

# Compositional Changes in African Walnut (*Tetracarpidium conophorum*) across Different Germination Periods

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## Research Article

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# Abstract

African walnut (*Tetracarpidium conophorum*) is an underutilized oilseed rich in protein, polyunsaturated fatty acids and essential minerals, yet its optimal processing conditions remain inadequately defined. Limited data exist on time-dependent compositional changes during germination, creating uncertainty about balancing nutritional enhancement with lipid stability. This study investigated the effect of varying germination periods on proximate composition, mineral profile, antinutritional factors, antioxidant properties, lipid quality indices, fatty acid distribution and amino acid profile of African walnut flour. It was hypothesized that germination duration significantly ( $p < 0.05$ ) modifies nutritional quality and oxidative stability parameters.

Fresh seeds were sorted, soaked (24 h) and germinated for 24, 48, 72 and 96 h, with ungerminated seeds as control. Dried samples were milled and analyzed in triplicate using standard AOAC methods. Proximate composition, minerals (AAS), antinutrients, antioxidant assays (TPC, TFC, DPPH, FRAP), lipid quality indices (PV, AV, TBA, IV), GC–MS fatty acid profiling, and amino acid analysis were conducted. Data were subjected to one-way ANOVA and Duncan's test ( $p < 0.05$ ).

Germination significantly enhanced protein from 22.37% (control) to 29.38% at 48 h, while fat increased to 28.73% at 96 h. Carbohydrate declined from 53.16% to 35.42%, indicating reserve mobilization. Alpha-linolenic acid rose to 59.06% at 96 h, confirming enrichment of omega-3 fractions. DPPH activity improved markedly (15.80% at 24 h vs 0.29% control). However, peroxide value increased from 3.00 to 5.00 mg/g and acid value peaked at 7.74, indicating greater oxidative susceptibility with prolonged germination.

Moderate germination (48 h) optimized protein enrichment, improved antioxidant activity and maintained comparatively lower oxidative indices. Controlled germination is therefore recommended to enhance nutritional functionality of African walnut flour while preserving lipid stability for functional food applications.

## Introduction

African walnut is one of the underutilized oilseeds native to West and Central Africa and is usually eaten in Nigeria and neighboring countries. The seeds are traditionally consumed boiled or roasted and are known for their appreciable contents of protein, lipids, dietary fibre and essential mineral [1, 2]. The lipid fraction of African walnut contains high levels of unsaturated fatty acids, especially oleic and linoleic acid which are desirable from a nutritional point of view. In addition, the essential amino acid content of the protein of African walnut has been reported, which suggests the importance of this species in the dietary protein quality of mostly plant-based diets [3, 4].

Despite these nutritional attributes, the utilization of African walnut is limited due to the presence of antinutritional factors that act as barriers, in this case phytates, tannins and oxalates which can reduce bioavailability of minerals and digestibility of proteins [5]. Furthermore, the quality and stability of lipids may also be affected by processing and storage conditions and there may be possible implications

involving oxidative deterioration and nutrient losses [6]. These factors indicate the need for suitable processing strategies that can increase the nutritional quality and the stability of the seed constituents [7].

Germination is a simple and low cost bioprocessing technique which is known to alter biochemical composition of the seeds through the activation of endogenous enzymes [8]. During the germination process, storage proteins are hydrolyzed to free amino acids and peptides by proteases which may enhance the digestibility of protein and amino acids [9].

In germination, the kilning step is by far the dominant user of energy and has, thus, become the main target in reducing the carbon footprint of germinating operations. [58].

The germination of plant seeds often leads to the synthesis of bioactive phytochemicals from scratch, which protects plants from various external challenges and could be considered health-promoting ingredients [56].

Germination usually affect the chemical composition, carbohydrate fractions, digestibility, antinutritional factors and mineral content of legumes and cereals [10].

Concurrently, there is heightened activity of phytase involved in degradation of phytates and enhanced mineral bioavailability [11]. Studies on legumes and oilseeds have reported improvements on protein quality. [12].

Although the nutritional composition of African walnut, in both raw and processed forms has been reported [1], systematic investigations of the relationship between different germination periods and the compositional attributes of African walnut still remain scarce. Available studies often target one processing condition, or do not comprehensively assess the time-dependent changes in proximate composition, mineral content, antinutritional factors and antioxidant properties during germination. This is a knowledge gap in the process of promoting underutilized indigenous oilseeds as affordable sources of nutrients. [13]

This study investigated the changes in composition of African walnut (*Tetracarpidium conophorum*) in defined germination periods particularly, proximate composition, mineral content, selected antinutritional factors and antioxidant properties. The findings provide an empirical basis on the germination associated biochemical modifications of the African walnut and establishes a scientific basis for the identification of germination conditions that may enhance the nutritional quality and possible functionality of African walnut.

## Materials and Methods

### Materials Procurement

Fresh and mature seeds of African walnut were bought from Oja'ba market in Ibadan, Oyo state, Nigeria [14]. Food grade additives and processing ingredients in the formulation of functional food prototypes were procured to the certified food ingredient suppliers. Analytical grade chemical and reagents were

purchased from the Food Chemistry Laboratory, Department of Food Science, Ladoke Akintola University of Technology, Ogbomoso, Nigeria. The instrumentation included digital weighing balances, Soxhlet extractors, UV-Visible spectrophotometers, muffle furnace and convection drying ovens, gas chromatography-mass spectrometry equipment, Kjeldahl apparatus and an amino acid analyzer.

### Sample Preparation & Germination Procedure

The walnut seeds were first subjected to manual sorting process in which the broken seeds, damaged seeds and even the seeds with mould were removed and lastly was washed using clean water in order to obtain the seeds free of dirt and other contaminants. Germination was performed according to the method of [16] but with minor changes. Specifically, received and cleaned seeds was soaked in distilled water at ambient temperature ( $28 \pm 2$  °C) for 24 hours to start the process of enzymatic activity[15]

The seeds were then placed on wet jute sacks and incubated at 24, 48, 72 and 96 hours in a dark and ventilated room with water in order to germinate. In case of germination distilled water was regularly sprayed every 12 hour to maintain the moisture over optimal level. At the end of every periods of germination, germination itself was arrested at 55 °C in the oven under drying conditions of 12 hours. Dried germinated seeds were dehulled and ground through a hammer mill followed by sieving to obtain the fine flour of 250µm mesh. The flour was stored in airtight containers at 4 °C to be further tested.[16]

## Proximate Composition Analysis

The proximate composition was analyzed using the standard methods. Quantities of moisture content was determined by drying of the samples in the oven at 105 °C to the constant weight. Incineration was done in a muffle furnace at 550 °C in order to measure ash content [17].the extraction of crude fat was done with help of Soxhlet apparatus as a solvent it takes the form of n-hexane. The Kjeldahl method of determining the crude protein was followed where the nitrogen content was multiplied by a conversion factor of 6.25 to obtain crude protein [18]. Crude fibre was determined in a sequential acid-alkali digestion technique and burned. Carbohydrate content was calculated by difference using the following formula: Carbohydrate (%) = 100 - (Moisture + Ash + Protein + Fat + Fibre.[19]).

## Mineral Analysis

Mineral composition (Ca, Mg, K, Na, P, Fe) was analysed using the atomic absorption spectrophotometry (AAS) after dry-ash digestion [20]. The samples were ashed at a temperature of 450 °C and dissolved in a dilute hydrochloric acid, filtered and diluted to desirable proportions before it was analysed [21]

## Phytochemical Determination

Phytochemical Determination

Anti-nutritional factors were determined by established methods [2]. Acknowledgement Vanillin - HCl assay was used to determine level of tannin using absorbance of 500 nm [22]. The method of determining the phytate content was performed using ferric chloride-thiocyanate technique. Quantification of oxalate was determined after precipitation of oxalate as calcium oxalate and determinate using titration with potassium permanganate [23]. Extraction of alkaloids using acidified ethanol then followed, precipitation using ammonium hydroxide and gravimetric measurement of alkaloids [24].

## Antioxidant Activities Measurement

Four assays were employed for testing the antioxidant activity. Total phenolic content (TPC) was calculated by using Folin-Ciocalteu method and was expressed as mg gallic acid equivalents per gramme. Total flavonoid content (TFC) was determined using aluminum chloride colorimetric method and expressed in mg of quercetin equivalent/g. Radical Scavenging activity of DPPH was calculated in percentage inhibition of the absorbance at 517nm. Ferric reducing antioxidant power (FRAP) was shown in mg/g [25].

