# Effect of traditional household processing techniques on phenolic compounds, antioxidants activity and γ-aminobutyric acid of cowpea (*Vigna unguiculata*) pods

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## Abstract

The influence of common home processing methods was investigated on the color characteristics, phenolic component, antioxidant activity (2,2-diphenyl-1-picrylhydrazyl [DPPH] and ferric-reducing antioxidant power [FRAP] activity) and the levels of γ-aminobutyric acid (GABA) in cowpea pods. The processing methods significantly increased the total phenolic content, total flavonoid content and the activity of antioxidant compounds (DPPH and FRAP). The GABA content sharply decreased in pods after boiling and drying treatment. However, it significantly increased after fermentation. The fermented cowpea pods displayed the highest content of phenolics, flavonoids, GABA and anthocyanin as well as antioxidant activity. Hence, these traditional domestic preparation methods could be recommended for the food industry.

*Keywords:* antioxidant activity, cowpea, GABA, phytochemical, traditional processing

## Introduction

Cowpea (*Vigna unguiculata*) belongs to the genus *Vigna*, subfamily Faboideae of the pea family Leguminosae or Fabaceae. Cowpea is native to Sub-Saharan Africa and is widely grown and produced there as well as in other semi-arid tropical and subtropical areas (Brink and Belay, 2006). It consists of two cultivar types: one has long pods used as vegetables, and the other is used for fodder (Morris and Li Wang, 2018). However, different parts of the plant, such as seeds, green pods and leaves, can be used (Lazaridi *et al.*, 2017), but it is commonly consumed as dried seeds. According to Iqbal *et al.* (2006), cowpea is high in proteins, fiber, minerals and vitamins.

According to Affrifah *et al.* (2022), cowpea is rich in indispensable amino acids. Furthermore, it has been observed that cowpea seeds contain a high quantity of phenolic acids and flavonoids as bioactive compounds with high antioxidant activity. By preventing the onset of numerous diseases, such as atherosclerosis, cancer and other chronic human diseases, phenolic and antioxidant substances are vital and crucial compounds for human health (Awika and Duodu, 2017).

As stated formally, the compounds of phenolic nature and those with antioxidant qualities in the lipophilic fraction of 56 vegetables are higher than those in the hydrophilic fraction. Hence, cowpea was considered as one of the vegetable samples with a higher antioxidant activity of 17.01 μmol Fe (II) g fresh weight (FW) and a phenolic content of 8.28 mg gallic acid equivalent (GAE)/g (Deng *et al.*, 2013). However, cowpea (raw) seeds contain many biofunctional, non-nutrient

compounds, such as phytic acid, trypsin and chymotrypsin enzyme inhibitors, classified as antinutritional factors. Accordingly, different traditional and innovative processing methods of cowpea seeds were recommended to enhance their nutritional quality. These methods utilized whole cowpea seeds, flour and processed meals (Affrifah *et al.*, 2022).

In general, processing methods were observed to significantly enhance the capacity of phytochemical (anthocyanin, flavonoid and phenolic) and antioxidant compounds in seeds. Barros *et al.* (2017) investigated changes in antioxidant capacity during cooking of different cowpea cultivars. The authors stated that the total phenolic and antioxidant activity was decreased after cooking (boiling) grains of cowpea cultivars, which could be due to the migration of these compounds through leaching in cooking broth. The fermentation process increased the digestibility of proteins, amino acids and essential dietary minerals in seeds as well as their bioavailability (Hassan *et al.*, 2021). According to Kapravelou *et al.* (2020), natural fermentation significantly improved the nutritional value of cowpea seeds and the digestibility of their essential amino acids. Traditional food preparation methods, such as blanching, drying and fermentation, significantly improved nutritional value of cowpea seeds (Affrifah *et al.*, 2022; Barros *et al.*, 2017).

