Evaluation of the Vitamins and Possible Nutrient Composition of Local and Improved Specie of Guava Flesh (*Psidium guajava*)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Original Research Article

ABSTRACT

Background and Objectives: Guava (*Psidium guajava*) belongs to the family Myrtaceae. It has been cultivated in Nigeria one of the most common fruits in Nigeria. It has become popular because of its availability almost throughout the year. The objective of this study is to evaluate the nutritional and vitamin contents of the flesh of the local and improved guava species.
Materials and Methods: Proximate components were determined using the AOAC method for the moisture, ash, crude lipid, nitrogen content (crude protein) and Carbohydrate. Vitamins A, B_1, B_2, B_3, B_6 and B_{12} were analyzed using standard methods.
Results: The result showed that moisture and carbohydrate content of the improved white guava flesh is low compared with the local white guava species (9.8825 and 14.4015%) and (56.378 and 62.802%), respectively. The ash, fat, fibre and protein content of the improved white guava flesh are high compared with the local guava species (11.25 and 5.15%), (5.55 and 1.335%), (6.6145 and 6.5865%) and (10.325 and 8.225%). Improved White guava flesh is relatively low in Vitamin A, vitamin B_3, vitamin B_6 and vitamin C compared to local white guava flesh, while the local white guava flesh is relatively poor in vitamin B_1, vitamin B_2 and vitamin E compared to improved white guava flesh.

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> **Conclusion:** The present findings suggest guava flesh as a considerable source of nutrients in the diet and may have health and economic benefits due to its vitamins, and nutritional composition.

1. INTRODUCTION

There is growing interest and concern among people from all walks of life in foods and their relationship to nutrition and health. Fruits form part of the balanced diet, which the human body need and they are used for prevention deteriorating health condition [1].

Guava (*Psidium guajava*) is one of such fruits and it belongs to the family Myrtaceae. It has been cultivated in Nigeria as one of the most common fruits [2] and it's available almost throughout the year. It also grows well in zones of scanty rainfall areas such as in the north-eastern part of Nigeria [3]. *Psidium guajava* is an evergreen shrub with a musky special odour when ripened [4]. The fruit contains several small seeds and consists of a fleshy pericarp and seed cavity with pulp [5].

It contains antibacterial and antimicrobial compounds[6] and had shown a high anti-diabetic activity[7]. Guava contains antioxidants, phytochemical, essential oils, polysaccharides, minerals, vitamins and enzymes[8]. Guava is also a very good source of bioactive components. However, in Nigeria the local breeds (Fig.1) cultivated by people are being replaced by improved breed (Fig.2) as a result of the size of the improved breed and pest infestation that commonly attacks the local breeds guava. Thus, this research aims at evaluating the nutritional composition and vitamin contents of the flesh of the local and improved guava species.

2. MATERIALS AND METHODS

2.1 Sample Collection and Preparation

The guava samples were bought from Eke Awka and Eke Amaenyi market both in Awka South local government area, Awka, Anambra State, Nigeria. This research project was conducted from May-June, 2019.

The composite samples of each species of the fruit were prepared separately. Each sample was washed thoroughly with distilled water, cut into smaller sizes and dried in the oven for 5-7 days at $40^{°}C$ after which the flesh was ground into fine powder. The already grounded flesh was transferred into an airtight container and properly labeled for further analysis.

2.2 Proximate Analysis

The methods of the Association of Official Analytical Chemists (AOAC, 1984) AOAC[9] were used for the determination of moisture, ash, crude lipid, Carbohydrate and crude protein (nitrogen content).

Fig. 1. Local breed White apple
Psidium guajava

Fig. 2. Improved breed White Malaysian
Psidium guajava

2.3 Determination of Crude Protein

The crude proteins of the samples were determined based on the principle that the total protein content of a sample can be quantified from its nitrogen content. One gram each of the samples were weighed out and introduced separately into the 100ml Kjeldahl digestion flask. Concentrated sulphuric acid, H_2SO_4 (20ml) was added and mixed gently by swirling under tap water. Ten gram of anhydrous sodium

sulphate and 1g of copper sulphate were mixed together and 3g of the mixture was introduced into the separate digestion flasks. Anti-bumping granules were added into the mixture to prevent splashing during digestion. The entire mixture was boiled gently in the different kjeldahl flasks in a fume cupboard until charred particles disappear and a clear green solution was obtained. Each digest was made up to 100ml with distilled water.