## Quality analysis of the lipids

Extracted oil samples were determined in terms of lipid quality parameters. Acid value was conducted by titration with potassium hydroxide and the results obtained were used as mg KOH/g oil. The main oxidation products were assessed by measuring of peroxide value. Thiobarbituric acid reactive substances (TBARS) was assessed in order to assess secondary lipid peroxidation. Wijs method was used to determine iodine value to determine degree of unsaturation. The refractive index and specific gravity were carried out at 40 degree Celsius[26].

## Amino Acid Determination

The amino acid composition of African walnut-flour germinated for 48 hours was determined using the Applied Bioics PTH Amino Acid Analyzer (Model 120A) contained with the followed standard hydrolysis and quantification protocols. Precisely 0.683 g of fat-free sample was hydrolyzed in 6N HCl at 110C for 24h under vacuum in the sealed tubes to avoid the oxidative damage on the sample. The hydrolysate was then filtered, neutralised and diluted five times before loading 60 microliters into the analyzer[27].

Quantification was performed on the basis of height and width of peaks, formed for each amino acid according to the chromatogram[28]. The calculation of concentration (g/100 g protein) was done using the formula:

Concentration = (Net Height\*Width at Half Height\*Standard Factor\*C),

where C is a correction coefficient which is calculated as:

$C = (\text{Dilution Factor} \times 16) / (\text{Sample Weight} \times \text{Nitrogen\%} \times \text{Volume Loaded})$  [27].

The nitrogen content of the sample was found to be 2.92% and norleucine was chosen as the internal standard for calibration purpose. Each amino acid was individually identified by retention time and quantified with respect to known standard curves generated from authenticated amino acid references[27].

The analysis was performed on essential and non-essential amino acids and results were presented in g/100g. Weight, which is part of the protein content, should also be accurate when evaluating the protein quality of the germinated flour of African walnuts[29].

## Statistical Analysis

Each of the experiments was conducted in 3 replications. The results were presented in the form of mean + standard deviation. Data were analysed with the use of one way analysis of variance (ANOVA) and values with statistically significant difference among means determined using Duncan's multiple range test ( $p < 0.05$ ). Statistical calculations were done with the use of IBM Corp. statistics package for social sciences (SPSS) software version 25.0 (Armonk, NY, USA).

## Samples Narration

The analysis of experiment was made of five sample groups of African walnut flour with different time fermentation aimed at analysing the gradual impact of germination time on nutritional and functional attributes of the seeds. Sample AWS was made of African walnut seed fermented in 24 hours which has shortest period of germination. In Sample BWS the fermentation was in 48 hours and in Sample CWS fermentation was in 72 hours. Sample DWS was represented by the maximum time of fermentation that was 96 hours. Control Group of EWS, in the form of non-fermented seeds of African walnuts was used in order to provide a point of reference in sampling. This systematic variation allowed this to be evaluated comprehensively because it was in order to investigate the extent of biochemical and functional alterations occasioned by varied levels of germination.

## Results and Discussion

### Proximate Composition for Germinated and Non-Germinated African Walnut Flour

Table 1 showed that germination had a significant impact ( $p < 0.05$ ) on the proximate composition of the *Tetradlepidium conophorum* flour reflecting progressive activation of metabolism under sprouting. Moisture content was found to increase with the germination time and reached the maximum value at 96 h. This increase is attributed to seed imbibition and loosening of the cellular matrices due to enzymatic activation with increased water retention even after drying. Similar increases in residual moisture after sprouting have been described for germinated oilseeds and legumes in which metabolic reorganisation of the seeds changes the seed microstructure and hygroscopic properties[30, 31].

Crude protein content increased significantly with early germination and peaked at 48 h and then declined slightly at longer sprouting time. The apparent increase is attributed very much to a concentration effect caused by the depletion of carbohydrates by means of respiration, when combined with synthesis of enzymatic proteins necessary in seedling development[32]. The action of protease at the state of germination a hydrolyzual delayed in aqueous state to the proteases peptites and free amino acubes, means for this increases the testable nitrogen activates and improves the extractibility of protein[33]. The drop in germination observed with the prolonged germination may be due to the use of amino acids for growth and potential leaching losses [34]. These results indicate that 48 hours germination is the optimum for the enrichment of protein in African walnut flour.

Crude fat content was significantly increased with the time of germination especially at 96 h. From the above data, even though lipid reserves contain energy reserves in the sprouting, it is possible that the increased amount is due to the increase in extractability due to the breakdown of cells and thus freeing bound lipids rather than synthesising lipids de novo[35]. Enzymatic hydrolysis of triacylglycerols by lipases may promote the release of oil during extraction with solvent [36]. Similar trends have been described in germinated sesame and sunflower seeds in which structural modification led to an improvement in oil recovery[36].

Ash content gradually declined and go on with the process of germination, probably because of the leaching of minerals in soaking or metabolic utilisation in enzymatic processes. Comparable losses of total ash during sprouting have been reported widely [37]. However, decreases in total mineral content do not mean necessarily a decrease in bioavailability, because phytate decomposition during germination may result in an increase in mineral absorption[11].

Carbohydrate content showed a significant loss corresponding to the increasing time of germination as the result of amylase reaction for starch hydrolysis and utilization of soluble sugars for respiration and radicle growth. The significant decrease at 96 h provides evidence of enhanced metabolic gradients and reserves mobilization. Such deplete of carbohydrate during sprouting is a steady phenomenon in all legumes and oilseeds[38].

Overall, germination resulted in extensive biochemical remodelling of African walnut flour. Among the values of germination time examined, 48 h is suggested to be nutritionally beneficial because it resulted in maximal protein improvement with moderate compositional changes, whereas an extended duration of germination (96 h) was associated with increased carbohydrate depletion and moisture increase that might have implications for storage stability.

Table 1  
Proximate Analysis of the Walnut Samples.

| Samples | Moisture(%)              | Protein(%)                | Fat(%)                     | Ash(%)                   | Fibre(%)                 | Carbohydrate(%)           |
|---------|--------------------------|---------------------------|----------------------------|--------------------------|--------------------------|---------------------------|
| AWS     | 6.32 ± 0.08 <sup>c</sup> | 24.25 ± 0.04 <sup>c</sup> | 19.23 ± 0.46 <sup>b</sup>  | 3.93 ± 0.12 <sup>c</sup> | 1.39 ± 0.00 <sup>a</sup> | 44.87 ± 0.43 <sup>c</sup> |
| BWS     | 3.94 ± 0.53 <sup>b</sup> | 29.38 ± 0.04 <sup>e</sup> | 20.67 ± 1.15 <sup>c</sup>  | 3.10 ± 0.53 <sup>b</sup> | 1.68 ± 0.01 <sup>c</sup> | 41.24 ± 1.11 <sup>b</sup> |
| CWS     | 7.07 ± 0.53 <sup>d</sup> | 26.05 ± 0.04 <sup>c</sup> | 20.33 ± 0.58 <sup>bc</sup> | 2.47 ± 0.15 <sup>a</sup> | 1.48 ± 0.02 <sup>b</sup> | 42.59 ± 0.74 <sup>b</sup> |
| DWS     | 8.23 ± 0.09 <sup>e</sup> | 24.04 ± 0.03 <sup>b</sup> | 28.73 ± 0.40 <sup>d</sup>  | 2.20 ± 0.10 <sup>a</sup> | 1.37 ± 0.00 <sup>a</sup> | 35.42 ± 0.38 <sup>a</sup> |
| EWS     | 1.57 ± 0.12 <sup>a</sup> | 22.37 ± 0.04 <sup>a</sup> | 16.23 ± 0.76 <sup>a</sup>  | 4.57 ± 0.12 <sup>d</sup> | 2.10 ± 0.01 <sup>d</sup> | 53.16 ± 0.81 <sup>e</sup> |

Mean in the same columns with different superscript were significantly different (P < 0.05)

Keys:

AWS = Walnuts fermented for 24 hr

BWS = Walnuts fermented for 48 hr

CWS = Walnuts fermented for 72 hr

DWS = Walnuts fermented for 96 hr

EWS = Non fermented (Control)

## Anti-Nutritional Composition of Germinated and Non-Germinated Walnut Flour (African)

As seen from Table 2, germination had a significant (p < 0.05) effect on the levels of tannins and phytate, on alkaloids and oxalate of *Tetracarpidium conophorum* flour. The response of these antinutritional compounds to sprouting was compound specific instead of equally reductive.

Tannin contents in germinated samples increased significantly compared with the control (EWS) and the maximum content occurred at 24 h and 96 h. Although germination often is related to decreases in some antinutrients, an increase in phenolic compounds during sprouting has been widely reported[39].

