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Impact of Washing and Fermentation on the Concentration of Pesticide Residues in Nigerian Staple Foods

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Abstract:- The use of pesticides in agriculture is linked to accumulation of toxic compounds in the soil, in food, and its accumulation in animal and human tissues. Pesticide residues are deposits of pesticides that remain on or in agricultural produce after harvest or during storage. This work examined the effects various processing steps on the levels of methoxychlor, dieldrin, p,p'-DDE and α-chlordane residues in common staples; maize, rice, beans and cassava. The treatments administered included cold water washing, hot water washing, and fermentation for 3 and 5 days. The Quick, Effective, Cheap, Easy, Rugged and Safe (QuEChERS) technique was adopted for extraction of pesticide residues while gas chromatography tandem mass spectrometry was used for quantification of the residues in the samples. The processing steps applied had various effects on the level of residues ranging from -149% to 100% reduction in level of pesticide; in rice processing, washing with hot water had the greatest impact in reducing methoxychlor (55.5-97.1%) and α-chlordane (95.5%). Fermentation for 5 days had the greatest impact in reducing methoxychlor residue from cassava (13.6-26.3%), while fermentation of maize for 3 or 5 days had similar impact in reduction of the level of methoxychlor (35.5-79.5% and 39.1-78.4% respectively) and α-chlordane (100% and 100% respectively). Hot water washing of beans was most effective in eliminating methoxychlor (98.4%), p,p'-DDE (100%), α-chlordane (100%) and dieldrin (100%). Reduction in hazard index was also observed with processing, as a result of reduction in amount of pesticide going into the diet.

Keywords:- *Organochlorines, Washing, Fermentation, Nigerian Staples.*

I. INTRODUCTION

Agricultural products such as maize, beans, rice, and cassava are very strategic in the drive towards food security in sub-saharan Africa. However, their post-planting and post-harvest stages could be bedeviled by pests which eventually lead to low produce quality and output. This is an alarming issue which calls for control. For this reason, pesticides are applied on plants in the field or on the product after harvest. Pesticides are chemicals used to protect crops against weeds, insects, fungi, and other pests. Pesticide residues refer to the pesticide content that may remain on or in agricultural products after being applied which could be of toxicological effect to one-health. The general population is exposed to pesticide residues either through consumption of treated food or close proximity to the areas treated with those pesticides. Most of these chemical residues especially derivatives of chlorinated pesticides could bioaccumulate causing health hazards. Pesticides on farm may be washed into the water bodies and other vegetation thereby posing negative threats on aquatic life and other plants (Stephen and Benedict, 2011).

The staple under study are major parts of the diet of humans. Maize is a major source of carbohydrate especially in the developing world. They are often treated with pesticides to avoid pest infestation during storage (Ogah *et al*., 2012). Beans are leguminous crops that are widely grown for human and animal consumption in the tropics and subtropics. Despite the importance of the bean crop for human health and needs, it is plagued by insect infestations from the field to the storage facility. Insects are a major contributor to low yields in African bean crops, affecting every tissue component and developmental stage. Infestation pressure is responsible for over 90% of yield loss in extreme infestations (Isegbe *et al*., 2016). Cassava has been found to be an important food source for millions of people in the tropical regions of Africa, Latin America and Asia (Atehnkeng *et al*., 2006). Although the use of pesticides on cassava is not so common, it could be imparted on by soil

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uptake of pre-planting herbicides and pesticides used on other crops where mixed farming is being practiced. According to Jones (1995), rice is a major commodity in world trade cultivated throughout the tropics. Rice is commonly cultivated in low land areas with high water harboring potential; hence it could be indirectly impacted by pesticide associated with water run-offs.

The safety of food has significant impact on food security (SDG 2) and human health (SDG 3), hence the increasing concern on safe food consumption. Residual chemicals are persistent organic contaminants that have serious lethal effect on human health when exposed beyond certain levels. In order to mitigate the health impact associated with the consumption of pesticide residues in food, this work examined the effect of washing, soaking, and fermentation processes on the residual content of methoxychlor, p,p'-DDE, dieldrin and α-chlordane in rice, maize, cassava and beans. It also took into account the effect of processing on the hazard index of residues in food.

