Beta-Carotene Content of Selected Maize Genotypes and Its Retention After Processing and Preparation of Different Nigerian Maize Foods

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Abstract
Biofortification of maize genotypes with micronutrients such as beta - carotene is a promising solution to the eradication of Vitamin A deficiency (VAD) in developing countries like Nigeria. A critical factor to consider for retention of beta - carotene in food products is the method/technique of processing such food crops. Processing maize for food exposes the grains to environmental factors such as air, light and temperature which may reduce beta - carotene content. The research question asked in this work says, is there significant difference in beta-carotene content of the product of different maize samples processed by different methods? The aim of this study is to determine beta - carotene concentration in raw samples of the selected maize genotypes and its retention after processing using different methods. The beta - carotene content in the selected maize genotypes before and after processing were estimated via spectrophotometry. The results show that beta - carotene content of raw samples of selected maize genotypes ranged from 20.74 – 40.42 µg/g and beta - carotene retention in the maize food products ranged from 3.55 – 42.84 µg/g. The maize varieties with significantly higher beta - carotene contents in their raw samples were EB1, SUW and PRO. While other maize varieties showed reduction in their beta-carotene after processing, the beta-carotene content in SUW maize variety was more than 100 % relatively to its raw sample after boiling and roasting. Based on the treatment/processing methods, boiled and roasted maize had significantly higher ($p<0.05$) beta - carotene retention than their Agidi, Pap and moin-moin counterparts. Pap recorded more than 70% loss of beta - carotene. Processing methods during preparation of maize food products contributed to degradation of beta - carotene content. This study suggests that boiling and roasting are the best processing methods for optimum retention of beta - carotene in maize food products. People should use SUW maize variety for maize food to obtain maximum utilization of beta-carotene. More investigations should be made to identify the properties that enhanced beta – carotene retention in SUW maize variety and such properties should be improved upon in other maize varieties for improvement in their beta-carotene content after processing. Maize breeder technologists should work on other maize genotypes to enhance their beta-carotene retention after processing.

Keywords: *beta-carotene content, retention, maize variety, processing methods*

INTRODUCTION

Billions of people suffer from “hidden hunger”, which is caused by lack of micronutrients in their diet (De Moura *et al.,* 2015). Micronutrient - rich diets include fruits, vegetables, and animal products, that are not affordable by the poor. The daily diet accessible to the poor mainly consists of a few low - cost staple foods like maize, cassava and rice, which are mainly white and devoid of carotenoids (De Moura *et al.,* 2015). Micronutrients are compounds required in small amounts to drive metabolic processes and beta -carotene is a notable example (Barra *et al*., 2021). Beta - carotene is a precursor of vitamin A hence its role in maintaining visual health. In addition, research has linked improved neurological function to consumption of beta - carotene rich diets among numerous benefits (Grune *et al*., 2010).

Carotene an orange or red crystalline hydrocarbon pigment like $C_{40} H_{56}$ that occurs in subcellular organelles (plastids) and can be easily converted to vitamin A. Beta - carotenes are isomers of carotene found in dark green or dark yellow vegetables and fruits (Khoo *et al*., 2011). Maize (*Zea mays*) is the most significant grain crop and staple in sub - Saharan Africa. Nigeria is second after South Africa among Africa's top ten (10) maize producers, with an anticipated output of 10.8 million tons in 2014 (FAOSTAT, 2014; Udensi and Omovbude, 2018).

Maize is an important staple crop widely grown and consumed after rice in Nigeria. Maize, as a cereal crop, contains approximately 65 percent carbohydrate and low amount of protein, minerals, and vitamin B contents (Iken *et al.,* 2002). Maize is usually consumed as boiled or roasted and generally made into a variety of tasty traditional Nigerian dishes, such as Akamu and Agidi. The fermented maize gruel (Pap), which is consumed by all tribes as a kind of infant nutrition or weaning food, is known by different names in Nigeria's three major tribes: Yoruba (Ogi), Igbo (Akamu), and Hausa (Koko) (Omololami *et al.,* 2021). Utilization of maize is categorized as: basic human food, animal feeds and industrial raw materials for brewing, ceramics, and biofuel (Udensi and Omovbude, 2018).

