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EVALUATION OF THE REMOVAL OF ORGANOCHLORINE PESTICIDES FROM COWPEA (*VIGNA UNGUICULATA*) BY THE TRADITIONAL FOOD PROCESSING METHODS IN NIGERIA

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ABSTRACT

Daily consumption of cowpea and reported use of pesticides in its preservation could mean daily poisoning, but traditional methods of handling cowpea before consumption could alter the mean concentration of the these pesticides. The effect of washing, heating and dehulling on some organochlorine pesticides (aldrin, o,p'-DDT, endosulfan, heptachlor epoxide and lindane) in cowpea was evaluated. Analysis of the mean concentration of these pesticides were determined using Gas chromatography equipped with Restek column coupled with Mass Spectrometry (GC-MS). Soaking and washing reduces the mean concentration of aldrin, o,p'-DDT, endosulfan, heptachlor epoxide and lindane but not significantly. However, boiling reduces the mean concentration significantly, when compared with the unprocessed cowpea (P value < 0.001). Dehulling the testa eliminated the organochlorine pesticides in the cowpea cotyledon. This study showed that various methods of processing cowpea drastically reduce the occurrence of organochlorine pesticide arising from intake or consumption of cowpea.

KEYWORDS: Cowpea; Pesticide Removal; Dehulling; Soaking and Washing; Boiling.

INTRODUCTION

Removal of pesticides is either Adventitious or Intentional, Adventitious removal is used to designate any loss occurring as a by-product of normal food processing procedures, while Intentional removal of pesticide necessitates processing techniques deliberately designed to remove pesticides and their degradation products.[1] An example of adventitious removal is the traditional food processing methods which are simple household procedures utilized in the process of preparing cowpea meals. They vary from cleaning, sorting, soaking, washing, dehulling and boiling. These methods have been reported to alter anti-nutrients, nutrient content, pesticides and its residues or improve nutrient bioavailability.[2,3]

Cowpea (*Vigna unguiculata* L. Walp), family leguminosae, is grown for its palatable seeds.[4] It is an alternative and rich source of protein when compared with animal sources.[5] It originates from sub-Sahara Africa[6] with Nigeria as the highest producer and consumer. In some households, cowpea is a daily delicacy, prepared by boiling, dehulled and made into paste (moi-moi) or beans cake. A major limitation during the production and storage of cowpea is the attack by several insect pests[7], which include Aphid, Thrips and Weevil.[8] This constraint has been managed by farmers and traders through the application of pesticides which have improved the yield and protected the grain from attack. However these pesticides have been implicated in incidences of food poison.[9]

Pesticide poisoning could either be due to accidental or deliberate. The incidence of food poison in Nigeria have been reported following the detection of pesticides in twenty eateries in 2010.[10] In Borno State, (Northeastern part of Nigeria) incident of pesticide food poisoning was reported in 2008, after a meal of cowpea that was suspected to have been preserved with pesticides.[11] Also, numerous food items exported from Nigeria to the United Kingdom and European Union have either been rejected due to high level of pesticides and their residues.[12] Though the use of these chemicals have been outlawed for decades in many developing countries[13] (UNEP, 2007), but are still being utilized in agriculture in Nigeria surreptitiously under anonymous trade names, due to their low cost[14] and their capacity to improve shelf life of agricultural products.[15,16] The unwarranted

and indiscriminate application of organochlorine pesticides, may cause its retention in food materials and in the process gain assess into the human body via consumption[17], thus causing chronic abnormalities due to its high toxic nature and also destroying the environment and biodiversity.[18] The prolonged accumulation of small doses of these pesticides can cause disorders overtime while triggering unwanted effects immediately at high dose levels.[19] Thus the removal of these residues from food commodities using different home processing methods has become imperative before consumption. This study aims to evaluate the effect of the traditional and home preparation of cowpea by soaking or washing, boiling for 15 minutes and dehulling on the concentration of pesticides in cowpea samples.

