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Screening for Genetically Modified Maize in Raw and Processed Foods Sold Commercially in Southern Nigeria Border States

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Abstract

Background and Objective: Due to the increasing number of Genetically Modifided Organisms cultivation and delayed approval of biosafety law in Nigeria, it became necessary to screen maize products in order to determine the identity of the consumed foodstuffs. The study was designed to screen for the presence of regulatory genes (35*S* promoter and *NOS* terminator) and common transgenes in food products sold in Southern Nigeria.

Materials and Methods: DNA was extracted from the raw and processed maize foods sold commercially in Southern Nigeria using the Cetyltrimethylammonium bromide method, followed by qualitative Polymerase Chain Reaction (PCR) to detect genetically modified maize. The recombinant DNA target sequences were detected with specific primers for CaMV 35S, nopaline synthase terminator, Bt-176 and NK603 genes. Certified reference materials were used as positive controls while organic maize grains and absence of template DNA served as negative controls.

Results and Conclusion: Bt-176 maize event (for insect resistance) was detected in two samples while the NK603 maize event (for herbicide tolerance) was detected in three samples. Four imported raw maize samples, four cereal food brands (two Nigerian made, two imported) and three imported canned corn brands were genetically modified and unlabelled. The results showed that Nigerians were already consuming GM maize before the biosafety law was enacted.

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1. Introduction

The development of new technology and inventions in genetic engineering have given rise to genetically modified organisms (GMOs). These organisms carry genetic material that has been altered by the insertion or deletion of genes in order to confer pest resistance and herbicide tolerance, or to improve the quality of their produce [1]. The use of GMOs in foods products or as food is getting more wide spread over the years. A huge variety of food crops has been genetically modified to contain beneficial traits [2].

The biosafety assessment of GMOs is required for their environmmental impact and also for the health of the consumers. It was demonstrated that unauthorized and potentially unsafe GM products may sometimes be found in the market [3]. Although the global area with GM crops has been increasing steadily, the European Union continues to be a region where the commercial cultivation of GM crops is very limited. Regulations on genetically modified food and feed have been in operation in the European Union since April 18th, 2004 [4].

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However, in Nigeria, the biosafety law was only enacted on April 20, 2015. The infiltration of unapproved and spurious varieties of GM crop seeds into the market has been the reason for controversies regarding their acceptance amongst the farmers because planting of these varieties leads to crop failures and huge commercial losses. In fact, there is a need for GM testing and detection, which will help to identify GMOs and stop the infiltration of spurious GM varieties into the market [1]. In order to guarantee consumers' freedom of choice, the use of GMOs and GMO-derived products in the food chain is subject to precise regulations in several countries.

While the labelling of foods and derived products are mandatory in the European Union with a threshold of 0.9% most African countries are still lacking such regulations [5]. Hence, platforms for the development of methodologies for GMO detection and quantification have risen all over the world in order to reveal the adventurous presence of GMOs in different matrixes and to comply with the respective regulations of labelling [6]. The future of genetically engineered foods and crops in Africa will depend heavily on the choices that African governments make regarding the regulation of this technology [7]. Most African countries and particularly Nigeria has a significant delay in the field of transgenic plants due to delayed approval of biosafety law.

In Nigeria, biotechnology is expanding, and GMOs are gradually becoming a reality for the consumer. However, it is imperative to have methods that are able to identify and quantify routinely the content of transgenic DNA in different matrixes (raw material and processed food products). The aim of this study, therefore, was to check for GM foods in the Nigerian food system, with special reference to GM maize foods in order to determine if Nigerians were already consuming GMOs before the approval of biosafety law. The study was carried out from August 2011 to October 2013.

2. Materials and Methods

2.1. Sample collection

Samples of raw maize, as well as processed foods containing maize, were collected randomly from all senatorial zones of the Border States of Southern Nigeria. The samples collected were milled in a laboratory food blender (Philips laboratory blender), labelled and stored at +4°C until analyzed. The analyzed products are shown in Table 1.

