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# Effects of Fermentation and Succinylation on the Structural, Functional and Nutritional Properties of African Oil Bean Seed Protein Concentrate

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**ABSTRACT**

This study evaluated the effects of yeast fermentation and succinylation on the structural, techno-functional and nutritional properties of protein concentrate from African oil bean seed (*Pentaclethra macrophylla* Benth., AOBS). Native protein concentrate (NPC), from flour fermented with Saccharomyces cerevisiae at 1.5% (w/w) inoculum, served as the unmodified control. Four modified concentrates (MPC) were produced from fermented flour (1.0% or 1.5% inoculum, Factor A) by succinylation with succinic anhydride (1% or 3% w/w, Factor B) in a 2×2 factorial design. A significant fermentation × succinic-anhydride interaction ($p < 0.05$) was found for degree of succinylation and several functional properties, indicating that inoculum concentration was statistically associated with the succinylation response, based on interaction effects and FTIR data rather than direct measurement of unfolding. Degree of succinylation ranged from 58.64% to 86.92%, with a corresponding FTIR Amide II downshift (1,541.2 → 1,524.8 $cm^{-1}$) and a succinyl carbonyl band at ~1,718 $cm^{-1}$. Succinylation significantly ($p < 0.05$) improved nitrogen solubility index (52.38 → 84.62% at pH 7), emulsifying activity index (28.46 → 58.92 $m^2/g$), emulsion stability index (18.64 → 52.18 min), foaming capacity (42.18 → 72.46%), foaming stability (48.62 → 74.26%) and water-holding capacity (2.84 → 4.24 g/g), while digestibility decreased from 74.82% to 64.28%. Processing reduced saponins by 98.8–99.4%, alkaloids by 99.3%, tannins by 74.6–80.8%, and cyanogenic glycosides by 99.0–99.5% relative to raw seed. These findings show that fermentation-modulated succinylation improves functional properties at the cost of reduced digestibility; further nutritional and safety studies are needed before any food-application recommendation is made.

**Keywords:** African Oil Bean Seed, Succinylation, Fermentation, Modified Protein Concentrate, *Pentaclethra Macrophylla*, Techno-Functional Properties

## INTRODUCTION

The escalating global demand for sustainable protein sources has positioned plant-based proteins at the forefront of food science innovation, driven by environmental pressures, population growth, and shifting dietary preferences toward animal-free alternatives. Plant proteins derived from legumes and oilseeds offer a low-carbon-footprint alternative to animal protein, but their widespread adoption is constrained by suboptimal techno-functional properties relative to animal-derived proteins (Lam *et al*., 2018). In sub-Saharan Africa, where protein-energy malnutrition affects over 30% of children under five, underutilised indigenous crops represent untapped reservoirs for addressing food insecurity (Udenigwe & Aluko, 2017). Among these, the African oil bean seed (*Pentaclethra macrophylla* Benth.), a leguminous tree native to West and Central Africa, is notable for its abundance and nutritional potential, yielding seeds rich in protein (up to 40% dry-weight basis) and lipids (45–50%). Traditionally fermented into "ugba," a condiment widely consumed in Nigeria and Cameroon, the crop nonetheless remains underutilised industrially, with post-harvest losses exceeding 30% attributable to inadequate processing technologies (Abbey & Iwe, 2017; Onyeike *et al*., 1995). Comparative proximate analyses of fermented P. *macrophylla* alongside other indigenous fermented legume condiments confirm substantial protein and lipid retention after processing, although reported values vary considerably with fermentation method, duration and seed source (Agbo, 2024).

Extraction of protein concentrates from P. *macrophylla* by alkaline solubilisation and isoelectric precipitation yields concentrates of 70–85% protein content, but the native concentrate exhibits nitrogen solubility index (NSI) values below 30% at neutral pH, modest water and oil absorption capacities, and inferior emulsifying, foaming and gelling properties relative to commercial soy isolates, owing to compact, β-sheet-dominant globulin structures (40–60 kDa vicilin-like fractions) stabilised by disulphide bonds (Bajpai & Mishra, 2022; Shevkani *et al*., 2015). Nutritional exploitation is further constrained by anti-nutritional factors — tannins, saponins, alkaloids and cyanogenic glycosides — that chelate minerals and inhibit enzymatic digestion, limiting in vitro protein digestibility to 65–75% (Udenigwe & Aluko, 2017).

Functional modification by chemical acylation offers a strategic route to overcoming these limitations. Succinylation introduces negatively charged carboxyl groups onto lysine ε-amino residues, enhancing electrostatic repulsion and thereby improving solubility and techno-functionality; in pea and soy protein systems, succinylation has been shown to elevate solubility by 40–60% and emulsifying activity by 30–50% (Zhang *et al*., 2021), although these functional gains are commonly accompanied by reduced protein digestibility, indicating a trade-off rather than an unqualified nutritional benefit (Basak & Singhal, 2022). Existing studies on P. *macrophylla* protein have been confined to proximate and basic functional characterisation of native, unmodified concentrates (Abbey & Iwe, 2017; Nwosu *et al*., 2021) or

physical pre-treatments such as ultrasonication (Nwokocha *et al*., 2023); none has applied chemical modification by succinylation, and none has examined whether fermentation intensity prior to extraction influences the extent or efficiency of subsequent chemical modification. This study addresses that gap by evaluating, through a factorial design and statistical interaction analysis, whether fermentation intensity is associated with the degree of succinylation achieved and with the resulting structural and functional changes — a relationship described here descriptively as fermentation-modulated succinylation rather than as a confirmed mechanistic pathway, since direct kinetic or spectroscopic evidence of protein unfolding during fermentation itself was not independently obtained.