Germination activates the enzymes of the phenylpropanoid pathway - especially phenylalanine ammonia-lyase (PAL) leading to the synthesis and release of soluble phenolics de novo [40]. Similar increments in total tannins, bound phenolics have been obtained in germinated legumes and oilseeds upon enhanced extractability after the disintegration of cells. While the protein digestibility may be decreased by tannins

due to the formation of complexes, tannins also provide a substantial extent of antioxidant activities suggesting a double nutritional implication[41].

Phytate content, as presented in Table 2, indicated a fluctuating, but generally high pattern of some germinated samples, especially samples at the 72 hour and 96 hour time periods. This finding is contradictory to the widely documented decrease of phytate throughout sprouting as a result of endogenous phytase activation[42]. However, a transient increase in the measurable phytate has been explained by the increased extractability in early germination stages after the softening and breakdown of the matrix related to protein-phytate complexes[43]. It is also possible that the hydrolysis of phytate in African walnut is depicted by a non-linear pattern of degradation, with the peak phytase activity lying outside the selected sampling intervals[44].

Significantly high level of alkaloids was observed at 72hr which may imply the activation of secondary metabolite biosynthesis with sprouting. Germination is a metabolically intensive response with oxidative stress and cellular signalling, and may result in the stimulation of synthesis of defensive phytochemicals including alkaloids[45]. Similar increases in some secondary metabolites throughout sprouting have been mentioned in soybean and other oilseeds[30] .

There was relatively little variation in oxalate content between periods of germination. Since the metabolism of oxalate is not directly related to primary reserve mobilisation, the low fluctuation indicates relatively stable metabolism during the sprouting stage. However, submerged as low as modest amounts of oxalate can affect the bioavailability of calcium due to chelation[46].

Overall, the results in Table 2 show that germination does not grow antinutritional compounds universally in African walnut. Instead, sprouting stimulates complex reprogramming of the metabolic processes which may lead to a higher extractability or transient synthesis of certain secondary metabolites. These results indicate the necessity to optimize the time of germination to achieve compromises between enhancement of protein quality and acceptable concentrations of antinutritional constituents.

Table 2  
Antioxidant Properties of the Walnut Samples.

| Samples | Tannin(mg/g)             | Phytate(mg/g)            | Alkaloid(mg/g)           | Oxalate(mg/g)             |
|---------|--------------------------|--------------------------|--------------------------|---------------------------|
| AWS     | 0.98 ± 0.14 <sup>d</sup> | 0.49 ± 0.01 <sup>c</sup> | 0.33 ± 0.01 <sup>b</sup> | 0.24 ± 0.00 <sup>bc</sup> |
| BWS     | 0.28 ± 0.04 <sup>b</sup> | 0.18 ± 0.01 <sup>b</sup> | 0.19 ± 0.00 <sup>a</sup> | 0.25 ± 0.01 <sup>c</sup>  |
| CWS     | 0.84 ± 0.04 <sup>c</sup> | 0.72 ± 0.00 <sup>e</sup> | 0.48 ± 0.01 <sup>c</sup> | 0.24 ± 0.01 <sup>b</sup>  |
| DWS     | 0.98 ± 0.01 <sup>d</sup> | 0.60 ± 0.01 <sup>d</sup> | 0.34 ± 0.02 <sup>b</sup> | 0.23 ± 0.00 <sup>b</sup>  |
| EWS     | 0.11 ± 0.00 <sup>a</sup> | 0.12 ± 0.00 <sup>a</sup> | 0.19 ± 0.01 <sup>a</sup> | 0.10 ± 0.01 <sup>a</sup>  |

Mean in the same columns with different superscript were significantly different (P < 0.05).

Keys:

AWS = Walnuts fermented for 24 hr

BWS = Walnuts fermented for 48 hr

CWS = Walnuts fermented for 72 hr

**DWS** = Walnuts fermented for 96 hr

**EWS** = Non fermented (Control)

### **Germinated and non-germinated African walnut flour Antioxidant properties.**

The antioxidant value results in Table 3 showed the significant ( $p < 0.05$ ) modulation of ferric reducing antioxidant power (FRAP), total phenolic content (TPC), total flavonoid content (TFC), and DPPH radical scavenging activity after germination.

As shown on Table 3, total phenolic content significantly increased in 24 hours and 72 hours compare to the control. Germination has been known to stimulate the biosynthesis of phenolics through activation of phenylpropanoid pathway and enhanced PAL activity [39]. Additionally, the release of bound phenolics from the seed matrix is improved by enzymatic softening of the matrix during sprouting and this increases the measurable TPC [47]. The higher values of total tannins found in the samples of Table 2 are associated with such an increase, supporting the correlation between germination and increase in phenolic content.

Total flavonoid content has been found to be maximum between 24 hours and moderate reductions for prolonged germinating. Flavonoids are protective factors against the effects of oxidative stress during early seedling growth[48]. However, in extended sprouting this might cause their metabolic usage in reactive oxygen species (ROS) scavenging with resulting lower measurable concentrations.

DPPH radical scavenging activity was significantly higher in germinated samples than for the control, especially at 24 h. This enhancement is consistent with enhanced hydrogen donating capacity as well as negara antioxidant neutralising of free radicals. Numerous studies have reported enhanced DPPH activity of germinated legumes and oilseeds owing to enhanced phenolic content and enhanced antioxidant enzyme activity[49].

Interestingly, the FRAP values were the highest in the control and relatively lower in most germinated samples with a slight higher value at 72 h. This divergence between FRAP and DPPH response is a difference in antioxidant mechanisms between the two types of assays. Although DPPH mainly tests the capacity of free radical scavenging, FRAP detects the electron donating capacity in an acid environment. [50] Differentiating effects of structural changes in phenolic compounds during germination may therefore affect the outcome of these assays differentially.

Collectively, Table 3 shows that the early to moderate germination improves the antioxidant potential of African walnut flour especially in the phenolic availability and in the radical scavenging activity. However, prolonged sprouting can disturb the balance between the antioxidant mechanisms, which makes such controlled germination duration highly important.

Table 3  
Phytochemical Properties Walnut samples.

| Sample | FRAP<br>(mg/g)           | Total Phenolic<br>(mg/g) | Flavonoid<br>(mg/g)       | DPPH<br>(%)               |
|--------|--------------------------|--------------------------|---------------------------|---------------------------|
| AWS    | 0.33 ± 0.01 <sup>b</sup> | 0.75 ± 0.03 <sup>c</sup> | 0.57 ± 0.02 <sup>d</sup>  | 15.80 ± 0.61 <sup>c</sup> |
| BWS    | 0.26 ± 0.03 <sup>a</sup> | 0.30 ± 0.01 <sup>a</sup> | 0.29 ± 0.01 <sup>ab</sup> | 12.16 ± 0.42 <sup>a</sup> |
| CWS    | 0.43 ± 0.00 <sup>c</sup> | 0.73 ± 0.00 <sup>c</sup> | 0.28 ± 0.02 <sup>a</sup>  | 14.41 ± 0.25 <sup>b</sup> |
| DWS    | 0.29 ± 0.01 <sup>a</sup> | 0.61 ± 0.06 <sup>b</sup> | 0.39 ± 0.06 <sup>c</sup>  | 12.64 ± 0.79 <sup>a</sup> |
| EWS    | 1.26 ± 0.03 <sup>d</sup> | 0.28 ± 0.00 <sup>a</sup> | 0.31 ± 0.01 <sup>b</sup>  | 0.29 ± 0.01 <sup>d</sup>  |

Mean in the same columns with different superscript were significantly different ( $P < 0.05$ ).

Keys:

AWS = Walnuts fermented for 24 hr

BWS = Walnuts fermented for 48 hr

CWS = Walnuts fermented for 72 hr

DWS = Walnuts fermented for 96 hr

EWS = Non fermented (Control)

### Mineral Composition of Germinated and Non-Germinated African Walnut Flour.

As presented on Table 4, germination significantly ( $p < 0.05$ ) changed mineral composition of *Tetracarpidium conophorum* flour. The control sample (EWS) showed the highest levels of important minerals such as potassium, phosphorus and calcium whereas the germinated samples generally showed decreased levels with time-dependent variation.

The reduction in potassium and sodium concentration in germinated samples is likely to represent losses due to leaching during extended periods of soaking and hydration as observed for other sprouted oilseed and legumes[51]. These minerals are highly water soluble and freely diffuse into the soaking medium when processing with a sprouting protocol that has repeated hydration steps. Although mineral concentrations in the total were decreased, this does not necessarily indicate impairment of the nutritional

value as germination is known to improve mineral bioavailability through enzymatic degradation of phytate [52]. Phytate-mineral complexes are degraded by activated phytases during seed germination and free chelated minerals are liberated and made more available physiologically[11].

