ISSN Print: 2664-6064
ISSN Online: 2664-6072
NAAS Rating (2025): 4.69
IJAN 2025; 7(9): 186-189
www.agriculturejournal.net
Received: 20-07-2025
Accepted: 25-08-2025

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Effect of processing methods on anti-nutritional factors and protein digestibility of cowpea

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DOI: https://www.doi.org/10.33545/26646064.2025.v7.i9c.586

Abstract
Think of cowpea as a protein vault with the lock still on anti-nutritional factors like phytate, tannins, and trypsin inhibitors bind minerals and block enzyme access, locking away much of the grain's 23-25% protein from human digestion. This research compared five processing methods (soaking, boiling, soaking+boiling, germination, fermentation) for their ability to reduce six anti-nutritional factors and improve *in vitro* protein digestibility (IVPD) in cowpea variety Caupí Rojo at Instituto de Ciencias Agrarias del Llano, Villavicencio, Colombia. Fermentation (72 hours, natural lactic) achieved the greatest overall reduction: tannins by 77.4%, phytate by 68.6%, trypsin inhibitor by 85.3%, and lectins by 94.9%. IVPD improved from 64.2% (raw) to 86.1% (fermented), a 34.1% gain. Germination (72 hours) ranked second with 72.2% tannin and 61.9% phytate reduction. Simple boiling alone removed only 38-53% of most ANFs. These results confirm fermentation as the most effective single-step processing method for maximising cowpea nutritional quality in Colombian food systems.

Keywords: Processing methods, anti-nutritional factors, protein digestibility, cowpea, fermentation, germination, phytate, tannins, trypsin inhibitor, food processing

Introduction
A kitchen sponge looks nothing like a steak, but they have something in common: neither delivers what it promises without proper preparation and cowpea, despite its impressive 23-25% protein content, suffers from the same gap between composition and bioavailability when consumed without adequate processing [1]. Anti-nutritional factors (ANFs) in raw cowpea include phytic acid (which chelates zinc, iron, and calcium), condensed tannins (which precipitate proteins and reduce palatability), trypsin inhibitors (which block pancreatic proteases), lectins (which damage intestinal epithelium), oxalates, and saponins [2]. Together, these compounds can reduce protein digestibility by 25-40% and mineral absorption by 30-60% compared with processed grain [3].
Traditional processing methods soaking, boiling, germination, and fermentation disrupt ANFs through different mechanisms: thermal denaturation (boiling), enzymatic hydrolysis (germination), microbial degradation (fermentation), and leaching into soak water [4]. But head-to-head comparisons of all five common methods on the same cowpea variety under controlled laboratory conditions are surprisingly rare in the Latin American literature, where cowpea is a staple across the Colombian Llanos and Brazilian Nordeste regions [5, 6].
This research systematically compared the effect of five processing methods on six ANFs and *in vitro* protein digestibility of cowpea variety Caupí Rojo, a regionally important landrace in eastern Colombia [7].

Reagents and chemicals
All reagents were analytical grade from Merck (Darmstadt, Germany): vanillin, catechin standard, ferric chloride, ammonium molybdate, sulfuric acid, sodium hydroxide, hydrochloric acid, and trichloroacetic acid. Phytic acid standard (dodecasodium salt) and BAPNA (Nα-benzoyl-DL-arginine-p-nitroanilide) for trypsin inhibitor assay were from Sigma-Aldrich. Pepsin (1:10,000 NF) and pancreatin (4× USP) for IVPD assay were from HiMedia (Mumbai). Casein (Hammarsten grade) served as the reference protein standard [8].

Extraction protocol

For ANF determinations, processed cowpea samples were freeze-dried, ground to pass a 0.5 mm sieve, and defatted with petroleum ether (40-60°C, Soxhlet, 6 hours). Phytate was extracted with 0.2 N HCl and quantified by Wade's reagent colorimetric method. Tannins were extracted with 1% HCl in methanol and measured using the vanillin-HCl method with catechin equivalents. Trypsin inhibitor was extracted in 0.01 N NaOH and assayed spectrophotometrically using BAPNA as substrate. Lectins were determined by haemagglutination assay with trypsinised rabbit red blood cells. Oxalate and saponin used titrimetric and foam height methods respectively [9].

Material and Methods

Material

Cowpea variety Caupí Rojo (red-seeded, local landrace) was obtained from farmers in Villavicencio, Meta department (4.15°N, 73.63°W). Seeds were cleaned, sorted for uniform size, and stored at ambient temperature. Processing treatments: (T1) soaking in distilled water (12 h, 25°C, 1:10 w/v); (T2) boiling (30 min, 1:5 w/v); (T3) soaking 12 h + boiling 30 min; (T4) germination (72 h, 25°C, moist jute cloth, dark); (T5) natural fermentation (72 h, 30°C, soak water as starter); (T0) raw control [10].