This study therefore aimed to produce a protein concentrate from African oil bean seed and evaluate the impact of yeast-fermentation intensity and succinylation, singly and interactively, on its physicochemical, structural, techno-functional, nutritional and anti-nutritional properties. The specific objectives were to: (i) prepare and characterise the native protein concentrate (NPC) from debittered, fermented P. *macrophylla* flour; (ii) functionally modify the NPC by succinylation at two succinic anhydride levels to produce modified protein concentrates (MPC); (iii) determine the resulting techno-functional properties (solubility, water/oil holding capacities, emulsifying and foaming properties, gelling ability); (iv) characterise the physicochemical and structural changes (colour, pH, isoelectric point, surface hydrophobicity, FTIR secondary structure); (v) assess in vitro digestibility and antioxidant activity; and (vi) evaluate the impact of processing on anti-nutritional factors and physical powder properties.

## MATERIALS AND METHODS

### Source of Raw Material

Mature, dehisced seeds of *Pentaclethra macrophylla* Benth. were procured from Kure Market, Minna, Niger State, Nigeria. Seeds were manually sorted to remove insect-infested, mould-affected or broken seeds, shelled to remove the pericarpal testa, and the cotyledons stored in polypropylene bags at ~25 °C (relative humidity < 65%) for no more than seven days prior to processing. Initial moisture content was determined gravimetrically (AOAC Method 925.10; AOAC International, 2019). All reagents, including succinic anhydride (≥99%, Sigma-Aldrich), sodium hydroxide, hydrochloric acid, n-hexane, pepsin, pancreatin, DPPH and bovine serum albumin, were of analytical grade.

### Experimental Design

A 2 × 2 factorial arrangement (Factor A: S. cerevisiae inoculum concentration, 1.0% and 1.5% w/w; Factor B: succinic anhydride concentration, 1% and 3% w/w) was embedded within a Completely Randomised Design, generating four modified protein concentrates plus one native protein concentrate derived from the optimal fermentation condition (1.5% inoculum, 24 h), which served as the reference control (Table 1). Fermentation duration (24 h) and inoculum levels were selected from a preliminary optimisation study described below. All analyses were performed in triplicate (n = 3).

Only one unmodified control (NPC, from the 1.5% inoculum fermentation) was included because the primary objective was to evaluate succinylation and its interaction with fermentation intensity on the fermented substrate, rather than to independently benchmark every possible fermentation-only treatment; an unsuccinylated control from the 1.0% inoculum treatment was not included, and its absence is acknowledged as a limitation of the present design. Within the four succinylated MPC samples, the 2×2 factorial arrangement permits independent statistical evaluation, by two-way ANOVA, of the main effects of inoculum concentration (Factor A), succinic anhydride concentration (Factor B), and their interaction; one-way comparisons of individual MPC samples against the single NPC control, however, necessarily reflect the combined effect of fermentation and succinylation and cannot fully isolate the contribution of fermentation intensity alone.

**Table 1: Experimental Design — Treatment Samples with Fermentation and Succinylation Conditions**

| Sample | Sample Code | Inoculum (%) | SA Level (% w/w) | Succinylated? |
|---|---|---|---|---|
| 1 | NPC | 1.5 | — | No (reference control) |
| 2 | MPC-1.0/1%SA | 1.0 | 1 | Yes |
| 3 | MPC-1.0/3%SA | 1.0 | 3 | Yes |
| 4 | MPC-1.5/1%SA | 1.5 | 1 | Yes |
| 5 | MPC-1.5/3%SA | 1.5 | 3 | Yes |

NPC = Native Protein Concentrate; MPC = Succinylated Modified Protein Concentrate; SA = Succinic Anhydride (% w/w Relative to Protein Mass). All Fermentations at 28 °C for 24 h

### Preliminary Fermentation Optimisation Study

Fermentation duration and inoculum level for the main experiment were selected from a preliminary 2 × 3 factorial optimisation study (inoculum 1.0%, 1.5% and 2.0% w/w × duration 16 h and 24 h; three independently prepared replicates per treatment) that assessed phytic acid content and trypsin inhibitor activity (TIA) in debittered African oil bean seed flour (DAOBSF baseline: phytic acid 924.6 mg/100g; TIA 14.86 TIU/mg). Results are summarised in Table 2. The 1.5% inoculum/24 h treatment (T4) achieved a favourable balance of phytic acid reduction (55.1%) and TIA reduction (54.0%) without the greater over-proteolysis observed at 2.0% inoculum (T6), and was therefore adopted as the optimal fermentation condition for the main experiment; the 1.0% inoculum/24 h condition was retained as the lower-intensity comparator to enable factorial evaluation of inoculum effects on succinylation and functional properties.

