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Research Article
Effect of Processing on Selected Varieties of Cowpea (*Vigna unguiculata* L. Walp)

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Abstract
Background and Objectives: Research has been focused on investigating the effects of processing on the nutritive composition of foods. This work quantitatively compared two selected varieties of cowpea (*Vigna unguiculata* L. Walp.) based on their chemical constituents (proximate, minerals and phytochemicals) and the effect of dehulling on the two. **Materials and Methods:** Brown beans and white beans were purchased from New Market, Wukari Local Government, Taraba State of Nigeria. Dehulled and unhulled portions of the cowpeas were milled into flour for analyses. Phytochemicals were determined using standard method whereas, mineral elements were determined by Atomic Absorption Spectroscopy (AAS). Proximate analysis of the leaves was done by the standard methods cited by Ojo and colleagues while SPSS was used for all statistical analysis. **Results:** All the phytochemicals (total phenolic content (TPC),flavonoids, phytates, oxalates and carotenoids) significantly decreased after dehulling in the two cowpea varieties although the red cowpea had significantly higher levels of phytochemicals than the white variety, both before (except for oxalates) and after dehulling. Generally, the white dehulling reduced mineral element content in the two cowpea varieties but the white cowpea showed higher levels of mineral elements than the red cowpea. Crude protein, fibre and ash were significantly decreased by dehulling whereas, carbohydrates increased in both the red and white cowpeas. The white cowpea had higher levels of protein, fat, fibre, ash and moisture than the red cowpea. **Conclusion:** Dehulling significantly reduces the nutritive composition of cowpeas, except for carbohydrates which increase after dehulling. The white cowpea variety in this research is a better source of protein, fat and fibre than red cowpea.

Key words: Phytochemicals, proximate, protein, anemia, minerals

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

From the most important cultivated legumes, cowpea (*Vigna unguiculata* L. Walp) has shown several agronomic, environmental and economic advantages, contributing to further improving the diets and incomes of peasant farming across Africa, Asia and South America[1,2]. *Vigna unguiculata* L. Walp is a herbaceous annual crop of the family *Fabaceae*[3,4]. It is favoured because of its wide adaptation and tolerance to several stresses. It is an important food source and is estimated to be the major protein source for more than 200 M people in Sub-Saharan Africa and is in the top ten fresh vegetables in the People's Republic of China[5].

Onyenekwe *et al.*[6] reported that cowpea seeds are characterized by a high proportion of carbohydrates, with starch being the main component and energy source. Similar to other legumes, cowpea starch is more slowly digested than starch from cereals. Based on the literature on assessment of the mineral composition of cowpea seeds widely varies depending on the unique cultivars evaluated[7]. These findings on the mineral composition of cowpea emphasize its value and reveal its recommended daily intake as other legumes[8]. Cowpea seed extracts possess more hypolipidemic activity than leaf extracts through the reduction of Total Cholesterol (TC) and Low-Density Lipoprotein (LDL)[9]. Cowpeas, being nutrient-dense have been used to improve the acceptability of some of the wheat-based cookies[10].

Mineral element deficiency has been an issue of concern as it leads to several diseases[11]. Also, oxidative stress, which can well be prevented by some phytochemicals, has been found to correlate with phytochemical deficiency[12]. Some food processing methods have been known to reduce the contents of such minerals and phytochemicals[13].

Research has revealed different methods of processing cowpea into food. In so many places like Wukari, Taraba State, Nigeria, it is commonly processed (dehulled) and ground to make Moi-Moi, Akara, etc. According to Madodé *et al.*[14], food processing techniques such as dehulling, boiling and soaking reduce the fermentability of indigestible carbohydrates in cowpea thus reducing associated flatulence. There is little or no literature with scientific proof of the effect of dehulling on the nutritional and phytochemical composition of different varieties of cowpea consumed in Wukari, Taraba State, Nigeria. This work aimed at quantitatively comparing the chemical constituents (proximate, minerals and phytochemicals) of two selected cowpea varieties (brown and white beans) and determining the effect of dehulling on the two. This will inform the effects of dehulling and the choice of the better variety for consumption as well.

MATERIALS AND METHODS

Study area: This study was carried out in the Department of Biochemistry, Federal University Wukari and Taraba, Nigeria. The research took about 5 months (extending from May through October, 2018).

