Effect of thermal and non-thermal processing on antioxidant potential of cowpea seeds

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ABSTRACT

Legumes are a good source of bioactive components, besides being nutrient dense. These components have antioxidant properties and have a protective role against free radicals. Legumes like cowpeas are usually consumed after certain processing, so the present study was intended to assess antioxidant property of selected four cultivars of cowpea after going through various thermal (autoclaving, microwave, boiling, and roasting) and non-thermal (soaking and fermentation) processings. Thermal processing reduced total phenolic content (TPC), total flavonoid content (TFC), and ferric reducing antioxidant power (FRAP) in all cultivars with respect to antioxidant activity of raw cultivars, while DPPH scavenging activity increased after all thermal treatments excluding few thermal processings. Non-thermal processing revealed increase in TPC, DPPH scavenging activity, and FRAP values, while TFC content showed a decreasing trend. A moderately high correlation between TPC and DPPH scavenging activity was observed in both kinds of thermal treatments indicating the role of phenolic compounds for antioxidant activity. It was concluded from the study that fermentation processing has promising effects on retention and enhancement of antioxidant activity of cowpea cultivars.

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Introduction

Legumes are economically nutrient-dense sources of protein, carbohydrate, dietary fiber along with minerals and vitamins. Besides these nutrients, legumes also serve as a rich source of bioactive components which include different type of phenolics and flavonoid-like compounds. Also, they have proven to be beneficial in working against oxidative damage associated with cardiovascular diseases and other lifestyle-related diseases. Various researches have clearly reflected the antioxidant properties of these biologically active components in various legumes and pulses by means of various antioxidant parameters like total phenolic content, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, ferric reducing antioxidant power (FRAP), and total flavonoid content (TFC).

Dry legumes are usually processed prior to consumption, so that they become chewable, palatable, and easily digestible. For instance, soaking, which is a preliminary processing step, helps to soften the legume seeds and cooking improves their nutritive value by reduction of antinutrients such as phytic acid and tannins along with increasing digestibility of complex carbohydrate and protein.

Cowpea (Vigna unguiculata (L.) Walp) is one of the most important and nutritious leguminous crops consumed worldwide. Like other pulses, they also contain many anti-nutritional factors which limits their consumption; therefore, efficient cooking techniques are used to bring some desirable changes to cowpea seeds. The seeds of cowpea contain various polyphenolic compounds which are known for their antioxidant property. Conventional methods of thermal processing like roasting,
boiling, and pressure cooking have been applied on seeds besides milling of pulse seeds to flour for
decades. Cowpeas are consumed worldwide in preparations of curries, soups, salads, and stews.
Some of the popular recipes of cowpea are Akara, Moyin-moyin, Rupiza, and Hoppin’ John.
Fermentation is also known to increase nutritional quality and remove undesirable factors of
cowpea. Onoja et al. [6] and Ritika et al. [7] utilized naturally fermented cowpea flour to develop
bread and noodles, respectively. Electro technologies like microwave treatment is also being adopted
as a popular method to ease cooking. All such processes can bring different physicochemical and
structural changes because availability of the nutrients not only depends upon content in the seed
but also on various interactive effects with other food constituents.

Processing also affects the antioxidant potential of legumes. Previous researches have explored effect
of cooking on the antioxidant activity of legumes and pulses [8–10], but data on antioxidant activities after
different kinds of processing at various duration and time intervals on certain Indian cowpea cultivars
are not available. So, the present study focuses on application of various thermal and non-thermal
processing treatments, at different time durations and intervals, on four selected cowpea cultivars grown
in India and the quantification of the changes that occurred with respect to the extent of antioxidant
activities, change in phenolic and flavonoid contents after the above-mentioned treatments.

Materials and methods

Procurements of cowpea cultivars

Four dried seeds of cowpea cultivars namely EC4216, BL2, Kohinoor, and Gomati were procured
from Indian Grassland of Forest Research Institute (IGFRI), Jhansi.

Processing of samples

Cowpea seeds were subjected to following thermal and non-thermal processing treatments:

Boiling

Boiling was done according to Hefnawy. [11] Whole cowpea seeds were taken and weighed (25 g). Distilled water was added in the ratio of 1:10 (w/v) and it was boiled on a hot plate for 90 min. Excess water left after boiling was drained off.

Autoclaving

Whole seeds of cowpea were weighed (25 g) and taken in a conical flask and distilled water was
added in the ratio of 1:10 (w/v). Flask was sealed with cotton plug along with brown paper and it was
heated in a vertical autoclave (Metrex) at 1.05 kg/cm² pressure, and a temperature of 121°C was
maintained. The samples were kept for varying time intervals: 15 min and 30 min, later the
remaining water was drained off. [12]

Microwave

Whole seeds were heated in a microwave oven (LG Grill Intellowave), previously set at 800 W. Distilled water was added in the ratio of 1:15 (w/v) for both 15 and 30 min time intervals. Water left in the container was later drained off. [12]
Roasting

Roasting was done according to the method of Ee et al.\textsuperscript{[13]} with some modifications. About 5 g cowpea seed sample was weighed and kept in hot air oven (Webcon) preheated to 160°C for 45 min.

Soaking

Cowpea seed samples were soaked in distilled water (1:10), at room temperature (25–30°C), for 6 h, and excess water was discarded.

