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Comparative Evaluation of Two DNA Isolation Protocols For PCR Detection In Processed Fruit Juices

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Abstract
Background: *Fruits are relatively easy to authenticate morphologically when intact and fresh. However, the act of processing them into juice gives rise to the possibility of substitution with cheaper products. For this reason, processed food products authentication is primarily significant for consumers, industries, and regulatory agencies. Effective, reliable, and rapid food authentication methods are valuable tools for identification of natural fruit pulp in reconstituted fruit juices to ensure juice quality and safety hence mitigate adulteration and fraud. Molecular-based methods have recently acquired immense priority for their ability to pick food material source at any stage along the food supply chain. This study focused on evaluation of two DNA isolation protocols from processed plant products specifically reconstituted juices. The robustness and sensitivity of the protocol for genomic DNA recovery from processed juices determines DNA quality and purity. This is because the degradation and chemical additives associated with processed fruit juice samples could directly act as PCR inhibitors.*
Results: *Two genomic DNA extraction protocols; CTAB and SDS were tested for isolation of DNA from processed fruit juices. The CTAB and SDS methods were able to recover genomic DNA of high quality and purity appropriate for application in various PCR analyses with little limitations in the CTAB protocol. The concentration of the DNA was determined using the Nano-drop spectrophotometer in µg/µl by calculating the absorbance at wavelengths (A260/A280nm: A260/A230nm). The quality of the extracted DNA was evaluated on 0.8% agarose gel electrophoresis stained with 1µl ethidium bromide and observation of bands integrity done in UV-trans-illuminator machine (Quantum ST4, France). PCR amplification was done using universal primers (rbcL-650 bp, psbA-323 bp) that target the plant chloroplast genome). DNA extracted from SDS method exhibited robustness and ease during PCR amplification process. The amplified bands quality and integrity were evaluated on 1.5% agarose gel stained with 1µl ethidium bromide.*
Conclusion: *Results from the study show an innovative experimental methodology that efficiently extracts, amplify, and identify natural fruit juice pulp by utilizing universal biomarkers to test for quality and authenticity of reconstituted fruit juices in Kenyan markets.*
Keywords: *rbcL, psbA, CTAB, SDS*

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I. Introduction

Processed food products authentication is primarily significant for consumers, industries, and regulatory agencies. This is due to the rapid globalization and advancement in local to international food markets. In addition, it is paramount to ensure that consumers are appropriately educated about the origin, type and composition of the available processed products in the market (Sovova& Ovesná, 2018). The necessity for precise, sensitive, elaborate, and reliable protocol for plant materials identifications especially in processed fruit products is on the rise due to current food scares and technological advancement in food production system. Furthermore, development of high added value products based on plants has increased concerns on adulteration hence effective methods for consumers and companies' protection are required (Panagiotis M *et al.,* 2014). Several analytical methods have been used in the authentication of processed fruit juices such as HPLC, GC-MS. These methods based on chemical profile are easily affected by factors such as cultivar, growing region, climate, harvest maturity, storage atmosphere (Eisele & Drake, 2005) and processing conditions (Thomaidis *et al.,* 2019). The afore-mentioned limitations have prompted the use of DNA based techniques (Manuela *et al.,* 2017). The techniques based on DNA analysis are becoming a custom for identification of raw materials

(Woolfe & Primrose, 2004), and hence could compliment the chemical collection of methods which are frequently ineffective in processed food products due to the complex nature of food matrices. Furthermore, techniques based on DNA analysis are more advantageous in accuracy, speed, short sample preparation, high sample throughput, inter-laboratory reproducibility, cost effective and robustness in contrast to biochemical and chemical methods, since the DNA is more resistant to industrial processing than other food markers (Marieschi *et al.,* 2016).In addition, DNA based detection methods, primarily the conventional RT-PCR are reliable in contrast to chemical analysis tools for fruit juice validation. This is mainly because of DNA's stability to various environmental conditions, farming modes and technology of production thus making it a good starting material for downstream analytical procedures (Madesis *et al.,* 2014).

