Journal of Agriculture and Food Research 2 (2020) 100086

Contents lists available at ScienceDirect

Journal of Agriculture and Food Research

journal homepage: www.journals.elsevier.com/journal-of-agriculture-and-food-research/

Influence of fermentation and germination on some bioactive components of selected lesser legumes indigenous to Nigeria

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ARTICLE INFO

Keywords:
Legumes
Total phenolic
Tannin
Anthocyanin
Carotenoid
Flavonoid

ABSTRACT

This study assessed the effects of fermentation time (2, 3 and 4 days) and germination time (2, 3 and 4 days) on total phenolics, tannin, anthocyanin, carotenoids and flavonoids contents of lesser legumes: cowpea (*Vigna unguiculata* L.), bambaranut (*Vigna subterranean* L.), red bean (*Phaseolus vulgaris*), pigeonpea (*Cajanus cajan*), African breadfruit (*Treculia africana*) seeds, African yam bean (*Sphenostylis stenocarpa*) seed, African oil bean (*Pentaclethra mycrophylla* Benth.) seed and groundnut (*Arachis hypogea* L.). The antioxidant and reducing powers of a three day germinated samples were also evaluated. The result revealed that fermentation reduced the total phenolics, tannin, anthocyanin, carotenoid and flavonoid contents of the samples with increasing fermentation time; however, red bean showed minimal increase with increasing fermentation time. There was significant ($p < 0.05$) increase in the total phenolics of all the samples with increasing germination time but, tannin and flavonoid showed significant ($p < 0.05$) reduction with increasing germination time. Germination significantly ($p < 0.05$) reduced the carotenoid and anthocyanin levels in all the samples, however, red bean, pigeonpea and African oil bean showed increases with increasing germination time. Samples evaluated exhibited significantly ($p < 0.05$) different antioxidant capacities. African oil bean and groundnut had the highest antioxidant activities 52.18% and 52.16%, respectively while, African yam bean seed was the lowest (19.85%). Similar trend was observed in the reducing power of the raw samples where groundnut, bambaranut and African breadfruit showed significantly ($p < 0.05$) higher reducing power. Three (3) days germination significantly ($p < 0.05$) increased the antioxidant capacities by 14.65%, 18.42%, 53.58%, 52.84%, 17.24%, 14.56%, 53.18% and 43.03% in African oil bean, bambaranut, cowpea, red bean, African breadfruit, groundnut, African oil bean and pigeonpea, respectively. Therefore, for increase antioxidant activity in lesser known legumes, germination is more preferred over fermentation and for maximum yield of total phenolics, three and four days germination time are recommended.

1. Introduction

Legumes serve as a large reservoir of bioactive compounds most especially the phenolics and these bioactives have been positively implicated in the management of degenerative diseases [1,2]. The health benefits of phytonutrients have led to increased research efforts on the possibilities of exploiting locally available and natural sources of bioactives for the dietary management of degenerative diseases. The rapidly increasing population of the third world countries calls for increase researches in providing alternative food sources with increase physiological health benefits. There are thousand lesser known plant food sources that might substantially add to the array of available nutrients most especially the protein need [3]. The lesser known legumes which are readily available and cheap, well adapted to extreme environmental conditions and highly resistant to drought, diseases and pest infestation are alternative sources to exploit.

Cowpea (*Vigna unguiculata* L.) belongs to the family Fabaceae and subfamily Fabiodeae. Most cowpeas are grown on the African continent, particularly in Nigeria and Niger, which account for 66% of world production [4–6]. It is a multipurpose crop and the entire plant can be used for either human or livestock consumption [7]. The matured seeds are majorly preserved as pulses and can be prepared into stews, soups, purees, casseroles and curries. It is the major ingredient used for the local production of *moimoi*, *akara and danwake* [8]. Furthermore, their

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https://doi.org/10.1016/j.jafr.2020.100086
Received 17 June 2020; Received in revised form 4 November 2020; Accepted 9 November 2020

immature green seeds and pods can be consumed as vegetables while the dry seeds can be processed into flour for reconstitution into local dishes. Bambaranut (*Vigna subterranean* L.) originated from West Africa and it is now widely grown across the world [9–11]. The pods mature underground like the peanut. Locally, the nuts are eaten as a snack which are roasted and salted; processed into a cake or eaten as a meal which is usually boiled into doneness similar to other beans [12,13]. In South Eastern Nigeria, the dried beans are used for the preparation of a cakey pudding (*okpa*) while, in North Central Nigeria, the beans are used for local delicacies such as *Sagidi*, *Kangu cake* among others [13]. Red bean (*Phaseolus vulgaris*) also known as the common bean and French bean is a herbaceous annual plant grown worldwide for its edible dry seeds or unripe fruit. The wild species is native to the Americas. It was originally believed that it had been domesticated separately in Mesoamerica and in the Southern Andes region, giving the domesticated bean two gene pools [6]. The bean is prepared into a paste called *anko* or red bean jam. Pigeonpea (*Cajanus cajan*) is an important food legume of the semi-arid tropics of Asia and Africa. The centre of origin is probably Indian peninsular, where the closest wild relatives (*Cajanus cajanifolia*) occur in tropical deciduous wood lands [14]. It is the second most important pulse crop next to chickpea [15]. Pigeonpea is both a food crop (dried peas, flour, or green vegetable peas) and a forage/cover crop [16,17]. It is eaten as a vegetable (immature green seeds) or as a pulse (dry seeds). Locally, the seeds are roasted and eaten as a snack or spiced with condiments and cooked to doneness and eaten as a meal, porridge or the flour incorporated into baked products [18,19]. The African breadfruit (*Treculia africana*) is an annual crop found mainly in the high rain forest zone of Southern part of Nigeria and other African countries [20,21]. The wild tree produces enormous seeds during its fruiting season (March to April) weighing five to 10 kg after processing [22,23]. The seeds serve as nutrient reserve most especially during scarce period when conventional sources of food are short in supply, usually before the rainy season sets in Ref. [23]. The seeds are locally prepared and consumed as a porridge meal or roasted and eaten as dessert snacks or turned into a flour and baked as a breadfruit cake [22–24]. African yam bean (*Sphenostylis stenocarpa*) seed is asserted to have originated from Ethiopia which later spread to many tropical African areas [25]. The above ground produces good yields (2000 kg/ha) of edible seeds while, the leaves are utilized as edible vegetable. It is grown for either seed or tuber but, in Nigeria the seed is valued more than the tuber; also, the crop is regarded as one of Africa's under-utilized plant species with potential to broaden man's food base [26]. The plant produces small tuberous root which contains more protein than sweet potatoes, potatoes and cassava roots [26,27]. In most West African communities, the seeds are boiled to doneness after long hours of soaking usually 12 h and eaten with other staples such as yam, plantain, cassava, corn/maize, etc. In addition, the seed is roasted and consumed as a snack [28]. The African oil bean tree (*Pentaclethra macrophylla* Benth) is a large wild woody plant that belongs to the family Leguminous and sub-family Mimosoidae. It produces seeds that are dorsa-ventrally flat, hard, brown in colour and about 6 cm wide [29]. The seeds are cited among the lesser known and under exploited legumes [30]. The major food processed from the mesocarp is "ugba", a ready to serve fermented product found in Eastern Nigeria. Additionally, the oily seed is cooked to doneness and consumed as a porridge in the Western Nigeria [31]. Groundnut (*Arachis hypogea* L.) also called peanut or monkey nut belongs to the family Leguminosae. The crop is asserted to have originated from Central American region from where it spread to other regions of the world [32]. Peanut is the most popular commercial crop in Nigeria and the country accounts for 41% of its total production in West African region [33]. Settaluri et al. [34] revealed that fifty percent of the nut is crushed into edible oil for industrial and domestic applications and the resulting by-product, groundnut cake is used as a protein source for livestock feeds or locally fried into a snack, *kwilikwili*.