However, the majority of maize grown and consumed is white and lacking in provitamin A carotenoids. It is largely consumed by the economically disadvantaged members of communities in food forms such as Agidi, Akamu and moin-moin oka owing to its affordability. This is evident by the high prevalence of vitamin A deficiency and its attendant consequences that characterize the aforementioned population (Nuss and Tanumihardjo, 2010; Kirthee *et al.,* 2014). This problem is prominent among the poor nations, where vitamin A deficiency (VAD) affects about 250 million children population and a significant proportion of pregnant women, causing night blindness and raising the likelihood of child and maternal death (De Moura *et al*., 2015). Vitamin A is a fat - soluble micronutrient that is required for gene expression, growth, fetal development, visual adaptation to darkness, and immune function (Rando, 1990; West *et al*., 1991; De Moura *et al.,* 2015).

Carotenoid, a natural pigment present in the hard endosperm of maize kernel with a little amount in the germ, is sensitive to isomerization, oxidation, and considerable loss during food processing due to heat, light, and air (Johnson, 2000; Rodriguez, 2001; Omololami *et al.,* 2021). There are two types of Carotenoids: provitamin A (beta – cryptoxanthin, alpha - carotene and beta - carotene), which have vitamin A activity, and xanthophylls (lutein and zeaxanthin), which are non - provitamin A but have anti - oxidative qualities. Carotenoids provide health benefits and also known to modulate enzyme activity, activate gene expression for protein formation, and has anti - oxidative properties which increases immune system performance, and lowering the risk of degenerative disorders. Therefore, all carotenoids that are precursors of vitamin A are classified as PVA carotenoids (Omololami *et al*., 2021).

Beta - carotene is a better source of vitamin A due to its superior conversion and provitamin activity. Beta - carotene is not toxic so it is considered a safe source of vitamin A (Sandesh *et al*., 2018). The main condition for provitamin A activity is that, carotenoid must possess at least one β - ionone ring residue (Fig 1).

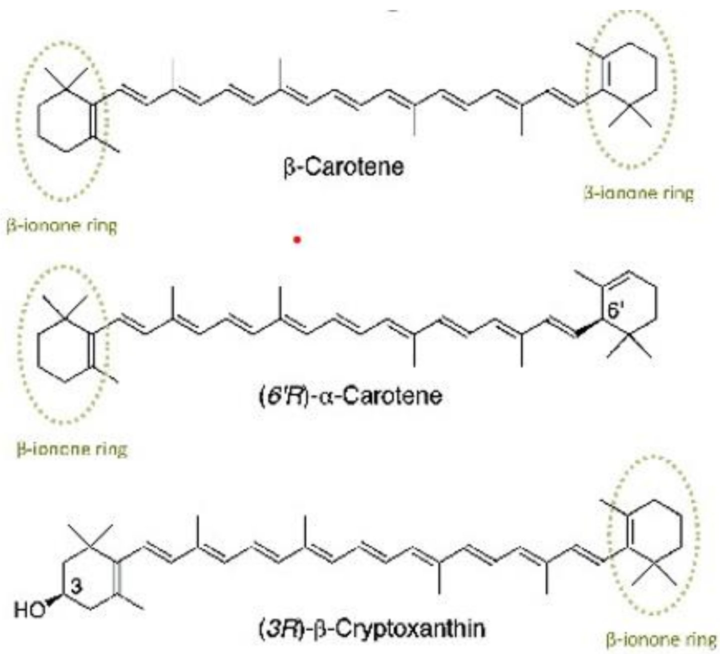

Figure 1: Chemical Structures of Provitamin - A Carotenoids (De Moura *et al*., 2015).

Carotenoid exposure to direct sunlight can isomerize all carotenoids. Also, when crop tissues are cut, chopped, shredded, cooked, or aged naturally their physical barriers become broken (De Moura *et al*., 2015). This makes the carotenoids vulnerable to oxygen and enzymes that act on it (Britton and Khachik, 2009).

Food processing is an important factor to consider in achieving hunger and malnutrition eradication goals. It disrupts the food matrix and exposes them to air, light which reduces nutrient (Alamu and Mooya, 2017). A study of ancient civilization demonstrates conclusively that throughout the historical record, humans conquered hunger and disease via food processing with complex techniques (Floros *et al*., 2010). Raw maize is therefore, usually been processed before consumption, thus, loss of micronutrients especially beta-carotene content in the maize is inevitable. Moreover, lack of provitamin A carotene in white maize and this loss of beta carotene in other types of maize during processing for food have compounded the challenge of VAD. The question in mind is that, how much of beta-carotene is retained after a raw maize is processed for consumption? Hence, it is important to evaluate the best processing techniques with higher retention level of beta-carotene to enable achievement of sustainability goals. We therefore, hypothesized that there is no significant difference in the beta carotene content of maize processed by different methods for consumption.