Fermentation

Cowpea seeds were finely grounded into flour in an electric blender and passed through a 0.5-mm mesh standard sieve. A total of 25 g of sample was packed in a sterilized container and distilled water was added in the ratio of 1:2. Samples were kept in an incubator set at 37°C for time intervals: 16, 24, 32, and 40 h and allowed to naturally ferment. Thereafter, fermented cowpea was dried onto sterilized petridishes in the hot air oven (Webcon) at 50°C and then powdered in a blender (Butterfly Grand) for subsequent analysis.

Sample preparation for analysis

Raw cowpea and hot air oven-dried (50°C) processed samples were finely grounded in the blender (Butterfly Grand) and passed through 0.5-mm mesh sieve. All the analyses were carried out in triplicates.

Antioxidant analysis of cowpea cultivars

Total Phenolic Content

Analysis of TPC was determined by Folin Ciocalteau method as described in ISO 2005.\textsuperscript{[14]} Methanolic extracts of samples were prepared by mixing 5 ml methanol in known weight of duplicate samples which were heated in water bath for 30 min at 70°C, and henceforth supernatant was collected. A total of 1 ml of methanolic extracts of cowpea seeds was taken in a test tube and 5 ml of diluted Folin Ciocalteau reagent (1:10 with distilled water) was added. Thereafter, 4 ml of sodium carbonate (7.5% w/v) was also added and mixed together. The tubes were kept in dark at room temperature for 60 min. The absorbance was measured in UV-visible spectrophotometer (Thermo Scientific, model-Evolution600) at 765 nm against blank as standard. A standard curve was prepared with “gallic acid,” and results were expressed in terms of mg per 100 g of polyphenol present in the sample.

Radical scavenging activity

The free radical scavenging activity of the extracts was measured using the DPPH radical (1, 1- diphenyl 2- picrylhydrazyl) by method of Brand-Williams et al.\textsuperscript{[15]} with slight modification. A total of 10 mg of cowpea seed flour was mixed with 10 ml acidified methanol and heated at 40°C in water bath for 20 min. On the whole, 100 µl of sample extract prepared was put in a test tube and then diluted with 2.9 ml of pure methanol. The resultant sample mix was mixed with 150 µl of DPPH solution (4.3 mg in 3.3 ml methanol) which also served as a control with same concentration. It was then incubated for 15 min in dark, and the decrease in absorbance was measured at 515 nm with the help of UV-visible spectrophotometer. The percentage radical scavenging activity was calculated using the following formula:

\[
\text{Control Absorbance – Sample Absorbance/Control Absorbance} \times 100
\]  
\text{(Eq.1)}
Ferric Reducing Antioxidant power (FRAP)

200 µl of methanolic extract of each cowpea seed flour sample was mixed with 1.3 ml of freshly prepared FRAP reagent and kept incubated at 37°C for 30 min. Absorption of samples was measured at 595 nm using spectrophotometer (Thermo Scientific, model-Evolution 600). The changes in the absorbance of test mixture were compared with standard mixture of heptahydrate ferrous sulfate (0.1 mm/L–1.0 mm/L). FRAP values were expressed as mmol of Fe (II) equivalent/g flour. [16]

Total flavonoid content

Ethanolic extracts (2 ml) were mixed with 150 µl of sodium nitrite (5%). After 5 min, 150 µl of aluminum chloride (10%) was added. Then after an interval of 10 min, 1 ml of 1M sodium hydroxide and 1.2 ml of distilled water were added in the mixture. The mixture was vortexed and incubated for 10 min, and then the absorbance was read at 510 nm by spectrophotometer. A calibration curve was prepared using a standard solution of quercetin (0.05–0.5 mg/ml). Final results were expressed as mg quercetin equivalents/g (QE) of sample. [17]

Statistical analysis

All the determinations were carried out in triplicates and results were presented as mean along with their standard deviation (mean ± SD). Statistical Package of Social Sciences (SPSS), a software programme for Windows (version 16.0), was used for conducting one-way analysis of Variance (ANOVA). The Duncan’s procedure was used to compare means and for determining significant difference at 5% significance level (p < 0.05). Pearson’s correlation coefficient (r) was also performed to evaluate the relationships among raw and processed cowpea cultivars.