**Table 2: Preliminary 2×3 Factorial Fermentation Optimisation Study — Effect of Inoculum Concentration and Duration on Phytic Acid (PA) and Trypsin Inhibitor Activity (TIA) of Debittered P. *macrophylla* Flour (Mean ± SD, n = 3)**

| Treatment | Inoculum (%) | Duration (h) | Phytic Acid (mg/100g) | TIA (TIU/mg) | PA Reduction (%) | TIA Reduction (%) |
|---|---|---|---|---|---|---|
| T1 | 1.0 | 16 | 682.4±8.2 | 10.24±0.28 | 52.8 | 31.1 |
| T2 | 1.0 | 24 | 524.8±7.6 | 8.36±0.24 | 43.2 | 43.7 |
| T3 | 1.5 | 16 | 546.2±8.0 | 7.84±0.22 | 40.9 | 47.2 |
| T4 ★ | 1.5 | 24 | 414.8±6.8 | 6.84±0.18 | 55.1 | 54.0 |
| T5 | 2.0 | 16 | 498.6±7.2 | 7.12±0.20 | 46.1 | 52.1 |
| T6 | 2.0 | 24 | 386.4±6.2 | 6.48±0.16 | 58.2 | 56.4 |

★ = selected optimal treatment. Reductions expressed relative to the DAOBSF baseline (phytic acid 924.6 mg/100g; TIA 14.86 TIU/mg).

**Preparation of Debittered and Fermented Flour**

Debittered African oil bean seed flour (DAOBSF) was prepared by soaking cleaned cotyledons in distilled water at 80 °C (grain:water = 1:3 w/v) for 16 h, cooking at 100 °C for 1 h, washing sequentially at 35 °C and 18 °C (grain:water = 1:15) for 5 h each, oven-drying at 50 °C for 24 h, milling, defatting by Soxhlet extraction with n-hexane (6–8 h), and sieving through a 100 µm mesh, adapted from Villacrés *et al*. (2020). For fermentation, dry S. cerevisiae (1.0 g or 1.5 g per 100 g DAOBSF) was dispersed in distilled water, mixed into the flour, and fermented at 28 ± 1 °C for 24 h under aluminium-foil cover, followed by oven-drying (40 °C, 24 h), milling and sieving to obtain DFAOBSF-1.0 and DFAOBSF-1.5.

**Extraction of Native Protein Concentrate and Succinylation**

Protein was extracted from each fermented flour by alkaline extraction–isoelectric precipitation (AE-IEP): dispersion in distilled water (1:10 w/v), pH adjustment to 9.0 ± 0.1 (0.5 M NaOH), stirring (200 rpm, 25 °C, 1 h), centrifugation (8,000 × g, 20 min, 4 °C), isoelectric precipitation of the supernatant at pH 4.5 ± 0.1, washing, neutralisation to pH 7.0, and freeze-drying (−50 °C, 0.01 mbar, 48 h). NPC was obtained from DFAOBSF-1.5 without further modification. For succinylation, each protein concentrate was dispersed in water (1:10 w/v), adjusted to pH 8.0 ± 0.1, and reacted with succinic anhydride (1% or 3% w/w relative to protein) added incrementally over 10 min at 25 °C, with the reaction continued for 60 min at pH 8.0 (adapted from Zhang *et al*., 2021). Products were purified by dialysis (MWCO 12–14 kDa, 4 °C, 48 h, three dialysate changes), neutralised to pH 7.0, and freeze-dried.

**Determination of Physicochemical, Structural and Techno-Functional Properties**

Degree of succinylation (DS) was determined by the trinitrobenzenesulfonic acid (TNBS) assay. Proximate composition (moisture, ash, crude fat, crude protein, crude fibre, carbohydrate) was determined by standard AOAC methods (AOAC International, 2019). Colour (CIE L*a*b*) was measured with a HunterLab colorimeter; dispersion pH, isoelectric point (by zeta-potential titration) and surface hydrophobicity ($S_0$, ANS fluorescence probe) were determined following Nwokocha *et al*. (2023). Secondary structure was assessed by FTIR spectroscopy (Amide I band curve-fitting) to quantify α-helix, β-sheet, β-turn and random-coil content, and to confirm succinylation via the Amide II downshift and the succinyl carbonyl band at ~1,718 $cm^{-1}$ (Kong & Yu, 2007). Molecular weight distribution was assessed by SDS-PAGE. Techno-functional properties were determined as follows: nitrogen solubility index (NSI) at pH 3.0–11.0 by the Bradford method (Zhang *et al*., 2021); water- and oil-holding capacities (WHC, OHC) gravimetrically (Stone *et al*., 2015); emulsifying activity and stability indices (EAI, ESI) turbidimetrically (McClements & Grossmann, 2021); foaming capacity and stability (FC, FS) by the whipping method (Verni *et al*., 2022); and least gelation concentration (LGC) by the tube-inversion method (Sá *et al*., 2020).

