97.5 ± 0.8	1.00	97.9–97.9	97.9 ± 0.9
2, 4′-DDE	0.06–19.0	0.06	1.00	114.3–114.6	114.5 ± 0.8	1.00	117.0–117.1	117.0 ± 1.7

DDT: dichlorodiphenyltrichloroethane; DDE: dichlorodiphenylchloroethane; LOD: Limit of detection. Recovery values are averages of eight replicates ± standard deviation.

Carrots (25 g) were fortified with working solutions with concentrations ranging from 0.1 to 0.5 μg kg^{-1} and most of the recoveries were above 70%. Pesticide residue mean recoveries for carrots varied from 65.2 to 118.6% and 81.2 to 124.8% at the concentration of 0.1 and 0.2 μg kg^{-1}, respectively. For concentrations of 0.3 and 0.4 μg kg^{-1}, the mean recoveries ranged from 82.3 to 128.7% and 94 to 135.5%, respectively. The recoveries ensured that the performance of the extraction method for samples were within acceptable limits (70–120%) for routine analysis [11]. Pesticide residue levels were not corrected for recovery, since most recoveries were above 70% (table 1).

Results and discussion

Occurrence of organochlorine pesticides in carrots from Kampala and Mukono districts

Detectable levels of organochlorine pesticide residues were found in less than half of carrots collected from markets in Kampala and Mukono (figure 1). Of the 200 pieces of carrots from Kampala analysed, 62% contained detectable levels of 2,4′-dichlorodiphenyl-chloroethane (DDE). Trace levels of DDE pesticide was also detected in 47% of samples from Mukono district. The incidence of 2,4′ and 4,4′- DDT in carrots from Kampala was 45 and 38%, respectively. On the other hand, the incidence of 2,4′-DDT and 4,4′-DDT in carrots from Mukono was 12 and 42%, respectively. Gamma (γ)-lindane was found in 27% and 24% of carrots from Kampala and Mukono, respectively. Alpha (α)-lindane was not detected in carrots from Mukono and only 18% contained trace levels of the pesticide in those from Kampala. Similarly, levels of dieldrin were below LOD in samples from Mukono and yet, a high proportion (42%) was in those from Kampala. The occurrence of α-endosulphan was 11% for samples from Kampala and 25% for those from Mukono.

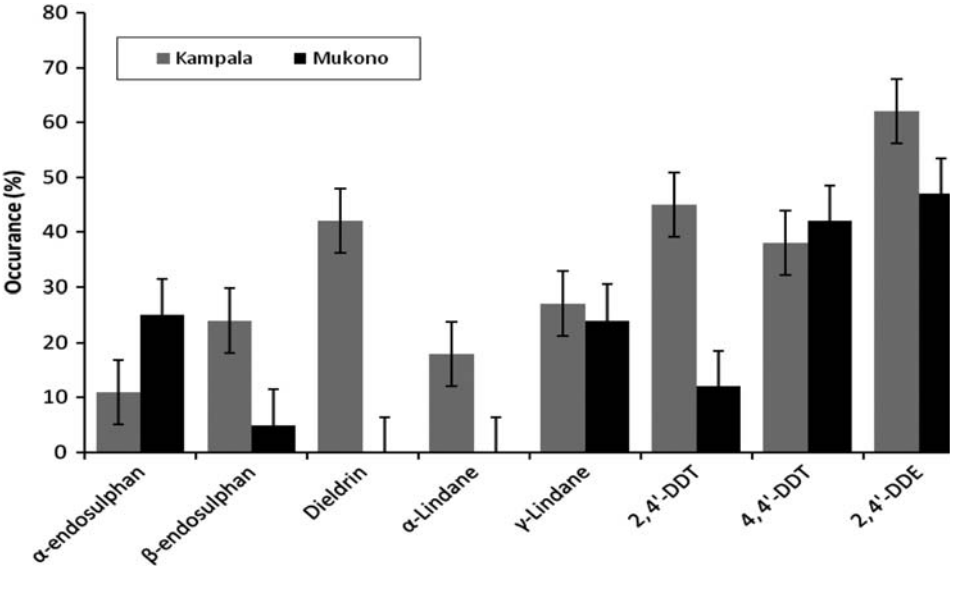

Figure 1. Occurrence of organochlorine pesticides in carrots from major markets from Kampala and Mukono districts. Error bars represent standard deviations for five determinations.