Even-though, molecular-based methods for instance PCR, are sensitive, robust, highly specific and takes shorter processing time, they are hindered by presence of inhibitors in processed food materials. Consequently, the success of DNA based protocols is highly depended on DNA extraction techniques. Thus, DNA extraction protocols generally require to be rigorously optimized to ensure an efficient recovery of DNA of suitable yield and quality able to be amplified from various materials and foodstuffs(Di Pinto e*t al.,* 2007).This is because every DNA based assay that determines food authenticity is dependent on downstream PCR-based molecular markers analysis(Asensio *et al.,* 2011).Recovering genomic DNA of high quality and quantity from small amount of tissue is a challenging undertaking. This is because genomic DNA extraction especially from processed food materials is always a significant starting point in molecular biology research hence, reproducible, reliable and achievable DNA based method solely depends on its pre-requisite step of DNA extraction (Pereyra *et al.,* 2012). A DNA recovery protocol should exhibit simplicity, robustness, timely and reproducibility in any molecular laboratory setting. Furthermore, it must be applicable across different states of sample types and generate genomic DNA of high quality and purity. The DNA quality is paramount for the subsequent molecular analyses since PCR amplification can be affected by presence of contaminants or inhibitors that later decreases the PCR sensitivity and efficiency. (Youssef M *et al.,* 2015). There are various procedures for DNA isolation which differ in parameters such as extraction buffer components, purification reagents and other steps; hence it is wise to select the appropriate protocol for the sample in question (Turci *et al.,* 2010). Given that different extraction protocols can be applied in recovery of genomic DNA showing diverse levels and yield, the quality and purity of each end results can considerably differ at some extent. For that matter, the main endeavor of any DNA extraction protocol should be to extract the DNA of high quality, quantity, and purity for the purpose of ensuing analyses such as PCR amplification (Branquinho *et al.,* 2012)). This is because, DNA isolation protocols can greatly affect PCR analyses in many ways either by; presence of PCR inhibitors in food matrix, excessive degradation of DNA molecules, and short average length of DNA fragment (Ruibal *et al.,* 2012). The PCR analysis allows identification of small traces of DNA residing in food in food matrices from primary components or contaminants. The aim of this study was to optimize and validate a robust, efficient, and sensitive DNA isolation protocol that utilizes universal and specific biomarkers to test for quality and authenticity of natural fruit pulp in reconstituted fruit juices. The main goal of this study was to validate a quick, reliable and sensitive protocol for isolation of amplifiable genomic DNA from selected processed fruit juices in the Kenyan markets. This molecular-based technique was to be applied to test for the quality and authenticity of natural fruit pulp in reconstituted juices.

II. Material And Methods

Study site and design

This was a cross sectional study involving processed fruit juices obtained from vendors/ traders in selected regions of Mt Kenya (Nyeri, Embu, Kiambu), Coast (Kwale, Kilifi, Mombasa) and Nairobi (South C) in Kenya.

http://fews.net/east-africa/kenya/enhanced-market-analysis/september-2018.

(Figure 1): A map showing the selected sampling regions for processed fruit juices

Mt Kenya (Nyeri, Embu, Kiambu)

Mount Kenya Region (Nyeri, Embu, Kiambu)

Nyeri is a town situated in the Central Highlands of Kenya. It is located at latitude: 0° 24' 59.99"N: 36° 56' 59.99" E. Embu is located approximately between latitude 0° 31' 5S and longitude 37° 27' 2E. Kiambu is located) at latitude: 1°10'0.01"S Longitude: 36°49'59.99"E.

Nyeri, Embu and Kiambu are among high fruit producing regions. The Delmonte fruit Juice processing Company is in Thika town in Kiambu County. The Karurumo self-help group fruit processing plant in Embu County which produces both fresh and processed fruit juices. The three towns provide direct market for both fresh and processed fruit juices from local vendors and industries.

Coast Region (Kwale, Kilifi, Mombasa)

The former coast province of Kenya; Located at latitude; 4.0584° S, 39.6677° E. The coastal regions especially, Kwale, Kilifi and Mombasa are highest fruit and fruit juice producing regions (Milly Fruit processor is in Mombasa). Coast region is economic hub with high population density. Therefore, provides a ready market and increased demand for fresh and processed fruit juices.

Nairobi; South C

This is both a residential as well as industrial estate in Nairobi, a capital city in Kenya.; its located on geographical coordinates 1° 19' 0" S, 36° 50' 0" E. The large population and industrialization in Nairobi increase demand of fruit juices produced in many regions around Kenya as a major consumer of the fruit juices.

Samples and sampling procedure

Four different species of fruit juices were randomly sampled as representatives of home- made, processed/reconstituted fruit juices for the study. Sampled fruit species were mango, orange, pineapple and apple. The choice of the fruit species was informed by the fact that they are the most popularly grown, readily available and easily processed to make fruit juices both locally and internationally. Juice extracted from intact fruits was used as positive controls for validation of experimental samples.

Sample Preparation

The samples collected from the sample sites were packed in properly sealed cartons and transported to KEBS molecular laboratory. Secondary sampling and coding was done where both processed and homemade juice samples were carefully transferred into 1.5 ml eppendorf and 15 ml falcon tubes labeled and stored in the freezer at -80°C for further analyses. In addition, for the controls fresh and intact fruits were bought from Nairobi market and supermarket, packed in the cool box at (-20ºC) and transported to the laboratory for analysis. The fruits were washed and chopped aseptically, then blended to extract juice.

DNA Extraction

Genomic DNA was extracted from processed fruit juices, homemade juices and fresh and intact fruit samples. The DNA was extracted using modified SDS and CTAB methods by Alice Muchugi et al., 2008 and (Edwards et al.,1991) and according to N.J. Gawel (1991) respectively.