In vitro protein digestibility (IVPD) was determined by sequential pepsin–pancreatin digestion (Udenigwe & Aluko, 2017). Antioxidant activity was assessed by DPPH and ABTS radical-scavenging assays and by the ferric-reducing antioxidant power (FRAP) assay (Benzie & Strain, 1996). Alkaloid, saponin, tannin and cyanogenic glycoside contents were determined gravimetrically/colorimetrically (Gemede & Ratta, 2014; Harborne, 1973; Obadoni & Ochuko, 2001) at each processing stage, from raw seed through debittering, fermentation, extraction and succinylation. Bulk density, Hausner ratio, Carr index and particle size (laser diffraction) were determined following Shevkani *et al*. (2015).

Protein recovery, defined as the proportion of total nitrogen in the starting fermented flour recovered in the final freeze-dried concentrate, was calculated as Recovery (%) = (protein content × concentrate yield) / (protein content of starting flour) × 100; recovery values for each treatment are reported alongside proximate composition in the Results. Removal of unreacted succinic anhydride and low-molecular-weight succinylation by-products was monitored by dialysate conductivity, with dialysis continued until effluent conductivity fell below 10 µS/cm; residual n-hexane from Soxhlet defatting was removed by air-drying the flour to constant weight, although residual solvent was not directly quantified and its confirmation by headspace gas chromatography is recommended for future validation. The TNBS assay for degree of succinylation was calibrated against an L-leucine standard curve (0–2 mM) run with each analytical batch, and DS values were corroborated qualitatively by the parallel, dose-dependent increase in FTIR succinyl carbonyl band absorbance (Table 4); this internal consistency between an amino-group-based assay and an independent spectroscopic signal supports, but does not by itself prove, the absolute accuracy of the reported DS values. FTIR Amide I curve-fitting followed established band-position assignments for α-helix, β-sheet, β-turn and random coil in globular plant proteins (Kong & Yu, 2007); as with all such curve-fitting approaches, the derived secondary-structure percentages are estimates subject to band-overlap uncertainty and are interpreted here as relative, treatment-wise trends rather than absolute structural quantification.

**Statistical Analysis**

Data are reported as means ± standard deviation (n = 3) and were analysed using IBM SPSS Statistics (Version 26.0). One-way ANOVA compared all five treatments for each parameter, with means separated by Duncan's Multiple Range Test (DMRT) at $p < 0.05$. For the four succinylated MPC samples, two-way ANOVA additionally tested the main effects of inoculum concentration (Factor A), succinic anhydride concentration (Factor B), and their interaction (A × B). Pearson correlation analysis was applied between degree of succinylation and selected functional properties.

Each treatment combination was prepared as three independently processed batches (biological replicates, n = 3) from separate fermentation and extraction runs; where an assay involved duplicate instrumental readings per batch (e.g., colorimetry, spectrophotometric absorbance), these technical replicates were averaged before statistical analysis, so that the reported n = 3 reflects independent biological replicates rather than repeated measurements of the same batch. The two-way ANOVA model applied to the four MPC samples was the fixed-effects factorial model $Yijk = \mu + Ai + Bj + (AB)ij + \varepsilon ijk$, where Ai and Bj are the main effects of inoculum concentration and succinic anhydride concentration respectively, (AB)ij is their interaction term, and εijk is random error.

## RESULTS AND DISCUSSION

### Proximate Composition and Protein Recovery

**Table 3: Proximate Composition of NPC and Modified Protein Concentrates from *Pentaclethra macrophylla* (Mean ± SD, n = 3, % Dry Weight Basis Unless Stated)**

| Parameter | NPC | MPC-1.0/1%SA | MPC-1.0/3%SA | MPC-1.5/1%SA | MPC-1.5/3%SA |
|---|---|---|---|---|---|
| Moisture (%, w.b.) | 8.52±0.18$^{a}$ | 5.24±0.12$^{b}$ | 5.18±0.09$^{b}$ | 5.09±0.11$^{b}$ | 5.11±0.10$^{b}$ |
| Ash (%) | 3.14±0.08$^{c}$ | 4.82±0.11$^{a}$ | 4.74±0.09$^{ab}$ | 4.68±0.10$^{b}$ | 4.71±0.09$^{ab}$ |
| Crude Fat (%) | 2.18±0.06$^{a}$ | 1.85±0.05$^{b}$ | 1.62±0.04$^{c}$ | 1.59±0.04$^{c}$ | 1.54±0.03$^{c}$ |
| Crude Protein (%) | 68.45±0.84$^{d}$ | 72.18±0.92$^{c}$ | 74.86±1.02$^{b}$ | 74.12±0.98$^{bc}$ | 76.53±1.14$^{a}$ |
| Carbohydrate (%) | 34.94±0.62$^{a}$ | 19.64±0.48$^{b}$ | 16.28±0.42$^{c}$ | 13.78±0.38$^{d}$ | 14.52±0.40$^{cd}$ |
| Crude Fibre (%) | 4.82±0.12$^{a}$ | 2.31±0.08$^{b}$ | 2.05±0.07$^{bc}$ | 2.08±0.07$^{bc}$ | 1.93±0.06$^{c}$ |

Values sharing a superscript within a row are not significantly different ($p > 0.05$, DMRT)

Crude protein increased significantly ($p < 0.05$) from 68.45% (NPC) to 76.53% (MPC-1.5/3%SA), consistent with concentration of protein by dialysis-mediated removal of low-molecular-weight and carbohydrate impurities, together with the added mass of succinyl groups incorporated into the Kjeldahl-derived nitrogen value — a pattern consistent with comparable increases reported for succinylated pea isolate (Wan *et al*., 2018) and soy protein isolate (Lian *et al*., 2022). Moisture and crude fat decreased significantly with succinylation, while ash increased (3.14% to 4.65–4.82%), consistent with sodium succinylate salt formation during pH adjustment with NaOH (Basak & Singhal, 2022).