MATERIALS AND METHODS

Reagents and Chemicals

All reagents used for this study were of high grade and included analytical grade of anhydrous sodium sulphate (Na_2SO_4) and silica gel, obtained from Oxford laboratory (India). Dichloromethane and acetone were supplied by Labscan (Sigma- Aldrich, USA). In-house purification was used to obtain the distilled water for analysis. Reference standard pesticides for aldrin, o,p'-DDT, endosulfan, heptachlor epoxide and γ-HCH were obtained from Sigma-Aldrich Germany.

Study Area and Sample Collection

Cowpeas were obtained from local wholesale markets in Akwa-Ibom, Bayelsa, Ekiti, Lagos, Ondo, Edo and Rivers states. A total of 48 samples were obtained and an estimated 1.2 kg of cowpea sample were separately collected at random from each location with sterile polyethene zip-lock bags before they were taken to the Pesticide Research Laboratory of the Faculty of Pharmacy, University of Benin, Benin City, Nigeria and stored immediately at a temperature of 4^{o}C until analysed.

Experimentation

There were four experimental treatments viz:

(i) Hand-picked 50 g cowpea seeds were taken from each state and pulverized using a milling machine (Victoria), after which the powder was properly mixed and 10 g Sample were weighed into a white baff which was placed in Whatman pre-extraction thimble of a Soxhlet apparatus, as described by.[20] Extraction was done using dichloromethane (150 ml) at 60 ^{o}C for 3 hrs. Rotary evaporator was thereafter used to reduce the extract to 5 ml at 40 ^{o}C and subsequently dried with sodium sulphate (5 g). Clean-up was done using 60-120 mesh size activated silica gel (analytical grade) with dichloromethane. The combined eluent was dried in rotary evaporator and reconstituted with 1 ml dichloromethane for analysis (GC-MS).

(ii) Using a previously described method by Alfonso and coworkers (1998)[21] with slight modification, another hand-picked 10 g of cowpea were also taken from each location and weighed into 100 ml of distilled water in a beaker at ambient temperature and was allowed to stand for 30 minutes, then filtered and cowpea sample air-dried, pulverized, extracted, cleaned-up, concentrated and analyzed by GC-MS as described above

(iii) Hand-picked 10 g of cowpeas were taken from each location and weighed into 100 ml of boiling distilled water in beaker and allowed to boil for 15 minutes, after which the cowpea sample was air-dried and subject to the same method described earlier.

(iv) Handpicked 10 g of cowpea were taken from each location and weighed into 100 ml of distilled water in a 250 ml beaker and allowed to stand for 30 minutes. Thereafter the testa and cotyledons of the cowpeas were separated, air dried properly and pulverized (Victoria milling machine). The cotyledon were subjected to the same method of extraction as described earlier.

GC-MS Conditions and Analysis

The GC-MS model QP-2010 (Shimadzu) with spitless/split injector and equipped connected to Restek Stx-CL pesticide column (Length 30m, 0.25mm I.D, 0.25µm thickness) was used to identify the target OCPs. An energy of 70 eV was used to generate the mass spectra by monitoring ions (m/z = 50 - 450) in full-scan and selected recording mode (SIM). Injection volume and temperature 1 µL and 250 ^{o}C respectively. Ion and interface source temperature were 200 ^{o}C and 250 ^{o}C respectively, injection and detection temperatures were adjusted to 250 ^{o}C. The column temperature was set at 60 ^{o}C and increased immediately at the rate of 10 ^{o}C/min. to 180 ^{o}C where it was held for 2 min. This was increased by 15 ^{o}C/min., to 280 ^{o}C, and held finally for 4 min.

Quantification, identification and calculations of organochlorine in Cowpea

External standard method was used to determine the concentrations of aldrin, ortho, para'-dichloro-diphenyl-trichloro-ethane (o,p'-DDT), endosulfan, heptachlor epoxide and gamma-lindane (γ-HCH) in cowpeas, by extrapolating the peaks obtained to those obtained from standard calibration curve. Linearity was obtained from the correlation coefficient of the calibration curve. Retention time was also used for the identification of the pesticides obtained from the analysis with that of the standards according.[22] This was further confirmed by setting the mass spectrometry in the SIM mode.