Procurement of sample: Two varieties of cowpea (*Vigna* spp.) which are the brown beans and the Portuguese, also known as white beans were purchased from Wukari new Market in Wukari Local Government, Taraba State, Nigeria.

Sample preparation: Cowpea flour was prepared as described by Nahemiah *et al.*[15]. The seeds (3 kg) were steeped in clean tap water for 30 min at ambient temperature (32$\pm$2°C) and dehulled manually by gently pounding in wooden mortar and pestle followed by washing several times. The dehulled grains were oven-dried at 65°C for 1 hr to constant weight. The dried cotyledons were then milled in an attrition mill, sieved with a fine 150 µm sieve (Brabender OHG Duisburg) and then stored in an air-tight container until needed.

Determination of proximate composition: The prepared samples were quantitatively analyzed for moisture, crude protein, ash, crude fat, carbohydrate and crude fibre contents according to the methods cited by Ojo *et al.*[16] and results were analyzed using SPSS and expressed in Mean$\pm$Standard deviation.

Determination of mineral content: The prepared samples were quantitatively analyzed for magnesium, iron and phosphorous using the methods cited by Ojo *et al.*[16] and results were analyzed using SPSS and expressed in Mean$\pm$Standard deviation.

Determination of carotenoids content: Carotenoids content was evaluated according to the method described by Krishnaiah *et al.*[17]. A laboratory blender was used to homogenize a measured weight of the sample and the initial crude extract was obtained by subjecting the homogenate to filtration. The crude extract was properly mixed with 20 mL of ether, followed by treatment with 20 mL of distilled water in a separating funnel. The ether layer was recovered and dried in a vacuum desiccator within the temperature range of 35-50°C. Using 20 mL of ethanolic potassium hydroxide, the dry extract was saponified and the product leftover in a dark cupboard till the next day when carotenoid was extracted in 20 mL of ether and washed with two portions of 20 mL distilled water. The carotenoid extract (ether layer) was dried (using a desiccator), treated with light petroleum (petroleum spirit) and allowed to stand overnight in a freezer (-10°C). The precipitated steroid was expelled by centrifugation after 12 hrs and the carotenoid extract was evaporated to dryness in a weighed evaporation dish, cooled in a desiccator and weighed. The weight of the carotenoids was determined and expressed as a percentage of the sample weight cited by Krishnaiah *et al.*[17]:

$$\text{Carotenoid content (\%)} = \frac{\text{Weight of sample}}{\text{Weight of sample taken}} \times 100$$

Total phenolic content (TPC): Total polyphenols were determined following the Folin-Ciocalteu's method using gallic acid as standard as described by Chandra and Arora[18]. Folin-Ciocalteu's reagent (12.5 µL) along with 7% sodium carbonate (125 µL) was added to the guava extracts. Samples were then incubated for 90 min at room temperature. The absorbance was measured at 750 nm using a microplate reader (Synergy HT, Bio Tek Instruments, Winooski, VT, USA).

Determination of flavonoid content: The flavonoid content was determined as described by Madaan *et al.*[19] with slight modifications. Ten grams of the grain sample were extracted, respectively with 100 mL of 80% aqueous methanol at room temperature (30±2°C). The mixture was then filtered through a Whatman No. 42 grade filter paper into a weighed 250 mL beaker. The filtrate was transferred into a water bath, evaporated to dryness and weighed. The percentage flavonoid was calculated as described by Madaan *et al.*[19]:

$$\text{Flavonoids content (\%)} = \frac{\text{Weight of residue}}{\text{Weight of sample taken}} \times 100$$

Determination of phytate content: The phytate content was determined using the spectrophotometric method as described by Obadoni and Ochuko[20]. One-gram mass of the pulverized flour sample was dissolved in 25 mL of 0.5M HNO_3 and centrifuged at 4000 rpm for 10 min. One millilitre of 0.003 M ferric solution was added to the supernatant and left to stand for 15 min to allow chelation of the iron molecules by the indigenous plant phytate. At the end of the incubation, it was capped and heated for 20 min. Thereafter, 0.1 mL of 1.33 M NH_4SCN (Ammonium sulphocyanide) solution was added and absorbance was read at 456 nm. The amount of phytate was extrapolated from a standard calibration curve for calcium phytate as described by Obadoni and Ochuko[20]:

$$\text{Phytates content (\%)} = \frac{\text{Absorbance of sample} \times \text{Concentration}}{\text{Absorbance of standard}} \times 100$$