Presently, there is little or no information available to account for the retention level of beta - carotene in maize after it has been processed to different maize food products. Therefore, the retention of beta-carotene content after processing of maize into different food products must be quantified and data made available to researchers in order to guide breeders during breeding of biofortified maize to achieved target set by HarvestPlus to alleviate VAD. Also, to identify and recommend Nigerian maize food products with higher beta - carotene retention after processing.

The aim of this study is to investigate beta-carotene content of selected maize genotypes and their retention after processing and preparation of different maize foods consumed in Nigeria.

MATERIALS AND METHODS

Materials

The followings are the materials used in this work; vacuum filter, sieve, separating funnel, aluminum foil, blender and spectrophotometer. Chemical and reagents used include acetone, petroleum ether, sodium chloride and anhydrous sodium sulphate. All the chemicals were of analytical grade and obtained from reputable vendors. The kernels of five selected maize genotypes (SUWAN - 1 - SR - Y, Pro - Vit A - LF - QPM, IFE - HYB 4 Inbred line, Ebonyi 1 and Ebonyi 2) were obtained for planting. SUWAN - 1 - SR - Y (SUW) and Pro - Vit A - LF - QPM (PRO) were obtained from I. A. R. & T research farm in Ibadan, Nigeria. IFE - HYB 4 Inbred line (IFE) was gotten from I. A. R. research farm Ahmadu Bello University, Zaria, Nigeria while two local cultivars, Ebonyi 1 (EB 1) and Ebonyi 2 (EB 2) were obtained from traders at Eke market in Afikpo North, Ebonyi State, Nigeria. Planting was done on 2nd June, 2023 at Ebonyi State University Research Farm, CAS Campus in Nigeria. The maize was harvested manually with its husk on 13th September, 2023.

Methods

Collection of maize samples

Each maize genotype with its four replicates were harvested and pooled together to get a well-mixed sample and 100 g of each Maize sample were milled using blender and wrapped with aluminum foil. This was kept in an air - tight laboratory cupboard. All analysis was performed within 48 hours of sample preparation to keep carotenoids from being broken down by light and oxygen.

Processing of maize food products

Boiled Maize: Boiled maize was processed as described by Bhaskarachary *et al*. (2017). Four Maize were picked and the outer leaves were removed. Exactly 100 g of corn kernel with the cob was placed in a pot containing 500 mL of distilled water and the content was boiled for 2 hours. All other maize genotypes were separately boiled following the same procedure. The boiled maize samples were cooled and wrapped in aluminum foil.

Roasted maize: Four corn were selected and their outer leaves were removed. Exactly 100 g of corn kernels were placed on coals pre-heated with fire, corn was turned until it was completely roasted (Bhaskarachary *et al.,* 2017). All other maize genotypes were separately roasted following the same procedure. The roasted maize samples were cooled and wrapped in aluminum foil.

Akamu: Exactly 100 g of maize sample was washed in distilled water and steeped for 72 hours. The steeped maize was wet-milled into slurry with a blender. More water was added to the slurry, filtered and the shaft was removed. The akamu was prepared in a form of thin gel by addition of boiled water (Olaniran et al., 2020).

Agidi (Thick gruel): The preparation of agidi was done as described by Adeyemi and Oluwamukomi (1989) with little modifications. Exactly 100 g of clean maize was boiled in water for 30 minutes. The water content was drained and the boiled grains were wet-milled. A slurry of the milled sample was prepared and filtered. Excess water was removed from the filtrate after few minutes. Then 80 % of filtrate (slurry) was cooked with 20 % boiling water in an aluminum pot for 10 - 15 minutes while it was stirred continuously until it becomes thick (gel - like).

Maize moin-moin (steamed maize pudding): This was processed according to Otunola and Afolayan, (2018) description with minor modifications. Exactly 100 g of the maize was cleaned and soaked in 500 mL of water for three hours and then milled with a blender. The milled sample was placed in small plate containers and steam - cooked for an hour.