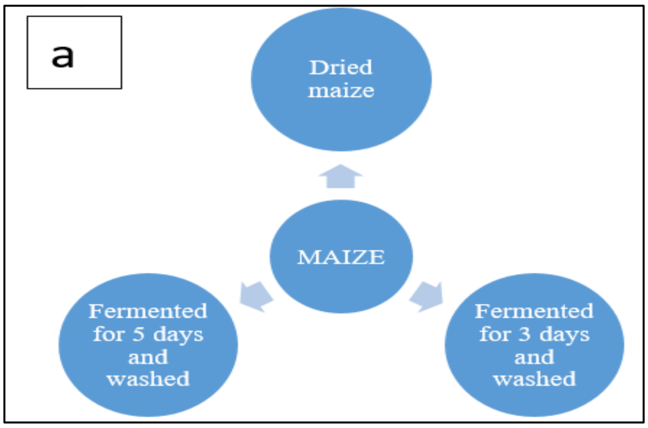
II. MATERIALS AND METHODS

A. Study Area, Sample Collection and Preparation

Food samples were taken from four towns in Kogi State, Nigeria. About 1kg of maize, cassava, beans and rice samples were purchased from open markets into ziploc bags.

B. Study Design

Samples were screened for the presence of pesticide residues. After obtaining the GC-MS/MS result and establishing samples that were contaminated, selected contaminated samples were processed to determine the effect of processing on level of residual pesticides. The processing steps are described below: fermentation/soaking for three and five days (cassava, maize), soaking in cold water for 5 minutes and washing off the water (beans, rice), allowing to boil in hot water for 5 minutes and washing off the water (beans, rice) and sun drying (cassava, maize). After processing, samples were subjected to extraction and quantification of pesticide residues, to determine the effect of processing on the levels of residual pesticides. Following processing, a total of 26 samples were analysed for residual pesticides.



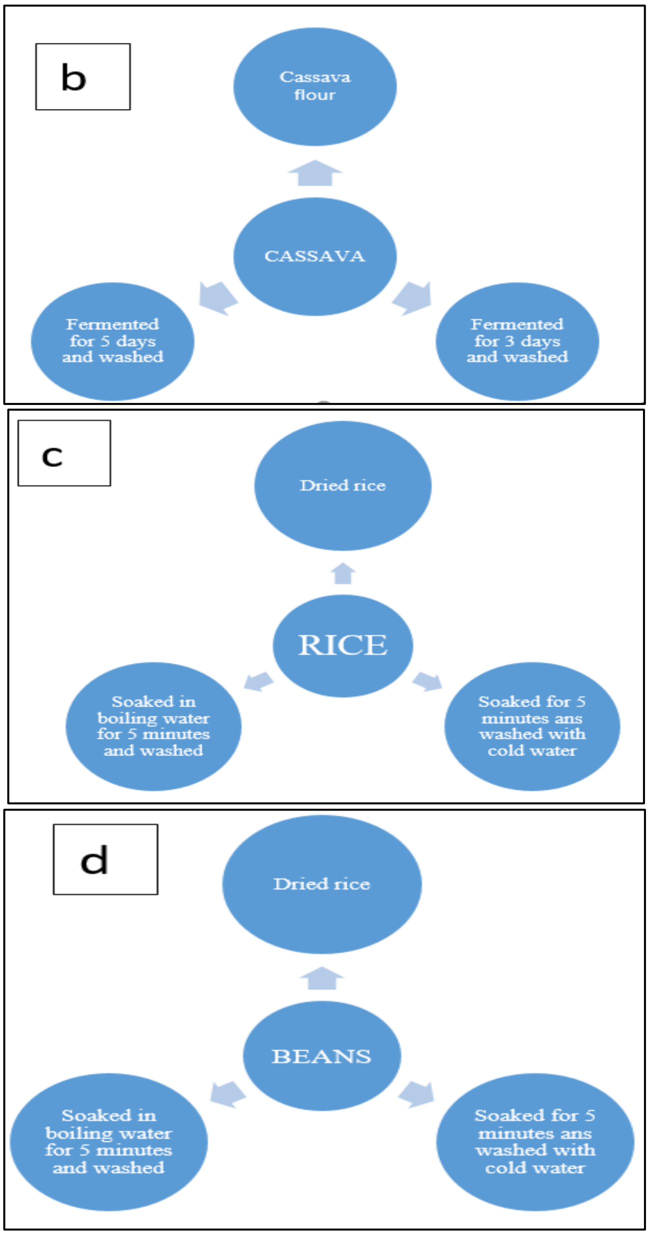


Fig 1: Optional Processing Steps Applied to Staples a-Maize, b-Cassava, c-Rice and d-Beans

C. Extraction and Quantification of Pesticide Residues

The Quick, Effective, Cheap, Easy, Rugged and Safe (QuEChERS) method was employed to extract the residual pesticides from the food samples. This involved a single extraction in acetonitrile with the addition of salt and buffers to aid the extraction of both non-polar and polar compounds. Gas chromatography was use to determine pesticides. The extract (2 µl) was injected into a TQ8040 Gas chromatography tandem Mass spectrometry equipped with; SH-RXi-55il MS-type fused silica capillary column (30m, 0.25mm id & film thickness 0.25µm), helium gas, flow control mode: linear velocity, one minute sampling time, 200 °C ion source temperature, 250°C interface temperature, CID gas: Argon gas. The pesticide residues assayed for were organochlorides; α- chlordane, Dieldrin, P, P'- DDE, and Methoxychlor.