Determination of oxalate content: The total oxalate content was determined using the method Adeniyi *et al.*[21] described. A mass (2 g) of the flour was weighed and digested with 10 mL 6M HCl for 1 hr, cooled and made up to the 250 mL mark in a volumetric flask for filtration. Part of the filtrate (125 mL) was poured into a beaker and 3-4 drops of methyl red were added. Following this, concentrated NH_4OH solution was added (in a drop-wise fashion) until the colour changed from salmon pink to a faint yellow colour. The pH of the solution was determined and each portion was heated to 90°C, cooled and filtered to remove the precipitate. To a heated (90°C) filtrate, 10 mL of 5% $CaCl_2$ (aq.) was added with continuous stirring, followed by decantation of the upper layer and complete dissolution of the precipitate in 10 mL of 20% (v/v) H_2SO_4 (aq.). The filtrate was diluted to the 300 mL mark and an aliquot of 125 mL of the filtrate was heated until near boiling, which was then titrated against 0.05 M standardized potassium tetraoxomanganate (VII) to give a pink colour (which persisted for the 30 sec) at the endpoint. The oxalate content was calculated by Adeniyi *et al.*[21]:

$$\text{Oxalates content (\%)} = \frac{T \times Vme \times Df \times 105}{ME \times Mf} \times 100$$

Where:

T = Titre value of $KMNO_4$
Vme = Volume-mass equivalent (that is, 1 mL of 0.05 m $KMNO_4$ = 0.00228 g of anhydrous oxalic acid)
Df = Dilute factor (Vt/A that is, total volume of titrated against/Aliquot used)
Mf = Mass of sample used
ME = Molar equivalence of $KMNO_4$ in oxalate concentration in g dm^{-3}

The AAS treatment results are presented below and the values are Mean±Standard Error (SE). These values were calculated from the equation described by Adeniyi *et al.*[21]:

$$\text{Concentration (mg kg}^{-1}\text{)} = \frac{\text{Concentration of sample (PPM)} - \text{Concentration of blank (PPM)} \times \text{Vol. (50 mL)}}{\text{Weight of sample (g)} \times 1000} \times 1000$$

RESULTS AND DISCUSSION

Results expressed the comparison of two varieties of cowpeas (white and red) at different processing levels (dehulled and unhulled), revealing their proximate, minerals, phytochemicals and energy contents. The DHR represents Dehulled Red, DHW represents Dehulled White, UHR represents Undehulled Red, UHW represents Undehulled White.

Table 1: Proximate composition of *Vigna unguiculata* L. Walp. samples (mg/100 g)

Samples	Crude protein	Fat	Fibre	Ash	Moisture	Carbohydrate (CHO)
UHR	24.13±0.005[c]	7.96±0.014[c]	4.95±0.007[b]	7.75±0.000[c]	9.50±0.000[d]	45.72±0.028[b]
UHW	26.92±0.005[a]	7.99±0.014[b]	5.03±0.000[a]	7.88±0.000[a]	9.70±0.000[c]	42.49±0.021[d]
DHR	21.76±0.010[d]	8.12±0.000[a]	3.65±0.000[d]	7.72±0.007[d]	9.75±0.000[b]	49.01±0.021[a]
DHW	24.64±0.010[b]	7.75±0.000[d]	4.51±0.007[c]	7.82±0.000[b]	9.81±0.014[a]	45.47±0.035[c]

Values are expressed in Mean±Standard errors of means, mean with same alphabets superscripts are not significantly different

Table 2: Mineral composition of *Vigna unguiculata* L. Walp. samples (mg/100 g)

Samples	Fe	Mg	p-value
UHR	10.46±0.005[c]	29.57±0.007[c]	71.04±0.010[c]
UHW	13.99±0.005[a]	28.44±0.014[d]	87.06±0.010[a]
DHR	8.88±0.010[d]	37.47±0.021[a]	65.35±0.005[d]
DHW	11.35±0.010[b]	36.33±0.007[b]	80.22±0.010[b]

Values are expressed in Means±Standard deviation. Mean with same alphabets superscripts are not significantly different

Proximate composition: Table 1 shows the proximate compositions of the *V. unguiculata* varieties before and after processing (dehulling). The result reveals that crude protein, fat, ash, fibre and moisture were significantly higher in the UHW than in the UHR. The UHR, however, had significantly higher CHO compared to the UHW. The UHR had significantly ($p<0.05$) lower crude protein, fibre, ash and CHO compared to the DHR. The fat and moisture composition, however, significantly increased after dehulling (DHR).