Phosphorus, mainly in binding form as phytate in oilseeds, decreased in most of the germinated samples, as expected, according to results published on the reduction of phytate reserves in the germinated sample obtained by phytase activation [53]. Concurrent reductions in phytate may play a role in improved phosphorous utilization when meat absolute concentrations are reduced. Magnesium showed a relative stability at intermediate germination duration (48 h), which suggests that mobilisation and redistribution is varied in different mineral species[54].

Calcium concentrations far decreased at a longer germination time (96h), potentially caused by interactions with oxalates and by leaching effects. Oxalates can actually form insoluble calcium oxalate complexes, and as such, decrease measurable calcium, a mechanism consistent with findings in the sprouted legumes[54]. Together, the data in Table 4 draw attention to the fact that absolute mineral content decreases with germination while physiological accessibility may improve, which warrants further investigation through in vitro bioaccessibility assays in the future in order to allow valid conclusions concerning the nutritional implications .

Table 4  
Mineral properties of the walnut sample.

| Samples | Potassium<br>(mg/g)        | Sodium<br>(mg/g)            | Phosphorus<br>(mg/g)        | Magnesium<br>(mg/g)         | Calcium<br>(mg/g)          |
|---------|----------------------------|-----------------------------|-----------------------------|-----------------------------|----------------------------|
| AWS     | 117.10 ± 0.01 <sup>a</sup> | 155.80 ± 0.63 <sup>a</sup>  | 292.50 ± 0.60 <sup>a</sup>  | 232.94 ± 1.56 <sup>b</sup>  | 95.97 ± 0.55 <sup>b</sup>  |
| BWS     | 145.89 ± 0.01 <sup>b</sup> | 192.80 ± 1.18 <sup>b</sup>  | 362.40 ± 0.60 <sup>b</sup>  | 289.84 ± 0.55 <sup>c</sup>  | 130.84 ± 0.53 <sup>c</sup> |
| CWS     | 118.70 ± 2.69 <sup>a</sup> | 162.40 ± 10.76 <sup>a</sup> | 304.50 ± 20.18 <sup>a</sup> | 243.55 ± 16.19 <sup>b</sup> | 165.89 ± 0.51 <sup>d</sup> |
| DWS     | 115.59 ± 2.69 <sup>a</sup> | 150.00 ± 10.76 <sup>a</sup> | 280.90 ± 19.90 <sup>a</sup> | 224.68 ± 1.78 <sup>b</sup>  | 61.68 ± 1.43 <sup>a</sup>  |
| EWS     | 368.30 ± 0.81 <sup>c</sup> | 200.39 ± 0.81 <sup>b</sup>  | 488.2 ± 5.11 <sup>c</sup>   | 185.60 ± 1.03 <sup>a</sup>  | 228.47 ± 0.53 <sup>e</sup> |

Mean in the same columns with different superscript were significantly different (P < 0.05)

Keys:

AWS = Walnuts fermented for 24 hr

BWS = Walnuts fermented for 48 hr

CWS = Walnuts fermented for 72 hr

DWS = Walnuts fermented for 96 hr

**EWS = Non fermented (Control)**

### **Effect of fermentation on Acid Value, TBA, Specific gravity and Yields of Germinated and Non-Germinated African Walnut Flour.**

The lipid quality parameters presented in Table 5 indicates significant ( $p < 0.05$ ) effect on the thiobarbituric acid (TBA) values, specific gravity and oil yield of the oil following germination.

As shown in Table 5, TBA values were the highest at 24 h (AWS) and 96 h (DWS), that is, formation of secondary lipid oxidation products was increased during the early and prolonged germination stage. TBA actually measures malondialdehyde and compounds produced from polyunsaturated fatty acid breakdown. The high values indicate intensified lipid hydrolysis and further oxidation of lipids. Germination is the activation of lipases that break down triacylglycerols to free fatty acids which are more prone to oxidation[59]. The oxidative susceptibility is probably enhanced by its high polyunsaturated fatty acid content of the African walnut oil.

Specific gravity indicated little change in the density characteristics of the oils extracted in the different samples, indicating that germination did not have a significant effect on the overall density characteristics of the extracted oils. This suggests that in spite of biochemical modifications, the bulk physical properties of the oil had been relatively stable[60].

Oil yield was increased at a significant level in the 24h and 96h germinated samples compared the control sample as shown in Table 5. This increase may be supposed to be due to improved extractability caused by the disintegration of cells and the weakening of structural barriers during sprouting. Enzymatic cell wall polysaccharide degradation enhances solvent penetration during Soxhlet extraction for increased measurable oil recovery[61]. Similar improvements of oil yield after germinating have been observed in sprouted oilseeds in which structural modifications favour the liberation of lipids.

Collectively, Table 5 shows that the germination can improve the extractability of the oil and may lower the oxidative stability, especially at long germination periods. Moderate germination periods may therefore give balanced choices between improving oil recovery and acceptable oxidative quality.

Table 5  
Lipid Quality Parameters of the Walnut.

| <b>Samples</b> | <b>TBA</b>        | <b>Specific gravity</b> | <b>Yield</b>       |
|----------------|-------------------|-------------------------|--------------------|
| AWS            | $4.25 \pm 0.03^d$ | $0.92 \pm 0.01^a$       | $22.00 \pm 1.00^d$ |
| BWS            | $0.59 \pm 0.13^a$ | $0.93 \pm 0.02^a$       | $20.63 \pm 0.42^c$ |
| CWS            | $2.83 \pm 0.07^b$ | $0.91 \pm 0.02^a$       | $15.50 \pm 0.50^b$ |
| DWS            | $3.20 \pm 0.04^c$ | $0.91 \pm 0.00^a$       | $22.90 \pm 2.26^d$ |
| EWS            | $2.88 \pm 0.21^b$ | $0.93 \pm 0.02^d$       | $13.73 \pm 0.02^a$ |

Mean in the same columns with different superscript were significantly different ( $P < 0.05$ )

Keys:

AWS = Walnuts fermented for 24 hr

BWS = Walnuts fermented for 48 hr

CWS = Walnuts fermented for 72 hr

**DWS** = Walnuts fermented for 96 hr

**EWS** = Non fermented (Control)

## **Oxidative and Chemical Stability Parameters of Germinated and Non-Germinated African Walnut**

As can be seen from Table 6, germination has a significant ( $p < 0.05$ ) effect on peroxide value (PV), acid value (AV) and iodine value (IV) of oil extracted from *Tetradlea oil*. These indices together give a picture of primary oxidation, the presence of hydrolytic degradation and degree of lipid unsaturation, thus forming an overall picture of the chemical stability during sprouting.

Peroxide value increased steadily with the increasing time of germination such that the maximum value was measured at 96 h (DWS) of the control (EWS). Peroxide value is used to measure hydroperoxides, which are one of the primary products of oxidation and products formed when unsaturated fatty acids react with molecular oxygen. The increase found in germinated sample indicates the enhanced oxidative activity during sprouting. Germination is followed by metabolic respiration with an increased oxygen uptake and by lipase mediated hydrolysis of triacylglycerols with the liberation of free fatty acids[62]. Free fatty acids, especially polyunsaturated fatty acids (PUFAs), are more vulnerable to oxidation attack because of possessing several double bonds[63]. Therefore, the high PV at long germination times probably indicates the cumulative impacts of higher unsaturation and higher enzymatic activity.

Acid value showed significant rise in 72 h (CWS) and 96 h (DWS) with the 48 h sample (BWS) showed the lowest AV among the germinated treatments. Acid value is a measure of free fatty acids added by the hydrolytic breakdown of ester bonds of triglycerides. During germination endogenous lipases hydrolyze the stored lipids to provide energy for seedling growth due to accumulation of free fatty acids[64]. The sharp increase of AV at prolonged germination seems to imply the enhanced hydrolytic rancidity after 48 h of germination. From a technological point of view, high acid values impair oil quality and shorten shelf-life and thus make longer germination less attractive from oil preservation.

Iodine value, used as a method of determining the degree of unsaturation, was higher in germinated samples compared with the control, especially at 24 h and 72 h. Increased iodine values are associated with an increase of the relative share of unsaturated fatty acids. Germination may preferentially use some of the saturated fatty acids as substrates of metabolism and therefore enhances the relative

representation of unsaturated fractions [65]. Alternatively, there may be structural disintegration of oil bodies at sprouting which may enhance extraction efficiency of polyunsaturated components. Although increased unsaturation increases the nutritional value especially with respect to omega-3 fatty acids, it also favours oil deterioration by oxidation, which explains the parallel rise in peroxide and acid values with the duration of sprouting [35].

Importantly the 48 h germination sample showed comparatively moderate peroxide and acid values and it appears that this period of time represents an optimal balance between enhanced unsaturation and acceptable oxidative stability. Thus, Table 6 shows that germination improves lipid characteristics from the nutritional point of view, but excessive sprouting deteriorates the chemical stability because of intensification of hydrolytic and oxidative processes.