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D. Data Analysis

Data obtained from the GC-MS/MS analyses were analyzed using Ms Excel 2013. Thereafter, figures and tables were used to present the results.

III. RESULTS AND DISCUSSION

A. Effect of Processing on Pesticide Residues in Food Samples

Figure 2 to 14 show the effect of various optional food processing steps on the concentration of four (4) pesticides residues while Table 1 show the percentage reduction in the concentration of the pesticide residues due to the corresponding treatments. The result shows that washing of rice after soaking for five minutes in cold water is able to reduce the concentration of pp'-DDE by 28.4%, methoxychlor by 44.4-86.2% and α-chlordane by 94.7%. Allowing rice to boil for five minutes before washing off the water was able to reduce the concentration of methoxychlor by 55.5-97.1% and α-chlordane by 95.5% but makes pp'-DDE more available (-149%). It further revealed that fermentation of cassava for 3 or 5 days has minimal effect on the reduction of levels of pesticide residues; its effect on methoxychlor was its reduction by -5.3-26.3% and 13.6-28.1% respectively. This may be due to slow water removal from cassava unlike the other staples where the water used for washing can be discarded almost completely.

The effect of processing of maize by 3- and 5-day long fermentation on the level of methoxychlor and α-chlordane in maize was high, result revealed similar range when the treatment lasted for 3 days or 5 days; 35.5-79.5% and 39.1-78.4% respectively for methoxychlor and 100% for α-chlordane. Cold water washing of beans was effective in removing high level (98%) of pp'-DDE residues from beans, hot water per-boiling was effective with the removal of 100% of p,p'-DDE, dieldrin and α-chlordane residues and about 98.4% of methoxychlor.

The effectiveness of washing on removal of pesticide residue could depend on the location of the residue, age, water solubility, temperature and the type of washing (Holland *et al*., 1994). It is also affected by the nature of sample, such as thickness, type, wax amounts on the cuticle, and surface area; pesticide characteristics; and retention time of various pesticide residues (Yang *et al*., 2022). According to Bonnechère *et al* (2012), the most effective technique for removing pesticide residues from agricultural products in households and commercial processing is washing. To support this statement, the study of Yang *et al*. (2022) examined various methods of washing on reduction of pesticides from leafy vegetables such as; alkaline water washing, blanching, boiling, washing with $NaHCO_3$, washing with detergent, washing with running water, washing with stagnant water, ultrasonic cleaning, and washing with vinegar, they concluded that washing under running water was the most effective method of all in pesticide removal.

Several other studies have established the important role of washing in removal of pesticide residues from various food matrices. Ryad and Mahmoud (2016) reported that washing of olives in cold water for 5 minutes removed 48% of cypermethrin, 26%-36% of chlorpyrifos, 67% of diazinon, 66% of profenfos, and 26%-39% of L-cyhalothrin. Lentza-Rizos *et al*. (2006) also reported that the residues of azoxystrobin on grapes were reduced by 75%.

During rice cooking, Medina *et al*. (2021) in their study concluded that pre-soaking rice with extra water before cooking proved to be the method that generates the greatest reduction in pesticide level, the authors thought that soaking probably contributes to increasing grain water absorption capacity, achieving a greater effect of destroying pesticides during cooking. Blanching (washing) of beans in warm water has shown effectiveness in reduction of DDT in beans by 50%, while washing removed 20% of DDT from potato (Holland *et al*., 1994). Soaking of beans resulted in 9.88% reduction while boiling resulted in 48.15% reduction in the concentration of pirimiphos-methyl (Aondona *et al*., 2019). Thus, supporting the effect of both cold and hot water washing treatment of beans found in this study, and suggesting that warm/hot water treatment of beans is more effective than cold water washing in removal of pesticide residues.