2.3. Reference materials

The Certified Reference Materials (CRMs) used were maize powder level 2, nominal 0.5% of Bt-176 maize for positive controls, which were obtained from the Institute for Reference Materials and Measurement (IRMM, Geel, Belgium) and stored at +4°C until use.

2.4. Total DNA extraction

Total DNA was extracted according to the method of Yoke-Kqueen et al. [2]. DNA from CRMs as well as from all investigated samples was extracted twice using independent procedures.

2.5. Oligonucleotide primers

Oligonucleotide primers for Polymerase Chain Reactions (PCR) were obtained from Alpha DNA (Canada). The primers were diluted to a final concentration of 10 μM with sterile double-distilled water and stored at -20°C until use. The sequences of oligonucleotide primers are given in Table 2.

2.6. Amplification of maize endogenous gene by PCR

Amplification was carried out using Thermo-cycler (Eppendorf Mastercycler Nexus Gradient GSX1, USA) with a final volume of 20.0 µl, comprising 13.5 µl of ultrapure water, 4.0 µl of FIREPol^R Master Mix (Solis BioDyne), 1.5 µl of DNA template, and 0.5µl of each forward and reverse primer pairs. Amplification was performed at initial denaturation at 95ºC for 8 min, followed by 40 cycles of 30 s at 95ºC, 35 s at 60ºC, 35 s at 72°C, and a final extension at 72°C for 7 min [2]. PCR Marker used was 100 bp DNA ladder from Promega Corporation, USA.

2.7. Amplification of regulatory genes for screening

The conditions for PCR amplification experiments for CaMV35S promoter and Nopaline Synthase (NOS) terminator employed in screening of GM maize food products were: 3 min initial denaturation at 95ºC followed by 40 cycles of 25 s denaturation at 95ºC, 30 s annealing at 62ºC, 45 s extension at 72ºC, and a final 7 min extension at 72ºC [12] using Eppendorf Thermocycler with a final volume of 20.0 µl comprising 13.5 µl of ultrapure water, 4.0 µl of FIREPol^R Master Mix (Solis BioDyne), 1.5 µl of DNA template, and 0.5 µl of each forward and reverse primer pairs.

2.8. PCR amplification of transgenic genes

PCR amplification of *5-enolpyruvylshikimate-3-phosphate synthase* (EPSPS) and the Cry1Ab genes was carried out using the Eppendorf Thermocycler (USA) with a final volume of 20.0 µl comprising 13.5 µl of ultrapure water, 4.0 µl of FIREPol^R Master Mix (Solis BioDyne), 1.5 µl of DNA template, and 0.5µl of each forward and reverse primer pairs. Conditions of amplification used were as follows: initial denaturation at 95ºC for 8 min, followed by 40 cycles of 30 s at 95ºC, 35 s at 60ºC, 35 s at 72ºC, and then a final extension at 72ºC for 7 min [12].

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Table 1. The analyzed product of maize samples and their origin

Food sample	Number and lanes of samples analyzed	Country of origin
Positive control	1	Belgium
Negative control	2	
Maize flour (grains)	3-28	Nigeria
Maize flour (grains)	29-30	Argentina
Maize flour (grains)	31-32	London
Cereal food	33,36,37,38, 39,47,49, 55,56	South Africa, Sourth Africa Nigeria, Benin, Spain, Nigeria, France, Indonesia, Nigeria respectively.
Corn snack	40, 41, 42, 50,62,63	Nigeria, Nigeria, Sourth Africa, Nigeria, Nigeria, Nigeria respectively.
Canned corn	34, 35,43,44,45	Thailand, Thailand, USA, England, Thailand respectively.
Custard powder	46,48,51,52,53, 54, 61	Nigeria.
Corn flakes	57,58,59,60	Nigeria, UK, Germany, Nigeria respectively.
Total	61	