Tchiagam *et al.*[22] reported similar results on crude protein contents of some cowpea varieties. Undehulled white variety (UHW) had higher crude protein than the unhulled red variety. This may be due to variations in genotypes and agronomic practices[23]. After dehulling, crude protein significantly ($p<0.05$) decreased in both varieties. This agrees with recent reports of Akinjayeju and Ajayi[24]. This suggests that the hulls also contain some proteins. Dietary proteins are essential to animals since they can't synthesize some essential amino acids. Thus, dehulling may place people at risk of a low protein diet.

Fibre is the part of plant based-diet which is resistant to enzymatic digestion and it includes cellulose, non-cellulosic polysaccharides (e.g., hemicellulose), pectic substances, gums, mucilages and a non-carbohydrate component lignin[25]. The UHW cowpea variety had significantly higher (5.03±0.00 mg/100 g) fibre than the UHR variety (4.95±0.01 mg/100 g). On dehulling, however, fibre significantly ($p<0.05$) decreased (to 4.51±0.01 mg/100 g in DHW and 3.65±0.00 mg/100 g). These results agree with the findings of Akinjayeju and Ajayi[24]. Abiodun and Adepeju[26] also reported a decrease in fibre content of Bambaranuts after dehulling. Consumption of foods rich in dietary fibre has been related to decreased incidence of several diseases[25]. Some health benefits of dietary fibre include: Lowering of blood cholesterol and sugar levels, reduction of risks of obesity, diabetes, constipation and heart complications, among others[25].

The whole seeds of the white variety had significantly ($p<0.05$) higher ash content (7.88±0.00 mg/100 g) than those of the red variety (7.75±0.00 mg/100 g). After dehulling, ash contents significantly decreased in both verities. Similar results have been reported in recent years[24,26]. Ash is the residual inorganic material, such as minerals, present in food that remains after heating removes water and organic material such as fat and protein. Ashcan includes both essential minerals, such as calcium and potassium and toxic materials, such as mercury[27]. The ash contents found in this research are higher than that of *Moringa oleifera* reported by Sodamade *et al.*[27].

Carbohydrates are essential macro-nutrients needed in animal nutrition. The most abundant carbohydrate in cowpea is starch. Carbohydrate was significantly ($p<0.05$) higher in UHR than in UHW cowpea variety, however, the carbohydrate content significantly ($p<0.05$) increased when dehulled in the two varieties. This could be due to the loss of proteins (during dehulling) which manifests as an increased percentage of carbohydrates. This agrees with the findings of Hoover *et al.*[28], who reported a carbohydrate yield of the range 17.78-22.93% in cowpea. Adebooye and Singh[29], however, had reported lower values for other cowpea varieties. Cowpea starches are increasingly concerned about the potential to incorporate into food products due to high amylose and resistant starch content[30].

Mineral composition: Table 2 affirms Fe and P to be significantly higher ($p<0.05$) in UHW than in UHR. The Mg, however, was found to be significantly higher ($p<0.05$) in UHR than in UHW. After dehulling, Fe and P significantly ($p<0.05$) decreased in both cases but Mg rather increased after dehulling (DHR).

Iron content in the UHW was significantly ($p<0.05$) higher (13.99±0.01 mg/100 g) than the undefiled red variety UHR (10.46±0.01 mg/100 g). However, dehulling reduced the iron content significantly ($p<0.05$) to 11.35±0.01 mg/100 g (for DHW) and 8.88±0.01 mg/100 g (for DHR). Processing also influences the mineral element content of Bambara nuts[31].

