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Upcycled coconut protein and its application in a plant-based milk alternative: Digestibility, nutrient bioaccessibility, and effect on gut barrier function

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Abstract

Upcycling food-processing by-products into protein-rich ingredients could improve the sustainability and nutritional diversity of plant-based foods. This study evaluated coconut protein concentrate (CPC), recovered from a virgin coconut oil processing by-product, as an ingredient in coconut–chickpea plant-based milk alternatives. CPC contained 82.6% protein and was blended with chickpea protein (CHP) at CPC ratios of 100:0, 0:100, 50:50 and 34:66. Formulations underwent simulated gastrointestinal digestion using the INFOGEST 2.0 protocol. Protein hydrolysis was assessed by SDS-PAGE, and bioaccessible amino acids and minerals by LC-MS and ICP-MS, respectively. Low-molecular-weight (< 3 kDa) digestate fractions were evaluated for effects on barrier integrity in Caco-2 monolayers by assessing transepithelial electrical resistance (TEER) and selected tight junction protein expression in Caco-2 monolayers. Major coconut and chickpea storage proteins were extensively hydrolyzed during intestinal digestion. CPC provided higher concentrations of most bioaccessible essential amino acids (EAAs) than CHP, while the 50:50 blend largely retained this EAA-rich profile, with total bioaccessible EAAs comparable to CPC. Mineral release was formulation-dependent: CPC provided more iron, copper and potassium, whereas CHP provided more zinc, manganese, calcium and magnesium, with the blends showing intermediate or ratio-dependent profiles. In healthy Caco-2 monolayers, none of the < 3 kDa fractions compromised barrier integrity, while CHP significantly increased TEER after 48 h. In the inflammation-induced leaky gut model, CHP produced the greatest barrier protection and recovery, with the 34:66 blend also significantly improving TEER, indicating partial attenuation of inflammatory barrier disruption. Under inflammatory conditions, CHP produced the strongest increase in ZO-1 abundance, with the 34:66 blend also showing partial attenuation of inflammatory barrier disruption. Total peptides from all formulations were detected in the basolateral compartment, indicating transepithelial transport. Overall, this study demonstrates a value-added route for upcycling an underutilized coconut-processing by-product into a protein-rich food ingredient, while strategic blending with chickpea protein enables nutritionally complementary plant-based beverages with ratio-dependent nutrient bioaccessibility and intestinal barrier activity.

1. Introduction

By 2050, meat production-related emissions may increase by 63% due to population growth and rising per capita demand, highlighting an urgent need for sustainable alternatives (Kumar et al., 2023). While cultured meat and insect proteins are potential alternative solutions, plant-based options currently hold the highest consumer acceptance (Onwezen *et al.*, 2021). The increasing adoption of plant-based alternatives fueled by health and environmental concerns offer lower-impact options to conventional animal products (Bryant, 2022). However, improving the sustainability of plant-based foods requires not only replacing animal-derived proteins but also using existing food resources more efficiently. In this context, upcycling converts underutilized food-processing by-products into valuable ingredients while preserving the resources invested in their production. Upcycling is estimated to divert 1.87 million tons of food waste from landfill annually, avoid 4.85 million tons of CO_2-equivalent emissions and conserve 446 billion gallons of water (Thorsen et al., 2024). One such opportunity is coconut skim milk (CSM), a protein-rich aqueous by-product generated during cream separation in VCO processing that is commonly discarded with processing wastewater but can instead be valorized through the recovery of coconut protein concentrate (CPC) (Villarino, Castulo and Tocmo, 2026).

However, despite their sustainability benefits, upcycled proteins may have nutritional limitations that constrain their use as standalone ingredients in plant-based foods. Hybrid or blended beverages provide a promising application route, as combining upcycled plant proteins with complementary protein sources may enhance nutritional quality, functionality, and sensory performance (Arbach et al., 2021). Despite market growth, most plant-based milk alternatives (PBMAs) remain nutritionally inferior to cow's milk in protein quality, amino acid balance, and nutrient bioavailability (Asif et al., 2026). Blending complementary plant proteins may partially overcome these constraints by improving amino acid compatibility and overall nutritional density while enhancing bioactivity (Roberts et al., 2026). However, such formulations present challenges due to the lack of empirical data available on nutritional outcomes of plant protein hybridization and the digestibility and nutrient bioaccessibility of hybrid products. Nevertheless, emerging research indicates hybridizing plant protein sources can improve protein and mineral balance, resulting in nutritionally more balanced products (Lee, Leong and Oey, 2024). Responding to these challenges and opportunities, this study combined CPC upcycled from CSM with chickpea protein in hybrid PBMA formulations to improve nutritional performance.

Coconut meat contains 4.3% protein composed of 36% globulin, 19% albumin, 10% glutenin-1, 4% glutenin-2 and 2% prolamin (Patil and Benjakul, 2018; Mat et al., 2022). Coconut protein is rich in glutamic acid and arginine, however, it is severely deficientin sulfur-containing amino acids (AA) likecysteine and methionine, as well as the amino acid lysine (Patil and Benjakul, 2018). In addition to its protein profile, coconut is rich in minerals, especially potassium and magnesium, contributing to its nutritional value (Appaiah et al., 2015; Kouadio Marcellin Konan et al., 2023). Chickpea (*Cicer arietnum L.*) is a popular legume consumed globally, with a protein content varying from 15–25% (Begum et al., 2023). Globulins constitute the predominant chickpea protein fraction (53–60%), followed by glutelins (18–24%), albumins (8–12%) and prolamins (3–7%) (Chang *et al.*, 2011; Begum et al., 2023). Lysine, leucine and phenylalanine were found to be chickpea's most abundant essential amino acids (EAA), occurring at levels exceeding FAO/WHO adult reference requirements (Gao et al., 2025). In addition, chickpeas are valuable sources of essential minerals, particularly potassium, phosphorus and magnesium, and provide iron and zinc, while naturally containing relatively little sodium (Erbaş Köse and Mut, 2020).

Assessing intestinal barrier responses is an important component of evaluating plant-based foods, particularly when protein ingredients undergo extensive processing and are formulated as potential alternatives to dairy proteins. AAs and di- and tripeptides released during protein digestion are absorbed primarily in the small intestine through specific amino-acid transporters and peptide transporter 1 (PEPT1), respectively (Miner-Williams, Stevens and Moughan, 2014). Tight junction proteins, including claudins, occludin and junctional adhesion molecules, regulate paracellular permeability, while scaffolding proteins such as zonula occludens-1 (ZO-1) connect these transmembrane proteins to the actin cytoskeleton (Chelakkot, Ghim and Ryu, 2018). AAs and bioactive peptides released during protein digestion may also modulate epithelial permeability and tight-junction integrity (Suzuki, 2020). Consequently, changes in the expression, localisation or function of these proteins can compromise barrier integrity and increase the passage of luminal antigens and other potentially inflammatory substances. When cultured on permeable inserts, Caco-2 cells differentiate into polarised, enterocyte-like monolayers with functional tight junctions and are therefore widely used to assess intestinal permeability and epithelial barrier function in vitro (Sambuy et al., 2005). This model provides a suitable platform for determining whether digestible fractions from the coconut-chickpea hybrid PBMA preserve, enhance or adversely affect intestinal epithelial barrier function.

Although coconut and chickpea proteins may provide complementary AA and mineral profiles, the nutritional and physiological effects of combining them in a hybrid beverage remain unclear. In particular, limited information is available on the digestibility and nutrient bioaccessibility of coconut protein recovered from CSM, an underutilised VCO processing by-product, or on the effects of digested PBMAs on intestinal barrier function. The present study therefore extended our previous work, which developed and characterised coconut-chickpea PBMA formulations and identified the 34:66 protein blend as consumer acceptable (Villarino, Castulo and Tocmo, 2026). Using the same beverage formulations, we investigated protein digestibility and nutrient bioaccessibility following simulated gastrointestinal digestion and assessed the effects of their digested fractions on epithelial barrier integrity using a Caco-2 model. This complementary analysis provides an integrated assessment of upcycled coconut protein as a nutritionally balanced ingredient for functional hybrid plant-based beverages.

2. Materials and methods

2.1. Materials and reagents

Rapeseed oil was purchased from Co-op (Reading, UK). Guar gum and soya lecithin were purchased from Special Ingredients (Chesterfield, UK). Chickpea protein (80%-60%) was procured from Natural Thrive (Natural Remedies 4U Ltd, Wednesbury, UK). Human salivary α-amylase (Cat. No. 1031), porcine pepsin (Cat. No. P7012 or P6887), porcine bile (Cat. No. B8631) and porcine pancreatin (Cat. No. P7545SDS) were from Sigma-Aldrich. Other reagents used for INFOGEST 2.0 were of analytical grade and were either purchased from Sigma-Aldrich or J. T. Baker.

2.2. Protein isolation and proximate composition analysis

CSM was collected from two processing batches (Fig. 1a) at a VCO processing facility located in Pagbilao, Quezon, Philippines. CSM was pasteurised at 63–65°C for 30 min, transferred to sanitised high-density polyethylene (HDPE) bottles, and frozen (-18 °C) until further use. CPC was subsequently extracted from the CSM as described in our recent work (Fig. 1b). The fat content of freeze-dried CSM and CPC powders was analyzed by WeibullStoldt extraction (acid hydrolysis) followed by solvent extraction of crude fat. Moisture content was determined by over drying. Briefly, samples were placed in a hot air circulating oven (102°C) until a steady weight loss occurred to estimate moisture content. The nitrogen content of the powders was analyzed using the Kjehldahl method. The value obtained was multiplied by a factor of 6.25 to convert it to crude protein. Total dietary fiber was determined by the enzymatic-gravimetric method (AOAC Method 991 – 43). Ash content was determined by incineration at 525°C. The carbohydrate content was determined by subtracting protein, fat, water, fiber, and ash contents from the total weight. Total fatty acids were analyzed by microwave digestion followed by gas chromatography with flame ionization detection (GC-FID). Total sugars were determined by high-performance liquid chromatography (HPLC) with pulsed amperometric detection. The gross energy value was estimated using the factors for protein (4 Kcal/g), fat (9 Kcal/g), and carbohydrates (4 Kcal/g).