According to the General Authority for Statistics (2019) bulletins, cowpea grows in different regions of Saudi Arabia, such as AlBaha, Najran and AlQssim (Ministry of Environment, Water, and Agriculture, 2019). Cowpea is considered as one of the typical traditional foods of these areas. It is commonly consumed in AlBaha (a southern province of Saudi Arabia) as boiled green pods. However, in other parts, cowpea meals are prepared in different traditional ways. Despite several investigations evaluating the nutritional value of cowpea, the evaluation of phytochemical compounds, antioxidant activity and γ-aminobutyric acid (GABA) as affected by traditional household methods, particularly blanching, drying and fermentation, has not been investigated. Therefore, determining the impact of conventional preparation methods (such as boiling, followed by drying and fermentation) on the color, total phenolic content (TPC), total flavonoid content (TFC), GABA and anthocyanin contents, and the antioxidant activity of cowpea was the aim of this study.

## Materials and Methods

### Samples collection and preparation

Local Saudi cowpea cultivar harvested in 2022 was obtained from a local market of the AlBaha region (South of Saudi Arabia). The pods were cleaned, inedible part was removed, and stored for the subsequent processing.

### Processing methods

Traditional household methods (blanching, drying and fermentation) were used to process cowpea pods. Boiling of the pods was done traditionally by following the conventional household method used in AlBaha. The raw cowpea pods were boiled in hot water at 1:2 (w/v) ratio for 45 min over medium heat. Following boiling, the water was decanted and the boiled pods were ground and stored in plastic containers at 4°C until further use. A portion of the boiled pods was dried in an oven at 55°C for 9 h before being ground into a fine powder and passed through a 0.5-mm sieve. The dried cowpea flour was kept at 4°C until further use. The cowpea was naturally fermented by adding water in a 1:3 (w/v) ratio and incubated at 37°C for 24 h. Raw pods were made from unprocessed seeds (as a control).

### Color measurement

A colorimeter (Chroma Meter CR 400; brand Minolta, Japan) was used to measure the color of raw and processed samples. A standard white reflector plate was used to calibrate the equipment. The following parameters were measured: $L^*$ (lightness), a*, either negative (green) or positive (red), and b*, either negative (blue) or positive (yellow). The following equation was used to calculate the total color difference (ΔE).

$$\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2}.$$

### Anthocyanin content determination

According to the method described by Egbuna *et al.* (2018), the anthocyanin content of control and processed cowpea samples was measured. Flour, 4 g, was added to 20 mL of methanolic (70%) solution, and the samples were allowed to extraction for 48 h at room temperature. A little above 1 mL of the extract was divided into two test tubes (A1 and A2), each of which contained 1 mL of ethanol HCL (0.01%) solution. Next, 10 mL of 2% HCL (pH 0.8) was added to test tube A1, and 10 mL of citric buffer (0.2-M $Na_2HPO_4$ and 0.1-M citric acid; pH 3.5) was added to test tube A2. For even mixing, both test tubes were rotated at 40 rpm for 2 min. At 520 nm, absorbance was finally measured with 70% methanol used as a blank. The following equation was used to determine the anthocyanin content (AC, μg cyanidin/g):

$$AC = \frac{(Aa - Ab) \times MW \times DF \times CF1 \times CF2}{\varepsilon \times l},$$

where $Aa$ = absorbance at 2% HCL solution, $Ab$ = absorbance at citric buffer solution, $MW$ = molecular weight of cyanidin-3-glucoside (449 g/mol), $DF$ = dilution factor (20 mL/4 g), $CF1$ = 106 (μg/g), $CF2$ = 1 L/1,000 mL, $\varepsilon$ = molar extinction coefficient of cyanidin-3-glucoside (26,900 L/mol.cm) and $l$ = path length (1 cm).

### Determination of GABA content

The technique first described by Zhang *et al.* (2014) was used to measure the amount of GABA in cowpea pods before and after processing. Deionized water (15 mL) was used to extract around 2 g of each sample, which were then centrifuged for 15 min at 10,000 rpm. Using a syringe filter (0.45 mm), the supernatant was filtered, and 0.5 mL of the filtered sample was combined with 0.2-M borate buffer at pH 9.0, 6% phenol and 9% NaClO (0.4 mL). The mixture was heated for 10 min, then cooled to the emergence of blue color. Spectrophotometry at a wavelength of 645 nm was used to quantify the samples of GABA. Various GABA concentrations were used to create a calibration curve ($R^2$ = 0.994).