Table 3: Phytochemical composition of *Vigna unguiculata* L. Walp. samples (mg/100 g)

Samples	TPC	Flavonoids	Phytates	Oxalates	Carotenoids
UHR	51.23±0.010[a]	5.12±0.014[a]	3.06±0.014[a]	3.77±0.007[b]	275.34±0.007[a]
UHW	48.35±0.015[b]	4.24±0.021[c]	2.45±0.007[b]	4.48±0.021[a]	237.14±0.021[b]
DHR	46.34±0.015[c]	4.77±0.007[b]	2.45±0.007[b]	3.33±0.014[c]	232.34±0.014[c]
DHW	35.34±0.010[d]	3.86±0.014[d]	1.44±0.014[c]	3.13±0.014[d]	192.25±0.021[d]

Values are expressed in Mean±Standard deviation, mean with same alphabets superscripts are not significantly different

This suggests that the cowpea seed hulls contain a significant amount of iron. Iron is helpful in bone formations, normal functioning of muscle and many others[32]. Iron serves important roles in the body and these include haematopoiesis, binding and transport of oxygen, DNA synthesis, electron transport, etc. Deficiency of iron in the body may lead to anaemia, which may, in turn, result in hypoxia and tissue infarction[33].

Phosphorus is the most abundant mineral element found in the varieties of cowpea. The significant ($p<0.05$) difference in phosphorus content observed between UHW (87.16±0.01 mg/100 g) and UHR (71.04±0.01 mg/100 g) might be due to genetic variations or agronomic practices[23]. The significant ($p<0.05$) decrease (to 65.35±0.01 mg/100 g for the "white" variety and 80.22±0.01 mg/100 g for the "red" variety) in phosphorus content after dehulling indicates that phosphorus is a component of the hulls. This is in line with the findings of Arinathan *et al.*[32]. Phosphorus is a notable component of comets, which have been believed to have played a great role in the emergence of life on earth[11]. Phosphorus complements the detection of glycine and is an indispensable element in all living organisms, which is found in adenosine 5 - triphosphate, in the backbone of DNA and RNA and cell membranes[11]. Phosphorus also plays a positive role in bone health[34]. Phosphorus deficiency leads to delayed vascular invasion and mineralization of growth plate cartilages[35].

Magnesium is important in tissue respiration, especially in oxidative phosphorylation leading to the formation of adenosine triphosphate (ATP). It is also involved in normal muscular contraction, magnesium relaxes the muscles[36,37]. The concentration of magnesium in the UHR was found to be significantly ($p<0.05$) higher than that of UDW by 4.0%. Magnesium concentration significantly increased ($p<0.05$) after dehulling in both DHR (by 27%) and DHW (by 28%). This finding is in line with that of Arinathan *et al.*[32]. These results, however are higher than those reported by Owolabi *et al.*[38] for both improved and local cowpea varieties in Samaru, Kaduna State of Nigeria. Magnesium is a constituent of bone and teeth and is closely associated with calcium and phosphorus. Magnesium is necessary for the release of parathyroid hormone and its action in the backbone, kidney and intestine and the reactions involved in converting vitamin D to its active form[39]. Magnesium deficiency results in uncontrollable twisting of muscles leading to convulsion and tetanus, which may both lead to death[40].

Phytochemical composition: Table 3 shows that, UHW has a significantly lower ($p<0.05$) composition of total phenolic content, phytates, flavonoid and carotenoid than the UHR but has a higher composition of oxalates than the UHR.

Phytochemicals were also determined in the two local cowpea varieties. Generally, the red variety was found to contain significantly higher ($p<0.05$) phytochemicals than the white variety, both before and after dehulling (Table 3). However, the white variety had significantly higher ($p<0.05$) contents of oxalates in both whole and dehulled samples.

Total phenolic contents (TPC) is an index of antioxidant power/activity of foods and it gives an estimate of phenolics like cinnamic acid, gallic acid coumaric acid, catechin, ferulic acid, resveratrol, etc.[41]. A statistically significant difference (2.88 mg/100 g) in TPC was observed between the UHW and UHR varieties with UHR having the highest TPC. This variation in TPC across varietal boundaries bears concordance with the reports of[12,42]. Dehulling reduced the total phenolic concentration of both varieties, although TPC in the red variety remained significantly higher in the white variety even after processing. Similar findings have been reported by Badifu[43] and Adebowale *et al.*[44]. Phenolic compounds like resveratrol are good antioxidants or free radical scavengers that mitigate oxidative stress and precipitation/exacerbation of some diseases[45,46]. The phenolic component of cowpea has been shown to vary with processing and also to have antioxidant activity, even boosting the enzymatic activities of GSH-Px and SOD[47].