Qualitative Analysis of Extracted DNA

Two conventional extraction protocols (CTAB & SDS) were applied to compare and determine the appropriate and robust method of D NA extraction specifically for processed fruit juices. The sensitivity, robustness and efficiency of each DNA recovery and purification method were determined by taking the measurements of recovered DNA concentration and its suitability for PCR amplification. The comparison of extraction and purification procedures was performed from freshly prepared, homemade, and reconstituted fruit juices. The concentration of the DNA was estimated by measuring absorbance at 260 nm (A260) and 280nm (A280) using the Nano-Drop 72020C Spectrophotometer (Genway Genova, UK) whereby each quantification was repeated twice. The yield, purity and quality of extracted DNA was determined by calculating the ratio of absorbance at 260 nm and absorbance at 280nm (A260/ A280) and the ratio at 260nm and 230nm (A260/A230), (considering that one absorbance unit is equal to 50ug/cm ^{3} DNA obtained as shown the formular-P* (DNA) = 50ug ×A260 ug/cm$^{3)}$. The ratio was around 1.8, which is the measure of a good quality genomic DNA extracted. Quality and yield assessments of all DNA samples was additionally carried out by resolving he DNA on 0.8% agarose gel, stained with Ethidium Bromide and bands obtained visualized using in gel documentation system (Incaba-biotec, 180711023, UK) & ((Quantum-ST4. 100-26 MIX France).

Primer Design and DNA Amplification

The available sequences of the coding genes (Ribulose Bisphosphate Carboxylase Large-rbcL) and (the non-coding plastid (trnH-psbA inter-genic spacer), were obtained from the National Centre For Biotechnology Information– Gen-bank database and alignment carried out using standard programs CrustalW available from the computational services of the (European Molecular Biology laboratory database (http://www.ebi.ac.uk/services). The alignment of sequences was for the search of conserved regions to ensure primers that are representative across different species/genomes were carefully selected.

PCR amplification

The rbcL and psbA-trnH genes in chloroplast genome were obtained from Gene Bank data base and used for amplification using Polymerase Chain Reaction (PCR). A preliminary experimental phase was done using DNA extracted from fresh fruit juices (controls) where DNA extracts were analyzed by PCR for evaluation of their suitability for amplification. The PCR process was done concurrently for multiple fruit species. The conventional PCR was applied (Veriti 96 well by Applied Bio-systems Ref 4375786). The reaction was performed in a final volume of 25µl using 12.5µl Taq DNA polymerase 2x Master Mix-Red (AMPLIQON DK-5230 Odense M, Denmark), 1µl of each primer, 1µl of 25mM MgCl, 1µl DMSO and 3.5µl of ddH_2O and 5µl of template DNA. The PCR cycling conditions were as follows; Initial incubation at 95ºC for 75 sec, a denaturation temperature of 95ºC for 30 sec, with a total of 35 amplification cycles, annealing at 58ºC for 45 sec, extension elongation at 72ºC for 1minute, and final extension at 72ºC for 5 minutes.

Agarose Gel Electrophoresis

The PCR-amplified variable sections of the DNA (Table 1) were resolved on 1.5% agarose gel at 90 volts for 40 minutes.

Table 1: Published Sequences of DNA primers and their sizes

Primer name	DNA Sequences (5'–3')	Product size(bp)	References
ITS2	5 forward CCTTATCATTTAGAGGAAGGAG 4 reverse TCCTCCGCTTATTGATATGC	221	(Yao et al., 2010).
rbcL	1 forward ATGTCACCACAAACAGAAAC 724 reverse TCGCATGTACCTGCAGTAGC	650	(Zhang, Jiang, Duan, & Zhou, 2016).
Mat K	390forward CGATCTATTCATTCAATATTTC 1326reverseTCTAGCACACGAAAGTCGAAGT	638	
psbA-trnH	PA forward GTTATGCATGAACGTAATGCTC TH reverse CGCGCATGGTGGATTCACAATCC	323	(Kress, Wurdack, Zimmer, Weigt, & Janzen, 2005).

III. Results

3.1: DNA extraction

The results are as reported in table2 and figures 1-4.

Table 2: DNA Concentration & Purity Determination

Method	**Fruit**	**A260/A280**	**A260/A230**	**ng/µl**
CTAB	Pineapple	1.91	2.18	99.99
	Pineapple	1.62	1.12	91.02
Mean (Average)		**1.77**	**1.65**	**95.51**
	Orange	1.65	1.260	119.48
	Orange	1.32	1.96	102.23
Mean (Average)		**1.49**	**1.61**	**110.86**
	Mango	1.35	2.00	115.81
	Mango	1.45	1.83	231.0
Mean (Average)		**1.40**	**1.42**	**173.41**
	Apple	1.088	1.587	105.57
	Apple	1.05	2.01	95.26
Mean (Average)		**1.07**	**1.80**	**100.42**
SDS				
	Pineapple	1.71	1.00	174.41
	Pineapple	1.82	1.56	147.68
Mean (Average)		**1.77**	**1.28**	**161.05**
	Orange	1.31	0.631	194.92
	Orange	1.62	1.12	207.84
Mean (Average)		**1.47**	**0.88**	**201.38**
	Mango	1.762	0.273	117.72
	Mango	1.706	1.036	540.0
Mean (Average)		**1.74**	**0.65**	**328.86**
	Apple	1.44	0.57	121.74
	Apple	1.36	0.345	231.32
Mean (Average)		**1.40**	**0.46**	**176.53**