Results and discussion

Total phenolic content

TPC in cowpea cultivars after thermal processing is exhibited in Table 1. A reduction in TPC was found in cowpea cultivars which were subjected to thermal treatments like boiling, autoclaving, microwave, and roasting for various duration and intervals of cooking by using different methods. Boiling of cowpea seeds for 90 min showed significant (p < 0.05) decrease in TPC of two of the studied cowpea cultivars (EC4216 and BL2), while Kohinoor cultivar showed 51.29% reduction after it was roasted for 45 min. The TPC content in Gomati cultivar was reduced by 82.82% and 86.10% after autoclaving for 15 and 30 min, respectively. The highest loss in TPC was recorded in autoclaving followed by microwave, boiling, and roasting treatments. Diminishing percentage of TPC during hydrothermal processing could be a result of leaching out of phenolic compounds from seed to water, which was perceived during the study. Similar kinds of results of decreasing TPC were also observed by Xu and Chang [9] on cool season food legumes and by Maheshu et al. [18] on pressure cooked field beans (Dolichos lablab). However, on extending the time interval up to 30 min in autoclaving and microwave process, a drastic leap in TPC was observed with respect to 15 min of autoclaving and microwave treatment in all cultivars of cowpea. This may be due to the fact that on increasing the duration, bound phenolics from the cowpea seeds might have been released and been collected at the seed coat which on determination of TPC had shown higher levels in respect to the same process carried out for 15 min. Ideally, the TPC of sample of 30 min of autoclaving/microwave should contain more TPC than raw samples. However, it was not reflected in the results. It might be due to leaching of soluble phenolics in boiling water, which were discarded as mentioned in methodology. Dewanto et al. [19] also explained that prolonged thermal processing can release more bound phenolics due to breakdown of the cellular components of the seeds, thus increasing TPC. Roasting of cowpea cultivars in hot air oven at 160°C for 45 min degraded the phenolic content
| Cultivars | Raw       | Autoclave 15 min | Autoclave 30 min | Microwave 15 min | Microwave 30 min | Boiling 90 min | Roasting 45 min | Soaking 6 h | Soaking 16 h | Soaking 24 h | Soaking 32 h | Soaking 40 h | Fermentation 15 min | Fermentation 30 min | Fermentation 45 min | Fermentation 60 min | Fermentation 90 min | Fermentation 120 min | Fermentation 180 min |
|----------|-----------|-----------------|-----------------|-----------------|-----------------|---------------|----------------|-------------|--------------|--------------|--------------|--------------|-------------------|-------------------|----------------|------------------|------------------|------------------|------------------|
| EC-4216  | 147.3 ± 2.49 | 77.3 ± 5.23     | 106.9 ± 6.30    | 69.9 ± 3.30     | 110.6 ± 1.39    | 50.7 ± 3.91    | 100.7 ± 5.99  | 99.7 ± 7.74  | 156.4 ± 8.51 | 150.8 ± 6.25 | 108.0 ± 7.19 | 92.9 ± 9.04   | 47.48%            | 27.40%            | 52.53%          | 24.88%          | 65.54%          | 31.64%          | 32.29%          |
| BL-2     | 187.3 ± 3.29  | 68.8 ± 6.24     | 82.9 ± 3.50     | 47.6 ± 1.46     | 643 ± 1.04      | 49.2 ± 6.25    | 70.2 ± 2.32   | 86.0 ± 2.92  | 159.1 ± 4.02 | 153.5 ± 13.17| 119.8 ± 7.34 | 96.5 ± 3.2     | 63.27%            | 55.70%            | 74.55%          | 65.65%          | 73.69%          | 62.47%          | 54.05%          |
| Kohinoor | 113.3 ± 2.39  | 64.9 ± 1.99     | 82.9 ± 3.50     | 67.7 ± 5.45     | 125.4 ± 3.64    | 109.5 ± 1.66   | 55.2 ± 5.43   | 77.8 ± 3.00  | 160.7 ± 11.36 | 154.4 ± 31.86| 99.2 ± 9.31  | 90.4 ± 2.5     | 42.73%            | 26.82%            | 40.21%          | 10.65%          | 3.36%           | 51.29%          | 31.34%          |
| Gomati   | 78.3 ± 0.47   | 13.4 ± 0.10     | 10.8 ± 0.64     | 23.0 ± 1.76     | 62.2 ± 5.74     | 36. ± 3.74     | 63.9 ± 4.19   | 51.9 ± 4.79  | 122.1 ± 8.86  | 104.1 ± 2.97 | 93.6 ± 9.90 | 90.4 ± 2.5     | 82.82%            | 86.10%            | 70.52%          | 20.56%          | 53.48%          | 18.28%          | 33.63%          |

All data are the mean ± SD of three replicates.
For each cowpea cultivars, means within a row not sharing a common letter differ significantly \( p < 0.05 \).
Values given in bracket are percentage differences calculated from raw values.
of the seeds due to high roasting temperature. Similar results were also obtained by Vadivel et al. [20] after open pan roasting of *Canavalia ensiformis*.

Table 1 shows the effect of non-thermal processing on TPC of cowpea cultivars. The soaking of cowpea reduced TPC in all cultivars. Totally, 54.05% reduction in TPC was observed in BL2 cultivar followed by Gomati, EC4216, and Kohinoor. Significant (*p* < 0.05) decrease was observed in all cultivars of cowpea after soaking, because the hydration of seeds resulted in loss of phenolic compounds from seeds due to their leaching out in water. Natural fermentation increased TPC in Gomati cultivar, while it led to decrease of TPC in BL2 cultivar.

However, in Kohinoor and EC4216 cultivar, TPC was increased, respectively, to 41.78% and 6.1% after 16 h of fermentation. Natural Fermentation for 16 h and 24 h was found to be adequate and an effective process for increasing the TPC in most of the cowpea cultivars under observation. An increase in TPC might be connected to lactic acid fermentation caused by microbial enzymes which causes depolymerization of higher molecular weight phenolic compounds to simple phenolic monomers like catechin. Metabolic activity of microbes can also change the level of bioactive compounds and also fermentation caused by lactic acid bacteria can further breakdown cell walls of seed and can lead to liberation or synthesis of various bioactive compounds. [21] Further, as explained by Liu et al. [22], breakdown caused by fermentation can ease the interaction between polyphenol and protein of cowpea flour, and therefore such protein bound polyphenols were changed to free phenolic compounds causing increase in TPC as observed in Table 1 leading to increased antioxidant activity. However, a gradual decrease was noticed when cowpea seeds were fermented for 32 h and 40 h when compared with raw as well as seeds fermented for previous durations. This may be due to the rise of polyphenol oxidase activity in fermented seed tissues. [23] Similar results were observed in grass pea (*Lathyrus sativa*) when it was inoculated with *L. plantarum* for fermentation of seed flour. [24] Thus, fermentation process for 16 and 24 h can lead to an increase in presence of total polyphenols which can also lead to increase in antioxidant activity of cowpea flours making it a better functional food.