Food treatments such as fermentation, germination, cooking, soaking and roasting affect both the nutritional composition as well as phytochemical profile. Alvarez et al. [35] and Weisburger [36] reported a rise in total phenolics of fermented legumes. The increase was attributed to the activities of polyphenol oxidases which catalyzed polyphenols to low molecular weight condensed tannins. However, Wollgast and Anklam [37] reported a loss in total phenolics during fermentation. The loss was attributed to leaching of some of the lipophilic polyphenols in the fermentation medium and their possible susceptibility to oxidation. The mechanism of loss in certain phytonutrients upon fermentation was further explained by Brouillard et al. [38], Giusti and Wrolstad [39], Cevallos–casals et al. [40] and Morata et al. [41]. The authors revealed that during fermentation process, adsorption mechanism between the fermenting flora and certain phytochemicals such as anthocyanin might be responsible for their depletion. Messen and Vuyst [42], Nazarni et al. [43] and Othman et al. [44] studied the influence of fermentation on flavonoids, tannins, alkaloids and polyphenol propanoids of some conventional legumes. The results revealed increases in the parameters studied which was attributed to favourable activities of microbial enzymes that produced more freely available form of the plant secondary metabolites. Lopez-Amoros et al. [45] examined the effects of germination on phenolic profile. The study showed that germination modified the phenolic profile of the legumes; influenced the functional properties as well as the antioxidant capacity of the resultant flour. Sokrab et al. [46] explained that germination solubilizes the phenolic content of germinated seeds leading to rise in its content. Furthermore, Sokrab et al. [46] and Duenas et al. [47] reported that germination leads to the solubilization of condensed tannins and the migration of phenolic compounds to the outer layer as indicated by the browning of the germinated seeds; and this leads to rise in the total phenolics of the germinated seeds. Furthermore, during seed germination, enzymes system are mobilized and activated; seed colour concentration is reduced most especially the hydrophilic component with resultant decrease in tannin, anthocyanin and total phenolics [46].

To advance the acceptability of local foodstuff, research studies have been ongoing in presenting lesser known legumes; their suitability in different food applications as well as their bioactive potentials. A number of studies have been conducted on the bioactive components of conventional legumes; however, there is dearth of information on the non-conventional ones. To present lesser known legumes, Oboh [48] evaluated the antioxidant properties of some commonly consumed and underutilised legumes in Nigeria; while, Ade-Omowaye et al. [49] profiled the nutritional composition of nine underexploited legumes indigenous to Southwest Nigeria. Also, James et al. [50] assessed the potentials of protein concentrate from seven legumes indigenous to northern Nigeria for different food applications. The result of the finding showed that, the concentrate has the functionality to be incorporated into different food systems. These are efforts in trying to present lesser known legumes. Therefore, this study assessed the effects of different treatments on some bioactive compounds in lesser legumes and evaluate their antioxidant potentials. This will establish their bioactive potentials as alternative food sources to be exploited.

2. Materials and methods

2.1. Materials

Indigenous and underutilised legumes for this study included cowpea (*Vigna unguiculata* L.), bambaranut (*Vigna subterranean* L.), red bean (*Phaseolus vulgaris*), pigeonpea (*Cajanus cajan*), African breadfruit (*Treculia africana*) seeds, African yam bean (*Sphenostylis stenocarpa*) seed, African oil bean (*Pentaclethra mycrophylla* Benth.) seed and groundnut (*Arachis hypogea* L.).

2.2. Source of raw materials

The samples were procured in the month of January 2018 from Umuahia Local Market, Abia State, Southeastern Nigeria. The seeds were botanically identified by the Department of Crop Production, Federal

University of Technology, Minna, Nigeria. Extraneous matters such as insect infected seed, sand and chaff were manually removed from the samples.

3. Methods

3.1. Treatments

3.1.1. Fermentation

Ten gram (10 g) of the whole seeds were fermented in 30 mL of tap water, that is 1:3 (w/v) for 2, 3 and 4 days at room temperature (28 ± 2 °C). After fermentation, the seeds were drained and oven dried at 80 °C for 24 h to a constant weight, milled into a powder of 0.5 mm size, kept in plastic bags and then stored at 4 °C for further analysis.

3.1.2. Germination

The intact and viable seeds were germinated in the dark at room temperature 28 ± 2 °C for 2, 3 and 4 days. This was done after sterilizing 10 g (10 g) of the seeds in ethanol for 1 min and soaking in 30 mL of distilled water (1:3 w/v) for 12 h. Germinated grains were oven-dried at 80 °C for 24 h to a constant weight, milled into a powder of 0.5 mm size, kept in plastic bags and then stored at 4 °C for further analysis [51].

3.1.3. Extract preparation for total phenolic, tannin and flavonoid quantification

Each sample (10 g) was transferred to dark-coloured flasks and mixed with 200 mL of solvents with different polarities (water, methanol, ethyl-acetate, acetone, petroleum ether), respectively and stored at room temperature. After 24 h the infusion was filtered through Whatman No. 1 filter paper and the residue was re-extracted with equal volume of solvents. The process was repeated for 48 h. At the end, supernatants were combined and evaporated to dryness under vacuum at 40 °C using rotary evaporator at kept in sterile sample tube and stored in a refrigerator at 4 °C.

3.1.4. Quantification of total phenolics

Folin-Ciocalteu method as described by Rajha et al. [52] was used. An aliquot of 10 μL of the sample solution was mixed with 100 μL of commercial Folin-Ciocalteu reagent and 1580 μL of water. After a brief incubation at room temperature (5 min), 300 μL of saturated sodium carbonate was added. The colour generated was read after 2 h at room temperature at 760 nm using a UV–Vis spectrophotometer (UV-9200, UK). The correlation between the absorbance and gallic acid concentrations creates a calibration standard curve. The phenolic compounds concentration of the samples was expressed as gallic acid equivalents in mg/L, then the Total Phenolic Compounds (TPC) were calculated by transforming milligrams of Gallic Acid Equivalent (GAE) per litre (mg GAE/L) into milligrams of GAE per 100 g dry matter (g GAE/100 g DM).

3.1.5. Quantification of total tannin

Total tannin was determined by Folin-Ciocalteu method. The extract (0.1 mL) was added to a volumetric flask (10 mL) containing 7.5 mL of distilled water and 0.5 mL of Folin-Ciocalteu phenol reagent, 1 mL of 35% Na_2CO_3 solution and the entire mixture was diluted to 10 mL mark of the volumetric flask with distilled water. The mixture was shaken well and kept at room temperature for 30 min. A set of standard solutions of gallic acid (20, 40, 40, 60, 80 and 100 μg/mL) were prepared and incubated for 90 min at room temperature and the absorbance for test and standard solutions were determined against the reagent blank at 550 nm with an ultraviolet/visible spectrophotometer. Absorbance for test and standard solutions were measured against the blank at 725 nm with an UV–visible spectrophotometer. The tannin content was expressed in terms of mg of GAE/100 g of extract [53,54].

3.1.6. Quantification of total flavonoids

The quantification was done as descried by Chen [55] with some modifications. The extract (1 mL) which contains flavonoid, 0.7 mL of 5% (w/w) $NaNO_2$ and 10 mL of 30% (v/v) ethanol were combined and stirred for 5 min and 0.7 mL of 10% $AlCl_3$ (w/w) was added and the mixture was swirled. In about six (6) min later, 5 mL of 1 moL/l NaOH was added. The mixture of the solution was diluted to 25 mL with 30% (v/v) ethanol prior to the measurement. After 10 min standing, the absorbance of the solution was measured at 500 nm with a spectrophotometer (Unico, WFJ2000, Shanghai, China). The total flavonoid content was expressed in mg rutin per g dry weight basis by comparison with rutin standard curve, and the yield of flavonoids was calculated using the following formula:

$$Y = \frac{(6.404A + 0.2806)BV}{(mg/100\ g)} \quad (1)$$

where: A – absorbance (500 nm); B – dilution factor; V – volume of the extracting agent (ml).