Extraction and evaluation of beta - carotene

The determination of beta - carotene content of maize samples was done as described by Rodriguez-Amaya and Kimura (2004). Five milligrams of the maize were ground with the aid of hyflosupercel (3.0 g) in 50 ml of cold acetone and filtered by vacuum filtration. The filtrate was extracted using 40 ml petroleum ether. Emulsion formation was prevented by addition of saturated sodium chloride. The lower aqueous phase was removed while upper phase was collected and filtered through 15 g of anhydrous sodium sulphate to eliminate residual water. The separating funnel was rinsed with petroleum ether and the flask was made up to 50 ml. Absorbance readings were taken at 450 nm using spectrophotometer and Beer - Lambert law was used to determine the total carotenoid content. The procedure was repeated for all maize genotypes and their food products. The experiment was done in triplicates.

$$\text{Total carotenoid content } (\mu g/g) = \frac{A \times \text{volume(ml)} \times 10^4}{A^{1\%}_{1cm} \times \textit{sample weight}(g)}$$

Where;

A = Absorbance

Apparent retention was determined according to Murphy *et al*. (1975), as stated below;

$$\%\ \text{Apparent Retention} = \frac{\text{Nutrient per g of cooked food (dry basis)}}{\text{Nutrient per g of raw food (dry basis)}} \times 100$$

Data analysis

The result of statistical analysis of data collected was recorded as mean ± standard deviation. Two – way ANOVA and LSD were done to determine if there is significant difference between treat groups (P<0.05) using R software version 4.3. The experiment was done in triplicates.

RESULTS

Table 1: Beta - carotene Contents of the Five Maize Varieties after Processing into Different Food Products

Sample	Beta - carotene (µg/g)	Beta - carotene retention (%)
IFE_Raw	36.58±0.01	100
IFE_Boiled	27.45±0.12	75.04
IFE_Roasted	16.89±0.13	46.17
IFE_Agidi	6.81±0.07	18.62
IFE_Pap	8.13±0.05	22.22
IFE_Moin-moin	7.85±0.13	21.46
SUW_Raw	39.24±0.10	100
SUW_Boiled	42.29±0.14	107.77
SUW_Roasted	42.84±0.02	109.17
SUW_Agidi	12.18±0.23	31.04

SUW_Pap	12.61±0.11	32.13
SUW_Moin-moin	21.45±0.03	54.66
EB1_Raw	40.42±0.52	100
EB1_Boiled	30.62±0.26	75.75
EB1_Roasted	38.54±0.05	95.35
EB1_Agidi	9.36±0.06	23.15
EB1_Pap	11.09±0.27	27.44
EB1_Moin-moin	14.55±0.10	35.99
EB2_Raw	20.74±0.11	100
EB2_Boiled	4.94±0.04	23.82
EB2_Roasted	12.37±0.09	59.64
EB2_Agidi	3.55±0.09	17.11
EB2_Pap	4.92±0.02	23.72
EB2_Moin-moin	6.28±0.07	30.28
PRO_Raw	37.56±0.02	100
PRO_Boiled	30.46±0.19	81.09
PRO_Roasted	20.31±0.02	54.07
PRO_Agidi	8.54±0.08	22.73
PRO_Pap	8.37±0.13	22.28
PRO_Moin-moin	10.41±0.12	27.71

Values are means ±S. D of triplicate determinations.

Beta - carotene retention of IFE maize variety after processing

The result showed that beta - carotene content in the raw IFE maize sample was 36.58 µg/g. After processing, the boiled and roasted IFE maize had 27.45 and 16.89 µg/g of beta - carotene respectively, while those processed into Agidi recorded the lowest beta - carotene content (6.81 µg/g) which is below Pap and moin – moin, 8.13 and 7.85 µg/g respectively. There was significant difference in the beta - carotene content of IFE maize variety processed into different maize food product (Table 2).

Beta - carotene retention of SUW maize variety after processing

The beta - carotene content of the SUW maize variety varied significantly ($p<0.05$) with the treatment methods used. The raw SUW sample recorded 39.24 µg/g beta - carotene content. Meanwhile, the roasted SUW maize had 42.84 µg/g and the boiled recorded 42.29 µg/g. However, there was significantly ($p<0.05$) low beta - carotene content in samples pre - processed into flour before final products (Agidi, pap, and moin - moin) which had beta - carotene content of 12.18, 12.61 and 21.45 µg/g respectively (Table 1), but there was no significant difference between pap and agidi (Table 2). SUW maize sample shows more than 100 % (107.77 and 109. 17) beta-carotene retention after boiling and roasting respectively (Table 1).