Table 6  
Peroxide, iodine and acid value of the walnut samples

| Samples | Peroxide (mg/g)           | Acid Value               | Iodine (mg/g)              |
|---------|---------------------------|--------------------------|----------------------------|
| WS      | 4.00 ± 1.00 <sup>ab</sup> | 5.54 ± 0.26 <sup>c</sup> | 165.84 ± 3.00 <sup>c</sup> |
| BWS     | 4.00 ± 1.00 <sup>ab</sup> | 1.94 ± 0.06 <sup>a</sup> | 127.84 ± 3.09 <sup>a</sup> |
| CWS     | 4.33 ± 0.58 <sup>ab</sup> | 7.74 ± 0.11 <sup>e</sup> | 163.55 ± 2.47 <sup>c</sup> |
| DWS     | 5.00 ± 1.00 <sup>b</sup>  | 6.09 ± 0.07 <sup>d</sup> | 151.50 ± 0.71 <sup>c</sup> |
| EWS     | 3.00 ± 1.00 <sup>a</sup>  | 3.61 ± 0.08 <sup>b</sup> | 122.29 ± 7.02 <sup>d</sup> |

Mean in the same columns with different superscript were significantly different ( $P < 0.05$ ).

Keys:

AWS = Walnuts fermented for 24 hr

BWS = Walnuts fermented for 48 hr

CWS = Walnuts fermented for 72 hr

DWS = Walnuts fermented for 96 hr

EWS = Non fermented (Control)

### Fatty Acid profile of Africa walnut ( *Tetracarpidium conophorum* ) oil

The composition of fatty acid profile in Table 7 confirms that oil from *Tetracarpidium conophorum* is consisted of unsaturated fatty acids mainly with alpha-linolenic and linoleic acid in all the stages of germination.

In the control (0 h), alpha-linolenic acid was the main predominant fatty acid, followed by linoleic acid with both palmitic and stearic as major saturated fatty acids. This profile is consistent with other earlier reports where African walnut has been shown to be a rich source of omega-3 polyunsaturated fatty acids [66]. The predominance of alpha-linolenic acid is indicative of the nutrition value of this oil, considering the proved cardioprotective and anti-inflammatory properties of omega-3 fatty acids [67].

Following germination interesting modifications were seen in the distribution of fatty acids. At the 24 h time point, there was a prominent appearance of oleic acid that suggested early lipid remodeling in the event of metabolic activation. Oleic acid (a monounsaturated fatty acid), which may be increased transiently by transient enzymatic conversion or by differential mobilization of fatty acid pools during transient initial sprouting. At 48h and 96h, alpha-linolenic acid was at higher relative proportions than that of the control which indicates that polyunsaturated fraction is enriched during extended germination[57].

The slight decrease in saturated fatty acids (palmitic and stearic acids) compared to unsaturated fatty acids is suggestive of selective mobilisation of the saturated lipids for energy production. In the process of germination, the enzymes involved in the metabolism of fatty acids are certain beta-oxidation, in order to provide acetyl-CoA for energy metabolism, it may affect the distribution of fatty acids[68]. The observed increase in unsaturation goes in direct correspondence with the increased iodine values observed in Table 6, which confirms consistency between the compositional and chemical parameters of stability.

However, the increase of polyunsaturated fatty acids also accounts for the higher susceptibility to oxidation for peroxide and TBA indices (Tables 5 and 6). PUFAs have several double bonds which are sites of activation (reactivity) of lipid peroxidation. Thus, although germination increases the nutritional quality by increasing omega-3 fatty acids, it also increases oxidative susceptibility[69].

The occurrence of minor components of fatty acid derivatives at 72 h (such as cyclopropane derivatives) may be due to a transient state of metabolic intermediates or rearrangements of their structure during remodeling of lipids. Such variations have been reported in germinated oilseeds undergoing to enzymatic activation and membrane restructuring[70].

Overall, the data in Table 7 shows that germination increased the relative amount of the nutritionally desirable unsaturated fatty acids, especially alpha-linolenic acid. However, the concomitant rise in oxidative indices implies that altering germination period to control it is needed to obtain maximum nutritional benefits without compromising chemical stability.

Table 7  
Fatty Acid profile in different hours 24rs

| Compound Name                                 | Abundance % | Molecular Weight | Molecular Formula | Common / Other Name                |
|-----------------------------------------------|-------------|------------------|-------------------|------------------------------------|
| <b>Profile for 0hr Fermentation (control)</b> |             |                  |                   |                                    |
| Hexadecanoic acid, methyl ester               | 4.35        | 284              | C18H36O2          | Palmitic acid, methyl ester        |
| Dodecanoic acid, 1,2,3-propanetriyl ester     | 23.61       | 639              | C39H74O6          | Trilaurin                          |
| 9,12-Octadecadienoic acid, methyl ester       | 21.41       | 294              | C19H34O2          | Linoleic acid, methyl ester        |
| 9,12,15-Octadecatrienoic acid, methyl ester   | 45.90       | 292              | C19H32O2          | alpha-Linolenic acid, methyl ester |
| Methyl stearate                               | 4.74        | 299              | C19H38O2          | Stearic acid, methyl ester         |
| <b>Profile for 24 hrs Fermentation</b>        |             |                  |                   |                                    |
| Hexadecanoic acid, methyl ester               | 4.04        | 270              | C17H34O2          | Palmitic acid, methyl ester        |
| 9-Octadecenoic acid (z), methyl ester         | 34.00       | 296              | C19H36O2          | Oleic acid, methyl ester           |
| 9,12,15-Octadecatrienoic, methyl ester        | 56.57       | 292              | C19H32O2          | alpha-Linolenic acid, methyl ester |
| Methyl stearate                               | 100.00      | 298              | C19H38O2          | Stearic acid, methyl ester         |
| <b>Profile for 48hrs Fermentation</b>         |             |                  |                   |                                    |
| Hexadecanoic acid, methyl ester               | 3.72        | 270              | C17H34O2          | Palmitic acid, methyl ester        |
| 9,12-Octadecadienoic acid, methyl ester       | 33.37       | 294              | C19H34O2          | Linoleic acid, methyl ester        |
| 9,12,15-Octadecatrienoic, methyl ester        | 56.75       | 292              | C19H32O2          | alpha-Linolenic acid, methyl ester |
| Methyl stearate                               | 4.95        | 298              | C19H38O2          | Stearic acid, methyl ester         |

| Compound Name                                     | Abundance % | Molecular Weight | Molecular Formula | Common / Other Name                             |
|---------------------------------------------------|-------------|------------------|-------------------|-------------------------------------------------|
| <b>Profile for 72hrs Fermentation</b>             |             |                  |                   |                                                 |
| Cyclopropaneoctanoic acid, 2-hexyl-, methyl ester | 1.21        | 282              | C18H34O2          | 2-Hexyl-cyclopropaneoctanoic acid, methyl ester |
| Hexadecanoic acid, methyl ester                   | 3.57        | 270              | C17H34O2          | Palmitic acid, methyl ester                     |
| 9,12-Octadecadienoic acid (z), methyl ester       | 32.60       | 294              | C19H34O2          | Linoleic acid, methyl ester                     |
| <b>Profile for 96hrs Fermentation</b>             |             |                  |                   |                                                 |
| 9,12,15-Octadecatrienoic, methyl ester            | 59.06       | 292              | C19H32O2          | alpha-Linolenic acid, methyl ester              |
| Methyl stearate                                   | 4.77        | 298              | C19H32O2          | Stearic acid, methyl ester                      |
| Hexadecanoic acid, methyl ester                   | 3.54        | 270              | C17H34O2          | Palmitic acid, methyl ester                     |
| 9,12-Octadecadienoic acid, methyl ester           | 35.64       | 294              | C19H34O2          | Linoleic acid, methyl ester                     |
| 9,12,15-Octadecatrienoic, methyl ester            | 56.62       | 292              | C19H32O2          | alpha-Linolenic acid, methyl ester              |
| Methyl stearate                                   | 4.21        | 298              | C19H38O2          | Stearic acid, methyl ester                      |

### Amino Acid profile of Africa walnut ( *Tetracarpidium conophorum* ) flour

AAs as shown in Table 8, the amino acid profile of germinated *Tetracarpidium conophorum* flour shows a nutritionally balanced distribution of essential and non-essential amino acids which is due to proteolytic and metabolic changes induced during sprouting. Leucine was one of the most abundant essential amino acids, which may be of nutritional importance considering its control role in muscle protein synthesis through activation of the mTOR pathway[71]. The relatively high leucine concentration meant that germinated African walnut protein could potentially have anabolic potential comparable to other plant derived oilseed proteins.