3.1.7. Extraction and quantification of total anthocyanin

Determination of total anthocyanin content (TAC) was done using pH differential method. In brief, ten (10) g of each sample (flour) was extracted with 60 mL acidified ethanol (37% HCl was added until the pH adjusted to 1.0) and the solution was stirred using a magnetic stirrer for 15 min at room temperature and then allowed for 24 h under dark conditions. After that, the solution was filtered with Whatman filter paper number 1. The filtrate was inserted into maceration bowl that has been covered in aluminium foil and dried until it becomes a paste. The extracts then weighed to calculate their yield as:

$$\text{Percentage yield}\ (\%) = \frac{(\text{Weight of extract})}{(\text{Weight of dry sample})}\ \text{X}\ 100 \quad (2)$$

Two hundred and fifty (250) mg of each extract was dissolved in ethanol p.a. 96% and acidified with 37% HCl to pH 1.0. After it was acidified, each 1 mL of the solution was put into two test tubes, 9 mL of buffer solution of KCl at pH 1.0 was added to test tube 1 and CH_3COONa at pH 4.5 buffer solution in test tube 2. In addition, an acid ethanol solution was prepared by adding HCl to pH 1.0. The solution was divided into two test tubes. In the first test tube I, 1 mL of ethanol pH 1.0 was added and 9 mL of buffer solution of KCl at pH 1.0 was added, while for test tube II, 1 mL of ethanol at pH 1.0 and 9 mL of CH_3COONa at pH 4.5 buffer was added. The solution is then divortexed for 20 s and silenced for 30 min in a dark room. The sample measurement was performed by taking 200 μL of sample solution with buffer (KCl, pH 1.0 and CH_3COONa, pH 4.5) and added to 1800 μL acid ethanol solution with buffer adjusted to the buffer used in the sample solution. The absorbance of each sample was measured both at 510 nm and 700 nm by spectrophotometer UV–Vis. TAC was expressed as cyanidin-3-glucoside equivalent, and calculated as follows:

$$\text{MAP}\ (\text{mg}/\text{L}) = \frac{(\text{A x MW x DF x 1000})}{(\text{molA x L})} \quad (3)$$

Where: MW and molA are the molecular weight and the molar absorptivity, respectively, of the pigment cyaniding-3-glucoside used as reference; MW = 449.2 g/mol and molA = 26,900 (26,900 L/(cm mol)).

Milligrams of MA (Monomeric Anthocyanin) per litre of extract (mg/L) were then transformed into Monomeric Anthocyanin Yield (MAY) which is milligrams per 100 g of the extract (mg/100 g).

The absorbance (A) of the diluted sample was calculated as follows:Where: A is absorbance calculated as:

$$A\ \ ¼\left[(Abs_{510} - Abs_{700})pH1.0 - (Abs_{510} - Abs_{700})pH4.5 \right. \quad (4)$$

3.1.8. Extraction and determination of total carotenoid

Each sample was extracted with acetone-methanol-petroleum ether at

(3:2:1, v/v/v) for 5 h in the dark, in order to avoid carotenoids degradation and oxidation. The crude extract was filtered, evaporated to dryness in a rotary evaporator and resuspended in ethyl ether. The concentration of total carotenoids in the extract was calculated by relating the absorbance reading A ($\lambda_{max} = 450$ nm) to the specific absorption (mean value $A_1^{1\%} = 2500$) of coloured carotenoids:

$$X = (A.Y.1000)(2500.100) = \frac{A.Y}{250} \quad (5)$$

Where, X was the weight of carotenoids in the sample (mg) and Y was the volume of the sample (100 g). The concentration of carotenoids was measured at 450 nm, in a spectrophotometer (Pharmaspec, Shimadzu UV-1700) [56].

3.1.9. 1,1-Diphenyl-2-picrylhydrazyl method (DPPH●) assay

The radical scavenging capacity of the samples was tested based on the procedure described by Siddiqua et al. [57]. Briefly, the reaction contained 1 mL of extracts, 3 mL of methanol, and 150 μL of DPPH● 0.1%. The absorbance was recorded at 517 nm after 30 min. The capacity of radical scavenging was calculated with the following formula: % $DPPH●_{scavenging} = [(Abs_{control} - Abs_{sample})/Abs_{control}]$ x 100%, where Abs_{sample} is absorbance of extract solution, and $Abs_{control}$ is absorbance of methanol in DPPH●.

3.1.10. Reducing power assay

For the assay of reducing power, the protocol of Singhal et al. [58] was used and described as follows. One millilitre of the filtrate was mixed with 2.5 mL of phosphate buffer (pH 6.6) and 2.5 mL of $K_3[Fe(CN)6]$ (1%), which was followed by incubation at 50 °C for 20 min. The reaction was then stopped by adding 2.5 mL of trichloroacetic acid (10%), followed by centrifuging at 3000 rpm (1000×*g*) for 10 min. The supernatant (2.5 mL) was mixed with distilled water (2.5 mL) and 5 mL of $FeCl_3$ solution (1%) and the absorbance was measured at 700 nm. In the reducing power assay, the more the absorbance of the reaction increased, the more reducing power was obtained. The percentage of the reducing power was calculated based on the following formula:

$$\text{Reducing power} = \left[(Abs_{extract} - Abs_{blank})/Abs_{blank}\right] \text{ X } 100\% \quad (6)$$

where $Abs_{extract}$ is absorbance of extracts, and Abs_{blank} is absorbance of water. The triplates determinations were expressed on percentage (%).

3.1.11. Reagents

The reagents used for the study were of analytical grade. Total phenolics standards of gallic acid was from Sigma Chemical Co. (St. Louis, MO, USA). The solvents employed for the extraction of the samples were pure water; and HPLC grades of acetone manufactured by Lobal Chemie Pvt. Ltd., India with CAS No. (64-17-5), ethanol manufactured by Guangdong Guanghua Sci-Tech. Co. Ltd. India with CAS No. (67-64-1) and methanol manufactured by Lobal Chemie Pvt. Ltd., India with CAS No. (67-56-1). The extraction solvents were procured from Finlab Abuja, Nigeria. The Folin reagent (Sigma Chemical Co., St. Louis, MO, USA) and sodium carbonate (Fluka, Buchs, Switzerland) were employed for the measurement of the total phenolic and tannin using the Folin-Ciocalteu method. The calibration curve was constructed with gallic acid (Sigma Chemical Co., St. Louis, MO, USA). Potassium chloride (Fluka, Buchs, Switzerland) and sodium acetate were used for total monomeric anthocyanin determination by the pH-differential method. The reagents were procured from Finlab Abuja, Nigeria.

3.1.12. Statistical analysis

The data obtained were in triplicates and the results were subjected to one-way analysis of variance and expressed as mean with standard deviation. The differences between means were separated by Duncan's Multiple Range Test using IBM SPSS Statistics Programme, Version 19.0 (Illinois, USA). Significant differences were expressed at 5% level.

4. Results and discussion

4.1. Effects of treatments on total phenolic content

Scientific evidences have shown that phenolic compounds confer physiological benefits such as antimicrobial, antimutagenic, antidiabetic, antioxidative, therapeutic among others. This underscores the need to harness their availability in treated lesser legumes. Vadivel and Biesalski [59] reported that the dietary intake of phenolics differ considerably among countries of the world and it is estimated that the daily intake of total free phenolics ranged from 20 mg–1 g. The TPC in this study were found to be 192.43 mg/100 g, 225.63 mg/100 g, 221.05 mg/100 g, 196.33 mg/100 g, 221.36 mg/100 g, 196.35 mg/100 g, 314.26 mg/100 g and 225.26 mg/100 g in cowpea bean, bambaranut, red bean, pigeonpea, African breadfruit, African yam bean seed, African oil bean and groundnut, respectively.