Statistical Analysis

Every experiment was repeated four times, sets of data were presented as Mean±Standard error of mean (SEM) and statistical significance was determined by GraphPad InStat 3 where the means were compared by one way ANOVA (all with post tests) and P was set at 0.05.

RESULTS

The mean concentration of the organochlorine pesticides in unprocessed cowpea showed that aldrin, o,p'-DDT, endosulfan, heptachlor epoxide and lindane were detected in selected markets in the six states (Table 1). Some of them exceed the maximum residual limit set by the European Union and the World Health Organization. The highest mean concentration of Aldrin (445.08±6.43 µg/kg), o,p'-DDT (18.57±5.22µg/kg), Endosulfan (107.00±18.00µg/kg), Heptachlor epoxide (10.21±1.71µg/kg) and Lindane 144.40±15µg/kg) were in Agenebode, Swali and Rumukoro markets. Table 2 shows the reduction in the mean concentration of aldrin, o,p'-DDT, endosulfan, heptachlor epoxide and lindane following soaking and subsequent washing. However, this reduction was not significant ($P \geq 0.05$). Table 3 shows significant reduction in the mean concentration, when compared with the unprocessed cowpea, ($P \leq 0.001$). Organochlorine pesticides were not detected in the cotyledon (cowpea without testa) following the removal of the testa as shown in Table 4. Significant reduction in the organochlorine pesticides was observed using the various traditional method (Table 5).

Table 1: Mean concentrations (in µg/kg) of organochlorine pesticide in cowpeas prior to processing.

States	Towns/Market	Aldrin	o,p'-DDT	Endosulfan	Heptachlor epoxide	Lindane
Edo	Agenebode	445.08±6.43	5.21±0.06	1.03±0.58	2.02±0.43	23.80±14.00
Bayelsa	Swali	1.72±0.79	18.57±5.22	1.28±0.47	1.18±0.84	41.20±23.80
Rivers	Rumukoro	1.85±0.43	10.23±2.03	107.00±18.00	10.21±1.71	144.40±15.00
Lagos	Agege	0.97±0.08	4.66±0.15	1.02±0.10	1.58±0.33	168.00±0.11
Ekiti	Agbado	0.82±0.02	0.09±0.04	0.89±0.08	1.01±0.28	1.88±0.02
Ondo	Ibaka	1.30±0.09	0.11±0.01	0.71±0.05	0.98±0.48	1.24±0.56

Note: Maximum residual limits (MRL)

Table 2: Mean concentration (in µg/kg) of organochlorine pesticides in cowpeas after soaking and washing in water.

States	Towns/Market	Aldrin	o,p-DDT	Endosulfan	Heptachlor epoxide	Lindane
Edo	Agenebode	432.23±7.10	4.70±0.02	0.83±0.60	1.98±0.53	18.42±5.03
Bayelsa	Swali	1.70±0.90	15.62±3.01	1.08±0.50	0.78±0.73	35.31±11.36
Rivers	Rumukoro	1.81±0.39	9.05±1.71	99.07±14.05	9.61±1.01	132.51±4.21
Lagos	Agege	0.93±0.09	4.27±0.20	0.90±0.07	1.14±0.29	155.32±0.24
Ekiti	Agbado	0.73±0.12	0.08±0.02	0.73±0.07	0.90±0.20	1.57±0.02
Ondo	Ibaka	1.01±0.09	0.10±0.01	0.64±0.03	0.83±0.21	1.18±0.26

ND= Not Detected

Note: Maximum residual limits (MRL)

Table 3: Mean concentration (µg/kg) of organochlorine pesticide in cowpeas after boiling for 15minutes.