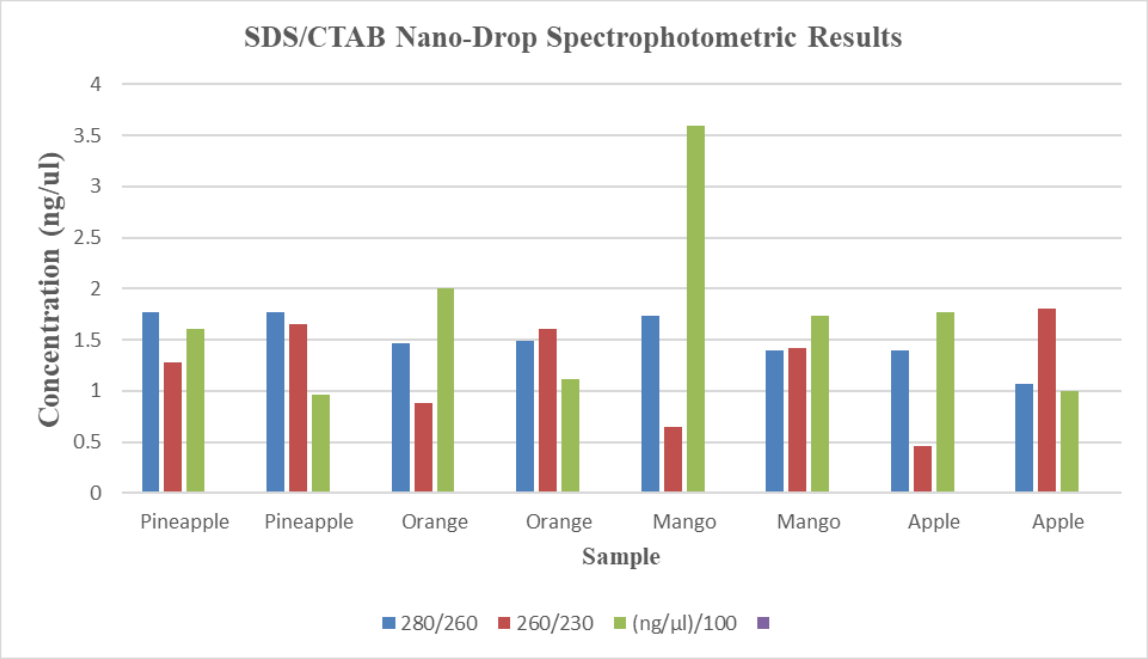

Figure2: DNA concentration for various processed fruit juices using the SDS/ CTAB isolation protocol.

The DNA extraction yield was measured in Nano-grams of DNA per micro-liter of sample. The Spectro-photometric analyses on DNA extracted by CTAB method indicated low DNA yield but high quality compared to SDS protocol, which reported high DNA concentration with varying qualities depending on the sample. According to CTAB protocol-the ratios from table 2 above showed that pineapple had the highest purity followed by mango, orange and the lowest apple. It is true because apple has a lot of pectin levels which affects DNA purity compared to the other fruits. The SDS protocol- ratios from the same table showed that pineapple had the highest purity levels followed by orange, mango and apple recorded the lowest purity due to the same reason. Using the SDS protocol mango had the highest DNA concentration, followed by orange, apple and pineapple recorded the lowest concentration.

The quality of the extracted DNA was evaluated on 0.8% agarose gel electrophoresis stained with 1µl ethidium bromide and observation of bands integrity done in UV-trans-illuminator machine (Quantum ST4, France). PCR amplification was carried out using universal (rbcL-650bp, psbA-323bp that targets the plant chloroplast genome) and species-specific primers. The amplified bands quality and integrity were resolved on 1.5% agarose gel stained with ethidium bromide.

For both protocols 0.8% and 1.5% gel concentration of gel was sufficient for evaluation of quality and integrity of both the genomic and amplified DNA bands, respectively. DNA extracted from SDS method exhibited robustness and ease during PCR amplification process. This was proved during extraction where only SDS protocol had the ability to extract DNA across the four fruit species, which CTAB did fail.