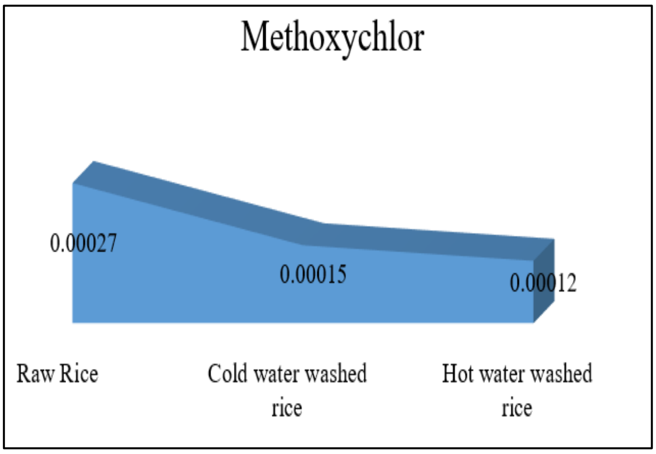

Fig 2: Effect of Processing on Level of Methoxychlorin Rice (1)

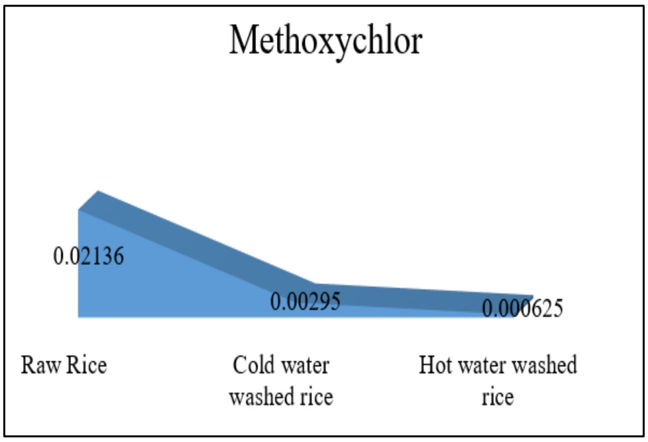

Fig 3: Effect of Processing on Level of Methoxychlor in Rice (2)

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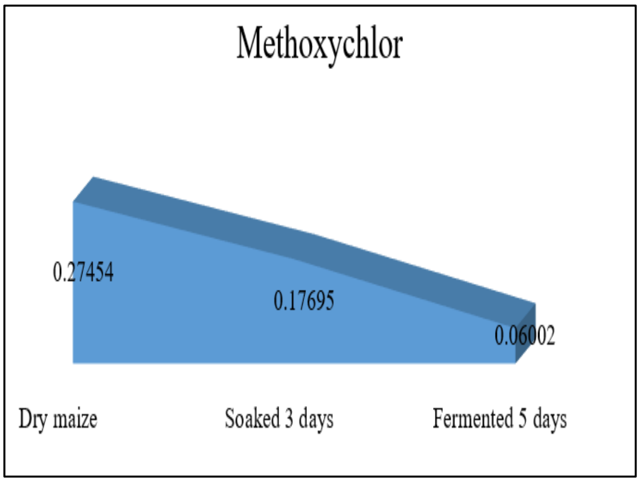