States	Towns/Market	Aldrin (µg/kg)	o,p'-DDT	Endosulfan	Heptachlor epoxide	Lindane
Edo	Agenebode	133.50±5.32	1.58±0.05	0.22±0.02	0.40±0.02	4.04±0.91
Bayelsa	Swali	0.31±0.03	3.12±2.24	0.27±0.02	0.34±0.03	8.24±2.64
Rivers	Rumukoro	0.33±0.04	2.06±0.12	10.70±3.03	2.04±0.09	26.88±4.70
Lagos	Agege	0.10±0.02	1.21±0.04	ND	0.32±0.08	33.60±0.21
Ekiti	Agbado	ND	ND	ND	0.20±0.02	0.38±0.03
Ondo	Ibaka	0.28±0.01	0.21±0.01	ND	0.11±0.02	0.30±0.01

ND= Not Detected

Note: Maximum residual limits (MRL)

Table 4: Mean concentration (in µg/kg) of organochlorine pesticides in cotyledon after dehulling of the cowpea.

States	Towns/Market	Aldrin	o,p'-DDT	Endosulfan	Heptachlor epoxide	Lindane
Edo	Agenebode	ND	ND	ND	ND	ND
Bayelsa	Swali	ND	ND	ND	ND	ND
Rivers	Rumukoro	ND	ND	ND	ND	ND
Lagos	Agege	ND	ND	ND	ND	ND
Ekiti	Agbado	ND	ND	ND	ND	ND
Ondo	Ibaka	ND	ND	ND	ND	ND

ND= Not detected

Note: Maximum residual limits (MRL)

Table 5: Percentage reduction and P-value of the decontamination methods per pesticides.

Pesticides	Markets	Prior to processing	Soaking (% Reduction)	Boiling (% Reduction)	P-value
Aldrin	Agenebode	445.08±6.43	432.23±7.10 (2.89)	133.50±5.32 (70.01)	0.0001***
	Swali	1.72±0.79	1.70±0.90 (1.16)	0.31±0.03 (81.98)	0.3244
	Rumukoro	1.85±0.43	1.81±0.39 (2.16)	0.33±0.04 (82.16)	0.0305*
	Agege	0.97±0.08	0.93±0.09 (4.12)	0.10±0.02 (89.69)	0.0002***
	Agbado	0.82±0.02	0.73±0.12 (10.97)	ND (100)	0.0003***
	Ibaka	1.30±0.09	1.01±0.09 (22.31)	0.28±0.01 (78.46)	0.0002***
o,p-DDT	Agenebode	5.21±0.06	4.70±0.02 (9.79)	1.58±0.05 (69.67)	0.0001***
	Swali	18.57±5.22	15.62±3.01(15.89)	3.12±2.24 (83.20)	0.0049**
	Rumukoro	10.23±2.03	9.05±1.71(11.53)	2.06±0.12 (79.86)	0.0012**
	Agege	4.66±0.15	4.27±0.20 (8.37)	1.21±0.04 (74.03)	0.0001***
	Agbado	0.09±0.04	0.08±0.02 (11.11)	ND (100)	0.0099**
	Ibaka	0.11±0.01	0.10±0.01 (9.09)	0.02±0.01 (81.82)	0.0001***
Endosulfan	Agenebode	1.03±0.58	0.83±0.60 (19.42)	0.22±0.02 (78.64)	0.1814
	Swali	1.28±0.47	1.08±0.50 (15.63)	0.27±0.02 (78.91)	0.0446*
	Rumukoro	107.00±18.00	99.07±14.05 (7.41)	10.70±3.03 (90)	0.0002***
	Agege	1.02±0.10	0.90±0.07 (11.76)	ND (100)	0.0001***
	Agbado	0.89±0.08	0.73±0.07 (17.98)	ND (100)	0.0001***
	Ibaka	0.71±0.05	0.64±0.03 (9.86)	ND (100)	0.0001***
Heptachlor epoxide	Agenebode	2.02±0.43	1.98±0.53 (1.98)	0.40±0.02 (80.20)	0.0037**
	Swali	1.18±0.84	0.78±0.73 (33.90)	0.34±0.03 (71.19)	0.3439
	Rumukoro	10.21±1.71	9.61±1.01 (5.88)	2.04±0.09 (80.02)	0.0002***
	Agege	1.58±0.33	1.14±0.29 (27.85)	0.32±0.08 (79.75)	0.0027**
	Agbado	1.01±0.28	0.90±0.20 (10.89)	0.20±0.02 (80.20)	0.0049**
	Ibaka	0.98±0.48	0.83±0.21 (15.31)	0.11±0.02 (88.78)	0.0264*
Lindane	Agenebode	23.80±14.00	18.42±5.03 (22.61)	4.04±0.91 (83.03)	0.0715
	Swali	41.20±23.80	35.31±11.36 (14.30)	8.24±2.64 (80)	0.0801
	Rumukoro	144.40±15.00	132.51±4.21 (8.23)	26.88±4.70 (81.39)	0.0001***
	Agege	168.00±0.11	155.32±0.24 (7.55)	33.60±0.21 (80)	0.0001***
	Agbado	1.88±0.02	1.57±0.02 (16.49)	0.38±0.03 (79.79)	0.0001***
	Ibaka	1.24±0.56	1.18±0.26 (4.84)	0.30±0.01 (75.81)	0.0311*