3.2: Genomic DNA

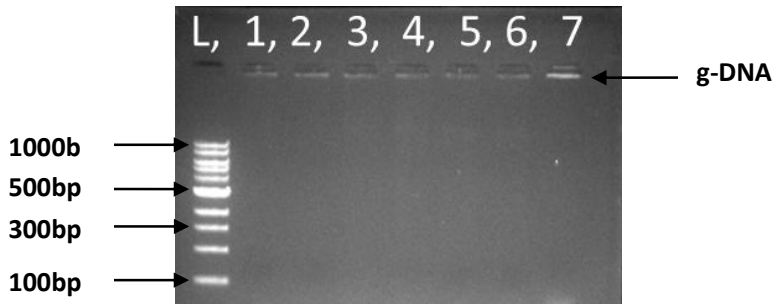

Figure 1: Gel electrophoresis results for gDNA extracted from the four fruit species; Orange, Mango, Pineapple and Apple using the modified SDS protocol. L-1kb ladder, 1&2-orange, 3&4-Mango, 5&6-Pineapple, 7-apple on 0.8% agarose gel size

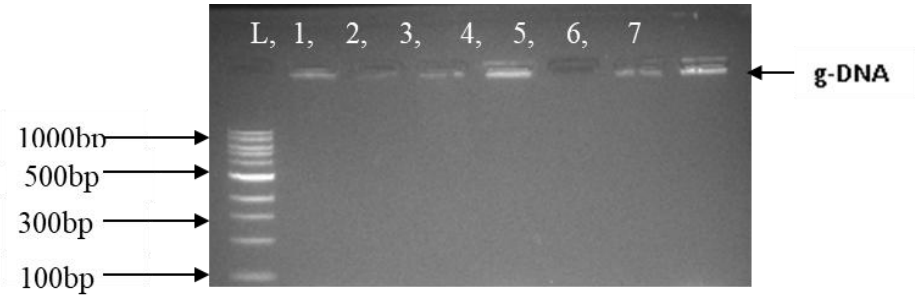

Figure 2: Gel electrophoresis results for g-DNA extracted from processed fruit juices in the selected regions; Orange, Mango, Pineapple and Apple using the modified SDS protocol. L-1kb ladder, 1&2-orange, 3&4-Mango, 5&6-Pineapple, 7-apple on 0.8% agarose gel size

3.3: The PCR Assay Analysis

A control experimental assay was carried out to confirm the efficiency of the primers before application in processed juice. This was done using the freshly prepared fruit juices by amplifying rbcL, Mat-k, ITS-2 and psbA-trnH genes as indicated on table 1 above. The rbcL and Mat-K primers target the plastid genome while ITS2 is a nucleo-chromosomal gene and psbA-trnH is a primer targeting an intron-based chloroplast gene. Comparing between CTAB and SDS protocols, the CTAB method failed to extract DNA in three of the four fruit species-Pineapple, orange and mango and even the amplification process whereas SDS protocol extracted DNA from all the fruit species and the results were amenable to the subsequent PCR processes.

3.4: PCR amplification

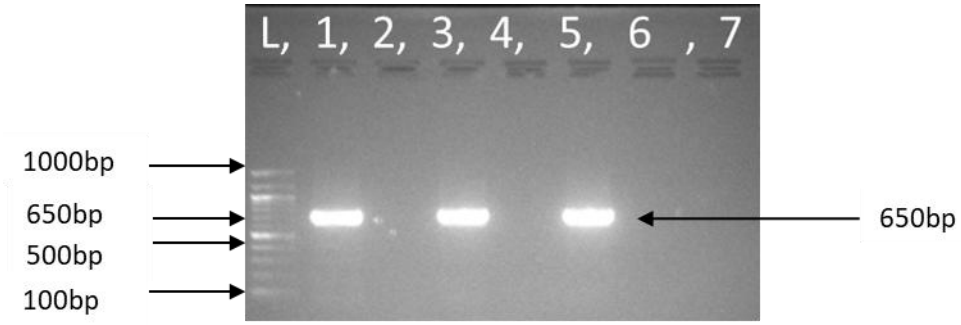

Figure3: PCR amplification result for g-DNA from orange using rbcL primer-650bp targeting chloroplast genome. Lanes; L-100bp ladder, 1, 3&5-or at 50ºC, 51ºC, &52ºC, 2, 4 & 6 are negative controls.

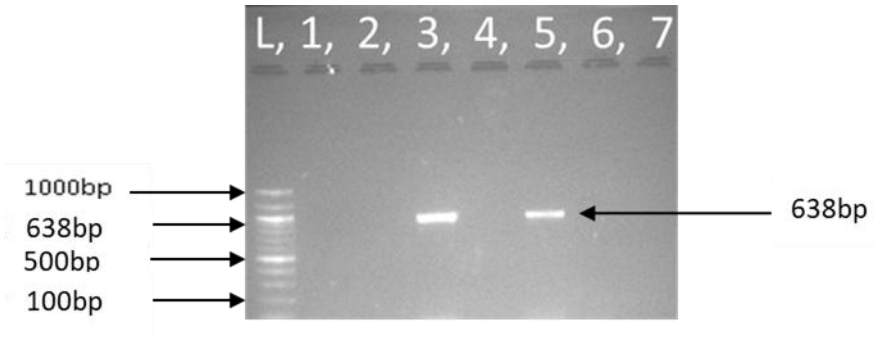

Figure4; PCR amplification results for g-DNA from orange using Mat-K primer-638bp targeting the chloroplast genome; Lanes; L-100bp ladder, 1, 3&5-or at 50ºC, 51ºC, &52ºC, 2, 4 & 6 are negative controls.