2.3. Analysis of selected techno-functional properties of coconut protein concentrate

2.3.1. Water holding and oil holding capacity

Water holding capacity (WHC) and oil holding capacity (OHC) of the samples were measured using a previously reported (Liu et al., 2025) with minor modifications. A 1.5 g portion of protein was placed in a centrifuge tube, added with 8 mL of deionized water or coconut oil, mixed thoroughly to hydrate the powder, and held at 25°C for 30 min. The suspension was centrifuged (Z 206 A, Hermle Labortechnik GmBH, Germany) at 4180×*g* for 10 min, after which the supernatant was carefully decanted. The remaining pellet was weighed, and WHC and OHC were calculated as the amount of water or oil retained per gram of protein using equation (Eq. 1) below:

$$WHC\ or\ OHC\ \ (\backslash \mathrm{raisebox} 1 ex\$ g\$ / \backslash \mathrm{raisebox} - 1 ex\$ g\$) = \frac{w_{final} - w_{initial}}{w_{initial}} \quad \text{(Eq. 1)}$$

where w_{final} represents the final weight of the protein sample and $w_{initial}$ represents the initial weight of the protein sample.

2.3.2. Foaming properties

The foaming properties, i.e., foaming capacity (FC) and foaming stability (FS) of the samples were evaluated following a previously reported method (Wang et al., 2022). A 50-mL protein solution (2%, w/v) was placed in a 250-mL beaker. The sample was mixed using a hand-held mixer (KD-319, Simplus, Ningbo, China) for 2 min. The foam was immediately transferred to a 100-mL graduated cylinder. The foam volumes were measured immediately after mixing (0 min) and after 30 min, denoted as V_0 and V_{30}, respectively. Foaming capacity (FC) and foaming stability (FS) were calculated using Eq. 2 and Eq. 3:

$$FC\ (\%) = \left(\frac{v_0}{V_i}\right) \times\ 100 \text{ (Eq. 2)}$$

$$FS\ at\ time\ t\ (\%) = \left(\frac{v_0}{v_{30}}\right) \times\ 100 \text{ (Eq. 3)}$$

v_i =initial suspension volume (before mixing)

v_o =suspension/foam volume after mixing)

v_{30} =suspension/foam volume after 30 min

2.4. Preparation of the hybrid coconut-chickpea beverages

The coconut-chickpea PBMA formulations analysed in this study were prepared as previously described in our sensory and flavour acceptability optimization study (Villarino, Castulo and Tocmo, 2026). Foundation recipes representing a low-protein hybrid plant-based milk beverage were prepared targeting a protein content of 2 g/100 mL (2% w/v) based on median protein content of PBMA sold in the Philippines (Anapi et al., 2025). All dried ingredients were weighed, and the powder mixes were stored in dry conditions. The amounts (g) of CPC and CHP were based on the percentages shown in Table 1. To reconstitute each beverage, a total volume of 250 mL was prepared in Milli-Q H_2O to contain 12.5 g of protein (5% w/v; the final weights of protein powder were added depending on their protein content determined by Kjeldahl method). Beverages were stored at 4 ◦C and allowed to rehydrate overnight. Four different samples with varied coconut and/or chickpea ratios were prepared (Table 1): 100% coconut protein (CPC), 100% chickpea protein (CHP), 50:50 CPC:CHP ratio, 34:66 CPC:CHP ratio. Preparation of hybrid beverages was based on our formulation and sensory optimization study (Villarino, Castulo and Tocmo, 2026). Beverage was blended to achieve homogeneous consistency and pasteurized in a water bath at 95°C for 10 minutes. Once cooled, the samples were then stored in a -20°C freezer.

Table 1
Recipes of the hybrid coconut-chickpea beverage formulations.

Ingredients	**Amounts (g/250 mL)**			
	100 CPC	**100 CHP**	**50:50 CPC: CHP**	**34:66 CPC: CHP**
CPC	7.36	0	3.43	2.25
CHP	0	6.41	3.43	4.29
White sugar	13.16	13.16	13.16	13.16
Vegetable oil	5.26	5.26	5.26	5.26
Soy lecithin	0.13	0.13	0.13	0.13
Gellan gum	0.1	0.1	0.1	0.1
Water	231.32	231.32	231.32	231.32
CPC: coconut protein concentrate; CHP: chickpea protein				

2.5. Simulated gastrointestinal digestion using the INFOGEST 2.0 static digestion protocol

To determine the digestibility and bioaccessibility of nutrients in the beverage formulations, samples were first subjected to a simulated gastrointestinal digestion (SGID) following the INFOGEST 2.0 protocol (Brodkorb et al., 2019). The oral phase was performed by addition of 5 mL of rehydrated beverage with 5 mL of simulated salivary fluids (SSF) containing 2.5 µL of 0.3 M $CaCl_2(H_2O)_2$, 0.25 mg of 1500 U/mg salivary amylase (EC 3.2.1.1) and Milli-Q H_2O. The pH was adjusted to 7 and the mixture incubated for 2 min at 37°C with shaking (120 rpm) using an IKA INC 125 FS digital (SP20) shaking-incubator (IKA England LTD, UK). In the gastric phase, 8 mL of simulated gastric fluids (SGF), 0.5 µL of 0.3 M

$CaCl_2(H_2O)_2$, 6.25 mg of 3200 U/mg porcine pepsin (EC 3.4.23.1, dissolved in SGF to reach a final concentration of 2000 U/mL) were added. The pH was adjusted to 2.5 with 5 M HCl, and Milli-Q H_2O was then added to reach a total volume of 10 mL. Samples were incubated at 37°C in a shaker-incubator (120 rpm) for 2 h, after which the pH was increased to 8.0 with 5 M NaOH to inhibit pepsin activity. In the intestinal phase, 16 mL simulated intestinal fluid (SSF) containing 300 mg of porcine bile (concentration of bile salts was 0.667 mmol/g, CAS no 8008637; 10 mM final concentration), 4 µL 0.3 M $CaCl_2(H_2O)_2$, and 10 mg pancreatin (EC 232.468.9, final concentration of 100 U/mL) were added to each sample. The concentration of bile was reduced to 2.5 mM to minimise the cytotoxicity of the digestates in the subsequent cell culture experiments (Kondrashina et al., 2024). The pH was adjusted to 7 with 5 M HCl, and Milli-Q H_2O was added to adjust the final volume to 40 mL. Samples were incubated at 37°C in a shaker-incubator (150 rpm) for 2 h. A blank (enzyme only control) were subjected to the same digestion process. Digestions were carried out in triplicate for each beverage, taking samples out after 2 h of gastric phase and at 0.5 h, 1 h, and 2 h of the intestinal phase. In the intestinal phase, samples collected were immediately placed in a 90°C water bath for 10 min to stop digestion. All samples were aliquoted and immediately frozen at -80°C until further analysis.

2.6. Protein digestion profiling by SDS-PAGE

Protein characterization was determined before and after *in vitro* digestion using SDS-PAGE under reducing conditions. Undigested samples and the digestates were precipitated with cold acetone for 1 h at − 20°C. Precipitated proteins were recovered by centrifugation (10,000×*g* at 25°C), re-solubilized in reducing 1× Laemmli buffer containing 5% β-mercaptoethanol and heated at 95°C for 5 min. An aliquot of 10 µL sample or standard molecular weight marker (Bio-Rad Precision, 10–250 kDa) was loaded into the gel and electrophoresis was conducted in a Mini-PROTEAN® Tetra Cell System (Bio-Rad, UK), which was initially run at 80 V for 15 min followed by a further 50 min at 150 V. Gels were then stained with 0.025% Coomassie Brilliant Blue R-250 solution (in 10% acetic acid aqueous solution) and rinsed with a destaining solution (40% methanol and 10% acetic acid in Milli-Q water) until protein bands could be seen clearly. Gel images were captured in ChemiDoc MP Imaging System (BIO-RAD, California, USA) and analyzed using BIO-RAD's ImageLab Software (Version 6.1).