Fermentation time (Table 1) showed both positive and negative effects on TPC. There was significant ($p < 0.05$) decrease with increasing fermentation time among samples. The values ranged from 5.29 to 10.38%, 3.57–3.76%, 7.46–7.78%, 3.72–3.92%, 0.91–1.88%, 0.23–1.31% and 5.62–6.15% in cowpea bean, bambaranut, pigeonpea, African breadfruit, African yam bean seed, African oil bean and groundnut, respectively. However, red bean showed significant ($p < 0.05$) increase in TPC regardless of the fermentation time. The loss in TPC with fermentation time agrees with the study of [35] in fermented wild beans. The loss might be attributed to the activities of polyphenol oxidases which are responsible for the catalyzing polyphenols to low molecular weight condensed polyphenols [36]. Wollgast and Anklam [37], also, indicated that the loss might be due to leaching of some of the components of lipophilic polyphenols in the fermentation medium and their possible oxidation. The increase in the total phenolics in red bean regardless of the fermentation time could be attributed to the favourable activities of microbial enzymes which in turn produce more freely available form of plant secondary metabolites such as flavonoids, tannins, alkaloids and polyphenol propanoids [42,43]. Furthermore, activities of polyphenol oxidase and proteolytic enzymes contribute to the simple phenolic conversion and de-polymerization of high molecular weight phenolic compounds [44]. Similar trend of increase in TCP with increasing fermentation time was reported by Ng et al. [60] in conventional legumes.

The production of phenolic compounds in plants during growth and development is a natural process. They help in protecting plants against biotic factors such as diseases, insects and environmental stresses [61,62]. Structural change in phytochemicals during germination process has been considered as a natural phenomenon in plants. In this study, all the legumes under investigation exhibited significant ($p < 0.05$) increase in TPC with increasing germination time (Table 2). The increase ranged from 2.59 to 2.69%, 7.01–8.73%, 3.24–5.95%, 1.42–2.15%, 10.80–10.91%, 0.41–0.66%, 1.24–3.41% and 4.21% in cowpea, bambaranut, red bean, pigeonpea, African breadfruit, African yam bean seed, African oil bean and groundnut, respectively. The increase in polyphenol content in plant materials after germination has been widely reported in oats [63]; peas and beans [45]; lupin seeds [47,64]; peanut [62] and chickpeas [65]. Lopez-Amoros et al. [45] reported that germination qualitatively and quantitatively modifies phenolic compounds in legumes and the change depends on the type of legume and germination conditions. These changes influence the functional properties of the legumes as well its antioxidant capacity. The rise in the phenolic content of germinated seeds has been explained. Sokrab et al. [46] reported that the increase in total phenolic could be as a result of solubilization of condensed tannins when the seeds were soaked in water and the migration of phenolic compounds to the outer layer as a result of germination as indicated by the browning of the germinated seeds [47] (see Table 3) (see Table 4) .

Table 1
Effect of fermentation and germination days on total phenolic content (mg/100 g).

T.	CPB	BBN	RBS	PGP	ABF	AYB	AOB	GGN
F (day)								
Raw	192.43^{a} ±0.00	225.65^{a} ±0.01	212.03^{b} ±0.01	196.33^{a} ±0.01	221.36^{a} ±0.01	196.35^{a} ±0.00	314.26^{a} ±0.00	225.26^{a} ±0.01
2	182.25^{b} ±0.00 (−5.29)	217.59^{b} ±0.01 (−3.57)	218.66^{a} ±0.02 (+3.03)	181.69^{b} ±0.01 (−7.46)	213.15^{b} ±0.00 (−3.22)	194.57^{b} ±0.02 (−0.91)	313.55^{b} ±0.00 (−0.23)	212.59^{b} ±0.01 (−5.62)
3	172.45^{c} ±0.00 (−10.38)	217.17^{c} ±0.02 (−3.76)	218.65^{a} ±0.00 (+3.04)	181.62^{b} ±0.00 (−7.49)	212.56^{c} ±0.01 (−3.98)	193.16^{c} ±0.01 (−1.64)	313.16^{c} ±0.01 (−0.35)	212.17^{b} ±0.02 (−5.81)
4	172.46^{c} ±0.01 (−10.38)	217.16^{c} ±0.01 (−3.76)	218.67^{a} ±0.02 (+3.04)	181.06^{c} ±0.01 (−7.78)	212.57^{c} ±0.01 (−3.97)	192.66^{d} ±0.01 (−1.88)	310.15^{d} ±0.01 (−1.31)	211.41^{c} ±0.00 (−6.15)
G (day)								
Raw	192.43^{a} ±0.00	225.63^{d} ±0.01	212.03^{d} ±0.01	196.33^{c} ±0.02	221.36^{a} ±0.01	196.35^{a} ±0.00	314.26^{c} ±0.00	225.26^{b} ±0.02
2	197.55^{a} ±0.00 (+2.59)	242.63^{c} ±0.00 (+7.01)	219.13^{c} ±0.01 (+3.24)	199.16^{b} ±0.01 (+1.42)	248.16^{b} ±0.01 (−10.80)	197.16^{b} ±0.01 (+0.41)	318.23^{b} ±0.34 (+1.24)	235.16^{a} ±0.01 (+4.21)
3	197.60^{a} ±0.01 (+2.69)	243.25^{b} ±0.00 (+7.24)	221.35^{b} ±0.00 (+4.21)	199.17^{b} ±0.02 (+1.43)	248.45^{a} ±0.00 (+10.90)	197.16^{b} ±0.01 (+0.41)	318.22^{b} ±0.33 (+1.24)	235.15^{a} ±0.01 (+4.21)
4	197.67^{a} ±0.02 (+2.65)	247.21^{a} ±0.01 (+8.73)	225.45^{a} ±0.00 (+5.95)	200.65^{a} ±0.00 (+2.15)	248.46^{a} ±0.01 (+10.91)	197.65^{a} ±0.00 (+0.66)	325.36^{a} ±0.27 (+3.41)	235.16^{a} ±0.01 (+4.21)

Values are means and standard deviations of three determinations. Values not followed by the same superscript in the same column are significantly different ($p < 0.05$). Key: ABF = African breadfruit, BBN = Bambaranut, RBS = Red bean, PGP = Pigeon pea, CPB = Cowpea, AYB = African yam bean seed, AOB = African oil bean and GGN = Groundnut, T = Treatment, G = Germination, F= Fermentation and (−/+) = % decrease/increase.

Table 2
Effect of fermentation and germination days on total tannin content (mg/100 g).