Protein recovery, calculated as the proportion of total nitrogen in the starting flour recovered in the freeze-dried concentrate (see Materials and Methods), averaged across the five treatments which consistent with typical alkaline extraction–isoelectric precipitation efficiencies reported for tropical legume protein concentrates (Shevkani *et al*., 2015).

### Degree of Succinylation and Structural Properties

**Table 4: Degree of Succinylation, Isoelectric Point, Surface Hydrophobicity and FTIR Secondary Structure of NPC and Modified Protein Concentrates (Mean ± SD, n = 3)**

| Parameter | NPC | MPC-1.0/1%SA | MPC-1.0/3%SA | MPC-1.5/1%SA | MPC-1.5/3%SA |
|---|---|---|---|---|---|
| Degree of Succinylation (%) | 0.00$^{e}$ | 58.64±0.84$^{d}$ | 72.31±1.02$^{c}$ | 75.48±1.08$^{b}$ | 86.92±1.24$^{a}$ |
| Isoelectric Point (pH) | 4.48±0.06$^{a}$ | 4.12±0.05$^{b}$ | 3.91±0.04$^{c}$ | 3.86±0.04$^{c}$ | 3.58±0.04$^{d}$ |
| Surface Hydrophobicity ($S_0$, ANS units) | 3812±48$^{a}$ | 3614±42$^{b}$ | 3248±38$^{c}$ | 3102±36$^{d}$ | 2784±32$^{e}$ |
| α-Helix (%) | 18.42±0.42$^{e}$ | 20.14±0.48$^{d}$ | 22.86±0.52$^{c}$ | 23.54±0.54$^{c}$ | 26.18±0.62$^{a}$ |
| β-Sheet (%) | 38.64±0.68$^{a}$ | 34.28±0.62$^{b}$ | 30.42±0.56$^{c}$ | 29.84±0.54$^{c}$ | 25.62±0.48$^{d}$ |
| Amide II Band ($cm^{-1}$) | 1541.2±0.8$^{a}$ | 1536.4±0.9$^{b}$ | 1531.8±0.8$^{c}$ | 1529.6±0.8$^{d}$ | 1524.8±0.8$^{e}$ |
| Succinyl C=O (~1718 $cm^{-1}$, rel. abs.) | 0.000$^{e}$ | 0.182±0.006$^{d}$ | 0.346±0.010$^{c}$ | 0.398±0.012$^{b}$ | 0.562±0.016$^{a}$ |

DS by TNBS assay; $S_0$ by ANS fluorescence probe; secondary structure by second-derivative curve-fitting of the Amide I band. Values sharing a superscript within a row are not significantly different ($p > 0.05$, DMRT)

Degree of succinylation increased from 58.64% (MPC-1.0/1%SA) to 86.92% (MPC-1.5/3%SA), with a significant fermentation × succinic-anhydride interaction ($p < 0.05$): MPC-1.5/1%SA (DS = 75.48%) exceeded MPC-1.0/3%SA (DS = 72.31%) despite using three times less succinic anhydride, indicating that substrate accessibility, not reagent dose alone, is statistically associated with succinylation efficiency. A plausible explanation, consistent with this interaction pattern and with the reduced β-sheet content reported below, is that greater protease and phytase activity at 1.5% inoculum produced more extensive unfolding of the compact 11S legumin structure — a phenomenon described here descriptively as fermentation-induced partial unfolding (FIPU) — thereby exposing additional lysine ε-amino groups for acylation (Emkani *et al*., 2022). Direct confirmation of this mechanism (for example, by intrinsic fluorescence or free-thiol quantification during fermentation itself) was not undertaken in the present study and is recommended for

future work; the interpretation offered here is inferential, drawn from the statistical interaction and the FTIR evidence rather than from a direct structural assay of the fermentation step.

FTIR analysis was consistent with the DS results: the Amide II band downshifted from 1,541.2 $cm^{-1}$ (NPC) to 1,524.8 $cm^{-1}$ (MPC-1.5/3%SA), and the succinyl carbonyl band at ~1,718 $cm^{-1}$, absent in NPC, grew in proportion to DS (Kong & Yu, 2007). β-Sheet content fell from 38.64% to 25.62% while α-helix and random-coil content rose correspondingly, a pattern consistent with charge-repulsion-driven unfolding of compact β-domains and within the 12–32% β-sheet reduction range reported for fermented legume proteins generally (Emkani *et al*., 2022). The isoelectric point shifted from pH 4.48 to 3.58 with increasing DS, consistent with conversion of lysine's positively charged ammonium groups to negatively charged succinyl carboxylates, while surface hydrophobicity decreased monotonically from 3,812 to 2,784 ANS units. This decrease is consistent with polar succinyl groups masking hydrophobic surface patches, as reported for succinylated soy protein isolate at comparable DS (Lian *et al*., 2022); this interpretation was not independently verified here by surface-specific hydrophobicity mapping and should be regarded as the most plausible explanation consistent with the available data rather than a directly demonstrated mechanism.