2.7. Analysis of mineral bioaccessibility by ICP-MS

Selected minerals were analyzed for their bioaccessible in the intestinal digestates by inductively coupled plasma-mass spectrometry (ICP-MS). Freeze-dried powder (50 mg) was weighed into a 15 mL glass boiling tube, mixed with 70% nitric acid (HNO_3), and left at ambient temperature for 24 h with the cap loosely fitted. Samples were then heated for 2 h on a heat block at 90°C in a closed fume hood and filtered (0.2 µm) to remove remaining fats and undissolved solids and diluted to 1:1 with 2% HNO_3. The mineral contents of the samples were injected into an ICP-mass spectrometer (iCAP Q ICP-MS, Thermo Scientific, USA) operated in kinetic energy discrimination mode. Calibration standards of selected minerals were used to ensure reliable quantification of mineral elements. To ensure analytical accuracy, raw data obtained from ICP-MS were subjected to an enzyme only blank-correction procedure. The concentration of each mineral within the enzyme blank (C_{blank}) was subtracted from measured sample concentration (C_{sample}) to account for potential enzyme-derived interference:

$$(C_{corrected}) = (C_{sample}) - (C_{blank})$$

The resulting blank-corrected values (ppb) were then converted to final mass concentrations (mg /200 mL) to obtain an estimate of how much minerals are present per typical plant-based beverage serving size and for standardized comparison across all formulations. This conversion accounted for the final digestion (V), sample mass (m), and the appropriate dilution factor (DF) according to the following expression:

The bioaccessibility (%) of the minerals were then calculated using the formula:

2.8. Analysis of bioaccessible amino acids by LC-MS

Bioaccessible AA in the digestates were quantified using liquid chromatography-mass spectrometry (LC-MS). To obtain absorbable fractions, digestates from the intestinal digestion stage were centrifuged at 4000×*g* for 10 min at 4°C to separate undigested particulates from the supernatant (containing the bioaccessible AA) prior to freeze-drying. The same steps were carried out for the enzyme only (SGID-E) samples. AA values obtained from SGID-E samples were subtracted from all samples to account for the AAs present in the enzymes added during simulated digestions. Freeze-dried digestates were prepared as a 2 mg/ml solution (HPLC-grade water). Samples were vortexed, homogenized, and filtered through a 0.22 µm PVDF syringe filter. Chromatographic separations were carried out on a Shimadzu UHPLC system equipped with a DGU-20A degassing unit (Shimadzu Corporation, Kyoto, Japan), with detection and mass analysis on a Triple Quadruple Mass spectrometer (TQMS) system (LCMS-8050). Samples (5 µL) were injected at a flow rate of 0.25 mL/min, and the separation was performed using a Discovery® HS F5 column, 150 × 2.1 mm, 3 µm (Supelco, Sigma-Aldrich; Cat. No. 567503-U) attached to a Discovery® HS F5 Supelguard™ cartridge, 20 × 2.1 mm, 3 µm (Supelco, Sigma-Aldrich, USA). The analytical column was thermostatted at 40°C (± 0.8°C). Water with formic acid (0.1%, v/v, Solvent A) and methanol with formic acid (0.1%, v/v, Solvent B) were used as solvents for the chromatographic separation. A gradient elution program was applied starting at 35% (Solvent B) and held for 11.0 min, then the proportion of Solvent B was increased to 95% by 15 min and maintained equal operation for 3.0 min, last the system was subsequently equilibrated to 35% (Solvent B), giving a total run time of 25 min. Multiple reaction monitoring (MRM) was optimised for each AA by adjusting collision energies. Transitions falling within predefined retention time windows were used for compound identification (qualifier), while a single transition was applied for quantification (quantifier). Quantification relied on standard

calibration curves generated from injections of increasing concentrations (0-100 µM) of an AA standard (AAS18, Supelco). Data analysis was performed using LabSolutions Insight quantitative analysis software (ShimadzuCorporation, Kyoto, Japan).

2.9. Culture and characterization of Caco-2 cell monolayer

Caco-2 cells purchased from the European Collection of Cell Cultures (Salisbury, UK) were cultured in Dulbecco's Modified Eagle Medium (DMEM) medium supplemented with 4.5 g/L glucose, 4 mM L-Glutamine, 1% MEM non-essential AA (NEAA), and 10% fetal bovine serum (FBS) (F7524, Sigma-Aldrich) at 37°C in a humidified atmosphere containing 5% CO_2. Sub-confluent cells were trypsinized using 0.25% Trypsin/EDTA (SM-2003, Sigma-Aldrich) and passaged at a ratio of 1:5 every 4 days. Cells between passages 4 and 10 were used for this study. To establish an intact monolayer, cells were seeded at a density of 20,000 cells/well in Corning HTS Transwell-12 system PET membranes (Corning BV, Amsterdam, Netherlands) with 0.4 µm pore size, 1.12 cm^2/well cell growth area and cultured as described above. Media in both apical and basolateral compartments was changed every 3 days.

2.10. Effect on barrier integrity under healthy and inflammation-induced leaky gut conditions

The integrity of the monolayer was determined at basal level by measuring transepithelial electrical resistance (TEER). TEER readings were obtained using an EVOM2 Voltohmmeter (EVOM; World Precision Instruments, Hertfordshire, UK) equipped with STX4 EVOM™ Electrode. TEER values correlate with the tightness of the confluent monolayer (Srinivasan et al., 2015). The background TEER (insert) was subtracted from total TEER (cell monolayer plus insert) to yield the monolayer resistance and then normalized to surface area by multiplying with the area of the insert (1.1 cm^2). A TEER of at least 800 Ωcm^2 (n = 12) was regarded as a tight monolayer (Bietto et al., 2026). The digestates were separated into soluble (potentially digestible/absorbable) and insoluble (indigestible/non-absorbable) fractions by centrifugation at 4000×*g* at 4°C for 20 min (Santos-Hernández et al., 2020a). Soluble fractions were ultrafiltered using a 3 kDa membrane (UFC5003, Merck, Darmstadt, Germany) following manufacturer's instructions. The dialysate was freeze-dried to a powder to create a uniform and stable test material for cell-based studies, resuspended in sterile deionized H_2O, and filtered through 0.2 µm PTFE syringe filters. Non-cytotoxic dose of 3-kDa fractions were determined by MTS assay, which measures the reduction of MTS by metabolically active cells to produce a water-soluble formazan product (Cory et al., 1991).

To test the effect of 3-kDa fractions on barrier integrity in healthy gut conditions, cells were treated with sterile-filtered 3-kDa fractions, SGID-E fractions as digestion control, or media as negative control. TEER was monitored over 48 h. To test the effect of 3-kDa fractions on barrier integrity in an inflammation-induced leaky gut condition, cells in transwells with TEER values of at least 800 Ωcm^2 were treated with an inflammatory cocktail (IC) consisting of IL-1β, TNF-α, IFN-γ, and lipopolysaccharide (LPS) (Putt et al., 2017). The use of IC represents intestinal barrier dysfunction associated with chronic inflammation. IL-1β, TNF-α, and IFN-γ were applied to the basolateral compartment at 25 ng/mL, 50 ng/mL, and 50 ng/mL, respectively in supplemented growth media. LPS (1 µg/mL) was applied to the basolateral compartment. Cells were pre-treated with or without 2 mg/mL of 3-kDa fractions for 16 h followed by addition of IC and a further 72 h of incubation. TEER was measured at times 0, 24, 48, and 72 h. Control monolayers contained DMEM only or the SGID-E fractions.

2.11. Effect on selected tight junction proteins

Caco-2 cell lysates were obtained using 1× RIPA buffer (Cat. R0278, Sigma-Aldrich, St Louis, MO, USA) supplemented with 1× protease inhibitor cocktail (P8340, Sigma-Aldrich, MO, USA) and 1× phosphatase inhibitor cocktail (P5726, Sigma-Aldrich). Protein concentration was measured with a Pierce™ Dilution-Free™ Rapid Gold BCA Protein Assay (A55860, Thermo Fisher Scientific, IL, USA) according to the manufacturer's instructions. Proteins were separated by SDS-PAGE) as described previously (Tocmo and Parkin, 2018). Proteins were transferred into a nitrocellulose membrane using Bio-Rad's Trans-Blot® Turbo™ Transfer System (CA, USA). After transfer, membranes were blocked by 5% non-fat milk (Cat. 9999, Cell Signaling Technology, MA, USA) for 1 h at 20–21°C, incubated overnight at 4°C with primary antibodies from ProteinTech (Manchester, UK): zonula occludens-1 (ZO-1, 20742-1-AP) and occludin (13409-1-AP), and beta-actin (20536-1-AP). After a 1-h incubation with anti-rabbit IgG, horseradish peroxidase (HRP)-conjugated secondary antibody (SA00001-2, ProteinTech), blot images were captured using a ChemiDoc MP Imaging System (BIO-RAD, California, USA) and intensities were quantified by densitometric analysis using analyzed using BIO-RAD's ImageLab Software (Version 6.1).

2.12. Statistical Analysis

Data from at least three independent experiments were expressed as mean values ± standard deviation (SD). All statistical analysis was performed using GraphPad Prism version 10.6.1. Data from at least three independent experiments were subjected to one-way ANOVA followed by a post hoc analysis with the Tukey's test to determine significant differences among treatments. Differences at $P < 0.05$ were considered statistically significant.

3. RESULTS AND DISCUSSION

3.1. Proximate compositions of coconut skim milk and coconut protein concentrates

The freeze-dried CPC isolated from CSM appeared as a crystalline powder with a predominantly yellowish-white to off-white color (Fig. 1). Proximate analysis on an as-is basis showed that CSM powder was a rich source of protein (26%) and carbohydrates (57%, as is) (Table 2). It also contained a substantial ash fraction (9.3%), indicating a potentially appreciable mineral content. The isolated CPC powder contained 82.6% protein, with moisture, total fat, total carbohydrates, dietary fiber, and ash contents of 2.7%, 3.4%, 5.3%, 0.9%, and 6.1%, respectively. Based on prevailing industry naming conventions for commercial plant-protein ingredients, its protein content exceeding 70% supports its classification as a protein concentrate.

3.2. Techno-functional properties of CPC

CPC exhibited moderate WHC and comparatively high OHC, together with strong foaming capacity and stability (Table 3). These properties are consistent with the functional potential previously reported for proteins recovered from coconut processing streams, although their magnitude varies substantially with raw material, extraction method, and processing history. Water absorption capacity (WAC) and oil absorption capacity (OAC) values of 0.78–3.43 and 2.26–3.40 g/g, respectively, have been reported for coconut milk- and oil-cake proteins (Rodsamran and Sothornvit, 2018). Liu et al. (2025)

Table 2

Proximate composition[a] of coconut skim milk and upcycled coconut protein concentrate.