Fig 4: Effect of Processing on level of Methoxychlor in Maize

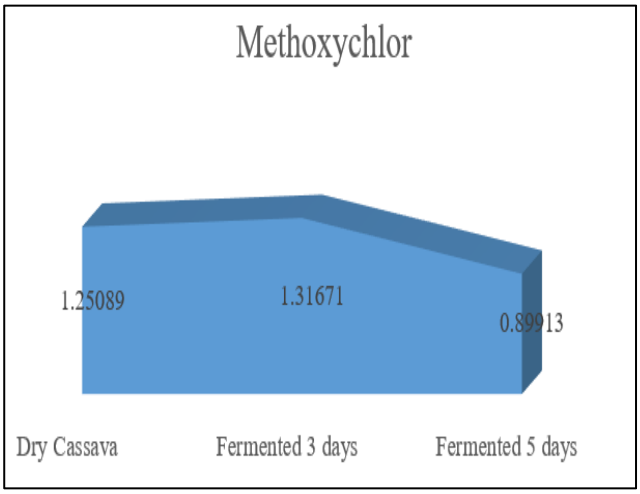

Fig 7: Effect of Processing on Level of Methoxychlor in Cassava

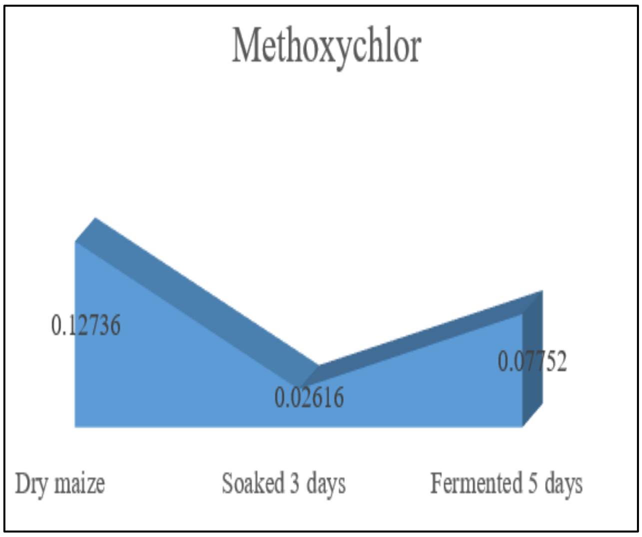

Fig 5: Effect of Processing on Level of Methoxychlor in Maize

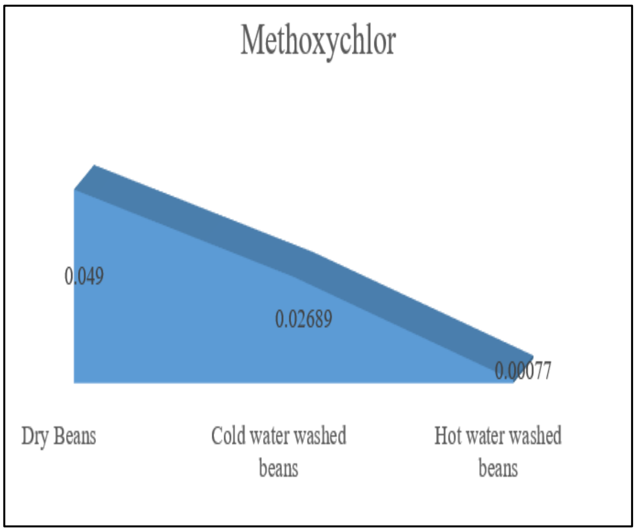

Fig 8: Effect of Processing on level of Methoxychlor in Beans

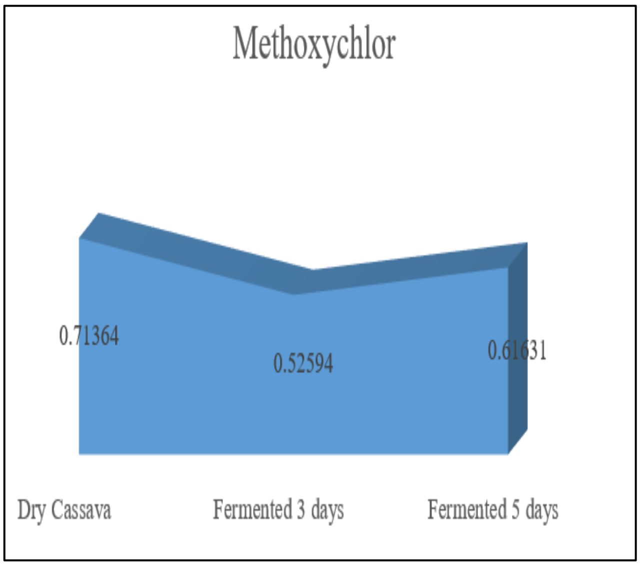

Fig 6: Effect of Processing on Level of Methoxychlor in Cassava

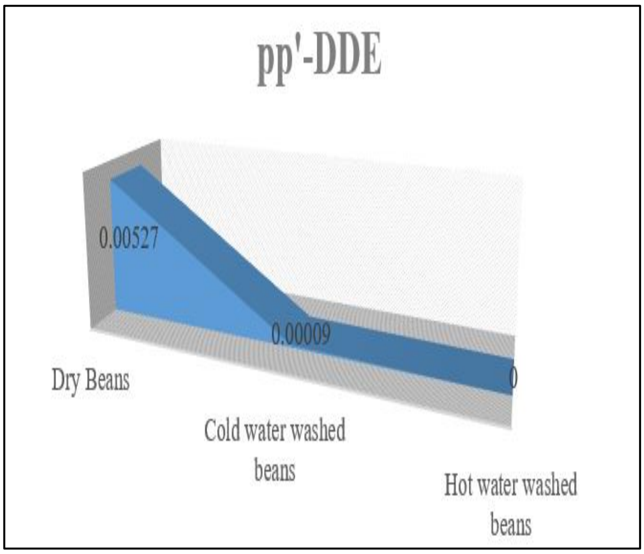

Fig 9: Effect of Processing on level of pp'-DDE in Beans

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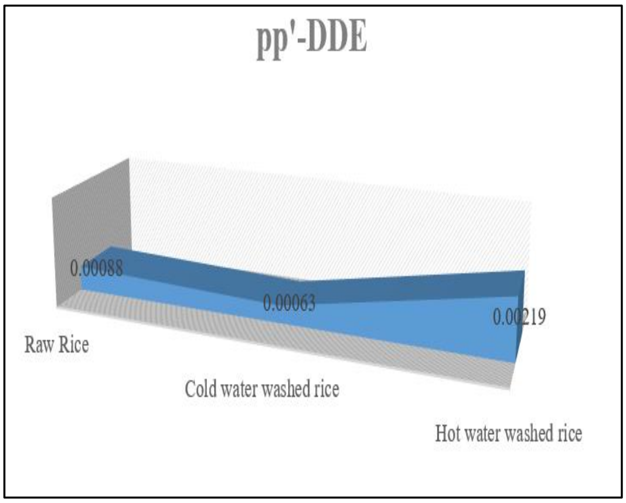