2.3.1 Distillation

Ten millitres of 2% boric acid was measured into a 250ml conical flask and three drops of mixed indicator (5 drops of methyl red and two drops of bromocresol green mixed together) were added. From the digest, 10ml was collected and placed in the distillation flask. The distillation apparatus was set up, and 30ml of 40% NaOH was added to the distillation flask slowly from a syringe. The distillation apparatus was heated for 25minutes. The receiver beaker containing 2% boric acid was removed from the set up and titration done on the distillate with 0.1N HCl until the end point (pink colour). The distillation procedure was done in triplicate for each sample.

Calculation:

$$\%Nitrogen = \frac{1.4 \times titre\ value \times dilution\ factor}{Weight\ of\ sample\ (mg)} \times \frac{100}{1}$$

$$\%\ Crude\ protein = \%Nitrogen \times 6.25$$

2.4 Determination of Moisture Content

This is the determination of water content of the samples.

2.4.1 Procedure

The silica dishes were washed, dried in an oven and cooled in a desicator and then weighed. Two grams of finely ground samples were weighed accurately into the silica dishes, each sample in triplicate. The dishes with the samples were weighed and transferred into an oven and heated at 105^0C for two hours.

After two hours, the dishes were brought out and allowed to cool in an activated gel desiccators. The dishes were reweighed and recorded. The dishes were transferred back into the oven and heated for 30mins, brought out and allowed to cool in desiccators, reweighed and recorded; this was repeated until a constant weight was achieved.

$$\%Moisture = \frac{X_1 - X_2}{Y} \times \frac{100}{1}$$

Where,

X_1 = Weight of sample + dish before drying
X_2 = Weight of sample + dish after drying
Y = Weight of sample (g)

2.5 Determination of Lipid Content

2.5.1 Procedure

The soxhlet extraction method was used to determine the lipid content of the samples. Two clean flat bottomed flasks were weighed separately and 250ml petroleum ether 60^0C – 80^0C was added into each of them. The flasks were connected to different extractors (containing thimble) and a known weight (5.0g) of the samples were put in the thimble and the reflux condenser with water inlet and outlet connected. Anti-bumping granules were added into the solvent system and cotton wool used to cover the mouth of the apparatus to prevent loss of the solvent. The set up was heated, and as the solvent boils, it evaporates and passes through the extractor to the condenser where the vapour cools and drops into the thimble in the extractor to extract the oil in the sample. When the solvent reaches the highest mark, it refluxes back into the flask by an automatic siphoning device. The extraction was done continuously for three hours. The extractant was distilled off and the flask reweighed after cooling in the desiccator. This procedure was done in triplicate.

Calculation:

$$\%Lipid = \frac{Weight\ of\ lipid \times 100}{Weight\ of\ sample}$$

$$i.e = \frac{Weight\ of\ flask\ and\ lipid - Weight\ of\ flask}{Weight\ of\ sample} \times \frac{100}{1}$$

2.6 Determination of Ash Content

2.6.1 Procedure

The crucibles were thoroughly washed, cleaned and placed in an oven for two hours and cooled in desiccators. The empty crucibles were transferred to the muffle furnance to burn off all the organic matter and to stabilize the weight of the crucibles at temperature of 550^0C, and cooled in desiccators. Two grammes each of the ground samples were weighed respectively into the labeled crucibles, each sample in triplicate. These were placed in a muffle furnance and incinerated at 550^0C for 3 hours. At the end of

the ashing period, the crucibles were removed using a pair of tongs and placed into desiccators to cool to room temperature and reweighed.

Calculations:

$$\%Ash = \frac{X_2 - X_1}{Y} \times \frac{100}{1}$$

Where,

X_1 = Weight of crucible
X_2 = Weight of crucible and ash
Y = Weight of sample

2.7 Determination of Crude Fibre

The samples were digested sequentially with acid and base, and the undigested substances constitute the crude fibre. Two grams of each sample were weighed separately into a 250ml conical flask and 100ml of 1.25% sulphuric acid solution was added to each flask. The sample was heated to boil for 30 minutes, and filtered with the Whatman No 1 filter paper with Buchner funnel connected to Buchner flask and the solution was washed with distilled water until traces of acid was undetected using a pH paper. The acid-extracted sample was transferred into 250ml conical flask and 100ml of 1.25%NaOH solution was added. The sample was heated to boil again for 30minutes and was filtered as above and washed with distilled water until base was undetected in the sample. The whole material was transferred into clean, dry weighed crucible and dried for 12 hours at 120^0C. After that, the crucible was placed into a muffle furnace at 550^0C for 12 hours and weight of crucible was recorded.