Table 2. Oligonucleotide primers used to detect species- specific or transgenic DNA sequences in maize products by Polymerase Chain Reaction (PCR)

Target	Sequence	Primer name	Amplicon length (bp)	Reference
Zein	AGTGCGACCCATATT CCAG GACATTGTGGCATCATCATTT	Zeo3 Zeo4	227 bp	[8]
NOS Terminator	GAATCCTGTTGCCGGTCTTG TTATCCTAGTTTGCGCGCTA	NOS-F NOS-R	180 bp	[9]
CaMV 35S Promoter	GCTCCTACAAATGCCATCA GATAGTGGGATTGTGCGTCA	35S-F 35S-R	195 bp	[9]
Cry1A	CGGCCCCGAGTTCACCTT CTGCTGGGGATGATGTTGTTG	CRYIA-F CRYIA-R	420 bp	[10]
EPSPS	CACTATCCTTCGCAAGACCCTTCC CTTCTGTGCTGTAGCCACTGATGC	HR-F HR-R	320 bp	[11]

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Table 3. Summary of results of Screening of maize samples from Southern Nigeria for zien gene, regulatory sequences of genetic modification, insect resistance (CryIA) and herbicide resistance (HR) genes

Food sample	Number of samples analyzed/ lanes	zien gene	35S	NOS	CRY	HR
Maize flour (grains)	3-32	26	4	4	0	3
Cereal Food	33,36,37,38, 39,47,49, 55,56	5	3	0	1	0
Corn snack	40, 41, 42, 50,62,63	2	1	0	0	0
Canned corn	34, 35,43,44,45	5	3	2	1	0
Custard powder	46,48,51,52,53, 54, 61	0	0	0	0	0
Corn flakes	57,58,59,60	0	0	0	0	0
Total	61	38	11	6	2	3

PCR marker used was 1kb DNA ladder (Promega, USA) with sizes ranging from 250 bp to 10000 bp.

2.9. Visualization on agarose gels

DNA was separated using 1.5% agarose gel and visualized under UV light after staining with ethidium bromide for molecular size estimations of the DNA [13].

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3. Results and Discusion

3.1. The presence of maize gene in foods (DNA amplifiability)

The zein gene was detected in thirty eight (38) samples as shown in Figure 1 with amplification occurring with the control primer pair. This resulted in an amplicon of the expected size in 38 out of the 61 samples and CRMs. No amplification was observed in the PCR control without DNA. The primer pair Zeo3/Zeo4 is specific for the zein gene and flanks part of exon number 3 of this gene. It gives rise to a 227 bp amplicon [8]. This product is detectable in both transgenic and conventional maize (maize-specific primer pair). Maize-specific primer pairs served as a control for the amplifiability of the isolated DNA and for the PCR procedure (PCR quality control).

It was observed that DNA amplification by all low processed maize samples yielded almost equal concentrations of PCR products while amplification by all the medium processed materials gave approximately the same intensity of PCR product but their bands were less intense than those of the low processed samples. Most of the highly processed samples did not amplify probably due to the heat and pressure to which these samples had been exposed. According to Jasbeer et al. [14], the genomic maize DNA integrity can be influenced by many factors, such as the quality of starting material, processing nature, storage condition, storage period and the matrix itself.