### Preparation of extracts

The raw and processed cowpea samples were shaken at room temperature for 24 h after being combined with methanol at a ratio of 1:2 (w/v) according to the procedure described by Talhaoui *et al.* (2014). Filter paper (Whatman No. 1) was used to filter the mixture. Leftovers were washed twice with methanol. The extracted samples were then dried with a rotary evaporator and conserved for further investigation.

### Determining total phenolic contents

The Folin–Ciocalteu (F-C) assay was used to quantify the samples' TPC following the procedure adopted by Waterhouse (2002). Briefly, 1.58 mL of water and 100 μL of Folin–Ciocalteu reagent were added to an aliquot (20 μL) of dried extract methanolic solution (ratio 1:10 w/v). Then, the mixture was thoroughly stirred for 10 min and 300 μL of 20% sodium carbonate solution was added. The mixture was incubated for 2 h at 20°C. The absorption was measured at 765 nm relative to a blank solution using an ultraviolet (UV) spectrophotometer. The calibration curve was created using different amounts of gallic acid dissolved in pure methanol ($R^2$ = 0.99743). The amount of total phenol in each gram of dried samples was calculated as milligrams of GAE (mg GAE/g dry weight [DW]).

### Determining total flavonoid contents

According to the colorimetric test (Kim *et al.*, 2003), TFC of cowpea extracts was calculated. Sodium nitrite (5%) solution, 300 μL, was added to 300 μL of aluminum chloride (10%) and 1 mL of methanolic extract. The mixture was incubated at a temperature of 25°C for 5 min. Then 2 mL of sodium hydroxide (1 mol/L) was added to the mixture. The reaction mixture's volume was immediately increased to 10 mL with distilled water and was vortexed completely. The solution's absorbance was measured at 510 nm. Different concentrations of quercetin were used to create a calibration curve ($R^2$ = 0.9761). The TFC was calculated as milligram of catechin equivalent per gram (mg QE/g DW) of dry weight sample.

### Determination of antioxidant activity

#### *Radical scavenging activity*

In order to calculate the antioxidant activity of sample extracts, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals were scavenged in raw and processed cowpea extract samples by following the method described by Chang *et al.* (2001). The extracts and deionized water (as a control) were incubated in 0.1 mL of 50-mM Tris-HCl buffer (pH 7.4) for 30 min at room temperature. Then using an UV-visible (UV-VIS PD-303 UV) spectrophotometer, the absorbance was determined at 517 nm. Trolox equivalent per gram of material (mg TE/g) was computed and used to express DPPH scavenging.

#### *Determination of ferric-reducing antioxidant power (FRAP)*

The FRAP of sample extracts was determined following Benzie and Devaki's (2017) approach. The methanol extract (0.5 mL, diluted for 10 times) was combined with 2-mL FRAP solution, followed by adding 10 mL of water; the mixture was allowed to settle down for 20 min. Finally, using an UV-VIS spectrophotometer to test absorbance at 593 nm against a blank, the result was reported as mg TE/g.

### Statistical analysis

The data were expressed as mean of three determinations. One-way ANOVA was used to evaluate the data, and significant differences ($p < 0.05$) were determined using the least significant difference (LSD). Using the XLSTAT software's PCA algorithms, multivariate analysis was carried out as described by Vidal *et al.* (2020). Using the XLSTAT program, the linear partial least squares regression (PLS) test verified the most effective conventional processing approach, according to Tenenhaus *et al.* (2005).