Component	Content (per 100 g)	
	Coconut skim milk	Coconut protein concentrate
Moisture	4.6 ± 0.0	2.7 ± 0.7
Protein	26.5 ± 0.1	82.6 ± 0.8
Fat	2.3 ± 0.2	3.4 ± 0.2
Fatty acid saturates	1.8 ± 0.1	2.6 ± 0.2
Fatty acids monounsaturates (*cis*)	0.4 ± 0.0	0.6 ± 0.0
Fatty acids polyunsaturates (*cis*)	0.1 ± 0.0	0.1 ± 0.0
Fatty acid (*trans*)	0.1 ± 0.0	0.1 ± 0.0
Carbohydrate (total)	57.3 ± 0.2	5.3 ± 0.8
Total dietary fibre	1.5 ± 0.5	0.9 ± 0.0
Ash	9.3 ± 0.0	6.1 ± 0.4
Total energy (kJ)	1495 ± 5	1602 ± 16
Total energy (kCal)	353 ± 1.5	378.5 ± 3.5

[a]mean values ± standard deviation (SD); n = 3

similarly reported WHC values of 1.76–2.38 g/g for proteins recovered from VCO-processing by-products (Liu et al., 2025). The WHC values reported in these studies are broadly comparable to those observed for CPC in the present study. In contrast, the OHC of CPC exceeded values reported for coconut proteins (Rodsamran and Sothornvit, 2018) and for desiccated-coconut and copra-meal protein isolates (2.12 and 2.71 g/g, respectively) (Liu et al., 2024), suggesting strong protein-lipid interactions that may favor lipid and flavor retention in formulated foods. CPC also showed greater foaming capacity and foaming stability than the ranges reported by Rodsamran and Sothornvit (2018) (28.33-85.00% and 16.67–43.33%, respectively), although direct quantitative comparison should be made cautiously because foaming behavior is strongly dependent on protein concentration, pH and assay conditions. Protein unfolding and exposure of hydrophobic regions promote adsorption at air-water and oil-water interfaces, providing a mechanistic basis for both foaming and emulsifying functionality of coconut proteins (Chambal, Bergenståhl and Dejmek, 2013; Rodsamran and Sothornvit, 2018), while processing-induced changes in protein conformation, hydrophobicity and aggregation can further modify these properties (Liu et al., 2024). Overall, the combination of moderate water retention, strong oil binding and high foam formation and stability demonstrates the techno-functional potential of CPC for incorporation into formulated foods, particularly applications requiring lipid retention, aeration and structural stability.

Table 3
Techno-functional properties of CPC.

Techno-functional properties	Mean values ± SD (n = 3)
Water-holding capacity (g H_2O/ g sample)	1.84 ± 0.48*
Oil-holding capacity (g oil/ g sample	4.57 ± 0.61*
Foaming capacity (%)	105.33 ± 3.06*
Foaming stability (%)	98.43 ± 0.57*
**Values are mean ± SD*	

**Significant difference between samples by independent samples t-test ($p < 0.05$).*

3.2. Digestibility of proteins in foundation beverage formulations

The SDS-PAGE profiles of CPC and CHP showed broadly overlapping molecular-weight distributions, with differences primarily in the relative intensity and prominence of individual protein bands (Fig. 2). CPC showed prominent bands at approximately 50 kDa, 34–36 kDa and 22–24 kDa, together with several lower-molecular-weight bands below 20 kDa. The bands in the ~ 50, ~34–36 and ~ 22–24 kDa regions are consistent with the reported subunit pattern of cocosin (11S globulin), the major coconut storage protein, which comprises approximately 86% of total coconut globulins (Garcia et al., 2005). The < 20 kDa bands may represent lower-molecular-weight coconut protein polypeptides and/or products of protein processing or partial proteolysis, although specific identities cannot be established from SDS-PAGE alone. CHP similarly displayed prominent bands within the ~ 45–65, ~ 30–40 and ~ 20–25 kDa regions, consistent with the expected distribution of chickpea globulins, including vicilin (7S) and the acidic and basic polypeptides of legumin (11S). Previous studies have likewise identified 45–65, 30–37 and 20–25 kDa as major band regions in chickpea protein (Chang et al., 2022; Di Francesco et al., 2024). The hybrid formulations retained bands across these overlapping molecular-weight regions, with variations in relative intensity reflecting contributions from both protein sources.

Following gastric digestion, the intensity of the major CPC bands decreased markedly, although faint residual bands remained (Fig. 2a), indicating substantial but incomplete pepsin hydrolysis. Cocosin comprises acidic and basic polypeptide chains associated through disulfide bonds and assembled into the compact oligomeric structure characteristic of 11S globulins (Garcia et al., 2005; Angelia et al., 2010). This compact structure may contribute to the persistence of faint protein bands following gastric digestion (Fig. 2a, lane 2), consistent with the relative resistance of specific regions of 11S globulins to pepsin hydrolysis reported for legumin from common bean (Santamaria et al., 2024). However, extensive hydrolysis of bands in lanes 3–5 (Fig. 2a, lanes 3–5) indicates the pancreatin enzymes achieved full hydrolysis of the major storage fraction.

During gastric digestion, CHP proteins remained faintly visible (Fig. 2b, lane 2), indicating partial resistance to pepsin hydrolysis. This may be attributed to the compact secondary structure of chickpea globulins, which can limit the accessibility of proteolytic cleavage sites, together with their propensity to aggregate under acidic gastric conditions (Contardo et al., 2025). Moreover, the high β-sheet content characteristic of many legume proteins may contribute to reduced proteolytic susceptibility; indeed, β-sheet content has been negatively correlated with in vitro digestibility in soy proteins (Yang et al., 2016). However, extensive hydrolysis of protein bands during the intestinal phase (Fig. 2b, lanes 3–5) indicates extensive hydrolysis by pancreatic proteases under intestinal conditions, consistent with the substantially greater intestinal digestibility reported for chickpea globulins (Contardo et al., 2025). Similarly, extensive hydrolysis of bands in the blended formulations indicates that combining CPC and CHP did not hinder intestinal proteolysis, with proteins from both sources remaining readily susceptible to enzymatic hydrolysis under intestinal conditions.

3.3. Bioaccessible amino acids in the beverage formulations after INFOGEST 2.0 digestion

Among the bioaccessible EAAs, phenylalanine and leucine were the most abundant in both CPC and CHP, with significantly higher levels in CPC (Table 4). This is broadly consistent with the amino acid profile reported previously (Thaiphanit and Anprung, 2014), in which leucine was the most abundant EAA in coconut protein powder, with phenylalanine also present at relatively high levels. Contrastingly, CHP has a slightly higher lysine content than CPC. Methionine, threonine, and histidine were the least abundant bioaccessible EAAs in both protein sources but occurred at significantly higher concentrations in CPC than CHP. Among the NEAAs, glutamic acid and arginine predominated, although their relative abundance differed between the protein sources: CPC contained significantly more arginine, whereas CHP contained more glutamic acid. Cysteine was not quantified because of limitations of the analytical method, although its oxidised dimer, cystine, was detected at low concentrations. The higher concentrations of bioaccessible EAAs in CPC than CHP may reflect differences in their intrinsic amino acid composition and/or the extent to which individual amino acids were released and solubilised during gastrointestinal digestion. Protein source, structural characteristics and processing history can influence susceptibility to gastrointestinal proteolysis and, consequently, the amounts and profiles of amino acids and peptides released during digestion (Santos-Hernández et al., 2020b; Rivera del Rio, Boom and Janssen, 2022).

Residual antinutritional components in chickpea protein, including protease inhibitors, phytate and phenolic compounds, may also influence protein digestion, although their contribution depends on their retention following protein extraction and processing (Mondor et al., 2009; Rivera del Rio, Boom and Janssen, 2022). Notably, total bioaccessible NEAA

Table 4
Bioaccessible amino acids (g/100 g) in the digestates* of the beverage formulations after the INFOGEST digestion.