**DPPH radical scavenging activity**

Radical scavenging activity of methanolic extracts of raw and thermally processed cowpea is given in Table 2, which was determined by DPPH radical. The presented results revealed an increasing trend in DPPH radical scavenging activity. Cowpea cultivars like Kohinoor and Gomati reflected significant (*p* < 0.05) increase in DPPH scavenging activity after all kinds of thermal processing. However, 15 min of autoclaving and microwave treatment of EC4216 cultivar, respectively, resulted in 0.99% and 3.28% decrease in percentage of radical scavenging activity. BL2 cultivar also showed a reduction of 1.36% after 30 min of autoclaving and up to 7.4% reduction after 15 min of microwave treatment. This increase in antioxidant activity can be explained on the basis of temperature and time of cooking. These phenolic compounds, especially tannins, are also likely to form insoluble complexes with protein of cowpea seed under some thermal and autoclaving conditions. As a result of such formation, some of the TPCs are retained in seed itself as depicted in Table 1.[5] Further, with increasing the duration up to 30 min of microwave, autoclaving, and also 90 min of boiling, a higher antioxidant activity was observed which could be possibly due to evaporation of most of the cooking water during the processing which led to concentration of phenolic compounds on the seed coat (Table 1). Similar kind of results were reported by Rocha-Guzman et al. [25] on beans (*L. Phaseolus vulgaris*), where a significant increase in DPPH radical scavenging was observed when beans were not soaked and cooking water was also not drained. Roasting treatment for 45 min might have formed Maillard products such as HMF (5-hydroxymethyl-2-furfuraldehyde) which rendered high-antioxidant activity to the cowpea seeds. [26] However, a reduction in antioxidant activity, as observed in two of the studied cultivars (EC4216 and BL2), can be explained as a result of leaching out of phenolic compounds from seeds due to heat applied by various methods (autoclave and microwave) as explained by Xu and Chang [9] in their study on effect of soaking, boiling, and
Table 2. Effect of thermal and non-thermal processing on DPPH radical scavenging activity (%) of cowpea cultivars.

| Cultivars | Raw     | Thermal processing | Non-thermal processing | Soaking 6 h | 16 h | 24 h | 32 h | 40 h |
|-----------|---------|--------------------|------------------------|-------------|------|------|------|------|
|           |         | Autoclave 15 min   | Microwave 15 min       |             |      |      |      |      |
| EC-4216   | 87.5 ± 4.5<sup>bc</sup> | 86.63 ± 1.80<sup>b</sup> | 94.56 ± 1.19<sup>d</sup> | 86.74 ± 1.80<sup>cd</sup> | 93.20 ± 0.67<sup>b</sup> | 92.78 ± 0.67<sup>b</sup> | 93.44 ± 0.67<sup>b</sup> | 93.20 ± 0.67<sup>b</sup> | 93.50 ± 0.67<sup>b</sup> | 92.82 ± 0.67<sup>b</sup> | 91.87 ± 0.67<sup>b</sup> |
|           |         | Autoclave 30 min   | Microwave 30 min       |             |      |      |      |      |
| BL-2      | 88.9 ± 4.8<sup>d</sup> | 87.69 ± 1.19<sup>ab</sup> | 82.31 ± 1.43<sup>d</sup> | 89.50 ± 2.81<sup>b</sup> | 95.33 ± 0.42<sup>bc</sup> | 94.88 ± 0.42<sup>cd</sup> | 94.88 ± 0.42<sup>cd</sup> | 95.33 ± 0.42<sup>cd</sup> | 93.53 ± 0.42<sup>cd</sup> | 93.53 ± 0.42<sup>cd</sup> | 95.33 ± 0.42<sup>cd</sup> |
|           |         | Boiling 90 min    | Roasting 45 min        |             |      |      |      |      |
| Kohinoor  | 79.7 ± 2.9<sup>a</sup> | 79.41 ± 7.01<sup>bc</sup> | 89.48 ± 8.19<sup>c</sup> | 88.59 ± 0.93<sup>a</sup> | 94.15 ± 0.12<sup>b</sup> | 93.57 ± 0.12<sup>b</sup> | 92.80 ± 0.12<sup>b</sup> | 80.12 ± 10.07<sup>a</sup> | 80.12 ± 10.07<sup>a</sup> | 80.12 ± 10.07<sup>a</sup> | 80.12 ± 10.07<sup>a</sup> |
|           |         | Soaking 6 h       | 16 h                  | 24 h        | 32 h | 40 h |      |      |
| Gomati    | 67.4 ± 1.92<sup>a</sup> | 79.41 ± 7.01<sup>c</sup> | 89.48 ± 8.19<sup>c</sup> | 88.59 ± 0.93<sup>a</sup> | 94.15 ± 0.12<sup>b</sup> | 93.57 ± 0.12<sup>b</sup> | 92.80 ± 0.12<sup>b</sup> | 80.12 ± 10.07<sup>a</sup> | 80.12 ± 10.07<sup>a</sup> | 80.12 ± 10.07<sup>a</sup> | 80.12 ± 10.07<sup>a</sup> |

All data are the mean ± SD of three replicates.