Calculation:

$$\%Crude\ fibre = \frac{X_2 - X_1}{Y} \times 100$$

2.8 Determination of Total Carbohydrate

The percentage carbohydrate of *Cucurbita pepo* and *Luffa cylindrica* were determined by the difference method.

Total carbohydrate = 100% - %Protein + %lipid + %moisture + %ash + %.

2.8.1 Vitamins

Vitamins A, B_1, B_2, B_3, B_6 and B_{12} were analyzed using the method described by Bayfield and Cole[10], vitamin C and E were analyzed by Kirk and Sawyer [11] method.

2.8.2 Vitamin A

The assay is based on the spectrophotometeric estimation of the colour produced by vitamin A acetate or palmitate with TCA.

2.8.3 Procedure

All procedures were carried out in the dark to avoid the interference of light. Liver homogenate (1.0ml) was mixed with 1.0ml of saponification mixture and refluxed for 20 minutes at 60□C in the dark. The tubes were cooled and 20ml of water was added and mixed well. The sample was exctracted twice with 10ml of (40°-60°C) petroleum ether. The two samples were pooled and washed thoroughly with water. Anhydrous sodium sulphate was added to remove excess moisture. An aliquot of the sample (1.0ml) was taken and evaporated to dryness at 60°C. The residue was dissolved in 1.0ml chloroform. Standards (vitamin A palmitate) of concentrations ranging from 0-7.5⌈g were pipetted out into a series of test tubes.

The volume in all the tubes was made up to 1.0ml with chloroform. TCA reagent (2.0ml) was added rapidly, mixed and the absorbance was read immediately at 620nm in a spectrophotometer (Genesys 10UV). The same procedure was repeated for the sample tubes also. Vitamin A content was expressed as ⌈g/g tissue.

Calculation:

Conc of vitamin A in sample = (Abs of sample / Abs of std) x conc of standard

2.8.4 Vitamin E

This was determined by the futter – mayercolometric method with association of vitamin chemist's(kirk and sawyer 1991). 1g of the sample was mixed with 10ml of ethanoicsulphuric acid and boiled gently under reflux for 30mins. It was transferred to a separating funnel and treated with 3x30ml diethyl ether and recovering ether layer each time, the ether extract was transferred to a dessicator and dried under for 30 mins and later evaporated to dryness at room temperature. The dried extract dissolved in 10ml of pure ethanol. 1ml of the dissolved extract and equal volume of standard

vitamin E were transferred to separate tubes. After continous addition of 5mls of absolute alcohol and 1ml of concentrated nitric acid solution, the mixtures were allowed to stand for 5mins and the respective absorbance measured in a spectrophotometer meter at 410nm with blank reagent at zero.

2.8.5 Vitamin C

This was determined by the titrimetric method reported by (Kirk and Sawyer). A weighted sample was homogenized in 6% EDTA/TCA solution. The homogenate was filtered and used for analysis. 20ml of 30% KI solution was added to it and it was titrated against 0.1M $CUSO_4$ solution. The end point was marked by aablackcolouration. A reagent blank was also titrated.

Vitamin C content was calculated based on the relationship below. 1ml of 0.1 mole $CuSO_4$ =.88mg vitC.

$$Vitamin\ C\ mg/100 = \frac{1 \times .88 \times titre - blank}{W}$$

2.8.6 Vitamin B_1 and B_2

1g of sample was weighed into a conical flask. This was dissolved with 100ml of deionized water. This was shaken thoroughly and heated for 5 minutes and allowed to cool and filtered. The filtrate was poured into cuvette and their respective wavelength for the vitamins set to read the absorbance using spectrophotometer

Vitamin B1 = 261nm
Vitamin B2 = 242nm

Calculations:

$$Concentration\ (mg\ \%) = \frac{A \times D.F \times volume\ of\ cuvette\ (5)}{E}$$

Where

A = absorbance
E = extinction coefficient = 25 for B1 and B
DF = dilution factor

2.8.7 Vitamin B_3 (Nicotinamide)

5g of sample was dissolved in 20ml of anhydrous glacial acetic acid and warmed slightly. 5ml of acetic anhydride was added and mixed. 2 -3 drops of crystal violet solution was added as indicator. Titrate with 0.1M perchloric acid to a greenish blue colour.