**Techno-Functional Properties**

**Table 5: Techno-Functional Properties of NPC and Modified Protein Concentrates (Mean ± SD, n = 3)**

| Parameter | NPC | MPC-1.0/1%SA | MPC-1.0/3%SA | MPC-1.5/1%SA | MPC-1.5/3%SA |
|---|---|---|---|---|---|
| **NSI at pH 7.0 (%)** | **52.38±0.96$^{e}$** | **61.24±1.08$^{d}$** | **72.86±1.24$^{c}$** | **76.18±1.32$^{b}$** | **84.62±1.48$^{a}$** |
| WHC (g water/g protein) | 2.84±0.06$^{e}$ | 3.18±0.08$^{d}$ | 3.64±0.09$^{c}$ | 3.82±0.10$^{b}$ | 4.24±0.12$^{a}$ |
| OHC (g oil/g protein) | 2.62±0.06$^{a}$ | 2.48±0.06$^{b}$ | 2.32±0.05$^{c}$ | 2.24±0.05$^{d}$ | 2.08±0.04$^{e}$ |
| EAI ($m^2$/g) | 28.46±0.62$^{e}$ | 36.82±0.78$^{d}$ | 44.28±0.92$^{c}$ | 47.64±0.98$^{b}$ | 58.92±1.18$^{a}$ |
| ESI (min) | 18.64±0.48$^{e}$ | 24.82±0.58$^{d}$ | 34.46±0.72$^{c}$ | 38.24±0.82$^{b}$ | 52.18±1.06$^{a}$ |
| Foaming Capacity (%) | 42.18±0.84$^{e}$ | 50.64±0.98$^{d}$ | 58.24±1.08$^{c}$ | 62.84±1.18$^{b}$ | 72.46±1.36$^{a}$ |
| Foaming Stability at 30 min (%) | 48.62±0.96$^{e}$ | 54.18±1.02$^{d}$ | 62.84±1.18$^{c}$ | 66.48±1.26$^{b}$ | 74.26±1.42$^{a}$ |
| Least Gelation Conc. (% w/v) | 12.0±0.0$^{a}$ | 11.0±0.0$^{b}$ | 10.0±0.0$^{c}$ | 10.0±0.0$^{c}$ | 9.0±0.0$^{d}$ |

NSI = Nitrogen Solubility Index; WHC/OHC = water/oil holding capacity; EAI/ESI = Emulsifying Activity/Stability Index. Values Sharing a Superscript Within a Row are not Significantly different ($p > 0.05$, DMRT)

Succinylation produced substantial improvements in nearly all techno-functional parameters. NSI at pH 7 rose from 52.38% (NPC) to 84.62% (MPC-1.5/3%SA), with a significant A × B interaction ($p < 0.01$) indicating that fermentation pre-conditioning amplified the solubility response to succinylation, consistent with the additional negative charge density introduced at each acylated lysine (Zhu *et al*., 2025). This 32-percentage-point gain falls within the range reported for succinylated legume proteins generally (Lian *et al*., 2022; Mune Mune *et al*., 2011); based on literature-reported values rather than direct side-by-side comparison in this study, the pH-11 value of 93.28% for MPC-1.5/3%SA (not shown) is comparable to commercial soy protein isolate benchmarks of 85–95% (Shevkani *et al*., 2015). WHC increased by 49.3% (2.84 to 4.24 g/g) with no significant A × B interaction, indicating additive contributions of the two factors, whereas OHC decreased over the same gradient — the expected consequence of polar succinyl groups masking hydrophobic oil-binding surfaces (Basak & Singhal, 2022).

EAI and ESI improved by 107% and 180% respectively, with a significant interaction effect ($p < 0.05$) reflecting the combined contribution of higher solubility, increasingly negative zeta potential (−18.4 to −44.2 mV, not shown), and partial unfolding exposing amphiphilic segments for interfacial adsorption (Lian *et al*., 2022). Based on literature benchmark values rather than a direct comparative assay, the resulting EAI of 58.92 $m^2$/g for MPC-1.5/3%SA approaches commercial soy protein concentrate benchmarks (50–58 $m^2$/g; Shevkani *et al*., 2015). Foaming capacity and stability improved additively (no significant interaction), consistent with foaming depending primarily on interfacial surface activity contributed independently by fermentation-derived structural flexibility and succinylation-derived charge density. Least gelation concentration decreased from 12.0% to 9.0% w/v with succinylation, indicating more efficient gel-network formation at lower protein concentration; again based on literature benchmarks rather than direct comparison, this range is comparable to commercial soy protein concentrate (8–12% w/v; Shevkani *et al*., 2015) and lower than reported values for native cowpea protein concentrate (14–18% w/v; Mune Mune *et al*., 2011).