However, the good amplifiable DNA of the processed food was in agreement with a study, which demonstrated good DNA amplification from highly processed food products using CTAB method [15, 16]. The presence of endogenous genes in the samples confirmed that the CTAB DNA extraction method used was adequate for the extraction of amplifiable maize DNA from the samples. For processed samples, which were not amplified, it is likely that either the DNA content was insufficient, or there were PCR inhibitors affecting them. Food samples comprise of a complex mixture containing PCR inhibitors that may compromise the amplifiability of DNA [17]. It was not possible to amplify the *zein* endogenous genes in the DNA isolated from corn flakes, corn starch, curstard and some corn snacks because of the presence of inhibitors of DNA polymerase. This agrees with a study by Ahmed [18], which revealed that heating and other processes associated with food production can degrade DNA. Methods based on PCR are not suitable for highly processed foods because the DNA might have been already fragmented to pieces smaller beyond what can be effectively detected by the PCR method [19]. From the gel images, it was observed that genomic DNA in some low processed food samples of maize did not amplify. This problem is easily overcome by selecting other extraction protocols. As demonstrated in another study, Jasbeer [14] reported that no single extraction method could produce consistently high amounts of amplifiable DNA in all the samples. Those samples that did not produce amplicons were eliminated.

3.2. Screening for CaMV35S promoter and NOS terminator sequence

The CaMV35S promoter gene (195 bp) was detected in 11 samples (29, 30, 31, 32, 33, 34, 35, 39, 40, 43, and 54) as shown in Figure 2. The samples which showed the presence of the CaMV35S segments, were further analyzed using appropriate primers for the presence of specific transgenic elements. PCR amplification using NOS-F and NOS-R primers gave an 180 bp amplicon in 11 (29, 30, 31, 32, 33, 34, 35, 39, 40, 43 and 56) maize samples as shown in Figure 3, indicating the presence of the NOS terminator sequence. These samples were analysed further using primers for the presence of specific transgenic elements. The CaMV35S promoter gene was detected in 11 out of the 38 maize samples, which produced amplicons with 35S primers. NOS terminator could also be detected in six samples as shown in Figure 3. All the eleven 35S and six NOS positive samples were further analyzed by PCR with specific primers for insect resistance and herbicide tolerance.

Transgenic maize (corn) has been deliberately genetically modified to have agronomically desirable traits. Traits that have been engineered into corn are resistance to herbicides (Glyphosate and Glufosinate-tolerant crops), and a gene that codes for the *Bacillus thuringiensis* toxin, protecting plants from insect pests. Herbicide and pest-resistant hybrids have also been produced. Corn varieties resistant to glufosinate (Liberty) herbicides and roundup have been produced [20]. Other commercialized herbicide-tolerant GM-maize varieties are classified into two types: glyphosate-tolerant and glufosinate ammonium-tolerant based on the inserted herbicide-tolerant genes. Examples are GA21 and NK603 varieties for the former, and Bt11, Event176, CBH-351 and T25 varieties, which have both herbicide and insect-resistant properties [20].

The samples that showed the presence of CaMV-35S and NOS segments were further analysed using appropriate primers for presence of specific transgenic elements

3.3. Trait-specific gene

Of the 11 GM positive maize food products, which had the regulatory (NOS terminator and/or CaMV 35S promoter) genes, the Cry protein (420 bp) that confers insect resistance, was detected in two GM maize samples (as shown in Figure 4). However, the EPSPS gene (320 bp) that confers tolerance to the herbicide glyphosate was detected in only three samples of raw maize using the HR-F and HR-R primers.