**Table 1. Effect of traditional processing methods on the color of cowpea pods.**

| Samples | *L** | *a** | *b** | Δ*E* |
|---|---|---|---|---|
| Raw | 41.40 ± 1.212[b] | 1.97 ± 0.643[a] | 25.95 ± 0.964[b] | 0.0 ± 0.000[d] |
| Blanched | 49.19 ± 1.149[a] | 1.35 ± 0.111[a] | 29.35 ± 1.271[a] | 8.52 ± 0.125[b] |
| Dried | 39.49 ± 2.357[c] | 1.37 ± 0.110[a] | 22.18 ± 1.422[c] | 4.27 ± 0.020[c] |
| Fermented | 49.61 ± 0.566[a] | -2.01 ± 0.448[b] | 29.64 ± 1.163[a] | 9.82 ± 0.060[a] |
| *F*-test | ** | ** | ** | ** |
| LSD 0.05 | 2.772 | 0.7532 | 2.290 | 0.132 |

*L*: lightness; Δ*E*: total color difference. Values are mean (±SD) of triplicate samples. Mean values in the same column without superscripts are not significantly ($p < 0.05$) different; ns: no significant difference at ($p < 0.05$) as assessed by least significant difference (LSD).

## Results and Discussion

### Influence of traditional processing on cowpea color

The effects of various home processing techniques on the coordinates of cowpea color (L*, a* and b*) and the overall difference in color (ΔE) of cowpea pods are shown in Table 1. In general, blanching, drying and fermentation significantly ($p < 0.05$) caused a change in color parameters, compared to raw samples. A higher increase was observed in blanched and fermented samples, particularly at a* and b* values. Blanching and fermentation had the respective maximum values for L* (49.19 and 49.61), compared to dried samples (39.49). Interestingly, a* and b* values followed the same manner as that followed by the L* value. The blanched and fermented samples had the highest values than the dried samples. Additionally, the total color difference (ΔE) was significantly different ($p < 0.05$) in processed pods. ΔE was 8.52, 4.27 and 9.82 in blanched, dried and fermented pods, respectively. A significant increase in ΔE was mainly due to the increased changes in L*, a* and b* values in blanched and fermented pods, compared to dried pods.

In this study, cowpea's traditional household cooking methods, particularly drying, were significantly influenced by its lightness, redness and yellowness. For instance, the L* and b* values of dried samples were considerably less than that of control and other treated samples. These results were also reflected in the dried samples' ΔE values, compared to different cooking techniques. It is possible that the observed increase in ΔE in fermented and blanched cowpea meal resulted from water replacing intercellular air and cellular material seeping into water because of ruptured cell membranes (Turkmen *et al.*, 2006).

### Influence of traditional processing on cowpea anthocyanin and GABA contents

The anthocyanin and GABA contents of control and processed cowpea samples are reported in Table 2.

**Table 2. Effect of traditional processing methods on anthocyanin and GABA contents of cowpea pods.**

| Samples | Anthocyanin (mg/g) | GABA (mg/g) |
|---|---|---|
| Raw | 3.69 ± 0.214[c] | 2.16 ± 0.05[b] |
| Blanched | 4.35 ± 0.547[b] | 0.90 ± 0.01[c] |
| Dried | 9.95 ± 0.040[a] | 0.65 ± 0.09[d] |
| Fermented | 4.50 ± 0.053[b] | 4.50 ± 0.02[a] |
| *F*-test | ** | ** |
| LSD 0.05 | 0.557 | 0.103 |

GABA: γ-aminobutyric acid. Values are means (±SD) of triplicate samples. Mean values in the same column without superscripts are not significantly ($p < 0.05$) different; ns: no significant difference at ($p < 0.05$) as assessed by least significant difference (LSD).

The anthocyanin content of cowpea pods was 3.69 mg/g. The content of anthocyanin increased significantly ($p > 0.05$) with each processing technique. The content of anthocyanin was similar in blanched and fermented samples (4.35 and 4.50 mg/g, respectively). However, in dried samples, it was significantly higher, at 9.95 mg/g (Table 2).

In control samples, the GABA content was 2.16 mg/g. Both blanching and drying processes caused a significant ($p < 0.05$) reduction in the GABA content of cowpea pods by 0.90 mg/g and 0.65 mg/g, respectively. However, fermentation significantly increased the content of GABA to 4.50 mg/g.