**In Vitro Digestibility and Antioxidant Activity**

**Table 6: In Vitro Protein Digestibility and Antioxidant Activity of NPC and Modified Protein Concentrates (Mean ± SD, n = 3)**

| Parameter | NPC | MPC-1.0/1%SA | MPC-1.0/3%SA | MPC-1.5/1%SA | MPC-1.5/3%SA |
|---|---|---|---|---|---|
| IVPD (%) | 74.82±0.94$^{a}$ | 70.24±0.88$^{b}$ | 67.48±0.84$^{c}$ | 68.14±0.86$^{bc}$ | 64.28±0.80$^{d}$ |
| DPPH Scavenging @1 mg/mL (%) | 42.64±0.86$^{e}$ | 47.18±0.92$^{d}$ | 52.84±1.02$^{c}$ | 55.46±1.08$^{b}$ | 62.38±1.22$^{a}$ |
| DPPH $IC_{50}$ (mg/mL) | 1.82±0.04$^{a}$ | 1.64±0.04$^{b}$ | 1.42±0.03$^{c}$ | 1.36±0.03$^{d}$ | 1.14±0.02$^{e}$ |
| FRAP (µmol TE/g) | 148.6±2.8$^{e}$ | 172.4±3.2$^{d}$ | 198.6±3.8$^{c}$ | 208.4±4.0$^{b}$ | 238.2±4.6$^{a}$ |

IVPD = in vitro protein digestibility; TE = Trolox equivalent. Values sharing a superscript within a row are not significantly different ($p > 0.05$, DMRT)

IVPD decreased significantly from 74.82% (NPC) to 64.28% (MPC-1.5/3%SA), a reduction of 10.54 percentage points. This reduction warrants particular attention, since chemical modification of a food protein that improves functional properties while measurably lowering digestibility represents a genuine trade-off rather than a straightforward improvement, and the nutritional implications of this trade-off should not be understated. The reduction is consistent with steric blockage of trypsin-accessible lysine–arginine cleavage sites by succinyl groups (Samtiya *et al*., 2020), though this specific mechanism was not directly confirmed by site-level analysis in this study. The magnitude observed is consistent with the 8–18% IVPD reduction typically reported for succinylation at DS above 55% (Basak & Singhal, 2022). All MPC samples retained IVPD above the ≥60% in vitro digestibility benchmark suggested by Sá *et al*. (2020) as a practical reference point for food-grade plant protein ingredients; it should be noted that no formal regulatory digestibility threshold exists for plant protein concentrates, and this value is used here only as an indicative, literature-derived benchmark rather than a universal nutritional-acceptability standard. Notably, MPC-1.5/1%SA retained an IVPD of 68.14% — only 4 percentage points below NPC — while delivering substantially improved solubility (76.18% vs 52.38%) and EAI (47.64 vs 28.46 m²/g), suggesting a comparatively more favourable digestibility–functionality balance among the MPC samples; recommending its use in infant or clinical nutrition would, however, be premature without dedicated in vivo digestibility, allergenicity and safety evaluation in the relevant population, and no such recommendation is made here.

Antioxidant activity improved concurrently with modification: DPPH scavenging at 1 mg/mL rose from 42.64% to 62.38%, and FRAP from 148.6 to 238.2 µmol TE/g, with a significant fermentation × succinylation interaction ($p < 0.05$) for most parameters. This pattern is consistent with the combined contribution of fermentation-generated bioactive peptides and succinylation-induced exposure of aromatic residues (tyrosine, tryptophan) from within the unfolded protein matrix (Senanayake *et al*., 2023; Zhu *et al*., 2025), although this combined mechanism was not independently isolated in the present design. The FRAP value achieved for MPC-1.5/3%SA exceeds comparable literature values reported for succinylated mung bean protein hydrolysate and fermented soy protein concentrate. It is also relevant to note that regulatory and toxicological data specifically addressing succinylated food proteins remain limited (Basak & Singhal, 2022), reinforcing the need for targeted nutritional safety evaluation before any food-grade application of the MPC samples is considered.

**Anti-Nutritional Factor Reduction**

**Table 7: Final Anti-Nutritional Factor Content in NPC and Modified Protein Concentrates, with Cumulative Reduction from Raw Seed (Mean ± SD, n = 3)**

| Parameter | NPC | MPC-1.0/1%SA | MPC-1.0/3%SA | MPC-1.5/1%SA | MPC-1.5/3%SA | Cumulative Reduction |
|---|---|---|---|---|---|---|
| Alkaloids (mg/100g) | 0.06±0.01$^{a}$ | 0.04±0.01$^{ab}$ | 0.04±0.01$^{ab}$ | 0.03±0.01$^{b}$ | 0.02±0.01$^{c}$ | 99.3% |
| Saponins (g/100g) | 0.08±0.02$^{a}$ | 0.06±0.01$^{ab}$ | 0.05±0.01$^{b}$ | 0.05±0.01$^{b}$ | 0.04±0.01$^{c}$ | 98.8–99.4% |
| Tannins (mg CE/100g) | 82.6±1.8$^{a}$ | 78.4±1.6$^{bc}$ | 72.8±1.5$^{c}$ | 68.6±1.4$^{d}$ | 62.4±1.2$^{e}$ | 74.6–80.8% |
| CNG (mg HCN eq./100g) | 0.08±0.01$^{a}$ | 0.06±0.01$^{ab}$ | 0.06±0.01$^{ab}$ | 0.04±0.01$^{b}$ | 0.04±0.01$^{b}$ | 99.0–99.5% |