For each cowpea cultivars, means within a row not sharing a common letter differ significantly (<i>p</i> < 0.05).

Values given in bracket are percentage differences calculated from raw values.
steaming on antioxidation activity of cool season legumes where they observed similar kind of loss in DPPH activity after pressure boiling at 15 psi for 15 min in legumes like green pea, yellow pea, and chickpea.

Table 2 shows effect of 6 h of soaking and fermentation for different hours of intervals. An increase in DPPH activity percentage was found after soaking and fermentation. Gomati cultivar exhibited greatest increase up to 31.39% followed by Kohinoor, EC4216, and BL2 after soaking. The results have been found similar to the results of DPPH IC \( \text{S}_{50} \) values reported by Boateng et al. \[17\] on dry pinto beans after soaking it for 22 h at ambience. However, our results are dissimilar with study of Xu and Chang \[9\], where a loss in DPPH activity was found in certain legumes after soaking as compared to raw values recorded. Natural fermentation for 16 h had shown an increase in antioxidant activity percentage in all cultivars of cowpea. However, the highest percentage increase up to 18.13% was observed in Kohinoor cultivar followed by Gomati (14.40%), EC4216 (7.65%), and BL2 (7.23%). Later, with increasing time interval, a decrease in antioxidant activity was found. These results are in agreement with those reported by Dajanta et al. \[27\] on naturally fermented soybean product called thua nao and also with Plaitho et al. \[28\] on fermented and pigmented rice, where an increase in DPPH activity was found. Natural fermentation is caused by action of certain microbes and also due to their enzymatic action on cowpea seeds flour. Fermentation therefore leads to breakdown of polyphenols of cowpea flour making total polyphenols and DPPH scavenging activity increase significantly from their respective raw values which has been corroborated in the study as explained in Table 1 and Table 2. However, with increasing duration of fermentation, a decrease was recorded after the passing of initial 16 h of fermentation which could be possibly due to utilization of antioxidant activity by free radicals generated by microorganisms. It can be concluded from the study that fermentation for 16 h is the best-suited processing technique for increasing antioxidant activity of cowpea cultivars.

**Ferric Reducing Antioxidant Power**

Raw cowpea cultivars and thermally processed cowpea seeds were evaluated for their capability to reduce TPTZ-Fe(III) complex to TPTZ-Fe(II) by means of FRAP assay (Table 3). FRAP assay as suggested by Halvorsen et al. \[29\] directly measures the antioxidants in a sample by the presence of reductones found as antioxidants. FRAP values of raw cowpea cultivars ranged from 5.09 to 7.89 mmol Fe (II) Eq/g. The reported results are lower than those reported by Zia-ul-Haq et al. \[30\] who evaluated values of four cowpea cultivars as 13.2–19.4 mmol Fe\(^{2+}\)/g. FRAP values of all studied cowpea cultivars were significantly \((p < 0.05)\) reduced after all kinds of thermal processing. Autoclaving for 15 min reduced FRAP activity compared to more than 30 min of autoclaving. Totally, 30 min of microwave treatment reduced more FRAP activity than 15 min of the same process in all cultivars except Gomati cultivar. Boiling for 90 min also minimized FRAP activity from raw values up to 72.7% as seen in Kohinoor cultivar. Roasting, for 45 min, had shown minimal losses of FRAP activity than any other processing method in all cultivars. Totally, 38.65% was maximum loss observed after roasting as noted for EC4216 cultivar. On the whole, 90 min of boiling treatment decreased to 72.7%, as observed in Kohinoor cultivar, and this was the highest percentage reduction recorded after all thermal processes. This finding revealed that hydrothermal processing had made reductones of raw cowpea seeds to leach out from seeds at each time-interval passed, like phenolic compounds have leached out from cowpea seeds to water during hydrothermal processing as observed in Table 1. The Ferric-reducing potential in roasting of all cowpea cultivars was maximum amongst all thermal processings carried out on them. Similar results of FRAP were given by Siddhuraju and Becker. \[8\] They reported the following order of FRAP activity: raw>dry heating>soaking followed by autoclaving in light- and dark-colored cowpea varieties.

Influence of non-thermal processing on cowpea cultivars like soaking and fermentations for various durations of hours on their FRAP values is given in Table 3. Soaking of cowpea seeds for 6 h reduced FRAP content in all cultivars which could be due to escape of secondary metabolites
Table 3. Effect of thermal and non-thermal processing on FRAP (mmol Fe (II) Eq/g) of cowpea cultivars.