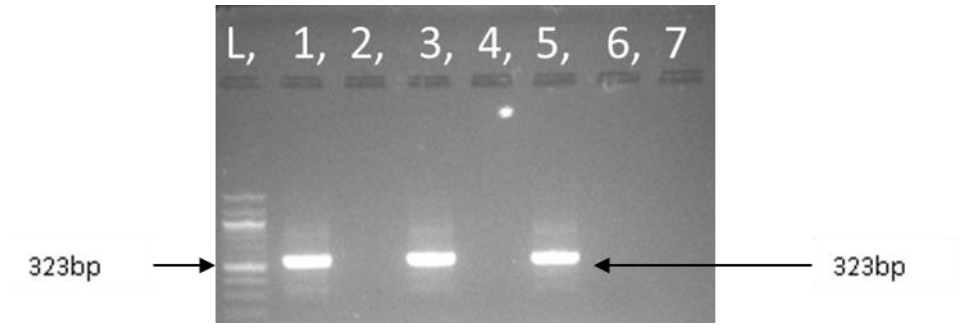

Figure 5; PCR amplification results for g-DNA from orange using psbA-trnH primer-323bp targeting the chloroplast genome: Lanes; L-100bp ladder, 1, 3&5-or at 50ºC, 51ºC, &52ºC, 2, 4 & 6 are negative controls.

The PCR amplification process was also applied to the homemade and processed fruit juice products purchased from the selected markets in Kenya. The results were as follows;

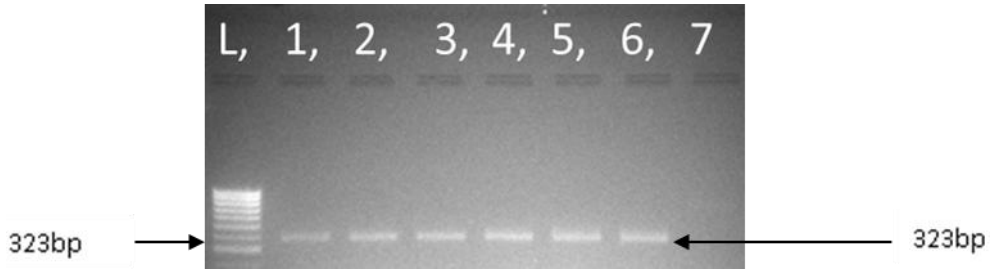

Figure 6; PCR amplification results for gDNA from mango reconstituted fruit juices from Coast using psbA-trnH primer-323bp targeting the chloroplast genome: Lanes; L-100bp ladder, 1-A1, 2-A2, 3-A36, 4+ve, 5-A48, 6-A23, 7-negative controls.

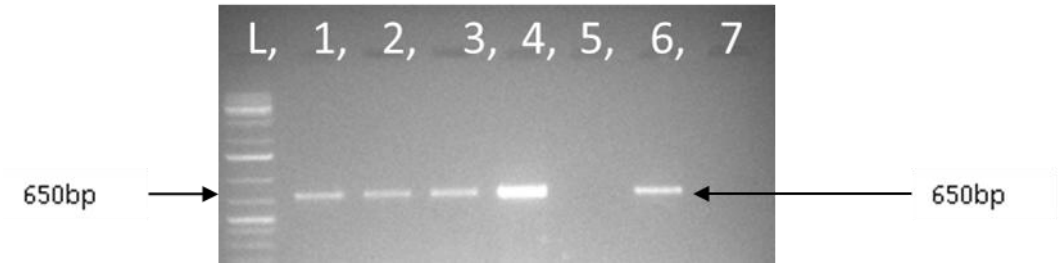

Figure 7; PCR amplification results for gDNA from mango reconstituted fruit juices from Coast using rbcLprimer-650bp targeting the chloroplast genome: Lanes; L-100bp gene rule ladder, 1-A1, 2-A2, 3-A36, 4+ve, 5-A48, 6-A23, 7-negative control.