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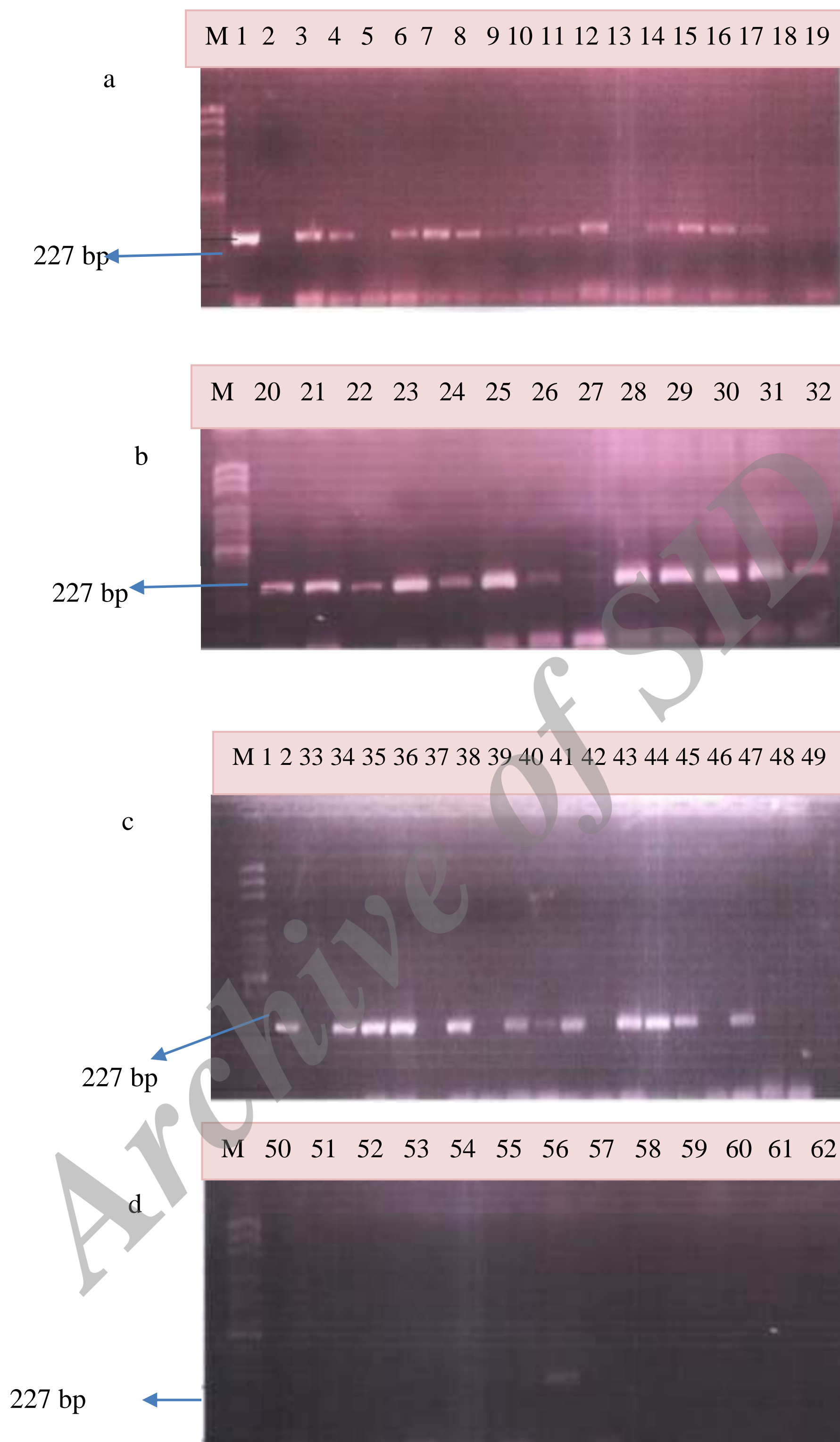

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Figure 1. Agarose gel electrophoresis of *zein* (maize-specific) gene fragment amplified by PCR from DNA of raw maize from Southern Nigeria using Zeo3 and Zeo4 primers. (a) lane M: 100 bp molecular weight marker, lane 1: CRM maize, lane 2: no template DNA (negative control), lane 3-19 maize samples. (b) lane M: 100 bp molecular weight marker, lane 20-32 Maize samples. (c) lane M: 100 bp molecular weight marker, lane1: CRM maize, lane 2: No template DNA (negative control), lane 33: cereal food, Line lane 34 and 35 canned corn, lane 36- 39: Cereal food, lane 40–42:corn snack, lane 43–45: canned corn, lane 46: custard powder, lane 47: cereal food , lane 48: custard, lane 49: cereal food. (d) lane M: 100 bp molecular weight marker, line 50: corn snack, lane 51-54: custards, lane 55 and 56: cereal food, lane 57-60: cornflakes, lane 61: custard, lane 62: corn snack.