Lysine was found in appreciable concentration with respect to many cereal proteins (Lysine is usually limiting). This increases the complementary value of African walnut flour in cereal-based formulations and aids the possible use of African walnut flour in improving the dietary protein quality[72]. The availability of

sufficient valine and isoleucine in combination with leucine is an indication of an adequate profile of branched-chain amino acids, having a role in metabolic regulation and nitrogen balance. Phenylalanine and histidine were also moderately represented favouring neurotransmitter synthesis and haemoglobin structure, respectively.

Methionine and cystine, the sulfur-containing amino acids, were at levels of comparative low when there the pattern commonly reported for plant proteins. Although sulfur amino acids would be considered a limiting record in legumes and oilseeds, their existence is considered extremely important for upkeep glutathione for formation and fight antioxidant. Tryptophan was found at the minimum essential amino acids, which is in accord with the observation of many plant-based protein source[73].

Of the non-essential amino acids, glutamic acid and aspartic acid were the predominant ones. High content of glutamic acid is a feature of oilseed proteins and is related to improved functional properties water binding, emulsification capacity and flavour enhancement[74]. The comparatively high level of arginine is also of interest, considering that its precursor is nitric oxide and it plays a significant role in the cardiovascular and immune systems. Oilseeds have been commonly known to be good sources of arginine, and the detected profile is in accordance with this biochemical property[75].

The distribution of amino acids shown in Table 8 is probably related to the biochemical events that take place during germination. Sprouting also activates endogenous proteases which break down storage proteins into peptides and free amino acids for embryonic growth[34]. Such proteolytic activity has been linked to an increased digestibility of the protein and an increase in amino acid bioavailability[34]. In addition, germination is known to reduce antinutritional factors such as phytates and protease inhibitors, and thus further increase the efficiency of protein utilisation[12].

Overall, the amino acid profile shown in Table 8 stores that germinated African walnut flour stores a reasonable essential amino acid spectrum profile, with special highlights in leucine, lysine and arginine. While the sulfur containing amino acids and tryptophan are potentially still on the marginal limits to FAO/WHO reference patterns, the protein fraction shows high potential for incorporation into the different composite plant-based food systems and functional formulations.

Table 8  
Amino Acid Profile

| 1             | 2               | 3         | 4               | 5     | 6 = (2x4x5xC)                 |
|---------------|-----------------|-----------|-----------------|-------|-------------------------------|
| Amino Acid    | Net height (mm) | NH/2 (mm) | Width@NH/2 (MM) | Sstd  | Concentration: g/100g protein |
| Leucine       | 62.6            | 31.30     | 1               | 5.91  | 3.66                          |
| Lysine        | 60.4            | 30.19     | 1               | 5.37  | 3.21                          |
| Isoleucine    | 34.7            | 17.35     | 1               | 6.63  | 2.28                          |
| Phenylalanine | 16.2            | 8.12      | 1               | 17.96 | 2.89                          |
| Norleucine    | 67.5            | 33.75     | 1               |       | INTERNAL STANDARD             |
| Tryptophan    | 10.9            | 5.47      | 1               | 5.32  | 0.58                          |
| Valine        | 50.9            | 25.47     | 1               | 5.92  | 2.99                          |
| Methionine    | 24.1            | 12.06     | 1               | 5.41  | 1.29                          |
| Proline       | 11.3            | 5.65      | 1               | 20.56 | 2.30                          |
| Arginine      | 13.6            | 6.80      | 1               | 17.42 | 2.35                          |
| Tyrosine      | 4.9             | 2.47      | 1               | 34.84 | 1.70                          |
| Histidine     | 23.4            | 11.70     | 1               | 6.47  | 1.50                          |
| Cystine       | 7.2             | 3.58      | 1               | 12.26 | 0.87                          |
| Alanine       | 42.3            | 21.14     | 1               | 7.68  | 3.22                          |
| Glutamic acid | 43.4            | 21.68     | 1               | 15.33 | 6.58                          |
| Glycine       | 67.2            | 33.59     | 1               | 4.81  | 3.20                          |
| Threonine     | 43.4            | 21.68     | 1               | 5.62  | 2.41                          |
| Serine        | 49.4            | 24.72     | 1               | 5.47  | 2.68                          |
| Aspartic acid | 90.2            | 45.08     | 1               | 6.28  | 5.61                          |

## Conclusion

Germination significantly altered the biochemical structure of the protein composition of *Tetracarpidium conophorum* with concerted effects on protein quality, lipid structure, and oxidative stability. Sprouting improved the nutritive quality of the walnut (increased polyunsaturated fatty acids, especially omega-3 fractions and balanced amino acid spectrum, with appreciable concentrations of leucine and lysine). These modifications are indicative of the processes of proteolysis and lipid remodeling in germination to bring more metabolic accessibility and nutritional density. However, the nutritional improvement was accompanied with increased oxidative susceptibility. Progressive germination increased peroxide and acid

values which suggested increased lipid hydrolysis and primary oxidation, especially at long sprouting time. Thus, the germination has a two-fold effect whereby it enhances the nutritional functionality while also decreasing the chemical stability by its uncontrolled nature.

From among the evaluated durations, moderate germination seems to yield the best balance between enhanced nutritional qualities and acceptable oxidative quality. These results place germinated African walnut in potentially interesting position as a functional ingredient in plant-based and composite food systems, while highlighting the need for process optimization to maintain lipid integrity.

## Recommendations

In order to apply these results to practical use, the following recommendations are made:

### Process Optimization

Germination time should be optimized in a controlled manner to ensure optimal nutritional gains whilst minimizing oxidative deterioration. Moderate durations of sprouting are preferable for industrial application.

Advanced Protein Quality Evaluation Determination of the DIAAS or PDCAAS values is also suggested to measure real biological protein quality and enhance the claims about nutritional improvement.

### Oxidative Stability Mitigation

Mitigation of oxidative instability (natural antioxidant incorporation, controlled atmosphere drying, or mild stabilization technique can be considered) of germinated products and further work should be conducted to understand the importance of shelf stability.

### Bioaccessibility and Validation of Functionality

It is important to assess in vitro digestibility, mineral bioavailability and antioxidant activity in simulated gastrointestinal conditions in order to determine functional benefits.

### Product Development Trials

Application studies through bakery applications, beverages and plant-based proteins formulations are in need to ensure techno-functional performance and consumers acceptability.

## Declarations

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There is national or local guideline for collection of walnuts in my region however necessary protocols were followed.

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## Author Contribution

All the authors reviewed the manuscript.Oloyede A.O wrote the literature review and recommendations, Adesola M.O reviewed the result and discussion.Abioye V.F wrote the abstract and conclusion Afolabi F.O wrote the introduction while Olaniyi A.A edited the manuscript

## References

1. Ayeni EA, Nuhu A. (2018) *Tetracarpidium conophorum* (African walnut) Hutch. & Dalziel: Ethnomedicinal uses and its therapeutic activities. Journal of Medicinal Plants for Economic