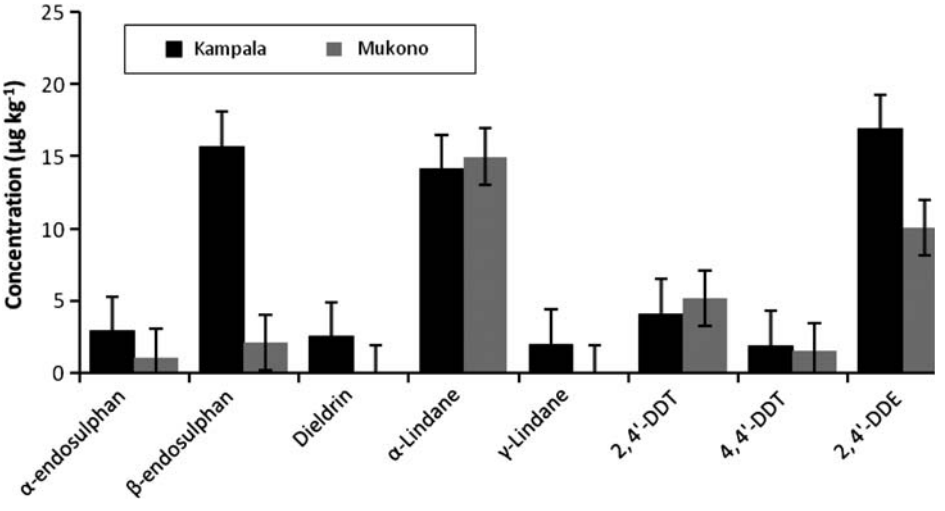

Figure 2. Residual levels (µg kg^{-1}) of organochlorine pesticides in carrots from Kampala and Mukono districts. Error bars represent standard deviations for five determinations.

About a quarter (24%) of carrots from Kampala contained a detectable concentration of β-endosulphan and only 5% was found in that from Mukono.

A high percentage of carrots from Kampala had detectable concentrations of organochlorine pesticide residues compared to that from Mukono. This may be due to the different farming practices in the two districts. Vegetable farms in Kampala operate at a more commercial level than in Mukono, where there is subsistence farming. Pesticide application is a common practice in Kampala to improve crop yields because of the high demands by consumers and prominent hotels. Dieldrin and lindane are applied against aphids and termites that attack tomatoes and other vegetable crops. We found that a reasonable proportion of carrots from Kampala and Mukono markets had very low levels of organochlorine pesticide residues below LOD.

Residual levels of organochlorine pesticides in carrots from Kampala and Mukono districts

Trace concentrations of α-endosulphan, β-endosulphan, dieldrin, 4,4′-DDT, 2, 4′-DDT, 2, 4′-DDE, α-lindane and γ-lindane were found in carrots from Kampala and Mukono (figure 2). Levels of α-endosulphan ranged from not detected to 2.9 µg kg^{-1} in carrots from Kampala. Samples from Mukono also contained fairly low amounts of α-endosulphan residues ranging from not detected to 1.1 µg kg^{-1}. The mean residual concentrations of β-endosulphan ranged from not detected to 15.7 µg kg^{-1} in carrots from Kampala and not detected to 2.1 µg kg^{-1} in those from Mukono. Samples from Nakasero and Nakawa markets contained higher ($p < 0.05$) amounts of β-endosulphan than those from Mukono and Seeta markets (table 2). No α-endosulphan residue was detected in carrots procured from Seeta market. Endosulphan sulphate was not detected in samples from all markets. Endosulphan is an abdomen and contact pesticide applied for controlling leaf hoppers, aphids and used to be sprayed by aircraft in East Africa against tsetse fly [13]. This could in part explain the detected residual amounts of endosulphan in carrots grown around Lake

Table 2. Organochlorine pesticide (μg kg^{-1}) residues in carrots from major markets around Lake Victoria basin.

	Markets in Kampala district		Markets in Mukono district		International Standards	
	Nakasero	Nakawa	Mukono	Seeta	CODEX	EUC
Pesticide	Pesticide residue concentration (μg kg^{-1})				MRL (μg kg^{-1})	
α-endosulphan	0.9 ± 0.8^{ab}	0.4 ± 1.2^{b}	1.1 ± 1.1^{a}	ND	200	50
β-endosulphan	9.8 ± 0.9^{a}	5.2 ± 0.1^{a}	1.6 ± 0.4^{b}	0.2 ± 0.9^{c}	200	50
Dieldrin	1.3 ± 1.8^{a}	0.5 ± 2.3^{a}	ND	ND	100	10
α-Lindane	5.2 ± 1.1^{b}	6.7 ± 0.4^{b}	4.1 ± 1.6^{b}	12.3 ± 1.9^{a}	NL	10
γ-Lindane	0.6 ± 0.1^{a}	1.0 ± 1.8^{a}	ND	ND	NL	10
2,4′-DDT	2.0 ± 1.0^{a}	0.9 ± 0.2^{b}	2.2 ± 0.3^{a}	1.8 ± 1.2^{a}	200	50
4,4′-DDT	0.8 ± 0.4^{a}	0.2 ± 0.1^{a}	0.8 ± 1.4^{a}	0.6 ± 2.1^{a}	200	50
2,4′-DDE	14.0 ± 1.3^{a}	5.2 ± 1.3^{b}	7.2 ± 0.1^{b}	2.9 ± 1.4^{c}	200	50