| Cultivars  | Raw  | Autoclave | Microwave | Boiling | Roasting | Soaking | Non-thermal processing |
|------------|------|-----------|-----------|---------|----------|---------|------------------------|
|            |      | 15 min    | 30 min    |         | 90 min   | 45 min  | 6 h                    |
|            |      | 15 min    | 30 min    | 90 min  | 45 min   | 90 min  | 45 min  | 6 h  | 16 h | 24 h  | 32 h  | 40 h  |
| EC-4216    | 7.89 ± 0.62 | 3.47 ± 0.03 | 3.64 ± 0.07 | 3.02 ± 0.08 | 2.72 ± 0.07 | 2.48 ± 0.07 | 4.84 ± 0.05 | 1.58 ± 0.05 | 8.10 ± 0.16 | 8.20 ± 0.15 | 8.46 ± 0.23 | 8.94 ± 0.27 |
|            |      | (56.02%)  | (53.86%)  | (61.72%) | (65.52%)  | (68.56%) | (38.65%)  | (79.97%)  | (2.66%)  | (3.92%)  | (7.22%)  | (13.30%) |
| BL-2       | 6.79 ± 0.36 | 3.34 ± 0.04 | 3.43 ± 0.16 | 2.72 ± 0.07 | 1.98 ± 0.08 | 2.56 ± 0.2 | 4.81 ± 0.11 | 1.49 ± 0.03 | 8.079 ± 0.11 | 8.137 ± 0.11 | 8.404 ± 0.19 | 8.71 ± 0.10 |
|            |      | (50.81%)  | (49.48%)  | (59.94%) | (70.83%)  | (62.29%) | (29.16%)  | (78.05%)  | (18.98%) | (19.83%) | (23.71%) | (28.27%) |
| Kohinoor   | 7.36 ± 0.32 | 3.25 ± 0.37 | 3.45 ± 0.16 | 2.88 ± 0.03 | 2.82 ± 0.01 | 2.01 ± 0.13 | 4.37 ± 0.08 | 1.79 ± 0.005 | 7.788 ± 0.36 | 7.813 ± 0.24 | 8.362 ± 0.13 | 8.507 ± 0.22 |
|            |      | (55.84%)  | (53.12%)  | (60.86%) | (61.68%)  | (72.69%) | (40.62%)  | (75.67%)  | (5.81%)  | (6.15%)  | (13.61%) | (15.38%) |
| Gomati     | 5.09 ± 0.20 | 3.32 ± 0.05 | 3.28 ± 0.14 | 2.62 ± 0.03 | 1.90 ± 0.14 | 2.08 ± 0.06 | 3.54 ± 0.15 | 0.99 ± 0.06 | 7.602 ± 0.20 | 7.797 ± 0.16 | 7.787 ± 0.26 | 8.00 ± 0.17 |
|            |      | (34.77%)  | (35.55%)  | (48.52%) | (62.67%)  | (59.13%) | (50.54%)  | (80.55%)  | (49.31%) | (53.04%) | (52.84%) | (57.17%) |

All data are the mean ± SD of three replicates.
For each cowpea cultivars, means within a row not sharing a common letter differ significantly (p < 0.05).
Values given in bracket are percentage differences calculated from raw values.
which had ferric-reducing ability. Sowndhararajan [31] also reported reduction in FRAP activity after soaking in *Vigna vexillata* seeds. However, an increase in ferric-reducing potential was observed with increasing duration of fermentation. All the cultivars had shown similar trend of increase in FRAP activity with increasing duration of fermentation. Fermentation up to 40 h significantly \((p < 0.05)\) increased FRAP values. Gomati cultivar has shown highest percentage increase (57.2%) after 40 h of fermentation. Dajanta et al. [27] worked upon antioxidant capacity of *thua nao* (naturally fermented soybean) and reported 24% increase in FRAP value from cooked and non-fermented soybeans. Naturally fermented cowpea seed flour aided the formation of ferric reductones that could react with free radicals to stabilize and terminate radical chain reaction during fermentation. [32] The results clearly indicate that increasing duration of fermentation helps in the formation of more ferric reductants that were quantified as FRAP activity. The assay does not measure the percentage inhibition of the free radical (like DPPH) by antioxidant so its result is directive toward the presence of reductones as antioxidants in a redox-linked colorimmetrical reaction. [29]

**Total flavonoid content**

TFC of raw cowpea cultivars and the thermally processed cowpea are given in Table 4. Up to 82.45% of loss of TFC in EC4216 was observed after 15 min of autoclaving followed by other hydrothermal processing. Roasting also caused minimum reduction in flavonoid content (25.92–33.52%). As explained by Boateng et al. [17], rate of increase or decrease in phenols and flavonoids primarily depends on the type of legume and preparation or procedure used. Hence, toasting or roasting resulted in better retention or sometimes increase in phenols and flavonoids. Autoclaving and microwave cooking was carried out for 15 min and 30 min, respectively; a lesser decrease in flavonoids was found when the duration of cooking time was reduced in all cultivars of cowpea. This could be due to breakdown of cellular matrix during increased time which helped in binding of the total phenolics with pectin or cellular networks and making them more extractable in the extraction solvent for TFC assay. [33] Among hydrothermal processing, 30 min of microwave treatment on cowpea seeds least affected the loss percentage (19.10–22.92%) of TFC while other processing significantly altered TFC. Autoclaving and boiling greatly affected TFC because cooking under pressure led to softening and rupturing of cell walls causing leach out of water-soluble polyphenols and flavonoids in cooking water, also prolonged duration of boiling of cowpea may have caused the observed reduction. Saikia and Mahanta [33] also explained that flavonoids and phenolics are heat-labile compounds of plants and the pattern of change in them depends on the severity of heat treatments and exposure to air/light. They concluded in their study on Assamese vegetables that microwave and steaming in most cases are better methods for retention of phytochemicals and antioxidants than boiling. Similar results were also reported by Doss et al. [34] on processed under-utilized tropical legume *Canavalia ensiformis* L. DC seeds.