GABA, responsible for significant health functions, is an inhibitory neurotransmitter in humans and animals (Komatsuzaki *et al.*, 2007). Generally, in plants, GABA concentration varies from 2 nmol/g to 700 nmol/g. However, owing to its extensive bioactivities, a rising awareness is observed about its availability in foods (Poojary *et al.*, 2017). Hence, several processing techniques have been performed to improve GABA content in foods.

Elbaloula and Hassan (2022) stated that germination of sorghum significantly enhanced GABA concentration in

sorghum grain, particularly under different salt concentrations. Moreover, fermentation was also detected as a positive method for enhancing GABA concentration in food (Liao *et al.*, 2013; Seo *et al.*, 2013). These statements were in agreement with our findings, which stated that higher values of GABA were noted in fermented cow pea samples. Anthocyanin was also affected by the traditional cooking methods of cowpea. It significantly increased in processed cowpea samples. Further, drying of cowpea almost increased the content of anthocyanin by 100%, compared to other household preparation methods.

### Influence of traditional processing on TPC of cowpea

The impact of conventional home approach on TPC (mg GAE/g) and TFC (mg QE/g) is shown in Figures 1a and 1b. The TPC of raw cowpea pods was 5.75 mg GAE/g. It was evident that TPC of samples increased due to typical processing techniques. TPC increased significantly ($p < 0.05$) during drying and fermentation, reaching 8.86 mg GAE/g and 7.56 mg GAE/g, respectively (Figure 1A).

Similarly, the traditional methods of preparation also significantly affected the TFC of cowpea pods ($p < 0.05$). Prior to processing, the TFC was 5.72 mg QE/g (Figure 1B). Balancing of pods caused a slight increment in TFC to 6.09 mg QE/g. However, sharp increase in TFC was observed when the pods were dried and fermented ($p < 0.05$), with the respective values of 27.61 mg QE/g and 35.89 mg QE/g (Figure 1B).

Increase in the TPC of processed samples could be due to bound phenolic compounds that were released and then collected by extending the time of the drying process, making them more extractable. Yadav *et al.* (2018) observed a similar result. The TPC was increased after using autoclaving and microwave treatment for 30 min, compared to 15 min.

The TPC values of raw pods reported in this study were lower than those (8.28 mg GAE/g) observed by Deng *et al.* (2013). Conversely, obtained results did not agree with the findings of Barros *et al.* (2017), who reported that the phenolic compounds were reduced after cooking (boiling) cowpea grains cultivars because of migration of soluble phenolic compounds through leaching in cooking broth. However, variation in TPC could be attributed to different factors associated with cowpea cultivars, maturity at harvest, environmental conditions, solvent type, assay procedure and interaction of phenolic compounds with other food contents (Marathe *et al.*, 2011). On the other hand, increase in TPC in fermented samples was caused by microbial enzymes that break down cell walls to release bioactive compounds (Hassan *et al.*, 2021).

Regarding TFC, the findings of this study differed from the results of Yadav *et al.* (2018), who observed that both boiling and fermentation had a negative effect on TFC. Instead, the authors attributed reduction to the leaching of flavonoids to cooking water and flavonoid consumption by growth microorganisms during fermentation. Nevertheless, the traditional household processing of cowpea meal significantly enhanced both TPC and TFC, particularly during drying and fermentation. Hence, both processing methods could be recommended for the food industry.