Fig 10: Effect of Processing on Level of pp'-DDE in Rice

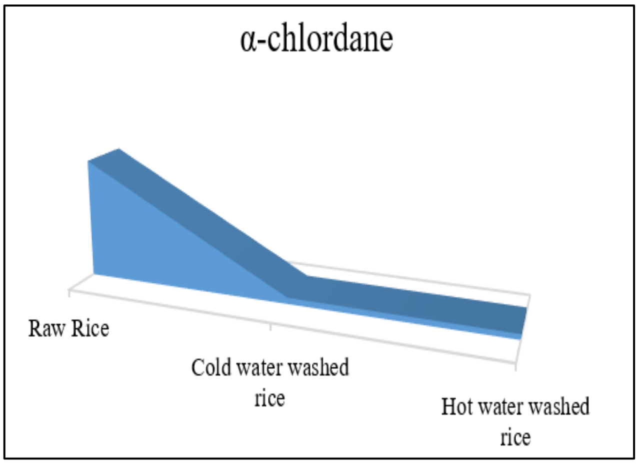

Fig 11: Effect of Processing on Level of α-Chlordane in Rice

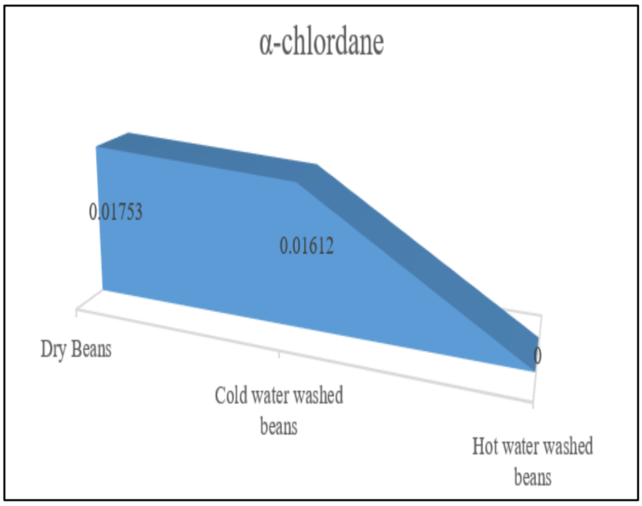

Fig 12: Effect of Processing on Level of α-Chlordane in Beans

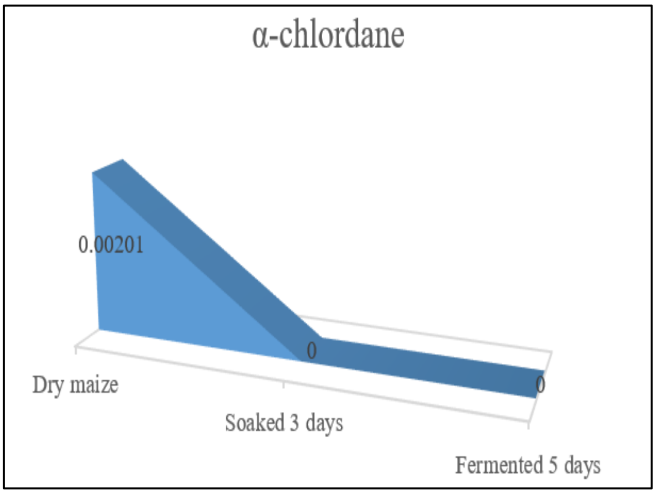

Fig 13: Effect of Processing on Level of α-Chlordane in Maize

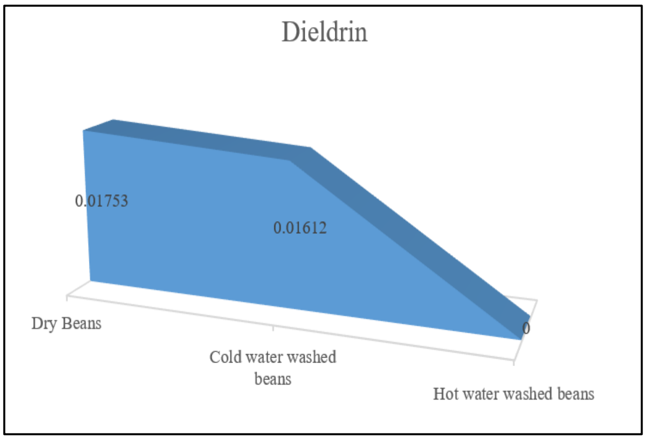

Fig 14: Effect of Processing on Level of Dieldrin in Beans

A variety of pesticides applied directly on crops undergo limited penetration, and are mostly confined to the outer surfaces, as such food processing steps such as washing, peeling and dehulling could be effective in removing them (Holland *et al*., 1994). Previous studies suggested that processing steps such as soaking and steaming at 100^{0}C lead to the degradation of pesticide (Kaushik *et al*., 2009; Pareja *et al*., 2011). Generally, surface residues will be more amenable to washing than systemic residues. Other factors that may impact residue removal by washing includes; the water solubility of the residue, the washing temperature, and length of time between application and processing (Holland *et al*., 1994). This study shows how important basic food processing steps are in reducing levels of various pesticides. The percentage reduction suggests that these pesticides will be bioavailable at levels for below their MRL if properly processed before consumption.

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Table 1: Effect of Processing (% Reduction) on Pesticide Residues in Food

Treatments	p,p'-DDE (%)	Methoxychlor (%)	Dieldrin (%)	α-chlordane (%)
Cold water washed rice	28.4	44.4-86.2	-	94.7
Hot water washed rice	-149%	55.5-97.1	-	95.5
Fermentation of cassava (3 days)	-	-5.3-26.3	-	-
Fermentation of cassava (5 days)	-	13.6-28.1	-	-
Fermentation of maize (3 days)	-	35.5-79.5	-	100
Fermentation of maize (5 days)	-	39.1-78.4	-	100