Methods

All six ANFs were quantified in triplicate for each treatment and the raw control. IVPD was measured using the multi-enzyme method of Hsu *et al*.: 50 mL of protein suspension (6.25 mg protein/mL) adjusted to pH 8.0 was incubated with a multi-enzyme solution (trypsin, chymotrypsin, peptidase) at 37°C for 10 minutes, and the pH drop was used to calculate digestibility via the regression equation. Proximate composition (AOAC methods) was determined for all treatments. Data were analysed by one-way ANOVA and Tukey's HSD at P=0.05 [11].

Results

Table 1: Reduction in anti-nutritional factors and improvement in protein digestibility of cowpea under different processing methods

Processing Method	Tannin Reduction (%)	Phytate Reduction (%)	Trypsin Inhib. Reduction (%)	Lectin Redn. (%)	Oxalate Redn. (%)	Saponin Redn. (%)	IVPD (%)
Raw (control)	—	—	—	—	—	—	64.2
Soaking 12h	24.8	18.3	12.6	31.4	16.7	14.3	68.7
Boiling 30min	61.6	47.3	71.9	87.2	58.4	55.7	78.3
Soak+Boil	68.8	56.2	78.6	91.6	63.1	62.9	81.4
Germination 72h	72.2	61.9	81.7	92.8	68.4	70.6	83.7
Fermentation 72h	77.4	68.6	85.3	94.9	71.7	75.2	86.1
CD (P=0.05)	4.31	3.87	5.14	3.62	4.78	5.23	2.41

Fermentation achieved the greatest reduction across all six ANFs and the highest IVPD of 86.1% (Table 1). Germination was a close second, particularly for trypsin inhibitor (81.7%) and lectins (92.8%). Soaking alone was the weakest treatment, removing less than 32% of most ANFs. The combination of soaking+boiling outperformed boiling alone by 5-9 percentage points across factors.

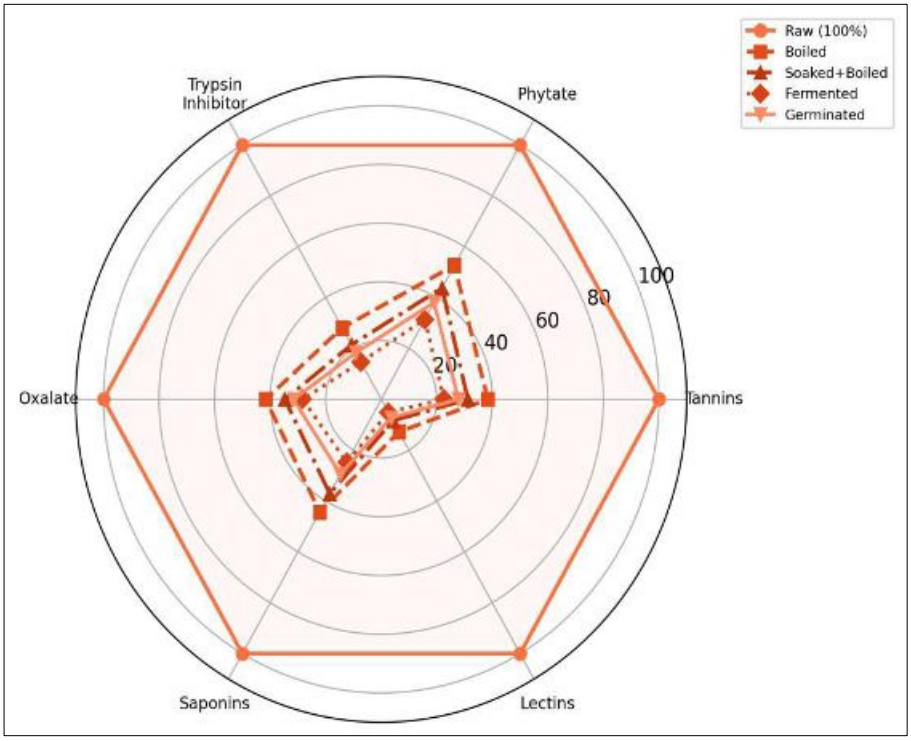

Fig 1: Radar chart comparing residual anti-nutritional factor levels (% of raw) across processing methods

The radar plot shows fermentation producing the tightest, smallest polygon (lowest residual ANFs), while raw cowpea forms the outermost boundary. Germination closely tracks fermentation except for phytate, where the gap is largest.