### Influence of traditional processing on antioxidant activity of cowpea

Figures 2A and 2B illustrate how the antioxidant activity of unprocessed and processed cowpea pods was

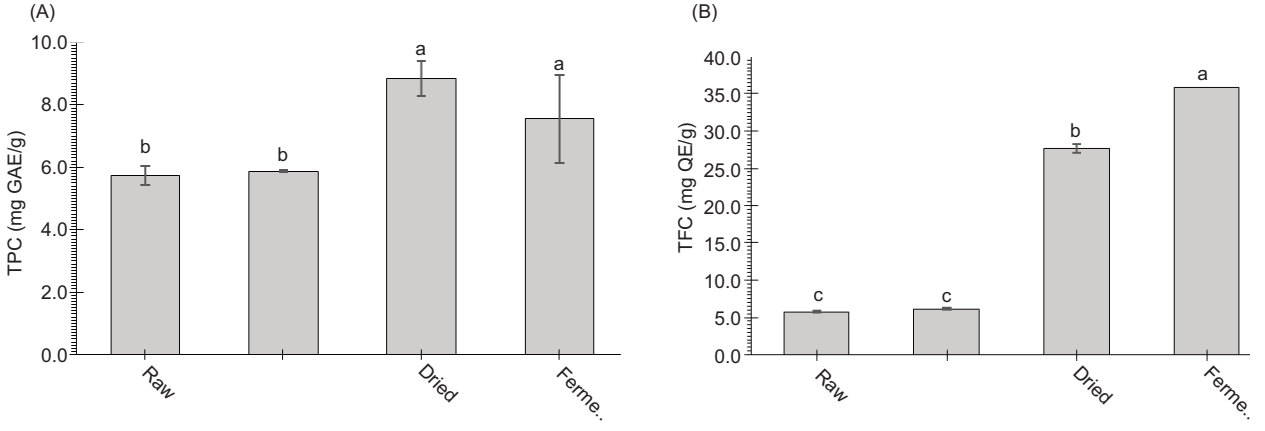


**Figure 1. Effect of traditional processing methods on the (A) total phenolic content (TPC, mg GAE/g) and (B) total flavonoid content (TFC, mg QE/g) of cowpea. Values are mean (±SD) of triplicate samples. Values with the same letters are not significantly different ($p > 0.05$) as assessed by least significant difference (LSD).**

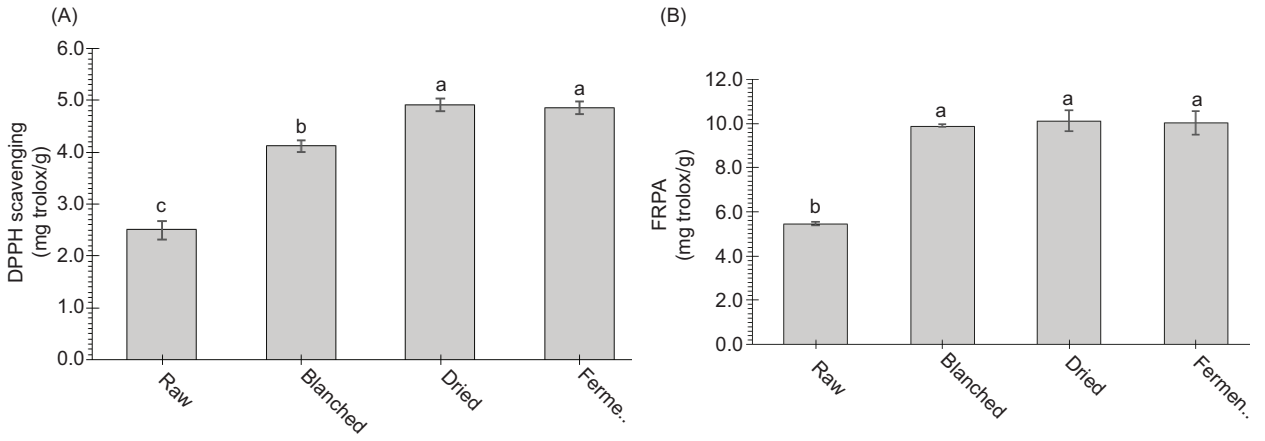


**Figure 2. Effect of traditional processing methods on the (A) DPPH scavenging assay (mg Trolox/g) and (B) ferric reducing power assay (FRAB, mg Trolox/g) of cowpea samples. Values are mean (±SD) of triplicate samples. Values with the same letters are not significantly different ($p > 0.05$) as assessed by least significant difference (LSD).**

evaluated in terms of FRAP and their capacity to scavenge free radicals. The impact of conventional blanching, drying and spontaneous fermentation on the DPPH scavenging activity of cowpea pods is shown in Figure 2A. Untreated pods had a DPPH of 2.5 mg TE/g. It was shown that the processing approach increased the DPPH activity of pods considerably ($p < 0.05$). Maximum DPPH activity was observed in dried (4.92 mg TE/g) and fermented (4.86 mg TE/g) samples.