T.	CPB	BBN	RBS	PGP	ABF	AYB	AOB	GGN
F (day)								
Raw	6.11^{a} ±0.01	7.96^{a} ±0.00	6.94^{a} ±0.01	7.01^{a} ±0.01	6.86^{a} ±0.01	5.98^{a} ±0.01	6.25^{a} ±0.00	9.34^{a} ±0.01
2	3.56^{b} ±0.00 (−41.73)	6.66^{b}± 0.02 (−16.33)	6.16^{b} ±0.01 (−11.24)	5.48^{b} ±0.01 (−21.83)	5.96^{b} ±0.01 (−13.11)	4.00^{b} ±0.00 (−33.11)	6.25^{a} ±0.00	6.15^{b} ±0.00 (−34.15)
3	3.15^{c} ±0.00 (−48.44)	3.95^{c} ±0.01 (−50.25)	5.16^{c} ±0.00 (−25.65)	3.96^{c} ±0.01 (−43.51)	3.87^{c} ±0.02 (−43.59)	3.95^{c} ±0.00 (−33.95)	6.11^{b} ±0.01 (−2.24)	3.02^{c} ±0.01 (−67.67)
4	2.96^{d} ± 0.00 (−55.55)	2.96^{d} ±0.01 (−62.81)	5.01^{d} ±0.01 (−27.81)	3.00^{d} ±0.01 (−57.80)	3.44^{d} ±0.00 (−49.85)	3.06^{d} ±0.01 (−48.83)	5.86^{c} ±0.01 (−6.24)	2.98^{d} ±0.01 (−68.09)
G (day)								
Raw	6.11 ±0.01	7.96^{a} ±0.00	6.94^{a} ±0.01	7.01^{a} ±0.01	6.86^{a} ±0.01	5.98^{a} ±0.01	6.25^{a} ±0.00	9.34^{a} ±0.01
2	6.11 ±0.00	6.66^{b} ±0.01 (−16.33)	6.37^{b} ±0.02 (−8.21)	6.36^{b} ±0.02 (−9.27)	4.12^{b} ±0.00 (−39.94)	3.59^{b} ±0.01 (−39.97)	6.24^{a} ±0.00 (−0.16)	4.25^{b} ±0.35 (−54.50)
3	6.10 ±0.01	3.96^{c} ±0.01 (−50.25)	6.26^{c} ±0.01 (−9.80)	3.85^{c} ±0.01 (−45.08)	4.00^{c} ±0.00 (−41.66)	3.43^{c}± 0.01 (−42.64)	6.20^{b} ±0.01 (−0.8)	4.14^{b} ±0.02 (−55.67)
4	6.10 ±0.00	2.96^{d} ±0.01 (−62.81)	6.16^{d} ±0.01 (−11.24)	3.47^{d} ±0.00 (−50.50)	3.45^{d} ±0.00 (−49.71)	3.26^{d}± 0.01 (−45.48)	6.02^{c} ±0.07 (−3.68)	4.14^{b} ±0.01 (−55.67)

Values are means and standard deviations of three determinations. Values not followed by the same superscript in the same column are significantly different ($p < 0.05$). Key: ABF = African breadfruit, BBN = Bambaranut, RBS = Red bean, PGP = Pigeon pea, CPB = Cowpea, AYB = African yam bean seed, AOB = African oil bean and GGN = Groundnut, T = Treatment, G = Germination, F= Fermentation and (−/+) = % decrease/increase.

4.2. Effects of treatments on total tannin content

Serrano et al. [66] reported that the mean daily intake of condensed tannin among the United States population is 53.6 mg/person/day; whereas, among the Spanish is put at 450 mg/person/day. Vadivel and Biesalski [59] reported that there are epidemiological data which strongly suggested that tannin intake might prevent the onset of chronic diseases, attributed to its antioxidant, anticarcinogenic, antimutagenic, antimicrobial antiviral and antidiabetic properties. Chava et al. [67] and Vadivel and Biesalski [68] revealed that high tannin content in seeds is associated with seed colour, cultivar, age of the plant, plant part, stage of development and environmental conditions. The tannin content of the legumes samples (Table 2) were found to be 6.11 mg/100 g, 7.96 mg/100 g, 6.94 mg/100 g, 7.01 mg/100 g, 6.86 mg/100 g, 5.98 mg/100 g, 6.25 mg/100 g and 9.34 mg/100 g in cowpea, bambaranut, red bean, pigeonpea, African breadfruit, African yam bean seed, African oil bean and groundnut, respectively. Groundnut had the highest tannin content (9.34 mg/100 g) while, Africa yam been seed had the lowest value (5.98 mg/100 g).

Fermentation significantly ($p < 0.05$) reduced the tannin content of legume samples with increasing fermentation time. The reduction ranged from 41.73 to 55.55%, 16.33–62.81%, 11.24–27.81%, 21.83–57.20%, 13.11–49.84%, 33.11–48.83%, 2.24–6.24% and 34.15–68.09% in cowpea, bambaranut, red bean, pigeonpea, African breadfruit, African yam bean seed, African oil bean and groundnut, respectively. It can be deduced that fermentation exhibited minimal tannin loss in African oil bean while the highest loss was recorded in groundnut on the fourth day (68.04%). It has been established that fermentation process creates a favourable environment for the generation of endogenous enzymes such as tannase, polyphenolase etc. These enzymes help in breaking down tannin and polyphenols resulting into their degradation, hence, lower recovery [69]. The result of this finding is in line with the report of [69] who reported 33–51%, 69–78%, and 68% tannin reduction in fermented wild legumes, *Bauhinia purpurea* and *Phaseolus vulgaris*, respectively.

Germination exhibits both increase and decrease in tannin content. For example, Fernandez-Orozco et al. [64] reported a high level of tannin increase (53%) in lupin sprout; also, Khattak et al. [65] reported a small rise in the tannin content of chick pea upon germination. A number of authors have observed high level of tannin increase in different germinated legumes [47,67,70]. However, Shimelis and Rakshit [69] reported

Table 3
Effect of fermentation and germination days on total anthocyanin content (mg/100 g).

T.	CPB	BBN	RBS	PGP	ABF	AYB	AOB	GGN
F (day)								
Raw	6.45^{a} ±0.00	8.94^{a} ±0.01	2.41^{a} ±0.01	2.52^{a} ±0.01	2.35^{a} ±0.00	2.30^{a} ±0.00	2.16^{c} ±0.00	12.42^{a} ±0.01
2	1.21^{b} ±0.01 (−81.24)	1.32^{b} ±0.00 (−85.23)	1.83^{b} ±0.01 (−24.07)	1.13^{b} ±0.00 (−51.16)	1.06^{b} ±0.02 (−54.89)	1.66^{b} ±0.01 (−27.83)	3.98^{b} ±0.01 (+45.73)	4.00^{b} ±0.01 (−67.79)
3	1.00^{c} ±0.00 (−84.50)	0.87^{c} ±0.02 (−90.27)	1.22^{c} ±0.00 (−49.38)	0.98^{c} ±0.01 (−61)	1.05^{b} ±0.01 (−55.32)	1.66^{b} ±0.02 (−27.83)	3.98^{b} ±0.01 (+45.73)	1.16^{c} ±0.01 (−90.66)
4	0.96^{d} ±0.01 (−85.12)	0.65^{d} ±0.02 (−90.27)	1.15^{d} ±0.00 (−52.28)	0.96^{c} ±0.01 (−61.90)	0.96^{c} ±0.01 (−59.15)	0.97^{c} ±0.02 (−57.83)	4.21^{a} ±0.01 (+48.69)	1.06^{d} ±0.02 (−91.47)
G (day)								
Raw	6.45^{a} ±0.00	8.94^{a} ±0.01	2.41^{d} ±0.01	11.51^{a} ±0.02	2.35^{a} ±0.00	2.30^{a} ±0.00	2.16^{d} ±0.00	12.42^{a} ±0.01
2	4.83^{b} ±0.01 (−25.12)	1.44^{b} ±0.00 (−83.89)	5.43^{c} ±0.04 (+55.62)	4.12^{b} ±0.00 (−64.21)	1.05^{b} ±0.00 (−55.32)	1.16^{b} ± 0.01 (−49.57)	4.45^{c} ±0.01 (+51.46)	1.04^{b} ±0.01 (−91.63)
3	4.76^{c} ±0.01 (−26.20)	1.72^{c} ±0.02 (−87.47)	6.49^{b} ±0.01 (+62.87)	3.00^{c} ±0.00 (−73.94)	0.98^{c} ±0.01 (−58.30)	0.98^{c}±0.01 (−57.39)	4.76^{b} ±0.01 (+54.62)	0.97^{c} ±0.02 (−92.19)
4	4.45^{d} ±0.00 (−31.01)	1.07^{d} ±0.02 (−88.03)	6.56^{a} ±0.01 (+63.26)	2.55^{d} ±0.01 (−77.85)	0.98^{c} ±0.01 (−58.30)	0.87^{d} ± 0.02 (−62.17)	6.56^{a} ±0.01 (+67.03)	0.88^{d} ±0.02 (−92.91)

Values are means and standard deviations of three determinations. Values not followed by the same superscript in the same column are significantly different ($p < 0.05$). Key: ABF = African breadfruit, BBN = Bambaranut, RBS = Red bean, PGP = Pigeon pea, CPB = Cowpea, AYB = African yam bean seed, AOB = African oil bean and GGN = Groundnut, T = Treatment, G = Germination, F= Fermentation and (−/+) = % decrease/increase.