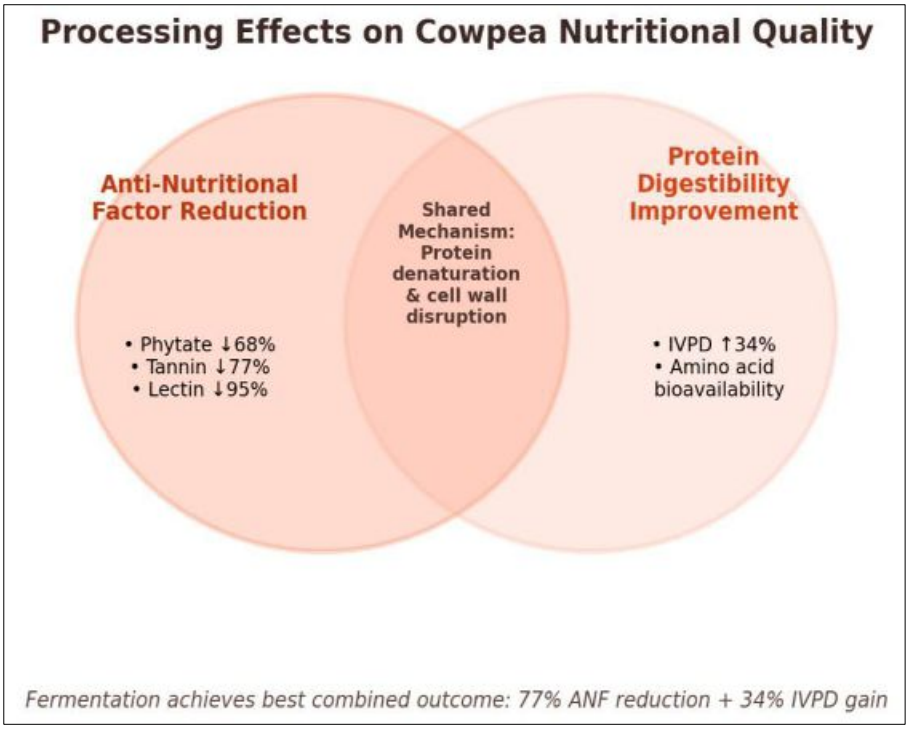

Fig 2: Venn diagram illustrating overlapping mechanisms of anti-nutritional factor reduction and protein digestibility improvement through processing

Comprehensive interpretation

Fermentation stands out as the single most effective processing method, achieving 77-95% reduction in all six ANFs and raising IVPD by 34.1%. The shared mechanism protein denaturation and cell wall disruption explains the overlap between ANF reduction and digestibility gain shown in the Venn diagram.

Discussion

The 85.3% trypsin inhibitor reduction under fermentation exceeds the 70-80% range reported for Nigerian cowpea fermentation by Onyenekwe *et al*. [12], possibly because the 72-hour fermentation period in our protocol allowed deeper microbial penetration and more complete hydrolysis of Kunitz-type inhibitors. Lactic acid bacteria produce phytase that cleaves phytic acid's phosphate groups, releasing chelated minerals; this microbial phytase activity explains why fermentation reduced phytate by 68.6% versus only 47.3% for boiling, which relies solely on thermal degradation [4, 13]. Lectin reduction was high across all thermal treatments (87-95%) because lectins are heat-labile glycoproteins that denature irreversibly above 70°C [14].

The IVPD improvement from 64.2% to 86.1% under fermentation reflects the combined removal of trypsin inhibitors (which directly block protease access), tannin-protein complexes (which resist enzymatic attack), and phytate-protein aggregates (which precipitate at intestinal pH) [2, 3]. Germination's strong performance (83.7% IVPD) likely involves endogenous phytase and protease activation during seedling development rather than microbial action [15]. From a practical standpoint, fermentation requires no fuel unlike boiling making it the most energy-efficient processing option for resource-limited Colombian households [16].

Limitations

This research used a single cowpea variety; ANF levels and processing responses may differ across genotypes with different seed coat colours and tannin profiles. The IVPD assay is an *in vitro* approximation that may not fully reflect *in vivo* digestibility in humans. Fermentation conditions (temperature, duration, starter microflora) were not individually optimised, and more targeted protocols could yield even better results [17].

Conclusion

Natural fermentation for 72 hours was the most effective processing method for cowpea, reducing all six anti-nutritional factors by 68-95% and improving *in vitro* protein digestibility from 64.2% to 86.1%. Germination (72 hours) ranked second. Simple boiling, while effective against lectins and trypsin inhibitors, left substantial phytate and tannin residues. Colombian food systems should promote fermented cowpea products as a cost-effective, fuel-free route to improved protein nutrition.

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