Amino acid	100 CPC	100 CHP	50CPC:50CHP	34CPC:66CHP
	Essential Amino Acids (EAA)			
Valine	$1.70^{a} \pm 0.16$	$1.19^{b} \pm 0.05$	$1.74^{a} \pm 0.03$	$1.18^{b} \pm 0.08$
Leucine	$8.61^{a} \pm 0.91$	$4.86^{b} \pm 0.45$	$8.23^{a} \pm 0.27$	$5.31^{b} \pm 0.29$
Isoleucine	$1.92^{a} \pm 0.17$	$1.30^{b} \pm 0.10$	$1.83^{a} \pm 0.04$	$1.49^{b} \pm 0.11$
Methionine	$0.69^{a} \pm 0.06$	$0.21^{c} \pm 0.06$	$0.59^{a} \pm 0.04$	$0.39^{b} \pm 0.03$
Phenylalanine	$10.83^{a} \pm 1.33$	$7.10^{b} \pm 0.34$	$9.38^{a} \pm 0.10$	$7.42^{b} \pm 0.53$
Threonine	$0.65^{a} \pm 0.05$	$0.32^{c} \pm 0.03$	$0.56^{a} \pm 0.03$	$0.43^{b} \pm 0.03$
Lysine	$1.83^{a} \pm 0.18$	$2.08^{a} \pm 0.04$	$1.87^{a} \pm 0.09$	$1.39^{b} \pm 0.08$
Histidine	$0.63^{a} \pm 0.07$	$0.15^{d} \pm 0.05$	$0.43^{b} \pm 0.01$	$0.27^{c} \pm 0.02$
Tryptophan	$1.70^{a} \pm 0.14$	$0.67^{c} \pm 0.11$	$1.36^{b} \pm 0.07$	$1.31^{b} \pm 0.04$
Total EAA	**$28.55^{a} \pm 3.07$**	**$17.88^{b} \pm 1.17$**	**$25.98^{a} \pm 0.46$**	**$19.19^{b} \pm 1.09$**
	Non-Essential Amino Acids (NEAA)			
Tyrosine	$4.24^{b} \pm 0.37$	$4.93^{ab} \pm 0.33$	$4.93^{ab} \pm 0.25$	$5.09^{a} \pm 0.21$
Cystine	$0.32^{a} \pm 0.04$	$0.20^{b} \pm 0.06$	$0.25^{ab} \pm 0.03$	$0.19^{b} \pm 0.02$
Proline	$0.10^{a} \pm 0.00$	$0.07^{b} \pm 0.01$	$0.11^{a} \pm 0.00$	$0.07^{b} \pm 0.00$
Glycine	$0.47^{a} \pm 0.02$	$0.33^{b} \pm 0.03$	$0.45^{a} \pm 0.04$	$0.41^{a} \pm 0.01$
Glutamic acid	$8.64^{c} \pm 0.12$	$12.81^{a} \pm 0.31$	$10.14^{b} \pm 0.10$	$12.10^{a} \pm 0.91$
Aspartic acid	$1.81^{a} \pm 0.07$	$1.75^{ab} \pm 0.16$	$1.84^{a} \pm 0.28$	$1.22^{b} \pm 0.28$
Alanine	$0.30^{a} \pm 0.02$	$0.21^{b} \pm 0.02$	$0.31^{a} \pm 0.01$	$0.22^{b} \pm 0.02$
Serine	$0.71^{ab} \pm 0.06$	$0.59^{c} \pm 0.01$	$0.79^{a} \pm 0.02$	$0.63^{bc} \pm 0.06$
Arginine	$14.13^{a} \pm 1.35$	$8.24^{c} \pm 0.47$	$11.33^{b} \pm 0.27$	$9.02^{c} \pm 0.34$
Total NEAA	**$30.71^{a} \pm 1.65$**	**$29.12^{a} \pm 0.61$**	**$30.15^{a} \pm 0.49$**	**$28.95^{a} \pm 1.60$**
Total AA	**$59.26^{a} \pm 4.72$**	**$46.99^{b} \pm 1.25$**	**$56.13^{a} \pm 0.94$**	**$48.13^{b} \pm 2.67$**

*Digestates after 2 h of simulated intestinal digestion were heated to 90°C to inactivate enzymes. The digestates were then centrifuged (4000 × *g*, 10 min) and the soluble fractions were analyzed for AAs by LC-MS.

concentrations were comparable between CPC and CHP (30.71 and 29.12 g/100 g, respectively), whereas total bioaccessible EAAs were substantially higher in CPC (28.55 vs 17.88 g/100 g). The 50:50 CPC:CHP formulation contained 25.98 g/100 g total bioaccessible EAAs, which. This differential pattern suggests that the higher bioaccessible EAA concentrations in CPC cannot be attributed simply to greater overall amino acid release, but may reflect differences in the underlying AA composition and/or digestive release of specific AAs.

Plant proteins can differ substantially in their EAA composition and digestibility; therefore, combining proteins with different AA profiles may provide a more complementary nutritional profile than reliance on a single source (Hertzler et al., 2020; Popova, Mihaylova and Lante, 2023). This may be particularly relevant to EAAs involved in muscle protein synthesis, such as leucine, which can occur at suboptimal levels in some plant proteins (Yang et al., 2012; Berrazaga et al., 2019). In the present study, blending CPC with CHP modified the distribution of bioaccessible EAAs

while retaining much of the EAA-rich profile of CPC, particularly at the 50:50 ratio. The 50:50 CPC:CHP formulation contained 25.98 g/100 g total bioaccessible EAAs, which was not significantly different from CPC (28.55 g/100 g) but was significantly higher than CHP (17.88 g/100 g). Importantly, this formulation maintained bioaccessible leucine, isoleucine, methionine, phenylalanine and threonine at concentrations statistically comparable to CPC, despite CHP constituting half of the protein blend. Lysine was also maintained at a concentration comparable to both individual protein sources. These findings indicate that the 50:50 formulation did not simply produce an intermediate AA profile, but enabled incorporation of CHP while largely preserving the higher bioaccessible EAA concentrations associated with CPC. Increasing the proportion of CHP to 66% shifted the profile towards that of CHP. The 34:66 CPC:CHP formulation had a total bioaccessible EAA concentration of 19.19 g/100 g, which was significantly lower than CPC and the 50:50 formulation but comparable to CHP. Nevertheless, incorporation of 34% CPC resulted in higher bioaccessible methionine, threonine, histidine and tryptophan than CHP alone, indicating that CPC contributed to improving several of the EAAs present at relatively low concentrations in CHP. Overall, the findings demonstrate that the nutritional effect of blending was ratio-dependent, with the 50:50 formulation providing the strongest retention of CPC's high bioaccessible EAA content, while the 34:66 formulation provided a profile more closely resembling CHP.

The findings are consistent with previous studies showing that protein blending can compensate for AA limitations in individual plant proteins. For example, blending lupin, pea or soy protein with potato or rice protein progressively improved AA scores because the component proteins had complementary AA profiles (Etzbach et al., 2025). Similarly, incorporating soybean or cowpea into cereal-based blends increased lysine and total EAA concentrations while retaining the relatively high leucine content of the cereals (Shuluwa et al., 2021). Soybean fortification of maize- and wheat-based products has likewise been shown to increase lysine and tryptophan, thereby improving EAA scores, PDCAAS and *in vivo* indices of protein quality (Acevedo-Pacheco and Serna-Saldívar, 2016). In the present study, the effect of blending differed somewhat from these classical examples of AA complementation. Rather than one protein strongly compensating for a limiting AA in the other, CPC contributed substantially higher concentrations of most bioaccessible EAAs, whereas CHP contained slightly more lysine. Notably, the 50:50 blend retained total bioaccessible EAA concentrations comparable to CPC, while the 34:66 blend increased methionine, threonine, histidine, and tryptophan relative to CHP alone. Thus, the principal nutritional benefit of blending was the diversification of protein sources while retaining, to varying degrees, the higher bioaccessible EAA concentrations contributed by CPC.

3.4. Bioaccessibility of selected minerals in the beverage formulations after INFOGEST 2.0 digestion

Following SGID, intestinal mineral release varied according to protein source and blending ratio (Table 5). CPC provided the highest amounts of bioaccessible iron, copper, and potassium, whereas CHP provided slightly more zinc, manganese, calcium, and magnesium. Blending produced a more evenly distributed mineral profile by combining the complementary strengths of the two protein sources. Both blends retained appreciable iron and magnesium, while the 34:66 formulation provided the highest zinc release and matched CHP for manganese. The 50:50 blend also contained more bioaccessible copper than CHP alone. Although blending did not maximize the intestinal release of every mineral, it reduced reliance on the mineral profile of a single protein source and produced PBMAs containing meaningful amounts of minerals. This supports previous evidence that combining coconut- and chickpea-derived ingredients can broaden the nutritional composition of plant-based beverages (Rincon, Braz Assunção Botelho and de Alencar, 2020).

Mineral bioaccessibility was strongly mineral- and formulation-dependent. CPC exhibited the highest percent bioaccessibility of zinc, iron, copper, and magnesium, whereas CHP showed higher manganese and potassium bioaccessibility. The blends again produced a broader, although not uniformly superior, pattern: the 50:50 formulation retained relatively high iron, calcium and potassium bioaccessibility, while the 34:66 formulation showed the highest manganese bioaccessibility and maintained comparatively high potassium bioaccessibility. These findings indicate ratio-dependent complementation, with the 50:50 blend favoring the relative release of iron and calcium and the CHP-rich blend favoring manganese and potassium. However, the lower bioaccessibility of zinc and magnesium in the blends may reflect mineral interactions with proteins and/or antinutritional components, including residual phytate potentially present in CHP (Mesfin, Belay and Amare, 2021). Phytate can form poorly soluble complexes with divalent mineral ions, although its inhibitory effect is particularly well established for zinc (Gupta, Gangoliya and Singh, 2015). Values exceeding 100% should not be interpreted as potential intestinal absorption exceeding the initial mineral content; rather, they may reflect enhanced release and solubilization of matrix-bound minerals during digestion, as well as analytical variation and potential mineral contributions from digestion reagents (Grundy et al., 2025; Aksoy et al., 2026).

These findings agree with previous formulation studies showing that blending plant sources can broaden the mineral profile of the resulting product. Combining chickpea and coconut extracts produced beverages with a favorable nutritional composition, with formulations containing 70–90% chickpea extract provided calcium concentrations comparable to cow’s milk and several commercial PBMAs (Rincon, Braz Assunção Botelho and de Alencar, 2020). Similarly, blending coconut and almond beverages significantly improved their micronutrient composition, with a 75:25 coconut:almond formulation producing the most balanced nutritional profile (Uche et al., 2025). Beyond beverages, a previous study demonstrated that cereal-legume blending increased calcium and zinc as the proportion of legumes increased, confirming that complementary raw materials can address mineral limitations in single-source formulations (Walle and Moges, 2017). Our results extend earlier compositional studies by showing that the benefits of blending are not limited to total mineral content but can persist in the intestinally bioaccessible fraction

as demonstrated through the INFOGEST digestion protocol. Nevertheless, neither blend outperformed both individual proteins for every mineral, and the reduced zinc and magnesium bioaccessibility observed in some blends indicates that complementation was mineral-specific rather than universally synergistic. This likely reflects interactions among proteins, phytate and divalent minerals during digestion and reinforces the need to evaluate mineral release in the final blended matrix rather than predict it solely from ingredient composition.