IV. Discussion

DNA extraction

DNA is among the most reliable molecules for food authentication process. However, its success is highly dependent on the extracted DNA's quality, quantity and purity. The DNA quality is vital and is determined by the DNA's degree of degradation which results to final average fragment length of DNA obtained. The DNA purity is equally important since when not achieved could compromise the amplification process. (Demeke & Jenkins, 2010), (Madesis et al., 2014). In the current study two conventional protocols were modified and applied specifically for DNA extraction and analysis of fresh, home-made and processed fruit juices in the study areas. The CTAB and SDS protocols were compared based on extraction efficiency, DNA purity, time and suitability for amplification process. These methods were able to extract genomic DNA from freshly prepared, homemade and reconstituted fruit juices. A preliminary analysis for determination of the DNA quality was performed by gel electrophoresis in 0.8% agarose. The outcome (in table 2) signifies that the DNA concentration calculated from the absorbance at 260nm/280nm obtained for both procedures (CTAB & SDS) is higher in SDS method as compared to CTAB for all the different samples. The ratios and concentration of DNA obtained from the two protocols differed greatly which concurs with results from (Abdullah et al., 2016) who

states that '' the DNA concentration varies among different samples majorly because of the difference in their chemical composition''. This is because quality DNA isolation depends on the part of the plant used or the species. Nevertheless, some of the results as observed from the 260nm/280nm ratios were not that different in the two protocols for example pineapple species. This can be attributed to the fact that absorbance measurements detect any molecules absorbing at a specific wavelength. Furthermore, little changes in the pH of the solution cause the absorbance ratio at 260nm/280nm to contrast. The presence of EDTA, used in the solution of DNA stabilization also might interfere with the absorbance in wavelengths of 280nm (Nunes et al., 2011). However, CTAB protocol failed to extract the DNA across all the four categories of fruit juices mentioned above. This is because the amount of DNA was always small and not adequate for further downstream analysis as reported by (Farah Izana et al., 2016). Consequently, the study progressed with the SDS method for extraction and amplification. Although both procedures involved extraction of DNA conventionally, that is using readily reconstituted laboratory reagents; CTAB used chloroform while SDS did not apply it.

PCR based protocols are rapid, cost effective, highly specific, and sensitive and characterized with high discriminatory power. The DNA isolation from food matrices is challenging due to natural existence of proteins, polysaccharides, polyphenols, lipid (Xue et al., 2013) and carbohydrates especially in plants (Yajun Wuet al., 2018). In current study the major challenge was the presence of food additives such as artificial flavors, excessive processing, sample exposure to high heat levels causing DNA structure dis-integration and fragmentation. They were solved by prior centrifugation of liquid sample before the exact extraction begins to remove the mixed food colors, the ethanol precipitation process was done twice using 70% ethanol to ensure complete eradication of contaminants. The SDS protocol was short, precise and rigorous with few steps which consolidated the amount of DNA obtained since we were dealing with already processed products which had little plant matter. These components need to be considered when developing a molecular based protocol since it solely depends on the efficiency of DNA extraction and purification technique used. Therefore, its application in food samples requires stringent extraction and purification strategies to ensure efficient recovery of DNA and removal of PCR inhibiting compounds since the chemicals used in DNA extraction protocol such as CTAB, phenol, and salts, which are considered strong inhibitors to Taq-polymerase (Di Pinto et al., 2007. In particular, the modified SDS protocol demonstrated high extraction efficiency in both fresh and processed fruit juices. It had the ability to isolate DNA across the four major fruit categories. Besides, the protocol was less time consuming taking a maximum of four hours due to short extraction steps and technically less demanding as compared to CTAB which requires a lot of reagents and long incubation steps. It also avoided the use of poisonous reagents such as phenol and chloroform which are hazardous, toxic, and expensive and need special facilities i.e., hoods for human health safety while working in the laboratory and environmental reasons (Sahu et al., 2012). The modified SDS method proved to be simple, sensitive, efficient, cheap, and reliable and provided the best PCR amplification process for analysis of fruit-based samples. Therefore, making it preferable when dealing with experiments with limited financial resources. The amount of DNA recovered by this protocol was of very high concentration in Nano-grams per micro-liters observed on Nano-drop Spectro-photometric measurement results (Table 1) and (chart 1). It also yielded high quality DNA as observed on the ratios obtained above. Even though (Di Bernadoet al., 2013) "states that the most suitable DNA extraction method strongly depends on the food matrix and that there is no "universal" method that could be used for all food samples", this study showed that the SDS protocol could be used to extract DNA from across a wide range of diverse processed fruit samples. However, the main drawback of the protocol was on the quality of DNA recovered since the fresh, homemade and processed fruit juices contained a lot of plant secondary metabolites such as polyphenols, polysaccharides, carbohydrates and other artificial additives. Also, brownish DNA pellets were observed indicating contamination by phenolic compounds. Results are in line with observation by (Moreira et al., 2011).