The unhulled red variety of cowpea (UHR) had significantly higher ($p<0.05$) phytate (3.06±0.01 mg/100 g) than UHW (2.45±0.01 mg/100 g). This difference may be attributed to genetic variations and environmental factors[23]. However, phytate reduced significantly ($p>0.05$) after dehulling, though it remained significantly ($p<0.05$) higher in DHR than in DHW. This is in line with the findings of Abiodun and Adepeju[26]. The decrease in phytate after dehulling may be attributed to the fact that the hulls contain a considerable

Table 4: Energy contents of *Vigna unguiculata* L. Walp. samples (Kcal/100 g)

Samples	Energy
UHR	501.35±0.015[c]
UHW	572.89±0.005[a]
DHR	435.56±0.015[d]
DHW	518.38±0.001[b]

Values are expressed as Mean±Standard deviations, mean with same alphabets superscripts are not significantly different

amount of phytate. Cooking and dehulling have been reported to reduce phytate levels in several plant products[26]. Phytate decreases calcium bioavailability and forms calcium phytate complexes that inhibit the absorption of Fe. Phytate contents vary with crop variety, climatic conditions, location, irrigation conditions, type of soil and the growing season of the plant[26,38]. Oxalate levels also reduced after dehulling, probable due to its solubility in water.

Carotenoids are also antioxidants that reduce or fight oxidative stress. Carotenoid significantly ($p<0.05$) varied, before dehulling (by about 38.2 mg/100 g) and after dehulling (by about 40 mg/100 g), between the two cultivars with the red having the highest concentration in either case. Abiodun and Adepeju[26] reported similar results. Carotenoids from fruits have been implicated in antioxidation, reduction and scavenging of free radicals[48,49].

Flavonoids have good antioxidant power and so abate oxidative stress[40]. The composition of flavonoids in the UHR variety was significantly ($p<0.05$) higher (up to 21%) than in the UHW variety. Dehulling significantly reduced the concentration in both varieties but the red variety maintained a higher flavonoid content than the white variety. Similar results have been reported in the literature[43].

Energy composition: Table 4 shows that, UHW had a significantly higher ($p<0.05$) energy content than the UHR. After dehulling, there was a significantly ($p<0.05$) great decrease in the energy content. UHR was found to have significantly higher ($p<0.05$) energy content than the DHR.

The energy of the white variety of cowpea was significantly ($p<0.05$) higher than the red variety when whole. UHW had an energy value of 572.89±0.00 Kcal/100 g while the UHR had 501.35±0.01 Kcal/100 g. However, dehulling reduced the energy of both varieties. The dehulled white variety (DHW) significantly ($p>0.05$) reduced to 518.38±0.00 Kcal/100 g so also with the dehulled red variety (DHR) which reduced to 435.56±0.01 Kcal/100 g. Nevertheless, the white variety still maintained significantly higher energy than the red variety even after processing. This is in line with the findings of Adebowale *et al.*[44], even though it is not in correspondence with that of Dhingra *et al.*[25].

CONCLUSION

Mineral element deficiency has been known to cause health challenges. Also, anti-nutrient factors in cowpea are hazardous to health. Phytochemical antioxidants are beneficial to health. The present study reveals that dehulling, which has commonly been practiced, reduces the nutritional values of such cultivars as studied here. The results of this work also indicate that the red cultivar is better in terms of phytochemical composition but the white is better in terms of mineral elements. These findings can help consumers and farmers make informed choices when purchasing or cultivating these local varieties. The two local cowpea varieties can be considered a good source of protein and carbohydrates.

SIGNIFICANCE STATEMENT

This study discovered the negative effect of dehulling on the nutritional composition of two selected local varieties of cowpea (*Vigna unguiculata* L. Walp.) that can be beneficial for counselling by dieticians, professional farmers and researchers. This study will help the researchers to uncover the critical areas of malnutrition-instigated diseases that many researchers were not able to explore. Thus, a new theory on the link between malnutrition and processing practices in Wukari may be arrived at.

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