Table 4
Effect of fermentation and germination days on total carotenoid content (mg/100 g).

T.	CPB	BBN	RBS	PGP	ABF	AYB	AOB	GGN
F (day)								
Raw	0.95^{a} ±0.01	1.94^{a} ±0.01	0.73^{a} ±0.01	0.82^{a} ±0.01	0.72^{a} ±0.00	0.67^{a} ±0.01	0.71^{a} ±0.01	2.46^{a} ±0.01
2	0.44^{b} ±0.02 (−53.68)	0.36^{b} ±0.01 (−81.44)	0.65^{b} ±0.01 (−10.96)	0.65^{b} ±0.00 (−20.73)	0.62^{b} ±0.01 (−13.87)	0.55^{b} ±0.01 (−17.91)	0.68^{b} ±0.01 (−4.23)	0.65^{b} ±0.01 (−73.58)
3	0.40^{c} ±0.00 (−57.89)	0.35^{b} ±0.01 (−81.96)	0.67^{b} ±0.00 (−8.22)	0.60^{c} ±0.00 (−26.83)	0.48^{c} ±0.00 (−33.33)	0.38^{c} ±0.01 (−43.28)	0.68^{b} ±0.01 (−4.23)	0.39^{c} ±0.01 (−84.15)
4	0.38^{c} ±0.00 (−60)	0.35^{b} ±0.01 (−81.96)	0.67^{b} ±0.00 (−8.22)	0.45^{d} ±0.01 (−45.12)	0.34^{d} ±0.01 (−52.78)	0.34^{d} ±0.00 (−43.28)	0.67^{c} ±0.01 (−8.46)	0.35^{d} ±0.01 (−85.77)
G (day)								
Raw	0.95^{a} ±0.01	1.94^{a} ±0.01	0.73^{d} ±0.01	0.82^{a} ±0.01	0.72^{a} ±0.00	0.67^{a} ±0.01	0.71^{d} ±0.00	2.46^{a} ±0.01
2	0.88^{b} ±0.00 (−7.37)	0.74^{b} ±0.01 (−61.86)	1.05^{c} ±0.00 (+30.48)	0.37^{b} ±0.00 (−54.88)	0.41^{b} ±0.00 (−43.06)	0.41^{b} ±0.00 (−38.81)	0.85^{c} ±0.01 (+16.47)	0.42^{b} ±0.01 (−82.93)
3	0.83^{ab} ±0.01 (−12.63)	0.40^{c} ±0.01 (−79.38)	1.12^{b} ±0.00 (+34.82)	0.37^{b} ±0.01 (−54.88)	0.42^{b} ±0.00 (−41.67)	0.36^{c} ±0.01 (−42.27)	0.89^{b} ±0.02 (+20.22)	0.40^{b} ±0.01 (−83.74)
4	0.80^{b} ±0.00 (−15.79)	0.39^{c} ±0.00 (−79.80)	1.92^{a} ±0.00 (+61.98)	0.35^{b} ±0.01 (−57.32)	0.35^{c} ±0.01 (−51.39)	0.34^{c} ±0.01 (−49.25)	1.14^{a} ±0.01 (+37.72)	0.40^{b} ±0.01 (−83.74)

Values are means and standard deviations of three determinations. Values not followed by the same superscript in the same column are significantly different ($p < 0.05$). Key: ABF = African breadfruit, BBN = Bambaranut, RBS = Red bean, PGP = Pigeon pea, CPB = Cowpea, AYB = African yam bean seed, AOB = African oil bean and GGN = Groundnut, T = Treatment, G = Germination, F= Fermentation and (−/+) = % decrease/increase.

96% tannin loss in germinated kidney beans. Similarly, Duenas et al. [47] and Chai [71] reported low level of tannin in lupin seeds and peanut during the first eight days of germination. In this study germination time significantly ($p < 005$) reduced the tannin content of all the samples except in cowpea where germination time had no influence on tannin. The percentage loss in tannin with increasing germination time ranged from 16.33 to 62.81%, 8.21–11.24%, 9.27–50.50%, 39.94–49.71%, 39.97–45.48%, 0.07–3.68% and 54.50–55.67% in bambaranut, red bean, pigeonpea, African breadfruit, African yam bean seed, African oil bean and groundnut, respectively. The reduction in tannin content with increasing germination times has been attribute to a number of factors. Shimelis and Rakshit [69] and Saharan et al. [72] explained that the observed reduction in tannin content after germination is attributed to the formation of tannin-protein, tannin-enzyme complexes in the plant matrix. In addition, the reduction might be due to leaching and binding of tannins with other organic substances such as carbohydrates [72]. Furthermore, during soaking period prior to germination, the enzyme polyphenolase might be activated which results into tannin hydrolysis with consequential loss [70,73].

4.3. Effects of treatments on total anthocyanin content

The composition of anthocyanin depends on some factors such as cultivar, climatic conditions, altitudes as well as storage conditions [74,75]. Food treatments exhibit either decrease or increase in anthocyanin. Tokusoglu and Yildirimz [76] and Sinha et al. [75] reported that cooking, steaming and frying decreased the anthocyanin content in food materials. Yang and Gadi [77] reported that oven drying decreased anthocyanin content while steaming showed increasing effect. In the study by Sinha et al. [75] it was reported that dehydration at 60 °C for 24 h reduced the anthocyanin content by 67%. Lemos et al. [78] assessed the effect of cooking techniques such as boiling, steaming and microwaving on the anthocyanin content of tubers. The result showed an increase in the anthocyanin content of the treated sample compared with the raw samples. In the same vein Leong and Oey [79] showed that processed summer fruits have more anthocyanin content then the raw ones. Anthocyanin are known to be unstable compounds. Food processing, time, storage and temperature are crucial factors that influence the stability of these compounds [80]. These factors can lead to several chemical and enzymatic reactions.

The result of this study showed that fermentation and germination times significantly ($p < 0.05$) affected the anthocyanin content of the samples. The anthocyanin content of the samples were found to be 6.45 mg/100 g, 8.94 mg/100 g, 2.41 mg/100 g, 2.52 mg/100 g, 2.35 mg/100 g, 2.30 mg/100 g, 2.16 mg/100 g and 12.42 mg/100 g in cowpea, bambaranut, red bean, pigeonpea, African breadfruit, African yam bean seed, African oil bean and groundnut, respectively. Fermentation time significantly ($p < 0.05$) reduced the anthocyanin content with increasing fermentation length. The percentage reduction ranged from 81.24 to 85.12%, 85.23–90.27%, 24.07–52.28%, 51.16–61.90%, 54.89–59.15%, 27.83–57.83% and 67.79–91.47% in cowpea bean, bambaranut, red bean, pigeonpea, African breadfruit, African yam bean seed and groundnut, respectively. The reduction with fermentation length agrees with the finding of [38–40]. Morata et al. [41] reported that adsorption mechanism between the fermenting flora and anthocyanin might be responsible for the decrease. Also, during fermentation anthocyanin is hydrolyzed to anthocyanidins which leads to its polymerization to form complex tannins [81]. Furthermore, during fermentation, natural enzymes such as glucosidase, polyphenol oxidase, peroxidase are activated. These enzymes might oxidize secondary metabolites e.g. anthocyanin thereby reducing their concentration. It can also be deduced that, the decrease in the anthocyanin content of the samples in this study could be attributed to its degradation during the adopted drying conditions (80 °C for 24 h) for sample preparations. However, fermentation times significantly ($p < 0.05$) increased the anthocyanin content of African oil bean from 45.73 to 48.69%. The increase in concentration with increasing fermentation time could be attributed to the abundance of acylated anthocyanin in African oil bean, which is reported to be more stable to enzymatic and oxidative reactions.