DISCUSSION

Some pesticides used in the storage of seeds persist for an extended period of time before being degraded while organochlorines that persist for a longer time may be converted to residues that could be more hazardous than the parent compound. These compounds could be present at the time of processing for meal. The different methods of processing could either reduce the concentration below the maximum residue limit or eliminate completely the pesticides or its residues.

Persistence of organochlorine pesticides on food items is a multifaceted issue affected by physicochemical properties, nature of applied formulation, adsorbents or solvents employed[23], characteristics of the host, its volatility, stability under ultraviolet radiation and relative tendency to bind to or dissolve in the food or plant items. These factors influence persistency and therefore ease of removal.[1]

Soaking and washing with water of cowpea reduce the concentration of the organochlorine pesticides but not significantly. These processing methods have been reported not to be a reliable means of removing pesticides from vegetables and fruits[2], due to their waxy nature. However, in some vegetables and fruits over 30-78 % removal rate have been reported.[24,25] In Chickpea, soaking was observed to eliminate almost all the pesticide residues. Most of the pesticides in these studies were observed to be organophosphate, carbamates and pyranthrin. The properties of organochlorine pesticides make them difficult to be easily dislodge by soaking. Washing may involve the application of certain amount of agitation, which could allow for effective removal of some of these pesticides. Water solubility of pesticide is a vital feature during washing process, however correlation between pesticide solubility and percentage removal has not been documented.[26] In the present study, water solubility was not found to be the important factor during washing operation. Residues of six pesticides on olives decreased after washing with no correlation to water solubility of the pesticides.

In a study carried out by Aondona and coworker (2019)[3], on the effect of traditional processing methods on pesticide residue dissipation in cowpea, showed that boiling cowpea significantly reduced pirimiphos-methyl and they ascribed this change to leaching. In this current study organochlorine pesticides were identified and treated with distilled water at a temperature of 100 ^{O}C for 15 minutes. It can be implied from the results that boiling has the capacity to reduce the concentration of these

pesticides residues to a safe level (maximum residual limits). This finding is in agreement with those of Kaushik and coworker (2016)[27], which stated that thermal degradation usually led to their effective reductions of pesticides.

The result of this study showed that dehulling of cowpea and subsequent testing of the cotyledon for organochlorine pesticides, showed the absence of organochlorine pesticides. Thus hot water treatment as dehulling are the effective methods of the decontamination of pesticide residues I cowpea. Implying that the pesticides may be limited to the outer part and did not penetrate the testa.

CONCLUSION

These study has shown the importance of processing cowpea before it is prepared made into a meal. Some of these processing methods have been shown to reduce the mean concentration of these pesticides below the maximum residual limit. It combination of heat application and dehulling appears to be these methods could reduce the mean concentration further below the set limit. Some cowpea delicacy have also been shown to be free from pesticides.

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