DDT: dichlorodiphenyltrichloroethane; DDE: dichlorodiphenylchloroethane; MRL: Maximum residue limits. NL: no limits; ND: not detected. EUC: European Union Commission. Values in rows followed by a different superscript letter (a, b, c) are significantly different ($p < 0.05$). Values are averages of eight replicates ± standard deviation.

Victoria basin. Carrots absorb residues of cyclodiene insecticides including endosulphan from the soil [6]. The lower concentration of α-endosulphan in carrots than β-endosulphan points to the fact that the former is more easily dissipated than the latter isomer [14]. The undetected amount of endosulphan sulphate, the most stable isomer, in carrots indicates that there is controlled application of the pesticide around Lake Victoria region.

Dieldrin residues were below detectable limits in carrots from Mukono district while trace levels (2.5 μg kg^{-1}) of dieldrin were detected in those from Kampala. Dieldrin is highly persistent in the environment and its absence could mean that no previous application was carried out in Mukono. In spite of the reported use of the pesticide, residual levels of dieldrin are still reported as being below LOD in fish, African fish eagle and marabou stork from Lake Victoria basin [15,16]. Low levels of dieldrin residue in the Lake Victoria environment are explained by the current reduced usage in contrast with former application against banana weevils [17]. Dieldrin can volatilize and be redistributed by air currents, thus contaminating areas far from its sources.

Mean levels of α-lindane (α-HCH) ranged from not detected to 14.1 μg kg^{-1} and not detected to 15.0 μg kg^{-1} in Kampala and Mukono, respectively. Meanwhile, the concentration of γ-lindane (γ-HCH) was 2.0 μg kg^{-1} in samples obtained from Kampala and was not detectable in those from Mukono. Levels of γ-lindane residue were higher in carrots from Seeta market than those from the other three major markets of Lake Victoria basin. The concentrations of HCH in carrots were within safe levels $\leqslant$10 μg kg^{-1}) for human consumption according to Codex Alimentarius [18] and the European Union Commission [19]. Levels of α-lindane residues were higher than that of γ-lindane in the carrots. Lindane consists of five isomers with α-lindane as a predominant isomer. The gamma-lindane isomer makes up only 12% of the commercial pesticide and is the most unstable isomer [20] despite its insecticidal property [21]. It therefore degrades easily. This explains its low concentration in carrots as compared to α-lindane [22].

Residual concentrations of 2,4′-DDT and 4,4′-DDT in carrots from markets around Lake Victoria region did not present important differences. Levels of 2,4′-DDT in carrots from Kampala district ranged from not detected to 4.1 μg kg^{-1}. Samples from Mukono also had levels below detection to 5.2 μg kg^{-1} of the pesticide. Residue amounts of 4,4′-DDT in carrots from Seeta market were the lowest (2.9 μg kg^{-1}) and those from Nakasero were the

highest (14.0 μg kg^{-1}) (table 2). Higher amounts of 2,4′-DDE were obtained compared to other DDT metabolites (figure 2). This suggests previous application of the pesticide but may indicate controlled application in the environment. Indoor DDT application has been used for malaria control in Uganda. Residual levels of DDT and its metabolites in carrots may be a result of drift or water runoff from indoor application. Trace levels of DDT metabolite, just like other organochlorine pesticides, were previously detected in fish, birds and animal products around Lake Victoria basin [15–17].

On the whole, carrots from the four major markets around Lake Victoria contained very low amounts of pesticide residues within the prescribed limits. Application of pesticides for agricultural production around the lake region did not seem to bring out any important differences. This could also be attributed to the low affinity of organochlorine pesticides for highly polar food systems. Alpha-lindane was particularly found in higher concentration in carrots from Seeta market (table 2). Similarly, DDE pesticide was higher in samples from Nakasero market. The difference is possibly a result of proximity of samples to sites of application and recent use. Nakasero market receives fresh vegetables from more commercial farmers around Kampala compared to other markets [23]. Much produce is brought to the market from commercial farmers who may be using different pesticides to control pests. This may be the reason for detection of pesticide residues in several samples from Kampala. By drift, organochlorines are transported to different places from the source of application. This may explain occurrence of similar residues in the different markets.