In the same vein, germination exhibited influence on the anthocyanin content of the samples. The reduction in the anthocyanin content with increasing germination times ranged from 25.12 to 31.01%, 83.89–88.03%, 64.21–77.85%, 55.32–58.32%, 49.57–62.17% and 91.63–92.91% in cowpea bean, bambaranut, pigeonpea, African breadfruit, African yam bean seed and groundnut, respectively. Yudiono and Kurniawati [82] reported a reduction in the anthocyanin profile of germinated pulse. During seed germination, different enzymes system are mobilized and activated and seed colour concentration is reduced most especially the hydrophilic component. These lead to the reduction in the anthocyanin content via oxidation and leaching. Highest percentage lost was observed in groundnut (91.63–92.91%), while, African breadfruit had the lowest lost (25.12–31.01%). There was significant increase in the anthocyanin by 55.62%, 62.87% and 63.26% in red beans at the end of second, third and fourth days of germination, respectively. Equally, African oil bean exhibited significant ($p < 0.05$) increase in the anthocyanin with increase in germination time by 51.46%, 54.62% and 67.03% at the end of second, third and fourth days of germination, respectively.

4.4. Effect of fermentation and germination on total carotenoid content

The carotenoid content of the legume samples were found to be 0.95 mg/100 g, 1.94 mg/100 g, 0.73 mg/100 g, 0.82 mg/100 g, 0.72 mg/100 g, 0.67 mg/100 g, 0.71 mg/100 g and 2.46 mg/100 g in cowpea bean, bambaranut, red bean, pigeonpea, African breadfruit, African yam bean seed, African oil bean and groundnut, respectively. The samples under investigation exhibited lower levels of carotenoid with groundnut (2.46 mg/100 g) having appreciable quantity. Carotenoids are lipophilic plant pigments that are present ubiquitously in nature. They are commonly used as natural pigments in foods and have important biological functions related to their pro-vitamin A activity, antioxidant activity, ability to regulate gene transcription enhancement of gap junction communication, phase II enzyme inducing activity and ability to enhance immense function [83].

Fermentation significantly ($p < 0.05$) reduced the carotenoid content of all the legumes. The carotenoid contents were significantly ($p < 0.05$) reduced from 53.68 to 60%, 81.44–81.96%, 8.22–10.96%, 20.73–45.12%, 13.87–52.78%, 17.91–43.28%, 4.23–8.46%, and 73.58–85.77% in cowpea bean, bambaranut, red bean, pigeonpea, African breadfruit, African yam bean seed, African oil bean and groundnut, respectively. Groundnut had the highest loss (73.58–85.77%) while, African oil bean (4.23–8.45%) had the least loss with increasing fermentation time. The reduction in the carotenoid content with increasing germination time agrees with [84] who reported that fermentation exact reducing effect on the carotenoid of plant materials, however, the mechanisms of the reduction is unknown.

Germination time exhibited both increase and decrease in the carotenoid content of the samples. There was significant ($p < 0.05$) decrease in the carotenoid content with increasing germination length from 7.37 to 15.79%, 61.86–79.30%, 54.88–57.32%, 41.67–51.39%, 38.81–49.25% and 82.93–83.74% in cowpea bean, bambaranut, pigeonpea, African breadfruit, African yam bean seed, and groundnut in the range of, respectively. The decrease in the carotenoid with fermentation time is in line with finding of [85] who reported 37.76% and 28.09% loss in carotenoid after days one and two, respectively in spouted lead tree. The mechanism of loss in carotenoid with increasing germination time is still unknown. Unlike in other legumes red bean and African oil bean exhibited increase in carotenoid with increasing germination length. There were increases of 30.48%, 34.82% and 61.98% at the end of second, third and fourth days of germination, respectively. In the same trend, germinated African oil bean showed increase of 16.47%, 20.22% and 37.72% after the second, third and fourth days of germination, respectively. The increasing level of carotenoid with germination time is attributed to the stability of carotenoid under germination conditions, the release and modification of carotenoid via chemical complexes by the activities of mobilized enzymes thereby creating its isomers and aiding its efficient recovery [70,86].

4.5. Effect of treatment on flavonoid content

The total flavonoid content of treated legumes is shown in Table 5. Treatments applied significantly ($p < 0.05$) influenced the total flavonoid. Groundnut had the highest content 27.16 mg/100 g. This was followed by bambaranut, pigeonpea, red bean, cowpea, African yam bean which had 14.97 mg/100 g, 11.51 mg/100 g, 11.50 mg/100 g, 11.32 mg/100 g and 11.22 mg/100 g, respectively. However, African oil bean seed (3.60 mg/100 g) and African bread fruit (5.21 mg/100 g) had the lowest total flavonoid content.

During fermentation, microbial enzymes such as *glucosidase, amylase, cellulase, tannase, esterase, invatase or lipases* is generated. This helps in the hydrolysis of glycosides and breakdown of plant materials and starch. Activities of these enzymes play a role in the disintegration of plant matrix and consequently facilitating the extraction of flavonoids [43,87]. However, Duenas et al. [47] reported that *β. glucosidase* of microbial origin could hydrolyze phenolics and flavonoids most especially the activities of *L. plantarum*. Therefore, activities of these microbial species can result into either increase or decrease in flavonoids. In this study, fermentation time exhibited significant ($p < 00.5$) decrease in the flavonoid content of all the examples. The percentage loss ranged from 45.30 to 57.77%, 76.95–88.03%, 69.13–70.00%, 69.16–81.23%, 68.46–72.53%, 71.75–75.40%, 1.67–18.61%, and 88.55–89.73% in cowpea, bambaranut, red bean, pigeonpea, African breadfruit, African yam bean seed, African oil bean and groundnut, respectively. It can be deduced that fermentation exhibited high decreasing effect on the flavonoid content in all the legumes. This results disagree with findings of [88] who reported that fermentation increased the total flavonoid content of fermented bambaranut and groundnut. The increase in the flavonoid content with increasing fermentation was attributed to the liberation of bound flavonoid component thereby increasing its bioavailability [89].

Tarsi et al. [90] and Lopez-Amoros et al. [45] indicated that germination modifies both the qualitative and quantitative phenolic profiles of

Table 5
Effect of fermentation and germination on total flavonoid content (mg/100 g).