Cold water washed beans	98	45	8.04	8.0
Hot water washed beans	100	98.4	100	100

Fermentation has been identified as a processing method that possess high potential for reduction of pesticide residues in food. One highly effective and promising method involves fermenting foods using natural microflora or intentionally introducing probiotic strains. Detoxification processes may entail enzymatic hydrolysis or oxidation of pesticides, breaking them down into less harmful substances (Armenova *et al*., 2023). In this study, fermentation was effective in reducing methoxychlor (35.5-79.5%) and α-chlordane (100%). In a study by Zhang *et al*. (2016), application of strains of the bacteria *Lactiplantibacillus plantarum* at room temperature for 10 weeks reduced phorate levels in corn silage by 24.9 - 33.4%. Đorđević *et al*. (2013) also reported 81% degradation of pirimiphos/pirimiphos-methyl by *Lactiplantibacillus plantarum* during wheat fermentation. In another study, *Saccharomyces cerevisiae* degraded glyphosate by 21% during bread fermentation within one hour (Low *et al*. 2004), *Saccharomyces cerevisiae* also reduced the concentration of endosulfan (70%), deltamethrin (63%), malathion (60%), propiconazole (52%), chlorpyriphos (51%), and hexaconazole (46%) during dough fermentation (Sharma *et al*., 2005). These reports and the finding in this work are indicative of the effectiveness of fermentation in reducing the level of residual pesticide.

B. Determination of Hazard Index before and after Processing

From appendix II, it is seen that the hazard index of pesticides also decreases as their concentration decreased. A reduced HI is implicated in reduction of potential health risk, toxicity, the duration of exposure, reduction in cumulative effect of pesticides, an indication of strength of regulation and education. This will also impact on the dietary intake of the pesticide residues in food.