The concentrations of organochlorines in carrot skin, flesh and whole parts

Levels of pesticide residues in whole carrots, flesh and skin were not significantly different ($p > 0.05$). Trace amounts of endosulphan, dieldrin, lindane and DDT metabolites in different carrot portions were in close range (figure 3). Residual amounts of α-endosulphan ranged from 1.6 μg kg^{-1} in whole carrot to 1.9 μg kg^{-1} in skin and flesh. Beta-endosulphan amounts were between 4.1 and 5.5 μg kg^{-1}. Dieldrin concentration was 0.5 μg kg^{-1} in both

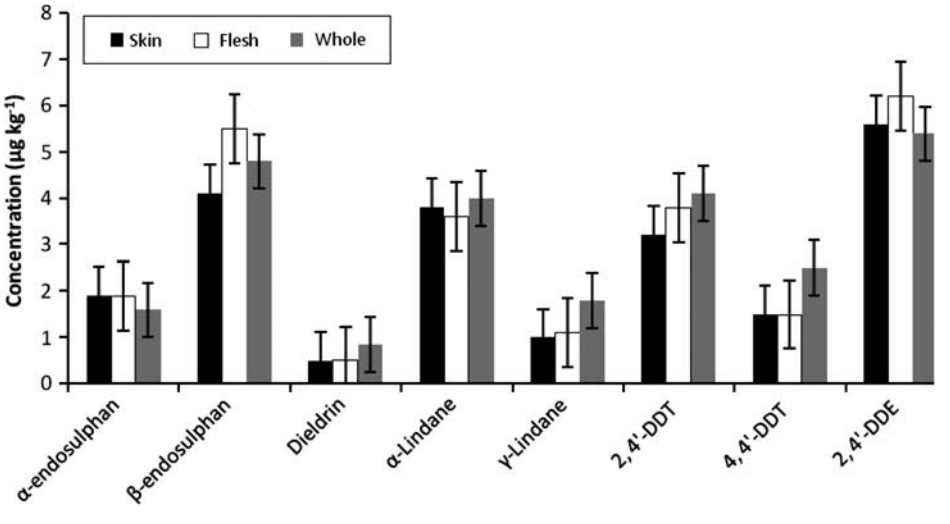

Figure 3. Concentrations (μg kg^{-1}) of organochlorines in the scraped skin, flesh and whole carrot. Error bars represent standard deviations for eight determinations.

skin and flesh, while whole carrot contained 0.85 $\mu g\,kg^{-1}$. The amounts of 2,4′-DDT, 4,4′-DDT and 2, 4′-DDE ranged from 3.2 to 4.1 $\mu g\,kg^{-1}$, 1.5 to 2.5 $\mu g\,kg^{-1}$ and 5.4 to 6.2 $\mu g\,kg^{-1}$, respectively. Residual level of α-lindane in carrot skin was 3.8 $\mu g\,kg^{-1}$ while flesh and whole carrot had 3.6 and 4.0 $\mu g\,kg^{-1}$, respectively. Gamma-lindane was 1.0 $\mu g\,kg^{-1}$ in skin while flesh and whole carrots had respectively, 1.1 and 1.8 $\mu g\,kg^{-1}$. This could be a result of low pesticide residue in the environment and may not be adequate to suggest any distribution pattern.

We observed a homogeneous distribution of organochlorine pesticide residues in skins, flesh and whole carrots (figure 3). The carrot skins contained a quantifiable concentration of pesticide residues. Peeling, therefore, reduces by a substantial amount the pesticides that could be consumed per carrot. Fresh carrots are mostly consumed without peeling because of their very thin skin. From this study, it is advisable to peel contaminated carrots so as to reduce the amount of pesticide ingested. The findings of this study are in accord with those of Randhawa *et al*. [24] who found that potato peeling results in a reduction in pesticide residues. In addition, washing fresh vegetables before peeling has been demonstrated to remove surface pesticides [25]. Accordingly, peeling removes a reasonable amount of pesticide residue that has penetrated the cuticles of vegetables. Bonnechère *et al*. have shown that levels of dimethoate and omethoate pesticide residues are in equal proportion in peels, flesh and whole fresh carrots [26]. This finding is comparable to the homogeneous distribution of α- and β-endosulphan, dieldrin, lindane, 2, 4′-DDT, 4, 4′-DDT and 2, 4′-DDE observed in this study. The low affinity of organochlorine pesticides for polar food systems could explain the observed distribution pattern in carrot portions. Peeling is ultimately important for removing surface pesticide residues in skins of carrots.

Conclusions

Fresh carrots procured from markets in the Lake Victoria basin are safe for human consumption. There may, however, be appreciable amounts of organochlorine pesticide residues on harvested carrots. Peeling raw carrots substantially reduces the pesticide residues contained in the skin. It is therefore advisable to peel carrots from contaminated environments to reduce the amount of pesticide residues that could be ingested.

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