Calculation:

$$Vitamin B_3 = \frac{titre\ value \times 0.0122}{0.1}$$

2.8.8 Vitamin B_6

5g of sample was dissolved in a mixture of 5ml of anhydrous glacial acetic acid and 6ml of 0.1m mercury II acetate solution. 2 drops of crystal violet was added as indicator and the mixture was titrated with 0.1m perchloric acid to a green colour end point.

Calculation: each meal of 0.1M perchloric acid is equivalent to 0.02056g of $C_8H_{11}NO_3HCL$

3. RESULTS AND DISCUSSION

The results of the proximate analysis in Fig. 3, showed that the improved white guava flesh contains percentages of moisture, ash, fat, fibre, protein and carbohydrate content of 9.8825, 11.25, 5.55, 6.6145, 10.325 and 56.378%, respectively. While local white guava flesh contains percentages of moisture, ash, fat, fibre, protein and carbohydrate content of 14.4015, 5.15, 1.335, 6.5865, 8.225 and 62.802%, respectively.

From the result of the proximate analysis done, the local white guava flesh contains high moisture and carbohydrate content than the improved white guava. While the improved White guava has higher compositions of ash, fat, fibre and protein content than the local white guava flesh.

The observed moisture content in both species of guava's flesh are relatively low compared to (80%) obtained by Ekpete and Edori[12]. The total carbohydrate and protein content values were higher than those reported by Jyorti et al., [13].

The higher level of carbohydrate can give consumers a boost of energy. Being a source of dietary fibre and protein, the guava flesh, especially the improved breed, can be a key to achieve good digestive health. Increased intake of guava in the diet can increase the intake of dietary fibre which helps to relieve many digestive problems including bloating, stomach cramps, and can be a source of amino acids in the body which helps in the body build up, cellular regulation and hormonal stimulation

which increases the body metabolic rate and helps fight diseases.

3.1 Vitamins Content

The results obtained for vitamin content of the improved White and local white *Psidium guajava* flesh are shown in Fig. 4 (a-e). Fig. 4a, showed the vitamin A and E content of improved and local white guava flesh (3.09 and 6.055mg/kg) and (3.265 and 1.0925 mg/kg) respectively. Fig. 4b., showed the vitamin B_1, B_2, and B_3 content of improved and local white guava flesh (1.2581 and 1.1568 mg%), (0.037 and 0.0275 mg%) and (0.4445 and 0.616 mg%) respectively. In Fig. 4c., the vitamin B_6 content of improved and local

white guava flesh is shown as (357.5 and 424.14 mg%), the vitamin C content of improved and local white guava flesh in fig.4d (13.11and 13.925 mg/g), and Vitamin B_{12} content of improved and local white guava flesh in fig.4e, (0.0865 and 0.0875 mg/100g).

From the result, it was observed that the improved White guava flesh is relatively low in Vitamin A, vitamin B_3, vitamin B_6 and vitamin C compared to local breed flesh, while local white guava flesh has relatively low content of vitamin B_1, vitamin B_2 and vitamin E compared to improved breed. Therefore, it entails that guava can be a source to supply these nutrients when consumed
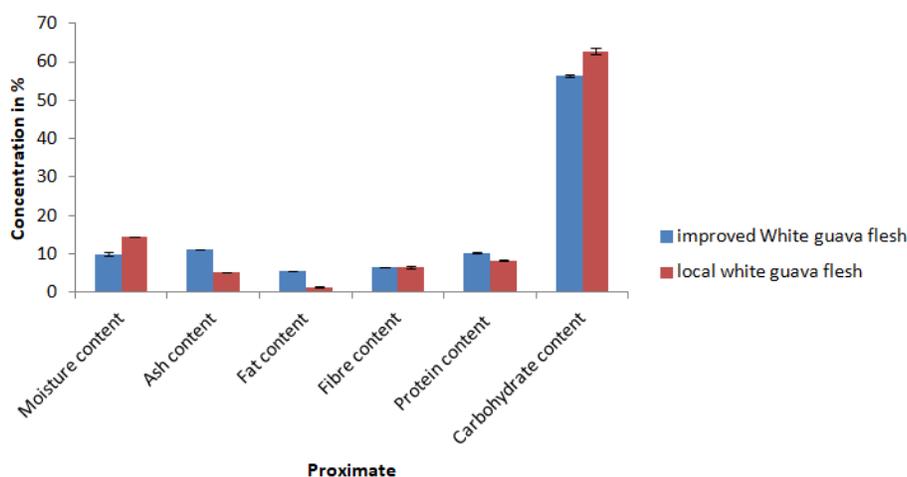
Fig. 3. Proximate components of improved and local white guava flesh