IV. CONCLUSION

This study showed that simple processing step which are sometimes not engaged such as washing with cold water, washing with hot water and fermentation were effective in reducing residue concentrations in p,p'-DDE, methoxychlor, dieldrin, α-chlordane from food despite their partial or very low solubility in water. In rice processing, washing with hot water had the greatest impact in reducing methoxychlor (55.5-97.1%) and α-chlordane (95.5%). Fermentation for 5 days had the greatest impact in reducing methoxychlor residue from cassava (13.6-26.3%), while fermentation of maize for three days or five days were not significantly different in reduction of the level of methoxychlor and α-chlordane. Hot water washing of beans was most effective in eliminating methoxychlor (98.4%) and dieldrin (100%). With this finding, the populace is encouraged to adopt the optional processing steps on food where and when applicable, as they have been seen to have residue minimization and elimination effects.

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APPENDIX 1

Levels of Pesticide Residues Resulting from Various Processing Treatments

Food	Code	Treatment	Methoxychlor	pp'-DDE	α-chlordane	Dieldrin
RICE	H1	Raw Rice	0.00027	ND	ND	ND
RICE	H2	Cold water washed rice	0.00015	ND	ND	ND
RICE	H3	Hot water washed rice	0.00012	ND	ND	ND
RICE	G1	Raw Rice	0.02136	0.00088	0.01122	ND
RICE	G2	Cold water washed rice	0.00295	0.00063	0.00059	ND
RICE	G3	Hot water washed rice	0.00063	0.00219	0.00051	ND
MAIZE	C1	Dry maize	0.27454	ND	0.00251	ND
MAIZE	C2	Soaked 3 days	0.17695	ND	0.00000	ND
MAIZE	C3	Fermented 5 days	0.06002	ND	0.00000	ND
MAIZE	D1	Dry maize	0.12736	ND	0.00201	ND
MAIZE	D2	Soaked 3 days	0.02616	ND	0.00000	ND
MAIZE	D3	Fermented 5 days	0.07752	ND	0.00000	ND
BEANS	A1	Dry Beans	ND	0.00527	ND	0.01753
BEANS	A2	Cold water washed beans	ND	0.00009	ND	0.01612
BEANS	A3	Hot water washed beans	ND	0.00000	ND	0.00000
BEANS	B1	Dry Beans	0.04900	ND	ND	0.01753
BEANS	B2	Cold water washed beans	0.02689	ND	ND	0.01612
BEANS	B3	Hot water washed beans	0.00077	ND	ND	0.00000
CASSAVA	E1	Dry Cassava	0.71364	ND	ND	ND
CASSAVA	E2	Fermented 3 days	0.52594	ND	ND	ND
CASSAVA	E3	Fermented 5 days	0.61631	ND	ND	ND
CASSAVA	F1	Dry Cassava	1.25089	ND	ND	ND
CASSAVA	F2	Fermented 3 days	1.31671	ND	ND	ND
CASSAVA	F3	Fermented 5 days	0.89913	ND	ND	ND

APPENDIX 2

Hazard Index of Pesticides before and after Treatment

FOOD	CODE	TREATMENT	Methoxychlor	pp'-DDE	α-chlordane	Dieldrin
			0.01	0.1	0.02	0.02
RICE	H1	Raw Rice	0.027			
RICE	H2	Cold Water Washed Rice	0.015			
RICE	H3	Hot Water Washed Rice	0.012			
RICE	G1	Raw Rice	2.136	0.0088	0.561	
RICE	G2	Cold water washed rice	0.295	0.0063	0.0295	
RICE	G3	Hot water washed rice	0.0625	0.0219	0.0255	
MAIZE	C1	Dry maize	27.454		0.1255	
MAIZE	C2	Soaked 3 days	17.695		0	
MAIZE	C3	Fermented 5 days	6.002		0	
MAIZE	D1	Dry maize	12.736		0.1005	
MAIZE	D2	Soaked 3 days	2.616		0	
MAIZE	D3	Fermented 5 days	7.752		0	
BEANS	A1	Dry Beans		0.0527		0.8765
BEANS	A2	Cold water washed beans		0.0009		0.806
BEANS	A3	Hot water washed beans		0		0
BEANS	B1	Dry Beans	4.9			0.8765
BEANS	B2	Cold water washed beans	2.689			0.806
BEANS	B3	Hot water washed beans	0.077			0
CASSAVA	E1	Dry Cassava	71.364			
CASSAVA	E2	Fermented 3 days	52.594			
CASSAVA	E3	Fermented 5 days	61.631			
CASSAVA	F1	Dry Cassava	125.089			
CASSAVA	F2	Fermented 3 days	131.671			
CASSAVA	F3	Fermented 5 days	89.913			