Table 2: Mean Separation of Beta - carotene Content of Maize Varieties Processed into Different Foods.

Treatment	IFE	SUW	EB1	EB2	PRO
Raw	36.58[a]	39.24[c]	40.42[a]	20.74[a]	37.56[a]
Boiled	27.45[b]	42.29[b]	30.62[c]	4.94[d]	30.46[b]
Roasted	16.89[c]	42.84[a]	38.54[b]	12.37[b]	20.31[c]
Pap	8.13[d]	12.61[e]	11.09[e]	4.92[d]	8.37[e]
Moin-moin	7.85[e]	21.45[d]	14.55[d]	6.28[c]	10.41[d]
Agidi	6.81[f]	12.18[e]	9.36[f]	3.55[e]	8.54[e]
LSD (p<0.05)	**0.1824**	**0.2246**	**0.4766**	**0.1321**	**0.1996**

Values within the same column with different letters are significantly different at p<0.05 (Tukey LSD test)

Beta - carotene retention of EB1 maize variety after processing

The percentage retention of beta - carotene among different food products processed from EB1 maize variety differed significantly ranging from 23.1 to 95.3 % in agidi and roasted samples respectively. Treatment with high percentage retention were boiling and roasting with 75.7 % and 95.35 % respectively (Table 1). There was significant difference for EB1 variety-derived food products based on the processing methods used (Table 2).

Beta - carotene content of EB2 maize variety

The beta - carotene content of raw EB2 maize variety was 20.74 µg/g, followed by the roasted and moin - moin samples having 12.37 and 6.28 µg/g respectively after processing. The boiled and pap sample recorded the same value (4.9 µg/g) while the lowest beta - carotene content 3.6 µg/g was recorded for agidi sample approximately (Table 2). There was no significant difference between boiled and pap processed maize food products (Table 2). It was only roasted EB2 that has beta-carotene retention above 50 % (Table1).

Beta - carotene content of PRO maize variety

The result showed that the raw PRO maize sample had beta - carotene content of 37.56 µg/g, followed by the boiled sample (30.46 µg/g), roasted (20.31 µg/g), moin - moin (10.41 µg/g) and agidi (8.54 µg/g). While pap recorded the lowest beta carotene content 8.37 µg/g. There was significant difference in the beta - carotene content of PRO maize variety processed into different maize food products with the exception of pap and agidi (Table 2).

DISCUSSION

This study provides detailed information on effect of food processing methods/techniques on beta - carotene retention in different maize samples used in formation of food products like roasted maize, boiled maize, pap, agidi and moin-moin.

We observed beta-carotene retention of about 107 – 109 % for SUW_Boiled and SUW_Roasted maize sample in our work (table 1). This shows an increase in beta-carotene content as compared to the raw maize sample. This increase in percentage concentration of beta-carotene of the processed SUW maize sample could be due to a release of more beta-carotene from the matrix as a result of moist heating by boiling, and roasting effect which might have burst open the matrix to release the content in the processed samples than in the raw sample. Sowa *et al*., (2018) earlier attests to this possibility of beta - carotene retention rates of greater than 100 % attributing it to changes in the food matrix, with cooking leading to better extractability of carotenoids after processing, or deactivation of oxidative enzymes that would degrade carotenoids, rather than true increase in carotenoid content.

Our EB1_Roasted maize sample beta-carotene retention was 95.35 % (table 1). This compares favorably to Kirthee *et al*. (2014) who reported that 95.5% beta - carotene was retained after cooking of Phutu (maize food products). Khachik *et al*. (1992a) reported that traditional blanching and cooking method significantly increased the concentration of carotenoids in numerous green vegetables. Although the reverse is the case of beta - carotene as it is susceptible to temperature, light and thereby likely to undergo oxidation (Sandesh *et al*., 2018).