Figure 2B displays the FRAP values of raw and processed pods. The procedure increased the FRAP of pods significantly ($p < 0.05$), as observed in the figure. It was 5.44 mg TE/g before processing but increased dramatically after blanching (8.88 mg TE/g), drying (10.11 mg TE/g) and fermentation (10.61 mg TE/g) of cowpea samples.

In general, the findings of this study demonstrated significant differences ($p < 0.05$) in DPPH and FRAP assays between raw and processed cowpea pod samples, particularly during fermentation. Therefore, improving the antioxidant activity of fermented cowpea pods could increase both TPC and TFC. Moreover, during fermentation, aglycone release from hydrolyzed phenolic glycosides by microbial enzymes contributed to increased antioxidant activity. Furthermore, fermentation encouraged the structural collapse of plant cell walls, releasing numerous antioxidant complexes (Hur *et al.*, 2014). Similarly, Adetuyi and Ibrahim (2014) discovered that the antioxidant activity was significantly increased in okra seeds after 48-h fermentation.

### Multivariate analysis

The principle component of analysis (PCA) biplot revealed an apparent clustering of the effect of traditional household preparation methods of cowpea on color, phytochemical compounds, antioxidant activity and GABA content (Figure 3). The plot showed that the first two principal components accounted for 48.01% of total variance. Principal component (PC1) showed 61.15% variation and PC2 accounted for 22.86% of total variation. The PCA factor showed that TPC, TFC, DPPH, FRAP, anthocyanin and color differences ($\Delta E$) were significantly associated for with PC1. In contrast, GABA was highly correlated with PC2. However, PCA displayed a strong positive correlation between TPC, TFC, DPPH, FRAP, anthocyanin, $\Delta E$ and GABA with fermented cowpea pods. These interpretations stated that fermentation improved the accumulation of health-promoting phytochemicals, antioxidant activity and GABA of cowpea pods.

The partial least squares (PLS) regression test analysis described the interactive impact of traditional preparation methods ($x$ variables) on the stated factors ($y$ variables) of cowpea samples (Figure 4). Referring to the PLS model, fermented cowpea pods showed a positive validation score for TPC, TFC, DPPH, FRAP, anthocyanin content, $\Delta E$ and GABA content, reflected as the most valid scores. This validation revealed that traditional fermentation could significantly improve the health-promoting metabolites of traditional cowpea meals.

## Conclusions

Cowpea pods contain a high amount of phenolic compounds with high antioxidant activity and GABA content. Significant improvement was observed in TPC, TFC, antioxidant activity (DPPH and FRAP), and anthocyanin and GABA contents during the traditional processing of cowpea, especially fermentation. As a result,