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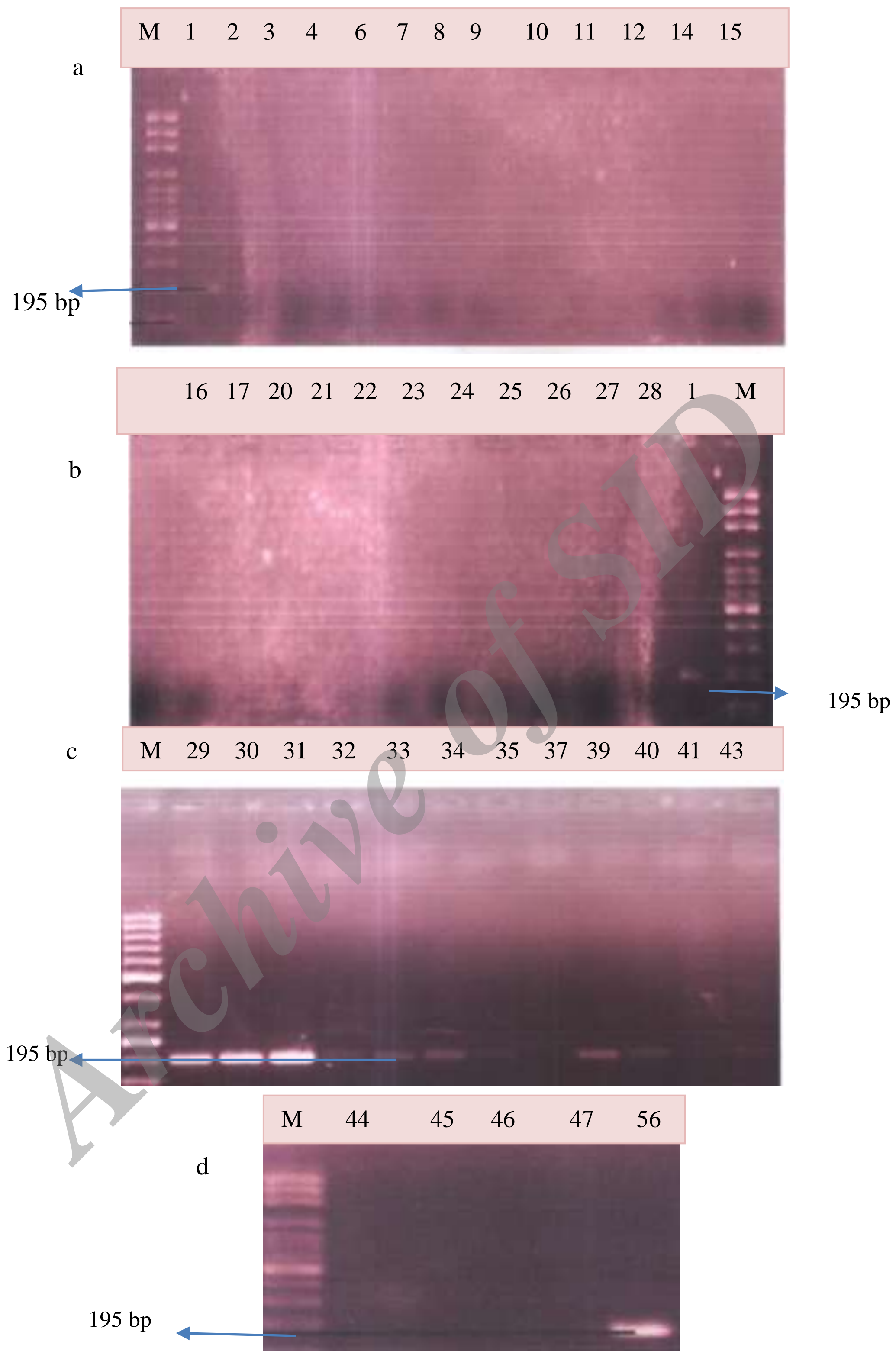