IFE_Boiled and EB1_Boiled maize samples gave beta-carotene retention of 75.04 and 75.75 % respectively after processing. This percentage retention is similar to Sowa *et al*. (2017) who observed 67 - 78 % retention in deep - fried maize, even though their methods of processing differ. Our results show that boiling and roasting different maize sample will give a higher content (75 % and above) of beta-carotene retention during processing as observed for IFE_Boiled, SUW_Boiled, SUW_Roasted, EB1_Boiled, EB1_Roasted and PRO_Boiled (Table 1) with the exception of IFE_Roasted, EB2_Roasted and PRO_Roasted whose beta-carotene retention is below 60 % (Table 1)

According to Munkhuwa *et al*. (2023), the disparity in retention percentages for similar food products from different varieties could be attributed to the differences in genotype between the different maize varieties. Eyinla *et al*. (2018) also, reported a similar observation on significant varietal effect on beta - carotene retention after processing, they observed that cassava varieties that had the highest beta - carotene content in the fresh roots did not necessarily have the highest content after processing.

Product oriented processing of maize sample leads to extremely low retention of beta-carotene in processing maize samples. All the different maize samples processed products like agidi, pap and moin-moin have < 35 % beta-carotene retention after processing (Table 1). A similar beta - carotene retention of 19 - 33 % was also reported in Ogi (Pap) powder (Omololami *et al*. 2021). In milled maize food products, the content of maize seed has more surface area exposed to direct heat than when roasted or boiled as seed. The prolonged time of exposure to heat is responsible for the low retention, since beta - carotene is sensitive to heat. Furthermore, an increased in the processing steps of provitamin A biofortified maize leads to reduction of carotenoids based on heat, prolonged exposure to light and physical friction (Omololami *et al*. 2021). The process of steeping in maize to produce pap could be accountable for the drastic degradation in beta - carotene. In our study, Pap making process leads to more than 70 % loss of beta - carotene.

Since, Pap (fermented maize gruel) serves as a weaning food for children in most developing countries, there is need for researchers to find ways of improving the concentration of beta - carotene in biofortified maize varieties so as to meet up with the target set by Harvestplus for VAD. It is also essential that food technologists improve on the processing method of Pap so as to mitigate the drastic loss of beta - carotene content.

There is significant difference in beta-carotene retention based on methods of processing for each group of maize samples as observed for IFE, SUW and EB1 (Table 2). However, there are no significant differences for EB2 when processed by boiling and pap production. More so, for PRO sample, there was no significant difference when processed into pap and agidi (Table 2)

In this study, beta - carotene as observed could have been degraded based on prolong exposure to air, light, high heat and other factors, this account for the variations in the beta - carotene retention based on different processing techniques/methods. It was equally observed that among the various maize genotypes, SUW has the highest retention from the various processing methods/techniques. The boiling and roasting processing methods recorded significantly higher beta - carotene retention compared with other methods.

CONCLUSION AND RECOMMENDATION

The data generated in this study reveal that recently developed biofortified maize varieties have an appreciable level of beta - carotene and that their beta - carotene contents depend on the maize variety. SUW maize cultivar's beta-carotene content is resistant to loss during processing. This cultivar should be given preference over others as potent source of beta-carotene.

The results also suggest that food processing methods have significant effect on beta-carotene retention due to exposure to oxidation, temperature, light and other environmental factors. The roasting method which is a heat processing method and the boiling method which is a moist heat processing method increased the beta - carotene retention level. These methods are seen to be the most appropriate processing method to retain beta-carotene micronutrient. Households and other users of maize should deploy these methods for processing their maize food products since it increased nutritional content. The choice of applying the methods for maize food production can help contribute to sustainability goal in eradicating micronutrients deficiency.

However, Pap which serves as a weaning food for children in most developing countries has lower beta - carotene retention due to the food processing method. This will not help Harvestplus to attain the target goals. It is therefore recommended that;

i. Researchers should investigate and develop a better methods of processing Pap to mitigate the more than 70 % observed loss of beta - carotene leading to low retention levels.
ii. Scientists should also increase beta - carotene content of biofortified maize during breeding so that maize food products like Pap will still have an appreciable level of beta - carotene retention after processing in order to alleviate VAD.
iii. Further research should be done on identifying maize varieties with appreciable level of beta - carotene content and the seeds of such varieties be made available to small holder farmers for cultivation.

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Author's contribution

Ile Bamidele Emakoji conceptualized this research idea, collected and processed maize samples, designed the methodology of the experiment, carried out the laboratory works and equally wrote the original draft and manuscript. The manuscript was edited and reviewed by Simon Drisu.

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