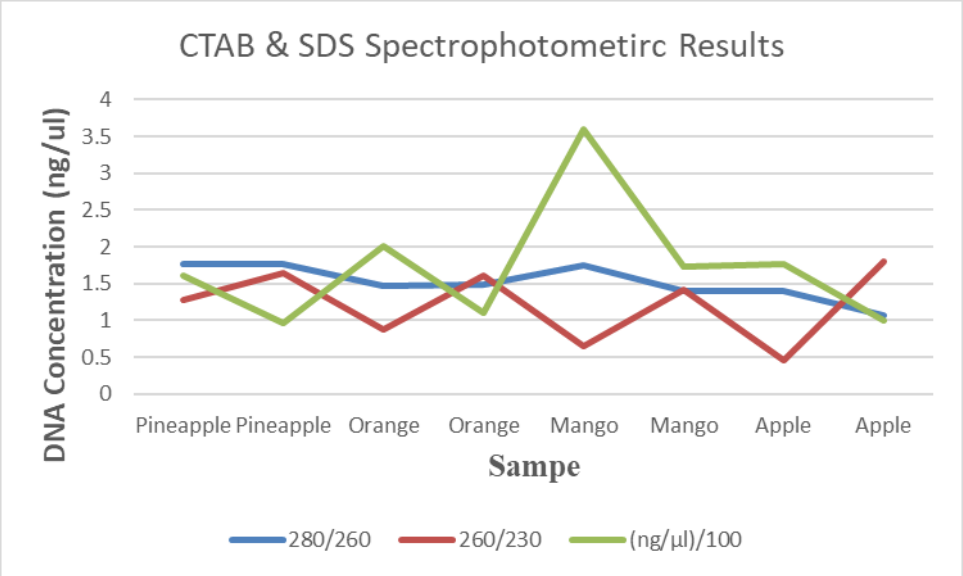

Chart 2: Comparison of Nano-drop spectrophotometric results for g-DNA extracted from processed fruit juices between SDS and CTAB protocol.

From the results in chart 2 above the SDS method manifested consistent increase in the quantity of DNA concentration recovered compared to CTAB. The SDS protocol had a slightly low-quality DNA compared to CTAB. This scenario was due to presence a lot of plant debris and food additives. The low Absorbance at ratio of A230/A260 was due to high levels of polysaccharides/carbohydrates which formed gel-like whitish mucus which affects the quality of DNA.

Statistical analysis

The statistical data was stored in an Excel database (Microsoft, Washington, United States). The statistical data was analyzed using the T-Test for the two means obtained from replicate data. The T-Test analytical method allowed me to calculate the significance difference (mean difference) between the absorbance ratios of spectrophotometric results of CTAB and SDS protocols. The size of each amplified DNA fragment was determined using molecular weight DNA marker extrapolation.

For example, when you take the CTAB mean for **Pineapple** sample and subtract from the same sample extracted using SDS (161.05-95.51= Mean difference (65.54).

Apple (176.53-100.42=Md,76.11. **Mango** (328.86-173.41=155.45), **Orange** (201.38-110.86=Md90.52). This result indicates that the SDS protocol was very rigorous and sensitive as compared to CTAB. This is because it had the highest difference DNA amount/concentration in comparison to CTAB.

The PCR Analysis

The polymerase chain reaction assays indicated that the genomic DNA extracted from freshly prepared and reconstituted fruit juice samples using the SDS protocols was suitable for amplification. However, no DNA was extracted using CTAB protocol across the four fruit juice categories. The study used SDS protocol for the whole process. All the four genes were tested for possible amplification efficiency. This study retained only two target genes rbcL and trnH-psbA due their efficiency in amplification process. The two primers rbcL and trnH-psbA successfully amplified the PCR products from all the four fruit juice species (figures 3-7).

V. Conclusion

The genomic DNA isolation protocols have a prodigious effect in not only the quality and quantity of DNA recovered but also cost and time. Between the two protocols in question, SDS method proved to be a simple, safe, reliable, timely and cost-efficient SDS DNA extraction protocol that provides high quality genomic DNA from fresh, homemade and processed fruit juices containing high concentrations of polysaccharide and polyphenolic compounds for fresh and homemade juices and other artificial and chemical additives for processed fruit juices. This protocol eliminates the need of using costly liquid nitrogen, environmentally hazardous phenol and chloroform to obtain high-quality genomic DNA. This proposed method facilitates the extraction of DNA even from highly processed fruit juices and their products. This research work achieved its aim of developing a modified, unbiased and rigorous DNA extraction protocol that extracted DNA across the four fruit species; mango, orange, pineapple and apple without the need to adjust a method to a specific plant

species or tissue for the interest of time and expense to be incurred. The modified and optimized SDS protocol enabled the recovery of high-quality g-DNA amenable to rbcL, trnH-psbA, Mat-K and ITS2 plant-based primers/barcodes amplification. DNA profiling has mainly been conducted using genomic DNA isolated from leaves in fruit tree species there very few reports of application for DNA recovered from processed fruits and their products. Results from this study will build onto future applications that aim to utilize molecular based DNA detection techniques from processed fruits and their products.

VI. Recommendation

The major shortcoming of the protocol was on the quality of DNA recovered. The fresh and processed fruit juices contained many plant secondary metabolites such as polyphenols, polysaccharides, carbohydrates and other artificial additives including food colors which may impart negatively on DNA quality. Hence, there is need to explore the use of antioxidants and other compounds which may aid in the removal of the contaminants. This will even ease the subsequent downstream processes such as PCR amplification and quantification. A further study on percentage quantification should be carried out on the processed fruit juices to confirm the authenticity of the declared quantities on their labels.

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