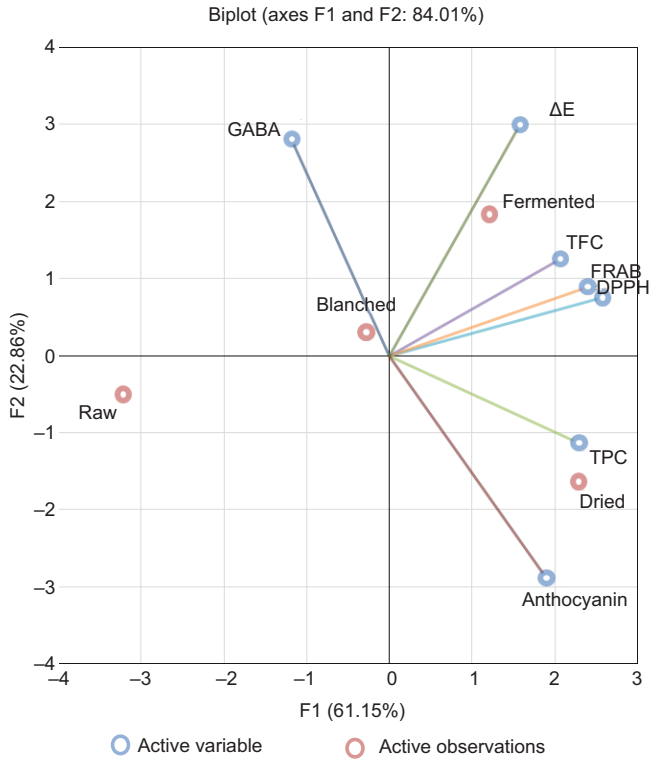


**Figure 3. The principle component of analysis (PCA) of TPC, TFC, DPPH, FRAB, anthocyanin content, GABA and Δ*E* of raw, blanched, fermented and dried cowpea pods.**

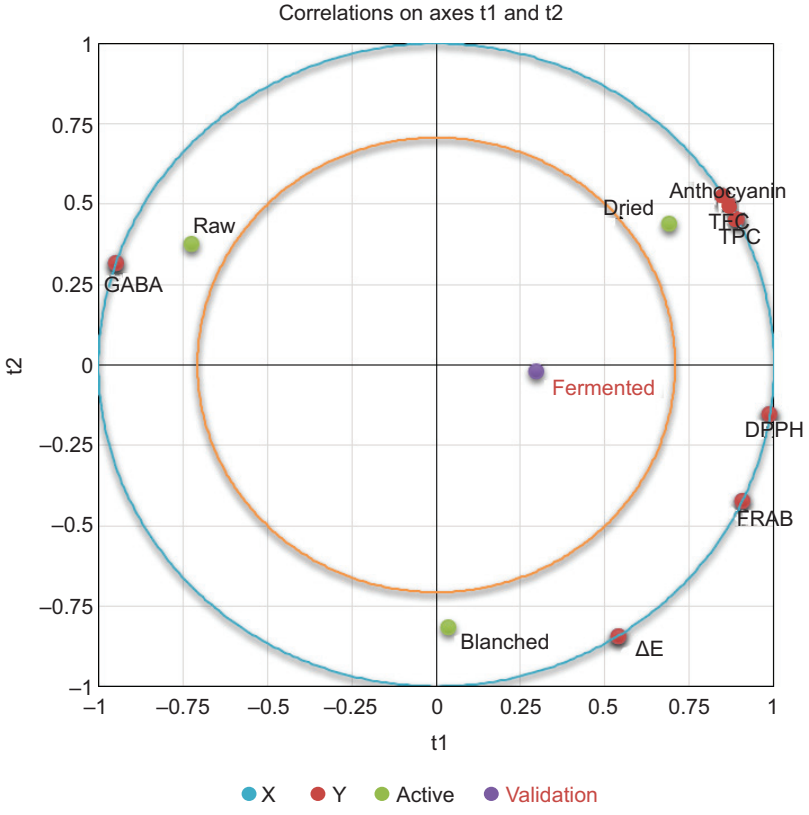


**Figure 4. The partial least squares (PLS) regression test analysis of TPC, TFC, DPPH, FRAB, anthocyanin content, Δ*E* and GABA content of raw, blanched, fermented and dried cowpea samples.**

these traditional methods, particularly fermentation, could be considered for use in the food industry as a traditional method for enriching food with secondary metabolism compounds and antioxidants.

## Data Availability Statement

The data used to support the findings of this study are included in the article.

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## Conflict of Interest

The authors declared no conflict of interest.

## Author Contributions

Fatima Ali AlGhamdi: Data curation, Visualization. Amro B. Hassan: Methodology, Writing review and editing. Nora Abdullah AlFaris: Data curation and review and editing last draft. Jozaa Zaidan AlTamiami Soft ware and Funding acquisition. All authors have read and agreed to the published version of the manuscript.

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