3.5. 3-kDa fractions of the PBMA digestates enhance barrier function in healthy and inflammation-induced leaky gut model

To determine whether low-molecular-weight digestion products influenced intestinal barrier

Table 5
Intestinal release (g/200 mL) and bioaccessibility (%) of selected minerals of the beverage formulations after INFOGEST 2.0 digestion.

Minerals	100 CPC		100 CHP		50CPC:50CHP		34CPC:66CHP	
	Intestinal release (mg/200 mL)	% Bioaccessibility	Intestinal release (mg/200 mL)	% Bioaccessibility	Intestinal release (mg/200 mL)	% Bioaccessibility	Intestinal release (mg/200 mL)	% Bioaccessibility
Zinc	0.32 ± 0.07	167 ± 55	0.37 ± 0.02	99 ± 32	0.32 ± 0.05	30 ± 11	0.40 ± 0.12	61 ± 21
Iron	1.11 ± 0.34	820 ± 206	0.83 ± 0.19	346 ± 64	0.81 ± 0.23	525 ± 73	0.93 ± 0.22	308 ± 61
Copper	0.54 ± 0.16	83 ± 35	0.16 ± 0.01	66 ± 3.3	0.23 ± 0.01	62 ± 15	0.15 ± 0.01	62 ± 11
Manganese	0.01 ± 0.003	47 ± 0.70	0.03 ± 0.01	60 ± 13	0.02 ± 0.00	42 ± 2	0.03 ± 0.00	77 ± 2.3
Calcium	0.13 ± 0.01	33 ± 35	0.21 ± 0.02	9.1 ± 4.5	0.15 ± 0.01	43 ± 10	0.13 ± 0.01	11 ± 3
Magnesium	18.42 ± 2.67	152 ± 17	19.78 ± 4.59	110 ± 19	16.15 ± 4.74	92 ± 4.8	14.13 ± 1.35	82 ± 12
Potassium	827.90 ± 43.88	76 ± 18	648.10 ± 127.90	87 ± 13	776.90 ± 109.60	81 ± 9.2	773.2 ± 67.37	89 ± 16

function, the soluble fractions of the INFOGEST digestate were ultrafiltered through a 3-kDa membrane, freeze-dried, and reconstituted for cell treatment. Caco-2 cells were cultured on transwell inserts until differentiated monolayers developed, as demonstrated by the progressive increase in transepithelial electrical resistance (TEER) from approximately 250 Ω·cm² at day 0 to approximately 2,600 Ω·cm² by days 14–19 (Fig. 3a). Cell viability was subsequently assessed across a concentration range of 1–4 mg/mL to identify a suitable non-cytotoxic treatment concentration (Fig. 3b). At 1 and 2 mg/mL, none of the beverage-derived fractions appreciably reduced cell viability relative to the untreated control. However, viability declined at 4 mg/mL, particularly following treatment with the CHP and 50:50 CPC:CHP fractions. Therefore, 2 mg/mL was selected for the barrier-function experiments, as it permitted assessment of bioactivity without an overt cytotoxic effect.

In a healthy Caco-2 model representing an intact monolayer, the 3-kDa fractions did not reduce TEER over the 48-h treatment period, indicating that the digestion products did not compromise basal barrier integrity at the selected concentration (Fig. 3c). The CHP fraction produced the clearest response, significantly increasing TEER to approximately 2,350 Ω·cm² after 48 h compared with approximately 2,000 Ω·cm² in the untreated control. The 34:66 CPC:CHP fraction also showed an upward trend, reaching approximately 2,200 Ω·cm², although this was not statistically significant. On the other hand, CPC and the 50:50 blend remained close to the control. These differences indicate that the response was dependent on protein source and blending ratio rather than simply on the total amount of digestion-derived material. The stronger effect of CHP may reflect the release of barrier-active chickpea peptides during digestion, although residual non-protein constituents in the 3-kDa fraction could also have contributed.

Exposure to the inflammatory cocktail (IC) caused a pronounced reduction in TEER, from approximately 1,500-1,600 Ω·cm² at baseline to approximately 800–900 Ω·cm² after 24 h, confirming successful disruption of the epithelial barrier (Fig. 3d). TEER remained low in the inflammatory-cocktail control throughout the subsequent 72 h. Pretreatment with the CHP fraction produced the greatest protection and recovery, significantly increasing TEER to approximately 1,200-1,250 Ω·cm² at 72 h. The 34:66 CPC:CHP fraction produced a smaller but significant improvement, reaching approximately 1,050 – 1,100 Ω·cm², whereas CPC and the 50:50 blend provided little protection. Importantly, the CHP-containing fractions did not completely restore TEER to the untreated-control level, indicating partial attenuation rather than preventing inflammatory barrier damage. The stronger effect of the 34:66 blend compared with 505:50 or CPC alone suggests the release of barrier-active chickpea peptides during digestion, although their activity was moderated by the proportion of the coconut fraction.

To investigate whether the TEER responses were accompanied by modulation of tight junction proteins, Caco-2 monolayers exposed to IC were treated with CPC, CHP or the 34:66 CPC:CHP 3-kDa fractions at 0.5 and 2.0 mg/mL. Cell lysates were analysed by immunoblotting for zonula occludens-1 (ZO-1) and occludin, with β-actin used as the loading control (Fig. 4). Densitometric values were normalised to β-actin and expressed relative to the untreated control. The 50:50 fraction was not included in this analysis, because it did not show an appreciable protective effect in the inflammatory TEER experiment. Exposure to IC reduced relative ZO-1 abundance from 1.0 in the untreated control to 0.8. Both CPC and CHP counteracted this reduction, although the CHP fraction produced the strongest response. CPC increased the ZO-1/β-actin ratio to 1.2 and 1.1 at 0.5 and 2.0 mg/mL, respectively, whereas CHP increased it to 1.3 and 1.6. The 34:66 blend showed a weaker response, with ratios of 0.8 and 1.0 at the corresponding concentrations. Thus, the improvement in TEER produced by CHP in Fig. 3d was accompanied by a concentration-dependent increase in ZO-1 abundance. In comparison, occludin was less affected by the inflammatory treatment, decreasing only from 1.0 to 0.9. CHP and the 34:66 blend increased the occludin/β-actin ratio to 1.0-1.1, while CPC restored it to 1.0 only at 2.0 mg/mL. The results therefore suggest that preservation or induction of ZO-1, rather than a large change in total occludin abundance, was more closely associated with the functional TEER response.

The stronger effect on ZO-1 than on occludin is biologically plausible because TEER is determined by the coordinated assembly, localisation and phosphorylation state of several junctional proteins and does not necessarily correspond directly to the total abundance of any single protein. Kiewiet et al. (2018), for example, found that a barrier-protective soy hydrolysate altered claudin-1 and claudin-2 expression without changing ZO-1 or occludin, despite protecting TEER (Kiewiet, Faas and de Vos, 2018). Conversely, Bitzer et al. (2016) also found that soy protein concentrate protected against DSS-induced Caco-2 permeability even though corresponding changes in *Cldn1* and *Ocln* transcript abundance were not detected *in vivo* (Bitzer et al., 2017). Collectively, these studies show that functional protection may involve changes in selected tight junction proteins, their cellular localisation or upstream inflammatory signalling rather than uniform increases in all junctional proteins (Moran, O'Neill and McLaughlin, 2012; Bitzer et al., 2017; Kiewiet, Faas and de Vos, 2018). These findings are broadly consistent with previous evidence that digestion- generated plant-protein fractions can protect intestinal epithelial monolayers. Bitzer et al. (2016) reported that soy protein concentrates attenuated DSS-induced permeability in differentiated Caco-2 cells, and that pepsin-pancreatin-digested soy protein retained this barrier-protective activity. Kiewiet et al. (2018) similarly found that pretreatment with a selected soy protein hydrolysate protected epithelial monolayers against decreases in TEER induced by the calcium ionophore A23187 and the membrane-disrupting peptide melittin, although the protection depended on the barrier-disrupting stimulus. This stimulus specificity may partly explain why the coconut, chickpea, and blended fractions differed in the present inflammatory model. Chickpea proteins can also release cytoprotective peptides. For example, Guo et al. (2014) showed that an antioxidative pentapeptide derived from chickpea protein hydrolysate protected Caco-2 and HT-29 cells against oxidative stress (Guo et al., 2014). Together, these studies support the possibility that small digestion-derived peptides contributed to the protective effects observed here, but peptide identification and pathway-specific experiments would be required to establish the active components and mechanisms (Guo et al., 2014; Bitzer et al., 2017; Kiewiet, Faas and de Vos, 2018). One limitation of the current study is that the peptide sequences present in the CHP and 34:66 fractions were not identified. However, we measured total peptide concentrations in the apical and basolateral compartments after exposure of Caco-2 monolayers to the 3-kDa fractions (Fig. 5). All beverage-derived fractions produced significantly higher apical peptide concentrations than the untreated control, with CHP showing the highest concentration (approximately 220 µg/mL), followed by the 34:66 blend (approximately 205 µg/mL). Peptides were also detected in the basolateral compartment for all formulations, demonstrating that a proportion of the low-molecular-weight digestion products crossed the Caco-2 monolayer. CHP produced the highest basolateral peptide concentration (approximately 65 µg/mL), while CPC, the 50:50 blend and the 34:66 blend produced broadly comparable concentrations of approximately 45–55 µg/mL. Basolateral peptide concentration following CPC treatment was also significantly higher than that of untreated control.