T.	CPB	BBN	RBS	PGP	ABF	AYB	AOB	GGN
F (day)								
Raw	5.21^{a} ±0.01	14.97^{a} ±0.01	11.50^{a} ±0.01	11.51^{a} ±0.02	11.32^{a} ±0.00	11.22^{a} ±0.01	3.60^{a} ±0.00	27.16^{a} ±0.01
2	2.85^{b} ±0.00 (−45.30)	3.45^{b} ±0.01 (−76.95)	4.16^{b} ±0.01 (−69.16)	3.55^{b} ±0.01 (−69.16)	3.57^{b} ±0.02 (−68.46)	3.17^{b} ±0.01 (−71.75)	3.54^{b} ±0.02 (−1.67)	3.11^{b} ±0.01 (−88.55)
3	2.82^{c} ±0.00 (−45.87)	2.96^{c} ±0.01 (−80.22)	3.55^{c} ±0.00 (−69.13)	2.96^{c} ±0.01 (−74.28)	3.25^{c} ±0.01 (−71.29)	2.90^{c} ±0.00 (−74.15)	2.95^{c} ±0.01 (−18.06)	3.00^{c} ±0.00 (−88.95)
4	2.20^{d} ±0.00 (−57.77)	2.99^{c} ±0.02 (−88.03)	3.45^{d} ±0.01 (−70)	2.16^{d} ±0.00 (−81.23)	3.11^{d} ±0.01 (72.53)	2.76^{d} ±0.01 (−75.40)	2.93^{c} ±0.01 (−18.61)	2.79^{d} ±0.00 (−89.73)
G (day)								
Raw	5.21^{a} ±0.01	14.97^{a} ±0.01	11.50^{a} ±0.01	11.51^{a} ±0.02	11.32^{a} ±0.00	11.22^{a} ±0.01	3.60^{c} ±0.00	27.16^{a} ±0.01
2	4.16^{b} ±0.01 (−20.15)	3.62^{b} ± 0.02 (−75.82)	6.13^{b} ±0.04 (−46.7)	4.12^{b} ±0.00 (−64.21)	3.12^{b} ±0.00 (−72.44)	2.95^{b} ±0.00 (−73.71)	3.93^{b} ±0.01 (+8.40)	3.16^{b} ±0.01 (−88.36)
3	4.03^{c} ±0.01 (−22.65)	2.87^{c}±0.02 (−80.83)	5.30^{c} ±0.07 (−53.91)	3.00^{c} ±0.00 (−73.94)	3.00^{c} ±0.00 (−73.50)	2.76^{c} ±0.01 (–75.40)	4.18^{b} ±0.22 (+13.88)	3.00^{c} ±0.00 (−88.95)
4	3.93^{d} ±0.01 (−24.57)	2.76^{d} ± 0.00 (81.56)	5.13^{d} ±0.04 (−55.39)	2.55^{d} ±0.01 (77.85)	3.00^{c} ±0.00 (−73.50)	2.75^{c} ±0.00 (−75.49)	6.15^{a} ±0.07 (+41.46)	2.65^{d} ±0.01 (−90.24)

Values are means and standard deviations of three determinations. Values not followed by the same superscript in the same column are significantly different ($p < 0.05$).
Key: ABF = African breadfruit, BBN = Bambaranut, RBS = Red bean, PGP = Pigeon pea, CPB = Cowpea, AYB = African yam bean seed, AOB = African oil bean and GGN = Groundnut, T = Treatment, G = Germination, F= Fermentation and (−/+) = % decrease/increase.

legumes. The degree of changes which affect the functionality of the resulting flour depends on the type of legumes and the germinating conditions. In this study germination time negatively affected the total flavonoid content of the legumes except in African oil bean, where there was a steady increase with increasing germination time. The decrease in the flavonoid content with increasing germination time ranged from 20.15 to 24.57%, 75.82–81.56%, 46.70–55.39%, 64.21–77.85%, 72.44–73.5%, 73.71–75.49% and 88.36–90.24 in cowpea, bambaranut, red bean, pigeonpea, African breadfruit, African yam bean seed and groundnut, respectively. While, in African oil bean seed there was increase of 8.40%, 13.88% and 41.46% at the end of second, third and fourth days of germination, respectively.

4.6. Antioxidant capacity and reducing power of the raw and 3-day germinated samples

The antioxidant activities of the raw and 3- days germinated legume samples were evaluated by the DPHH● method and the reducing power assay (Table 6). The result revealed that legume samples exhibited significantly ($p < 0.05$) different antioxidant capacities. Raw African oil bean and groundnut had the highest antioxidant activities 52.16% and 52.17%, respectively. These were followed by raw bambaranut (45.13%) and African breadfruit (35.80%) which ranked third and fourth in antioxidant capacities; however, African yam bean seed had the lowest antioxidant capacity (21.75%). The same trend was observed in the reducing power of the raw samples where groundnut, bambaranut and African breadfruit showed significantly ($p < 0.05$) high reducing powers, 35.17%, 32.27% and 29.86%, respectively; while, African yam bean seed had the lowest reducing power (21.63%).

Three (3) days germination significantly ($p < 0.05$) increased the antioxidant capacity by 53.58%, 18.42%, 52.84%, 43.03%, 17.24%, 53.18%, 14.65% and 14.56% in cowpea bean, bambaranut, red bean, pigeonpea, African breadfruit, African yam bean seed, African oil bean and groundnut, respectively. Cowpea bean had the highest percentage increase after 3 days germination (53.24%), while, groundnut had the least percentage increase (14.56%). After the three days germination, groundnut and African oil bean significantly ($p < 0.05$) had the highest antioxidant capacities 61.06% and 61.11%, respectively. This was followed by red bean (60.12%) and pigeonpea (57.84%). Antioxidant activity is closely related to phenolic content [63,91,92]. In this study groundnut contained the highest concentration of total phenolics, hence, this accounts for it highest antioxidant capacity over other legumes. Three days (3) germination significantly ($p < 0.05$) increased the reducing power of the legumes by 57.25%, 5.09%, 57.14%, 46.85%, 9.30%, 39.48%, 7.18% and 41.44% in cowpea bean, bambaranut, red bean, pigeonpea, African breadfruit, African yam bean seed, African oil bean and groundnut, respectively. Cowpea bean (57.25%) and red bean (57.14%) had the highest increase in reducing power after three days germination; while, bambaranut had the least percentage increase (5.09%). Therefore, at the end of three days germination, groundnut had significantly ($p < 0.05$) high reducing power (60.06%), while African breadfruit had the lowest value (32.92%).

Table 6
Antioxidant capacity and reducing power of the raw and 3-day germinated samples.

Sample	DPPH● of Raw (%)	DPPH● of 3-day germinated (%)	Reducing power of Raw (%)	Reducing power of 3-day germinated (%)
CPB	25.64^{f}±0.02	55.24^{d} ± 0.03 (+53.58)	23.54^{f}±0.02	55.06^{c}±0.08 (+57.25)
BBN	45.13^{b} ± 0.69	55.32^{d} ± 0.11 (+18.42)	32.27^{b} ± 0.02	34.00^{f}±0.00 (+5.09)
RBS	28.35^{e}±0.01	60.12^{b} ± 0.02 (+52.84)	25.49^{e}±0.01	59.47^{b} ± 0.20 (+57.14)
PGP	32.95^{d} ± 0.00	57.84^{c}±0.06 (+43.03)	26.10^{d} ± 0.00	49.11^{d} ± 0.01 (+46.85)
ABF	35.80^{c}±0.00	43.26^{f}±0.26 (+17.24)	29.86^{c}±0.01	32.92^{g} ± 0.07 (+9.30)
AYB	21.75^{g} ± 0.00	46.45^{e}±0.32 (+53.18)	21.63^{h} ± 0.01	35.74^{e}±0.04 (+39.48)
AOB	52.16^{a}±0.00	61.11^{a}±0.13 (+14.65)	22.63^{g} ± 0.01	24.38^{h} ± 0.40 (+7.18)
GGN	52.17^{a}±0.02	61.06^{a}±0.08 (+14.56)	35.17^{a}±0.01	60.06^{a}±0.08 (+41.44)

Values are means and standard deviations of three determinations. Values not followed by the same superscript in the same column are significantly different ($p < 0.05$).
Key: ABF = African breadfruit, BBN = Bambaranut, RBS = Red bean, PGP = Pigeon pea, CPB = Cowpea, AYB = African yam bean seed, AOB = African oil bean, GGN = Groundnut and (−/+ = % decrease/increase).

5. Conclusion

The results of the finding revealed that fermentation and germination influenced the bioactive components studied. There was reduction in total phenolic, tannin, anthocyanin, carotenoid and flavonoid with increasing fermentation and germination time. African oil bean exhibited

increased anthocyanin content with increasing fermentation time, while, red bean, pigeonpea and African oil bean exhibited increased anthocyanin, carotenoid and flavonoids with increasing germination time. African oil bean and groundnut showed superiority in antioxidant activities, while, African yam bean seed was the lowest. Three days germination significantly increased the antioxidant capacities of all the samples with African oil bean having the highest value while African yam bean seed having the least.

Declaration of competing interest

There is no conflict of interest or whatsoever from the authors involved.

Acknowledgements

The authors acknowledged the part funding provided by the Federal Government of Nigeria through the Tertiary Education Trust Fund.

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