- Development. <https://doi.org/10.4102/jomped.v2i1.47>
2. Alawode O, Olatunji IO, Abioye VF, Ayansola JH, Odedeji JO. Effect of boiling methods on the nutritional profile, phytochemicals, functional properties, lipid quality and consumer acceptability of African walnut (*Tetracarpidium conophorum*). *Bangladesh J Sci Industrial Res.* 2025;60:143.
  3. Adetunji JB, Adetunji CO, Olaniyan OT. (2021) African Walnuts: A Natural Depository of Nutritional and Bioactive Compounds Essential for Food and Nutritional Security in Africa. p 331.
  4. Mereles L, Smidt M, Velázquez E et al. (2022) Tropical Plant Species and Technological Interventions for Improvement. IntechOpen eBooks. <https://doi.org/10.5772/intechopen.98109>
  5. Zhang M, Cai S, Wang O, Zhao L, Zhao L. A comprehensive review on walnut protein: Extraction, modification, functional properties and its potential applications. *J Agric Food Res.* 2024;16:101141.
  6. Saikia D, Gupta S, Saini RK. Effect of processing techniques on in vitro protein digestibility, antinutrients, and functional properties of selected plant-based foods. *J Food Meas Charact.* 2025. <https://doi.org/10.1007/s11694-025-03896-1>.
  7. Valdés A, Sanahuja AB, Karabagias IK, Badeka AV, Kontominas MG, Garrigós MC. Effect of Frying and Roasting Processes on the Oxidative Stability of Sunflower Seeds (*Helianthus annuus*) under Normal and Accelerated Storage Conditions. *Foods.* 2021;10:944.
  8. Minchán-Velayarce HH, Bustos A, Paucar-Menacho LM, Vidaurre-Ruiz J, Schmiele M. (2025) New Frontiers in Cereal and Pseudocereal Germination: Emerging Inducers for Maximizing Bioactive Compounds. <https://doi.org/10.20944/preprints202507.1915.v1>
  9. Bueno DB, Júnior SI da, Chiarotto S, Cardoso ABS, Neto TM, de Reis JA, Glória GCL, Tavano MBA. OL (2019) The germination of soybeans increases the water-soluble components and could generate innovations in soy-based foods. *LWT* 117:108599.
  10. Şenlik AS, Alkan D. Improving the nutritional quality of cereals and legumes by germination. *Czech J Food Sci.* 2023;41:348.
  11. Lemmens E, Brier ND, Spiers K, Ryan CG, Garrevoet J, Falkenberg G, Goos P, Smolders E, Delcour JA. The impact of steeping, germination and hydrothermal processing of wheat (*Triticum aestivum* L.) grains on phytate hydrolysis and the distribution, speciation and bio-accessibility of iron and zinc elements. *Food Chem.* 2018;264:367.
  12. Borgonovi SM, Marzocchi S, Pasini F, Bordoni A, Barbiroli A, Marti A, Iametti S, Nunzio MD. Exploring Germination to Unlock the Nutritional Potential of Sorghum (*Sorghum bicolor*). *Molecules.* 2025;30:3622.
  13. Sharma S, Sahni P. Dynamics of Germination Behaviour, Protein Secondary Structure, Technofunctional Properties, Antinutrients, Antioxidant Capacity and Mineral Elements in Germinated Dhaincha. *Food Technol Biotechnol.* 2021;59:238.
  14. Manoharlal R, Saiprasad GVS. (2023) Contrasting germination behavior of nodding broomrape towards soybean seeds- and sprouts-extract is associated with their corresponding phytohormones, sugars and isoflavones contents. Research Square (Research Square). <https://doi.org/10.21203/rs.3.rs-2855675/v1>

15. Pătrașcu L, Vasilean I, Garnai M, Aprodu I. (2020) Physical pre-treatments as a tool for enhancing nutritional functionality of germinated legumes. *The Annals of the University Dunarea de Jos of Galati Fascicle VI – Food Technology* 44:127.
16. George N, Mildred N, Wanzala E, Munga J, Oduori C, Kinyuru J, Hudson N. Nutritional Composition and Anti-Nutrient Levels in Raw and Processed Varieties of Finger Millet Promoted for Nutritional Security. *Food Nutr Sci.* 2023;14:1183.
17. Muraguri EN, Wakibia J, Kinyuru J. Chemical Composition and Functional Properties of Selected Seaweeds from the Kenya Coast. *J Food Res.* 2016;5:114.
18. Ijarotimi SO, Keshinro OO. Determination of Nutrient Composition and Protein Quality of Potential Complementary Foods Formulated from the Combination of Fermented Popcorn, African Locust and Bambara Groundnut Seed Flour. *Pol J Food Nutr Sci.* 2013;63:155.
19. Josephine D, Francis A, Reindorf B, John B, David BK. Effect of drying on the nutrient and anti-nutrient composition of *Bombax buonopozense* sepals. *Afr J Food Sci.* 2019;13:21.
20. Moussa MID, Alashi AM, Sossa-Vihotogbe CNA, Akponikpè PBI, Baco MN, Djenontin AJ, Aluko RE, Akissoé N. Proximate Composition, Mineral Profile and Trypsin-Inhibitory Activity of West African Leafy Vegetables: Influence of Urea Micro-Dosing and Harvest Time. *Pol J Food Nutr Sci.* 2020;70:179.
21. Adeleke OR, Adiamo OQ, Fawale OS, Olamiti G. Effect of Soaking and Boiling on Anti-nutritional Factors, Oligosaccharide Contents and Protein Digestibility of Newly Developed Bambara Groundnut Cultivars. *Turkish J Agric - Food Sci Technol.* 2017;5:1006.
22. Bello FA. Storage effects and the postharvest quality of African star apple fruits (*Chrysophyllum africanum*) under ambient conditions. *Afr J Food Sci Technol.* 2015.  
<https://doi.org/10.14303/ajfst.2015.011>.
23. Ogbu JC, Etuk-Udo G, Ugoh SC, Odunsanya OO, Amos JR. (2024) Phytochemical and Antimicrobial Activity of African Star Apple [*Chrysophyllum albidum*]. *Research Square (Research Square)*.  
<https://doi.org/10.21203/rs.3.rs-5345688/v1>
24. Elshaafi IM, Musa KH, Sani NA. Effect of oven and freeze drying on antioxidant activity, total phenolic and total flavonoid contents of fig (*Ficus carica* L.) leaves. *Food Res.* 2020;4:2114.
25. Singh S, Chaudhary S, Singh K. (2025) Physicochemical Analysis and Quality Evaluation of Selected Edible Vegetable Oils. *BIO Web of Conferences* 178:2015.
26. Kambhampati S, Li J, Evans BS, Allen DK. Accurate and efficient amino acid analysis for protein quantification using hydrophilic interaction chromatography coupled tandem mass spectrometry. *Plant Methods.* 2019. <https://doi.org/10.1186/s13007-019-0430-z>.
27. Norikura T, Tatehana H, Izawa H, Saito C, Kon A. The Validity of Estimated Dietary Amino Acid and Protein Values via the Amino Acid Composition Table 2015. *J Nutri Sci Vitaminol.* 2019;65:219.
28. Burbano JJ, Cabezas DM, Correa MJ. Characterization and Techno-Functional Properties of High Protein Walnut Flour from an Oil by-Product. *Plant Foods Hum Nutr.* 2024;79:810.

29. Ikram A, Saeed F, Afzaal M, Imran A, Niaz B, Tufail T, Hussain M, Anjum FM. Nutritional and end-use perspectives of sprouted grains: A comprehensive review. *Food Sci Nutr*. 2021;9:4617.
30. Mridula D, Sharma M, Kochhar A, Prasath VA, Patra A, Pandiselvam R. Investigation on thin-layer drying kinetics of sprouted wheat in a tray dryer. *Qual Assur Saf Crops Foods*. 2022;14:12.
31. Romano A, Luca LD, Romano R. Effects of germination time on the structure, functionality, flavour attributes, and in vitro digestibility of green Altamura lentils (*Lens culinaris* Medik.) flour. *Food Funct*. 2024;15:3539.
32. Gao Z, Wang Z, Gao G, et al. GERMINATION AND IN VIVO AMINO ACID BALANCE STRATEGIES OF OAT (*Avena sativa*) SEEDS UNDER SALINEALKALI STRESS SIMULATED WITH NaCl AND NaHCO<sub>3</sub>. *Appl Ecol Environ Res*. 2025;23:2837.
33. Bera I, O'Sullivan M, Flynn D, Shields DC. Relationship between Protein Digestibility and the Proteolysis of Legume Proteins during Seed Germination. *Molecules*. 2023;28:3204.
34. Nemzer B, Al-Taher F. Analysis of Fatty Acid Composition in Sprouted Grains. *Foods*. 2023;12:1853.
35. Sagun JV, Yadav UP, Alonso AP. Progress in understanding and improving oil content and quality in seeds. *Front Plant Sci*. 2023;14:1116894.
36. Kassegn HH, Hshe BG, Meresa BK, Berhe MH, Tadesse HA. (2024) Germination Process Impact on Proximate, Inorganic, and Phytochemical Contents of Malt Barley, Abyssinian Purple-Colored Barley and Wheat. *Research Square (Research Square)*. <https://doi.org/10.21203/rs.3.rs-4761793/v1>
37. Botër M, Calleja-Cabrera J, Carrera-Castaño G, et al. An Integrative Approach to Analyze Seed Germination in *Brassica napus*. *Front Plant Sci*. 2019;10:1342.
38. Günal-Köroğlu D, Esatbeyoglu T, Çapanoğlu E. Effect of germination on the phenolic compounds: content, bioavailability, food applications, and health benefits. *J Food Meas Charact*. 2025. <https://doi.org/10.1007/s11694-025-03392-6>.
39. Longkumer IY, Akbar U, Singh J, Rasane P, Nanda V, Pandey VK, Kaur S, Abdi G. Elicitors enhance bioactive compound production in sprouts and improve health benefits. *Discover Sustain*. 2025. <https://doi.org/10.1007/s43621-025-01841-2>.
40. Ojo MA. Tannins in Foods: Nutritional Implications and Processing Effects of Hydrothermal Techniques on Underutilized Hard-to-Cook Legume Seeds-A Review. *Prev Nutr Food Sci*. 2022;27:14.
41. Bautista-Expósito S, Vandenberg A, Peñas E, Frías J, Martínez-Villaluenga C. Lentil and Fava Bean With Contrasting Germination Kinetics: A Focus on Digestion of Proteins and Bioactivity of Resistant Peptides. *Front Plant Sci*. 2021. <https://doi.org/10.3389/fpls.2021.754287>.
42. Duraiswamy A, A. NMS K, SJ, et al. Genetic manipulation of anti-nutritional factors in major crops for a sustainable diet in future. *Front Plant Sci*. 2023. <https://doi.org/10.3389/fpls.2022.1070398>.
43. Muleya M, Shumoy H, Muchuweti M, Vandamme P, Raes K. Enzymatic degradation of mineral binders in cereals: Impact on iron and zinc bioaccessibility. *J Cereal Sci*. 2018;82:223.
44. Díaz-Batalla L, Aguilar-Arteaga K, Castro-Rosas J, Falfán-Cortés RN, Navarro-Cortez RO, Gómez-Aldapa CA. Common bean (*Phaseolus vulgaris* L.) seed germination improves the essential amino acid profile, flavonoid content and expansion index. *Czech J Food Sci*. 2023;41:73.