The presence of peptides in the basolateral compartment suggests that digestion-derived peptides from both individual proteins and blended formulations were capable of transepithelial transport. The relatively high basolateral concentration produced by CHP is consistent with its stronger effects on TEER and ZO-1 abundance, although the present data cannot establish that the transported peptides were directly responsible for these effects. Moreover, because total peptide concentrations differed among the apical samples, absolute basolateral concentrations should not be interpreted as transport efficiency without normalisation to the corresponding apical concentration. Identification and quantification of individual peptide sequences by LC-MS/MS and testing of purified candidate peptides would clarify which peptides cross the epithelial barrier and whether they mediate the barrier-protective activity of CHP and the 34:66 blend.

4. Conclusion

This study demonstrates the potential of upcycled CPC, recovered from VCO processing by-products, as a functional ingredient for developing nutritionally complementary coconut-chickpea plant-based beverages. Simulated gastrointestinal digestion resulted in extensive hydrolysis of the major coconut and chickpea storage proteins during the intestinal phase. Blending modified the distribution of bioaccessible nutrients in a ratio-dependent manner. CPC provided substantially higher concentrations of most bioaccessible EAAs than CHP, and the 50:50 CPC:CHP formulation largely retained this EAA-rich profile, with total bioaccessible EAA concentrations comparable to CPC despite half of the protein being derived from CHP. The 34:66 formulation showed an EAA profile more closely resembling CHP, although incorporation of CPC increased

several EAAs that occurred at relatively low concentrations in CHP. The blends also combined minerals contributed by the individual protein sources, although mineral release and percentage bioaccessibility remained strongly dependent on blending ratio. Neither blend was universally superior across nutrients, highlighting the importance of assessing nutrient bioaccessibility in the final food matrix rather than predicting it from ingredient composition alone.

The < 3-kDa fractions generated during digestion did not compromise intact Caco-2 monolayers and showed formulation-dependent effects on intestinal barrier function. The chickpea fraction produced the strongest improvement in TEER under both basal and inflammation-induced conditions, accompanied particularly by increased ZO-1 abundance, while changes in occludin were comparatively modest. The 34:66 CPC:CHP fraction also partially promoted barrier recovery, indicating that barrier-protective activity was retained within this hybrid formulation. Peptides from all formulations were detected in the basolateral compartment, supporting transepithelial transport of low-molecular-weight digestion products, although the transported peptide sequences and their contribution to the observed barrier effects remain to be established. Overall, the findings support strategic, ratio-dependent blending of upcycled coconut protein with chickpea protein as an approach to diversify protein sources while retaining favourable bioaccessible nutrient profiles and potential intestinal barrier-protective activity. Further work should identify the bioactive peptides and their mechanisms and validate the nutritional and physiological performance of selected formulations *in vivo*.

Declarations

Ethics declaration

not applicable.

Competing Interests:

The authors declare no competing interests

Consent to Participate:

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Consent to Publish:

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Author Contribution

Conceptualization: R.T. Methodology: R.T., Y.W., C.V.B. Formal analysis: W.W., R.T., Y.W., A.G.E., and C.B.V. Investigation: R.T., W.W., A.G.E., L.T., and C.B.V. Writing-original draft preparation: R.T., L.T., W.W., and C.B.V. Writing-review and editing: R.T. Project administration: R.T. Funding acquisition: R.T. and C.B.V.

Data Availability

Included in the paper or Supplementary Information.

References

1. Acevedo-Pacheco, L., & Serna-Saldívar, S. O. (2016). In vivo protein quality of selected cereal-based staple foods enriched with soybean proteins. *Food & Nutrition Research*, *60*, 31382. https://doi.org/10.3402/fnr.v60.31382
2. Aksoy, A. S., et al. (2026). Effects of Different Cooking Methods (Pan-Frying, Sous-Vide, Boiling, and Deep Frying) on Mineral Composition and In Vitro Bioaccessibility of Beef, Lamb, and Chicken Livers. *Food Science & Nutrition*, *14*(6), e72011. https://doi.org/10.1002/fsn3.72011
3. Anapi, G. R., et al. (2025). Nutritional profile of selected plant-based milk alternatives sold in Metro Manila, the Philippines. *Philippine Journal of Science*, *154*(2), 235–245. https://doi.org/10.56899/154.02.02
4. Appaiah, P., et al. (2015). Physico-chemical characteristics and stability aspects of coconut water and kernel at different stages of maturity. *Journal of Food Science and Technology*, *52*(8), 5196–5203. https://doi.org/10.1007/s13197-014-1559-4
5. Arbach, C. T., et al. (2021). Recent patent applications in beverages enriched with plant proteins. *NPJ Science of Food*, *5*(1), 28. https://doi.org/10.1038/s41538-021-00112-4
6. Asif, N., et al. (2026). The rise of plant-based milk alternatives: exploring nutritional, health, and sustainability impacts. *Food Chemistry: X*, *34*, 103528. https://doi.org/10.1016/j.fochx.2026.103528
7. Begum, N., et al. (2023). Nutritional composition, health benefits and bio-active compounds of chickpea (Cicer arietinum L). *Frontiers in Nutrition*, 10–2023. 10.3389/fnut.2023.1218468. https://www.frontiersin.org/journals/nutrition/articles/
8. Berrazaga, I., et al. (2019). The Role of the Anabolic Properties of Plant- versus Animal-Based Protein Sources in Supporting Muscle Mass Maintenance: A Critical Review. *Nutrients*, *11*(8), 1825. https://doi.org/10.3390/nu11081825
9. Bietto, F., et al. (2026). Modeling a leaky gut In vitro: Sodium butyrate-induced barrier disruption of Caco-2 monolayers. *The Journal of Nutritional Physiology*. https://doi.org/10.1016/j.jnphys.2026.100021. 6.
10. Bitzer, Z. T., et al. (2017). Soy protein concentrate mitigates markers of colonic inflammation and loss of gut barrier function in vitro and in vivo. *Journal of Nutritional Biochemistry*, *40*, 201–208. https://doi.org/10.1016/j.jnutbio.2016.11.012
11. Brodkorb, A., et al. (2019). INFOGEST static in vitro simulation of gastrointestinal food digestion. *Nature Protocols*, *14*(4), 991–1014. https://doi.org/10.1038/s41596-018-0119-1
12. Bryant, C. J. (2022). Plant-based animal product alternatives are healthier and more environmentally sustainable than animal products. *Future Foods*, 100174. https://doi.org/10.1016/j.fufo.2022.100174. 6.
13. Chambal, B., Bergenståhl, B., & Dejmek, P. (2013). Coconut Press Cake Alkaline Extract—Protein Solubility and Emulsification Properties. *Food and Nutrition Sciences*, *04*, 29–37. https://doi.org/10.4236/fns.2013.49A2005
14. Chang, L., et al. (2022). Plant proteins from green pea and chickpea: Extraction, fractionation, structural characterization and functional properties. *Food Hydrocolloids*, *123*, 107165. https://doi.org/10.1016/j.foodhyd.2021.107165
15. Chang, Y. W. (2011). 'Characterization of protein fractions from chickpea (Cicer arietinum L.) and oat (Avena sativa L.) seeds using proteomic techniques', *Food Research International*, 44(9), pp. 3094–3104. Available at: https://doi.org/10.1016/j.foodres.2011.08.001
16. Chelakkot, C., Ghim, J., & Ryu, S. H. (2018). Mechanisms regulating intestinal barrier integrity and its pathological implications. *Experimental & Molecular Medicine*, *50*(8), 1–9. https://doi.org/10.1038/s12276-018-0126-x
17. Contardo, I., et al. (2025). Understanding the structural differences in chickpea globulins and their relationship with in vitro protein digestibility. *Food Research International*, *202*, 115702. https://doi.org/10.1016/j.foodres.2025.115702
18. Cory, A. H., et al. (1991). Use of an Aqueous Soluble Tetrazolium/Formazan Assay for Cell Growth Assays in Culture. *Cancer Communications*, *3*(7), 207–212. https://doi.org/10.3727/095535491820873191
19. Di Francesco, A., et al. (2024). Mass Spectrometry Characterization of the SDS-PAGE Protein Profile of Legumins and Vicilins from Chickpea Seed. *Foods*, *13*(6), 887. https://doi.org/10.3390/foods13060887
20. Erbaş Köse, Ö., & Mut, Z. (2020). Mineral Contents of Chickpea Cultivars (Cicer arietinum L.) Grown at Different Locations of Turkey. *Sains Malaysiana*, *49*, 293–303. https://doi.org/10.17576/jsm-2020-4902-07
21. Etzbach, L., et al. (2025). Beyond the sum of parts: Exploring the combination of plant protein ingredients for enhanced nutritional quality and techno-functionality. *Food Chemistry*, *492*, 145517. https://doi.org/10.1016/j.foodchem.2025.145517
22. Gao, Y., et al. (2025). Structures, functional properties and simulated digestion in vitro of Chickpea protein fractions. *European Food Research and Technology*, *251*(10), 3287–3303. https://doi.org/10.1007/s00217-025-04829-4
23. Garcia, R. N., et al. (2005). 11S and 7S Globulins of Coconut (Cocos nucifera L.): Purification and Characterization. *Journal of Agricultural and Food Chemistry*, *53*(5), 1734–1739. https://doi.org/10.1021/jf0402173
24. Grundy, M. M. L., et al. (2025). Bioaccessibility and associated concepts: Terminology in the context of in vitro food digestion studies. *Food Chemistry*, *485*, 144424. https://doi.org/10.1016/j.foodchem.2025.144424
25. Guo, Y., et al. (2014). The effects of an antioxidative pentapeptide derived from chickpea protein hydrolysates on oxidative stress in Caco-2 and HT-29 cell lines. *Journal of Functional Foods*, *7*, 719–726. https://doi.org/10.1016/j.jff.2013.12.013