CE = Catechin Equivalents; HCN eq. = Hydrogen Cyanide Equivalents. Cumulative Reduction Calculated relative to Raw-seed Baseline (Alkaloids 2.84 mg/100g; Saponins 6.82 g/100g; Tannins 324.8 mg/100g; CNG 8.24 mg HCN eq./100g). Values Sharing a Superscript within a Row are Not Significantly Different ($p > 0.05$, DMRT)

The debittering step (soaking and cooking) achieved the largest single-stage reductions in the freely water-soluble alkaloids (85.2%), saponins (88.0%) and cyanogenic glycosides (84.9%), while the partially cell-wall-bound condensed tannins were reduced more modestly at this stage (23.5%). Yeast fermentation at 1.5% inoculum contributed significantly greater reductions than 1.0% inoculum for all four compound classes ($p < 0.05$) — for example, 48.3% versus 33.7% additional tannin reduction from the debittered baseline — a pattern consistent with dose-dependent tannase and glycosidase activity of S. cerevisiae (Kasprowicz-Potocka *et al*., 2022), although enzyme activity itself was not directly assayed. Alkaline extraction and succinylation contributed further incremental reductions, bringing cumulative reductions from raw seed to 98.8–99.4% for saponins, 99.3% for alkaloids, 74.6–80.8% for tannins, and 99.0–99.5% for cyanogenic glycosides. Residual cyanogenic glycoside levels in all products (0.04–0.08 mg HCN eq./100g, equivalent to 0.4–0.8 mg HCN eq./kg) were well below the 10 mg HCN/kg maximum level established by the Codex Alimentarius Commission for edible cassava flour (Codex Alimentarius Commission, 2003). No specific regulatory cyanogenic-glycoside threshold exists for African oil bean seed or its protein concentrates, so this comparison is offered only as an indicative benchmark from the most closely related regulated commodity, not as a claim that a formal safety standard has been met. The substantially reduced tannin content in the MPC samples relative to raw seed and NPC is expected to translate into improved sensory acceptability, pending formal panel evaluation.

**Physical Powder Properties and Application Potential**

Loose bulk density decreased from 0.524 g/mL (NPC) to 0.426 g/mL (MPC-1.5/3%SA), consistent with, though not directly confirmed by, electrostatic inter-particle repulsion from surface carboxylate groups resisting powder packing; the Hausner ratio remained below 1.25 for all samples, indicating good flowability throughout (Kumar *et al*., 2022). Colour lightened (L* 62.84 to 66.70) with reduced redness and yellowness, consistent with disruption of tannin–protein chromophore complexes during succinylation and dialysis. Based on literature-reported benchmark values rather than direct side-by-side comparison, MPC-1.5/3%SA (NSI 84.62%, EAI 58.92 m²/g, FRAP 238.2 µmol TE/g) approaches the functional performance ranges reported for commercial soy protein concentrate (Shevkani *et al*., 2015), while MPC-1.5/1%SA shows a comparatively more favourable digestibility–functionality balance meriting further investigation for nutritionally sensitive food applications, subject to dedicated safety and in vivo digestibility evaluation. Given that yeast fermentation infrastructure is already culturally embedded in AOBS

processing through the traditional ugba industry, the succinylation route demonstrated here represents a scientifically promising, though not yet commercially validated, pathway for further development of P. *macrophylla* as an indigenous protein ingredient, pending the additional studies outlined in the Conclusion.

## CONCLUSION

This study shows that succinylation, modulated by fermentation intensity, substantially altered the structural and techno-functional properties of *Pentaclethra macrophylla* protein concentrate. A significant fermentation × succinic-anhydride interaction was observed for degree of succinylation, solubility, emulsifying properties and antioxidant activity, indicating that fermentation intensity is statistically associated with the extent of the succinylation response — consistent with, though not direct proof of, a fermentation-induced structural pre-conditioning effect. Succinylation improved nitrogen solubility index, emulsifying and foaming properties, water-holding capacity and antioxidant activity, at the cost of reduced oil-holding capacity and, notably, a reduction in in vitro protein digestibility from 74.82% to 64.28%; this digestibility–functionality trade-off is central to interpreting the practical value of the modification and should not be understated. Multi-stage processing achieved substantial reduction of the major anti-nutritional and bitterness compounds present in African oil bean seed. While the functional properties of the more extensively modified concentrates approach literature-reported benchmarks for commercial soy protein concentrate, claims of commercial readiness or direct nutritional equivalence are not supported by the present data and are not made here. Further work should include in vivo digestibility and nutritional safety studies, quantification of residual processing chemicals, consumer sensory evaluation, accelerated storage-stability testing, and evaluation of the modified concentrates' performance within representative food systems, before any recommendation for commercial or nutritionally sensitive application can be made.

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## CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interest associated with this study.

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