Effect of non-thermal processing like soaking and fermentation on TFC is presented in Table 4. Soaking for 6 h caused a significant \((p < 0.05)\) loss of flavonoids in all cultivars. Since most flavonoids are found basically in seed coat of cowpea seeds and are water soluble, therefore soaking lead to tenderization and solubilization of flavonoids into soaking water. [9] The present results are in agreement with Kaur et al. [35] who studied soaking effect on *Chenopodium quinoa* seeds. Natural fermentation of cowpea seed flour ranging from 16 h up to 40 h exhibited gradual and significance decrease from their respective raw flavonoid content in all cultivars of cowpea. It has been observed in previous studies on fermentation effect on flavonoids that fermentation has improving effect on flavonoid contents. [36–38] The contradictory results obtained could be due to lesser concentration of phenolics or flavonoid compounds or duration of fermentation process occurred. Usually microorganisms involved in fermentation cause break down of linkages of flavonoids making them easily available. However, increasing duration of fermentation allows microorganisms to utilize these available compounds as substrate for their growth, and therefore decreased levels of flavonoid are found. The present study results are found similar with work of Karimi et al. [39] on solid-state
Table 4. Effect of thermal and non-thermal processing on total flavonoid content (TFC) (mg QE/g) of cowpea cultivars.

| Cultivars | Raw | Thermal processing | Non-thermal processing |
|-----------|-----|--------------------|------------------------|
|           |     | Autoclave          | Microwave              | Boiling | Roasting | Soaking | Fermentation |
|           |     | 15 min 30 min      | 15 min 30 min          | 90 min 45 min | 6 h 16 h 24 h 32 h 40 h |
| EC-4216   | 1.026 ± 0.04² | 0.18 ± 0.03²  | 0.45 ± 0.01² | 0.68 ± 0.05² | 0.83 ± 0.04² | 0.54 ± 0.09² | 0.76 ± 0.08² | 0.42 ± 0.02² | 0.54 ± 0.02² | 0.41 ± 0.03² | 0.33 ± 0.03² | 0.31 ± 0.03² | 82.45% | 56.14% | 33.72% | 19.10% | 47.36% | 25.92% | 59.06% | 47.36% | 60.03% | 67.83% | 69.78% |
| BL-2      | 0.842 ± 0.012² | 0.173 ± 0.09² | 0.413 ± 0.045² | 0.531 ± 0.02² | 0.649 ± 0.06² | 0.506 ± 0.01² | 0.59 ± 0.04² | 0.5 ± 0.03² | 0.68 ± 0.05² | 0.51 ± 0.02² | 0.49 ± 0.02² | 0.35 ± 0.06² | 79.45% | 50.95% | 36.93% | 22.92% | 39.90% | 29.92% | (84.61%) | 39.42% | 41.80% | 58.43% |
| Kohinoor  | 0.981 ± 0.015² | 0.24 ± 0.019² | 0.368 ± 0.05² | 0.632 ± 0.08² | 0.777 ± 0.09² | 0.54 ± 0.02² | 0.673 ± 0.07² | 0.71 ± 0.06² | 0.82 ± 0.04² | 0.75 ± 0.07² | 0.62 ± 0.014² | 0.48 ± 0.015² | 75.53% | 62.48% | 35.57% | 20.79% | 44.95% | 31.39% | (72.62%) | 35.41% | (36.79%) | 51.07% |
| Gomati    | 1.053 ± 0.02² | 0.18 ± 0.02² | 0.18 ± 0.01² | 0.69 ± 0.02² | 0.83 ± 0.02² | 0.59 ± 0.03² | 0.70 ± 0.01² | 0.49 ± 0.04² | 0.64 ± 0.015² | 0.46 ± 0.011² | 0.44 ± 0.03² | 0.41 ± 0.02² | 82.90% | 82.90% | 34.47% | 21.17% | 43.96% | 33.52% | (53.46%) | 39.22% | (56.31%) | (58.21%) |

All data are the mean ± SD of three replicates.
For each cowpea cultivars, means within a row not sharing a common letter differ significantly \( p < 0.05 \).
Values given in bracket are percentage differences calculated from raw values.
fermentation on pistachio hulls. They reported decrease in flavonoid content of pistachio hull, fermented with all types of fungi from control pistachio hull. The loss of TFC in naturally fermented cowpea in the present study could also be due to growth of certain microorganisms that might have used flavonoids of cowpea for their growth.

**Correlational studies**

DPPH radical scavenging activity and FRAP assays determine antioxidant capacities by different mechanism, therefore these cannot be correlated. However, phenolics and flavonoid compounds are known to be potent antioxidants and therefore in the present work, TPC and TFC of selected cultivars of cowpea and their contents in respective thermal and non-thermal processing treatment were compared and studied in linear correlation model with the antioxidant activity parameters, DPPH radical scavenging activity, and FRAP.