Figure 2: Agarose gel electrophoresis of CaMV 35S promoter gene sequence amplified by PCR from DNA of maize food samples from Southern Nigeria, using 35S-R and 35S-L primers. (a) Lane M: 100 bp marker, Lane 1: Certified Reference Material (positive control), Lane 2: no template DNA (negative control), Lane 3: FUNAAB organic maize, Lane 4-15: maize samples. (b) Lane 1: reference material (positive control), lane 16 – 28: maize samples. (c) Lane M: Marker, lane 29-30: raw maize from Argentina and lane 31-32: raw maize from London, Lane 33: is cereals food, lane 34 and 35: canned corns, lane 37 and 39: cereals food, lane 40 and 41: corn snack, lane 43 canned corns. (d) Lane 44-45: canned corn, lane 46: custard powder, lane 47 and 56 cereal food

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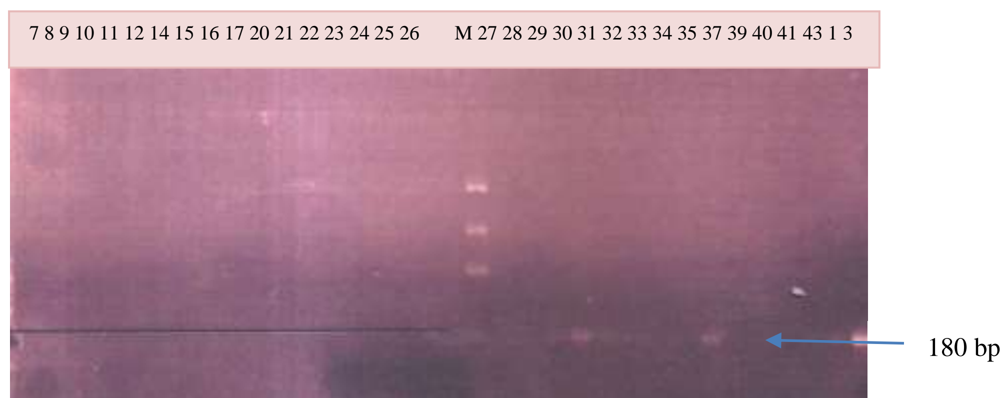

Figure 3: Agarose gel electrophoresis of Nopaline synthase (NOS) gene sequence amplified by PCR from DNA of maize food samples from Southern Nigeria, using NOS-F and NOS-R primers. Lane M: 1kb marker, lane 1: CRM, lane 3: FUNAAB organic maize, lane 4-28: raw maize from Southern Nigeria, lanes 29-30: raw maize from Argentina and lanes 31-32: raw maize from London, lane 33: cereal food, lane 34 and 35: canned corn, lane 37and 39: cereal food, lane 40 and 41 corn snack, lane 43 canned corn.