45. Hassan S, Zeng X, Khan MK, Farooq MA, Ali A, Kumari A, Mahwish, Rahaman A, Tufail T, Liaqat A. Recent developments in physical invigoration techniques to develop sprouts of edible seeds as functional foods. *Front Sustainable Food Syst.* 2022. <https://doi.org/10.3389/fsufs.2022.997261>.
46. Baranzelli J, Somacal S, Monteiro CS, et al. Grain Germination Changes the Profile of Phenolic Compounds and Benzoxazinoids in Wheat: A Study on Hard and Soft Cultivars. *Molecules.* 2023;28:721.
47. Fernie AR. (2023) Metabolomics and machine learning technique reveals that germination enhances the multi-nutritional properties of pigmented rice. *Research Square (Research Square)*. <https://doi.org/10.21203/rs.3.rs-2940671/v1>
48. Kim SM, Aung T, Kim MJ. Optimization of germination conditions to enhance the antioxidant activity in chickpea (*Cicer arietinum* L.) using response surface methodology. *Korean J Food Preservation.* 2022;29:632.
49. Kowalski RK, Belniak K, Kowalska G, Włodarczyk-Stasiak M, Mazurek A, Baj T. Hardtank® technology in extraction of caffeine and chlorogenic acid from coffee: analysis of chemical composition and antioxidant properties of coffee beverages. *Front Chem.* 2025. <https://doi.org/10.3389/fchem.2025.1681137>.
50. Avezum L, Rondet É, Mestres C, Achir N, Madodé YE, Gibert O, Lefèvre C, Hémerly Y, Verdeil J, Rajjou L. Improving the nutritional quality of pulses via germination. *Food Reviews Int.* 2022;39:6011.
51. Kumari S, Gray A, Webster K, Bailey KB, Reid M, Kelvin KAH, Tey SL, Chisholm A, Brown R. Does 'activating' nuts affect nutrient bioavailability? *Food Chem.* 2020;319:126529.
52. Sharma S, Anandkumar LHD, Tyagi A, Muthumilarasan M, Kumar K, Gaikwad K. An insight into phytic acid biosynthesis and its reduction strategies to improve mineral bioavailability. *Nucleus.* 2021;65:255.
53. Erba D, Angelino D, Marti A, Manini F, Faoro F, Morreale F, Pellegrini N, Casiraghi MC. Effect of sprouting on nutritional quality of pulses. *Int J Food Sci Nutr.* 2018;70:30.
54. Doria E, Pagano A, Ferreri C, Larocca A, Macovei A, Araújo S, de Balestrazzi S A. How Does the Seed Pre-Germinative Metabolism Fight Against Imbibition Damage? Emerging Roles of Fatty Acid Cohort and Antioxidant Defence. *Front Plant Sci.* 2019. <https://doi.org/10.3389/fpls.2019.01505>.
55. Karolkowski A, Guichard É, Briand L, Salles C. Volatile Compounds in Pulses: A Review. *Foods.* 2021;10:3140.
56. Zhang X, Zhang Y, Sun P, Su W, Qu Z, Dong Y, Du S, Yu X. Effect of germination pretreatment on the physicochemical properties and lipid concomitants of flaxseed oil. *RSC Adv.* 2023;13:3306.
57. Gu M, Kim H, Kim BH, Kim I, Hyun TK, Lee H, Lee JS. Changes in Phytochemical Content and Antioxidant Activities of Soybean Oil during Germination. *J Oleo Sci.* 2025;74:287.
58. Dugulin CA, Rouck GD, Cook DJ. Green Malt for a Green Future – Feasibility and Challenges of Brewing Using Freshly Germinated (Unkilned) Malt: A Review. *J Am Soc Brew Chem.* 2021;79:315.
59. Jedidi B, Mokbli S, Sbihi HM, Nehdi IA, Romdhani-Younes M, Al-Resayes SI. Effect of extraction solvents on fatty acid composition and physicochemical properties of *Tecoma stans* seed oils. *J King*

60. Assaf N, Metouekel A, Rhayem L, Guerin C, Tabib M, Liu S, Rinaldi M, Chiavaro E, Atheaux G, Grimi N. (2025) Comparative Study of Conventional and Emerging Extraction Methods for Papaya Seed Oil: Process Optimization and Quality Evaluation. Food and Bioprocess Technology. <https://doi.org/10.1007/s11947-025-04083-2>
61. Belt D, Grygier A, Siger A, Kmiecik D, Spasibionek S, Rudzińska M. Changes in Oil Quality and Peroxidase Activity during Germination of Rape Seeds and Mustard Seeds. Appl Sci. 2023;13:2196.
62. Miklaszewska M, Zienkiewicz K, Inchana P, Zienkiewicz A. Lipid metabolism and accumulation in oilseed crops. OCL. 2021;28:50.
63. Al-Taher F, Nemzer B. Effect of Germination on Fatty Acid Composition in Cereal Grains. Foods. 2023;12:3306.
64. Miray R, Kazaz S, To A, Baud S. Molecular Control of Oil Metabolism in the Endosperm of Seeds. Int J Mol Sci. 2021;22:1621.
65. Pei Q, Liu Y, Peng S. Fatty Acid Profiling in Kernels Coupled with Chemometric Analyses as a Feasible Strategy for the Discrimination of Different Walnuts. Foods. 2022;11:500.
66. Antora SA, Ho K, Lin C, Thomas AL, Lovell ST, Krishnaswamy K. Quantification of Vitamins, Minerals, and Amino Acids in Black Walnut (*Juglans nigra*). Front Nutr. 2022. <https://doi.org/10.3389/fnut.2022.936189>.
67. Yu L, Liu D, Yin F, et al. Interaction between phenylpropane metabolism and oil accumulation in the developing seed of *Brassica napus* revealed by high temporal-resolution transcriptomes. BMC Biol. 2023. <https://doi.org/10.1186/s12915-023-01705>.
68. Brito TRR, Valeriano HHC, Ítavo LCV, et al. Effect of including oilseed grains in bovine diets on fatty acid profile, lipid stability, and sensory aspects of burgers. Front Veterinary Sci. 2022. <https://doi.org/10.3389/fvets.2022.923937>.
69. Lin Y, Xu H, Yin G, Zhou Y, Lu X, Xin X. Dynamic Changes in Membrane Lipid Metabolism and Antioxidant Defense During Soybean (*Glycine max* L. Merr.) Seed Aging. Front Plant Sci. 2022;13:908949.
70. Shaikh N, Soomro AH, Miano TF, Tarar OM. Proximate Composition, Amino Acid Profiling, and Safety Assessment of Processed Natural Vegetation of Tharparkar. Pakistan J Biotechnol. 2024;21:237.
71. Nkwonta C, Auma CI, Gong YY. Underutilised food crops for improving food security and nutrition health in Nigeria and Uganda—a review. Front Sustainable Food Syst. 2023. <https://doi.org/10.3389/fsufs.2023.1126020>.
72. Chéreau D, Videcoq P, Ruffieux C, Pichon L, Motte J, Belaid S, Ventureira J, Lopez M. (2016) Combination of existing and alternative technologies to promote oilseeds and pulses proteins in food applications. OCL. <https://doi.org/10.1051/ocl/2016020>
73. Hoehnel A, Zannini E, Arendt EK. Targeted formulation of plant-based protein-foods: Supporting the food system's transformation in the context of human health, environmental sustainability and consumer trends. Trends Food Sci Technol. 2022;128:238.

74. Tapiero H, Mathé G, Couvreur P, Tew KD. L-Arginine. *Biomed Pharmacother.* 2002;56:439.