(b)

(c)

(d)
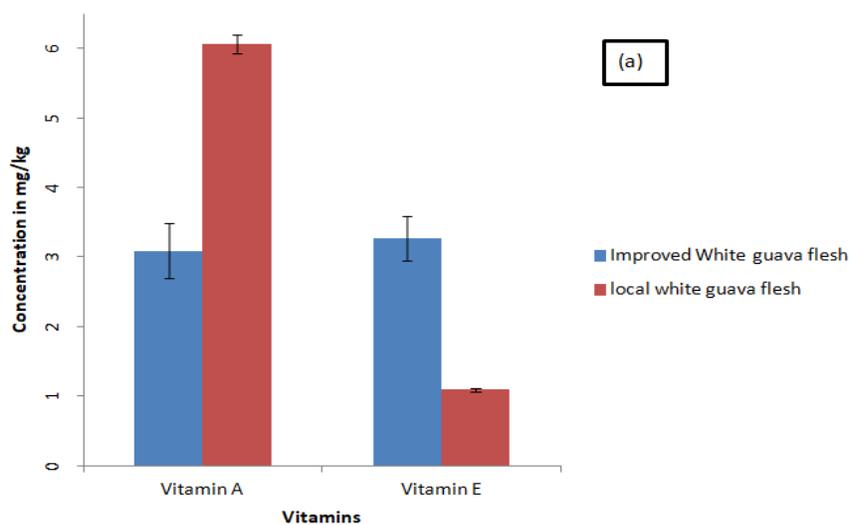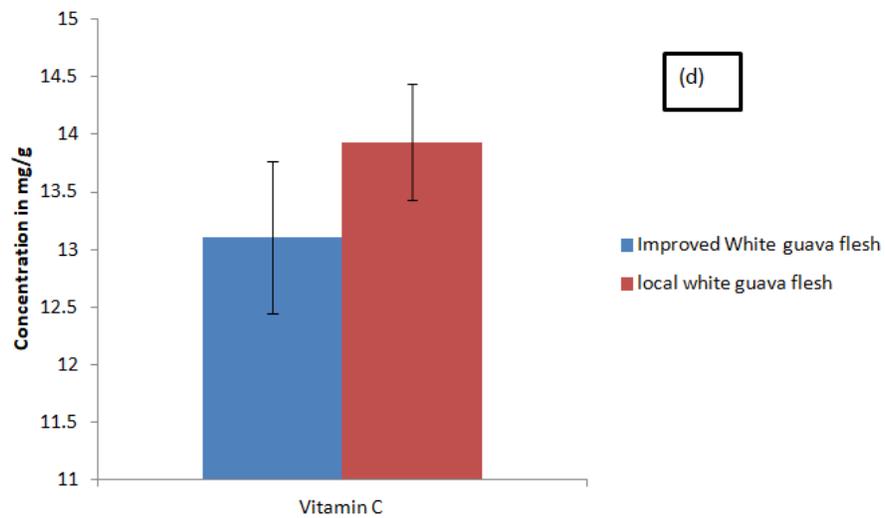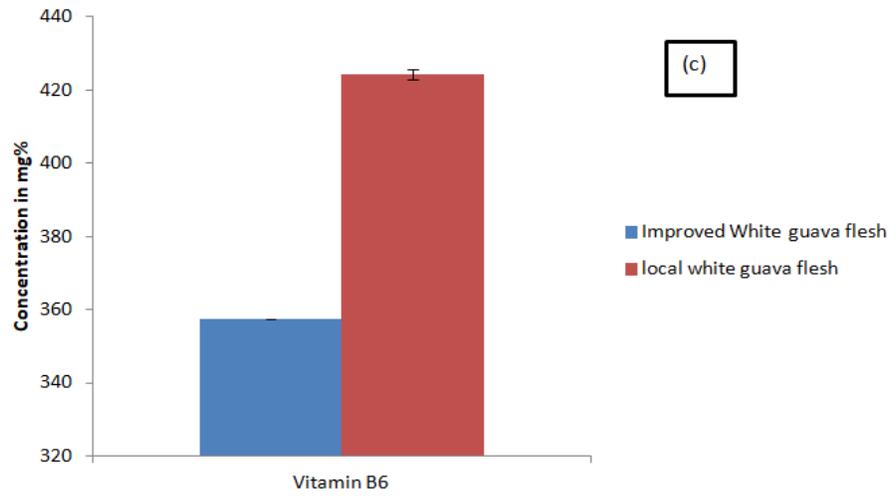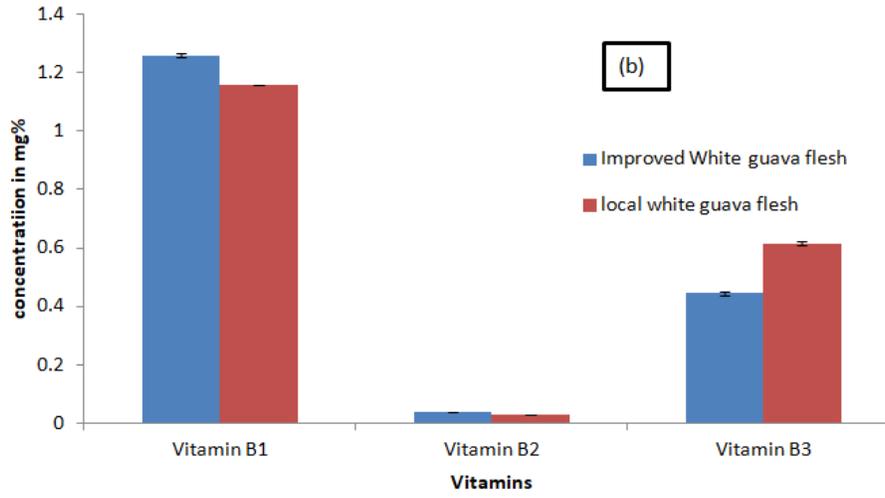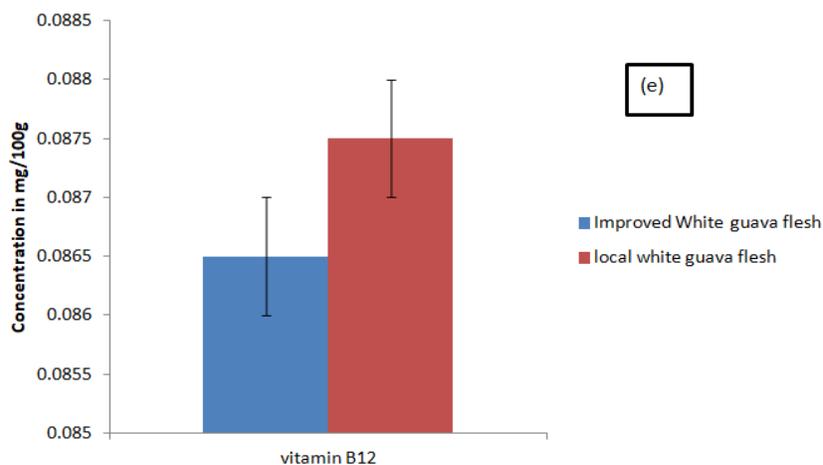
Fig. 4. Graphical representation of different vitamins (a) vitamin A and E, (b) Vitamin B$_1$, B$_2$ and B$_3$, (c) Vitamin B$_6$, (d) vitamin C and (e) vitamin B$_{12}$ compositions of improved and local white guava flesh

4. CONCLUSIONS

This study of these two guava species has highlighted the potentials of this fruit to serve as food. It has also shown the importance of the two species most especially the local white guava and that it should not be allowed to go into extinction as its cultivation is gradually declining observed by its reduced availability in the market.

SIGNIFICANCE STATEMENT

This study discover the guava especially the local white guava species has a high nutritive values that can be beneficial for the improvement of the health of its consumers and enhancing the establishment of its industrial products. This study will help the researcher to uncover the critical areas of different species of guava nutritive valus that many researchers were not able to explore. Thus a new theory on the production of local white guava species may be arrived at.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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