TPC of raw cultivars were positively correlated with DPPH scavenging activity (Table 5, \( r = 0.936 \)). Similar kind of strong coefficient of correlation (0.857) was also reported by Amarowicz et al. \[40\] between total phenolics and total antioxidant activity of their selected legume seeds. However, on autoclaving for 15–30 min, this correlation coefficient decreased (Autoclave 15, \( r = 0.246 \) and Autoclave 30, \( r = 0.062 \)). However, a strong correlation was found in other thermal processing (microwave 15, \( r = 0.979 \), microwave 30, \( r = 0.888 \), boiling, \( r = 0.792 \), and roasting \( r = 0.974 \)). Correlation study of TPC with FRAP values of raw cowpea showed moderate correlation (\( r = 0.573 \)) which also decreased after 15 min autoclave (\( r = 0.354 \)). However, a moderate to high correlation was observed in other thermal processing (autoclave 30, \( r = 0.912 \), microwave 15, \( r = 0.932 \), microwave 30, \( r = 0.993 \), boiling, \( r = 0.497 \), and roasting \( r = 0.538 \)). The results of this correlation coefficient at different processings indicates that change in antioxidant activity on application of different kinds of thermal treatments can be associated with change in phenolic composition of the cowpea cultivars, although a decrease in correlation (\( r \)) was also observed which may be due to cultivar variation and degradation of polyphenols due to thermal treatment which caused antioxidation effect. Similar results have also been reported by Vadivel et al. \[20\] and Chaieb et al. \[41\] who correlated TPC with antioxidant activities and explained a positive correlation between phenolic content and antioxidant properties of *C. ensiformis* seeds and 13 genotypes of Faba beans, respectively. However, a reduction in correlation coefficient was also observed by Sandhu et al. \[42\] after toasting treatment of oat flour.

Non-thermal treatment of cowpea cultivars like soaking and fermentation at various time durations showed strong positive correlation between TPC and DPPH radical scavenging activity (Table 6, soaking, \( r = 0.785 \), fermentation 16 h, \( r = 0.993 \), fermentation 24 h, \( r = 0.996 \), fermentation 32 h, \( r = 0.726 \), fermentation 40 h, \( r = 0.817 \)). A satisfactory correlation was shown between TPC and FRAP

**Table 5.** Correlation between antioxidant activity and total phenolic content and total flavonoid content of thermal processing of cowpea cultivars.

|          | Raw | A15 | A30 | M15 | M30 | Boil | Roast |
|----------|-----|-----|-----|-----|-----|------|-------|
| DPPH     | 0.936 | 0.246 | 0.062 | 0.979 | 0.888 | 0.792 | 0.974 |
| FRAP     | 0.573 | 0.354 | 0.912 | 0.932 | 0.993 | 0.497 | 0.538 |

|          | Raw | A15 | A30 | M15 | M30 | Boil | Roast |
|----------|-----|-----|-----|-----|-----|------|-------|
| DPPH     | 0.936 | 0.246 | 0.062 | 0.979 | 0.888 | 0.792 | 0.974 |
| FRAP     | 0.573 | 0.354 | 0.912 | 0.932 | 0.993 | 0.497 | 0.538 |

**Table 6.** Correlation between antioxidant activity and total phenolic content and total flavonoid content of non-thermal processing of cowpea cultivars.

|          | Raw | Soak | F16 | F24 | F32 | F40 |
|----------|-----|------|-----|-----|-----|-----|
| DPPH     | 0.936 | 0.785 | 0.993 | 0.996 | 0.726 | 0.817 |
| FRAP     | 0.573 | 0.734 | 0.753 | 0.557 | 0.701 | 0.587 |

|          | Raw | Soak | F16 | F24 | F32 | F40 |
|----------|-----|------|-----|-----|-----|-----|
| DPPH     | 0.936 | 0.785 | 0.993 | 0.996 | 0.726 | 0.817 |
| FRAP     | 0.573 | 0.734 | 0.753 | 0.557 | 0.701 | 0.587 |
(soaking, \( r = 0.734 \), fermentation 16 h, \( r = 0.753 \), fermentation 24 h, \( r = 0.557 \), fermentation 32 h, \( r = 0.701 \), fermentation 40 h, \( r = 0.587 \)). Bei et al. \(^{[43]}\) also found positive correlation between free, conjugated, and bound phenolics with DPPH scavenging activity and they also found pretty high correlation between content of phenolics like chlorogenic, vanillic, ferulic acid, and catechin and the antioxidant activities in the free phenolic fraction extract from fermented oats (\( r > 0.85, p < 0.01 \)).

No significant correlation was observed between TFC and DPPH radical scavenging activity. TFC and FRAP also did not show significant correlation except between TFC and FRAP at 30 min of autoclaving process (autoclaving 30 min, \( r = 0.877 \)). Lahouar et al. \(^{[44]}\) also reported a weak correlation between TFC and DPPH (\( r = 0.25 \)) and a moderate correlation between TFC and FRAP (\( r = 0.63 \)) of whole barley.

**Conclusions**

In the present study, assessment of antioxidant potentials was carried out on four selected cowpea (\( \textit{vigna unguiculata} \)) cultivars after various kinds of thermal and non-thermal processings were done. Thermal processings have been found to reduce TPC, TFC, and ferric reducing antioxidant potential. However, DPPH radical scavenging activity was found to have increased values after thermal processing as compared to their respective raw values. Among non-thermal processing, soaking reduced TPC, TFC, and FRAP, but fermentation increased TPC, DPPH, and FRAP. Therefore, fermentation of cowpea seeds has shown promising effect on retention and enhancement of antioxidant activity quantified as TPC, DPPH radical scavenging activity, and ferric reducing antioxidant potential of methanolic extracts of cowpea seeds. So, consumption of fermented cowpea would not only improve nutrient utilization but will also provide improved levels of potential antioxidants which will have beneficial effects on human health.

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