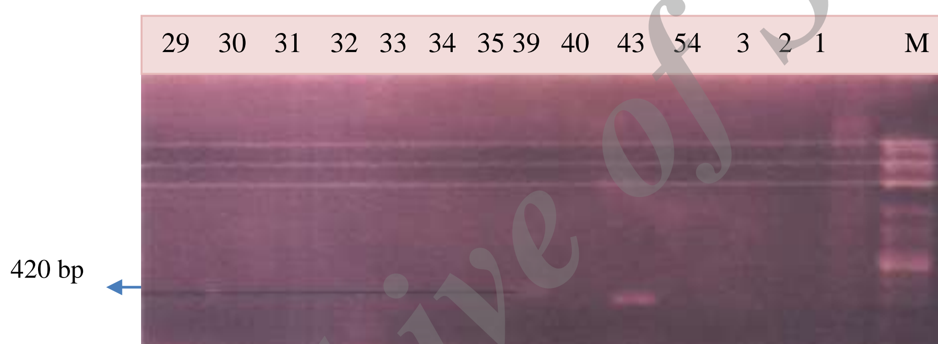

Figure 4: Gel electrophoresis of Cry1Ab gene fragment amplified by PCR from DNA of maize samples from Southern Nigeria, using CryIA-F and CryIA-R primers. Lane M: 1kb DNA marker, lane 1: Reference Material, Positive control. Lane 2: No template negative Control, lane 3: FUNAAB maize, lanes 29-30: samples which showed the presence of CaMV35S and/or NOS segments.

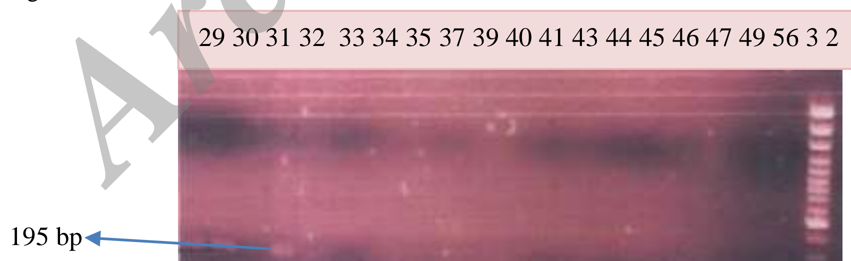

Figure 5: Gel electrophoresis of EPSPS gene amplified by PCR from DNA of maize samples from Southern Nigeria, using HR-F and HR-R primers Lane M: 1kb DNA marker, lane 1: Reference Material (Positive control), Lane 2: No template (negative control), Lane 3: FUNAAB organic maize (negative control), lane 29-56: samples which showed the presence of CaMV35S and/or NOS segments

Two (sample 39: cereal food and sample 43: canned corn) out of the 11 samples screened were found to be positive for the GM event Bt 176, as shown in Figure 4. The Glyfosate-resistance gene was detected in three samples of maize (imported from Argentina and London) as seen in Figure 5. This result is in line with the findings of Randhawa and Firk [21] who suggested that HR primer can be used in the routine screening of GM soybean and maize.

The detection limit is defined as the minimum amount of DNA necessary to yield a visible signal on agarose gel after amplification [16]. This is also in accordance with Miraglia et al. [22] who defined

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the limit of detection as the lowest quantities that can be reliably detected. Based on the definition, 0.5% Bt-176 from IRMM that was used as reference material was below the limit of detection as there was no visible signal on agarose gel after three times of amplification. However, in this study, some samples that were positive for 35S and NOS genetic elements, did not show positive signals for the Bt-176 maize line and NK603. This could be due to the presence of a GM maize line other than Bt-176 and NK603. Four GM-positive samples were detected from the imported raw maize samples; 3 cereal food brands (1 manufactured in Nigeria, 1 manufactured in Spain and 1 manufactured in South Africa), corn snack and 3 canned corn brands (1 manufactured in USA and 2 manufactured in Thailand).

As expected, the organic corn from the Federal University of Agriculture, Abeokuta, Nigeria, used as negative control, gave negative result. In most of the processed samples, no positive detetion was made. In the present study, GM DNA was detected in some of the processed and raw food products, which were gathered from a Southern Nigerian market. This study indicated that GM maize exists in the Nigerian food market, meaning that despite the fact that Nigeria had no biosafety laws as at the time when this study was carrried out, GM foods were already coming into the country.

4. Conclusion

The results of the study clearly demonstrated the presence of unlabelled GM maize in the Nigerian Market, indicating that Nigerians were consuming GM foods unknowingly, even before the biosafety law was enacted, emphasizing the need for implementing labelling systems for GM food products.

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6. Conflict of interest

Authors declare that there is no conflict of interest.

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