26. Gupta, R. K., Gangoliya, S. S., & Singh, N. K. (2015). Reduction of phytic acid and enhancement of bioavailable micronutrients in food grains. *Journal of Food Science and Technology*, *52*(2), 676–684. https://doi.org/10.1007/s13197-013-0978-y
27. Hertzler, S. R., et al. (2020). Plant Proteins: Assessing Their Nutritional Quality and Effects on Health and Physical Function. *Nutrients*, *12*(12), 3704. https://doi.org/10.3390/nu12123704
28. Kiewiet, M. B. G., Faas, M. M., & de Vos, P. (2018). 'Immunomodulatory Protein Hydrolysates and Their Application.', *Nutrients*, 10(7). https://doi.org/10.3390/nu10070904
29. Kondrashina, A., et al. (2024). Coupling in vitro food digestion with in vitro epithelial absorption; recommendations for biocompatibility. *Critical Reviews in Food Science and Nutrition*, *64*(26), 9618–9636. https://doi.org/10.1080/10408398.2023.2214628
30. Kouadio Marcellin, Konan, et al. (2023). Influence of Maturity Stage and Cultivar on the Proximate, Mineral and Amino-Acid Composition of Cocos nucifera L. Kernel from Côte d'Ivoire Coconut Germplasm. *Journal of Food and Nutrition Sciences*, *11*(5), 146–153. https://doi.org/10.11648/j.jfns.20231105.12
31. Kumar, P., et al. (2023). New insights in improving sustainability in meat production: opportunities and challenges. *Critical Reviews in Food Science and Nutrition*, *63*(33), 11830–11858. https://doi.org/10.1080/10408398.2022.2096562
32. Lee, P. Y., Leong, S. Y., & Oey, I. (2024). The role of protein blends in plant-based milk alternative: A review through the consumer lens. *Trends in Food Science & Technology*, *143*, 104268. https://doi.org/10.1016/j.tifs.2023.104268
33. Liu, X., et al. (2024). Effect of Dry Processing of Coconut Oil on the Structure and Physicochemical Properties of Coconut Isolate Proteins. *Foods*, *13*(16), 2496. https://doi.org/10.3390/foods13162496
34. Liu, X. Y., et al. (2025). Structural and techno-functional characteristics of protein from the by-product of virgin coconut oil produced by centrifugation using coconut milk. *Food Chemistry: X*, *28*, 102561. https://doi.org/10.1016/j.fochx.2025.102561
35. Mat, K., et al. (2022). Coconut Palm: Food, Feed, and Nutraceutical Properties. *Animals*, *12*(16), 2107. https://doi.org/10.3390/ani12162107
36. Mesfin, N., Belay, A., & Amare, E. (2021). Effect of germination, roasting, and variety on physicochemical, techno-functional, and antioxidant properties of chickpea (Cicer arietinum L.) protein isolate powder. *Heliyon*, *7*(9). https://doi.org/10.1016/j.heliyon.2021.e08081
37. Miner-Williams, W. M., Stevens, B. R., & Moughan, P. J. (2014). Are intact peptides absorbed from the healthy gut in the adult human? *Nutrition Research Reviews*, *27*(2), 308–329. https://doi.org/10.1017/S0954422414000225. 2015/01/27 edn.
38. Mondor, M., et al. (2009). Influence of processing on composition and antinutritional factors of chickpea protein concentrates produced by isoelectric precipitation and ultrafiltration. *Innovative Food Science & Emerging Technologies*, *10*(3), 342–347. https://doi.org/10.1016/j.ifset.2009.01.007
39. Moran, G. W., O'Neill, C., & McLaughlin, J. T. (2012). GLP-2 enhances barrier formation and attenuates TNFα-induced changes in a Caco-2 cell model of the intestinal barrier. *Regulatory Peptides*, *178*(1), 95–101. https://doi.org/10.1016/j.regpep.2012.07.002
40. Angelia, M. R., et al. (2010). Physicochemical and Functional Characterization of Cocosin, the Coconut 11S Globulin. *Food Science and Technology Research*, *16*(3), 225–232. https://doi.org/10.3136/fstr.16.225
41. Onwezen, M. C. (2021). 'A systematic review on consumer acceptance of alternative proteins: Pulses, algae, insects, plant-based meat alternatives, and cultured meat', *Appetite*, 159, p. 105058. https://doi.org/10.1016/j.appet.2020.105058
42. Patil, U., & Benjakul, S. (2018). Coconut Milk and Coconut Oil: Their Manufacture Associated with Protein Functionality. *Journal of Food Science*, *83*(8), 2019–2027. https://doi.org/10.1111/1750-3841.14223
43. Popova, A., Mihaylova, D., & Lante, A. (2023). Insights and Perspectives on Plant-Based Beverages. *Plants*, *12*(19), 3345. https://doi.org/10.3390/plants12193345
44. Putt, K. K., et al. (2017). Yogurt inhibits intestinal barrier dysfunction in Caco-2 cells by increasing tight junctions. *Food & Function*, *8*(1), 406–414. https://doi.org/10.1039/c6fo01592a
45. Rincon, L., Braz Assunção Botelho, R., & de Alencar, E. R. (2020). 'Development of novel plant-based milk based on chickpea and coconut', *LWT*, 128, p. 109479. https://doi.org/10.1016/j.lwt.2020.109479
46. del Rivera, A., Boom, R. M., & Janssen, A. E. M. (2022). Effect of Fractionation and Processing Conditions on the Digestibility of Plant Proteins as Food Ingredients. *Foods*, *11*(6), 870. https://doi.org/10.3390/foods11060870
47. Roberts, J., et al. (2026). Comparative effects of dairy, hybrid and plant-based protein blends (including fibre fortification) on amino acid profiles and gut microbiota adaptations: The Promephy study. *Current Research in Food Science*, *12*, 101359. https://doi.org/10.1016/j.crfs.2026.101359
48. Rodsamran, P., & Sothornvit, R. (2018). Physicochemical and functional properties of protein concentrate from by-product of coconut processing. *Food Chemistry*, *241*, 364–371. https://doi.org/10.1016/j.foodchem.2017.08.116
49. Sambuy, Y., et al. (2005). The Caco-2 cell line as a model of the intestinal barrier: influence of cell and culture-related factors on Caco-2 cell functional characteristics. *Cell Biology and Toxicology*, *21*(1), 1–26. https://doi.org/10.1007/s10565-005-0085-6

50. Santamaria, L., et al. (2024). Identification and Characterization of a Pepsin- and Chymotrypsin-Resistant Peptide in the α Subunit of the 11S Globulin Legumin from Common Bean (Phaseolus vulgaris L). *Journal of Agricultural and Food Chemistry*, *72*(26), 14844–14850. https://doi.org/10.1021/acs.jafc.3c08744
51. Santos-Hernández, M., et al. (2020a). Compared digestibility of plant protein isolates by using the INFOGEST digestion protocol. *Food Research International*, *137*, 109708. https://doi.org/10.1016/j.foodres.2020.109708
52. Santos-Hernández, M., et al. (2020b). Compared digestibility of plant protein isolates by using the INFOGEST digestion protocol. *Food Research International*, *137*, 109708. https://doi.org/10.1016/j.foodres.2020.109708
53. Shuluwa, E. M., et al. (2021). Amino acid profiles and in vitro antioxidant properties of cereal-legume flour blends. *Journal of Food Bioactives*, *14*, 94–101. https://doi.org/10.31665/JFB.2021.14271
54. Srinivasan, B., et al. (2015). TEER Measurement Techniques for In Vitro Barrier Model Systems. *SLAS Technology*, *20*(2), 107–126. https://doi.org/10.1177/2211068214561025
55. Suzuki, T. (2020). Regulation of the intestinal barrier by nutrients: The role of tight junctions. *Animal Science Journal*, *91*(1), e13357. https://doi.org/10.1111/asj.13357
56. Thaiphanit, S., & Anprung, P. (2014). 'Increasing the utilization of coconut (Cocos nucifera L.) wet processing waste: physicochemical and functional properties of coconut protein powder', *1st Joint ACS AGFD- ACS ICSCT Symposium*.
57. Thorsen, M., et al. (2024). Upcycled food: How does it support the three pillars of sustainability? *Trends in Food Science & Technology*, *143*, 104269. https://doi.org/10.1016/j.tifs.2023.104269
58. Tocmo, R., & Parkin, K. (2018). S-Alk(en)ylmercaptocysteine suppresses LPS-induced pro-inflammatory responses in murine macrophages through inhibition of NF-κB pathway and modulation of thiol redox status. *Free Radical Biology and Medicine*, *129*, 548–558. https://doi.org/10.1016/j.freeradbiomed.2018.10.424
59. Uche, C. P., et al. (2025). Chemical composition, protein digestibility and sensory acceptability of coconut-almond nut beverage blends: A plant-based alternative. *Food Chemistry Advances*, *9*, 101193. https://doi.org/10.1016/j.focha.2025.101193
60. Villarino, C. B. J., Castulo, A. N. M., & Tocmo, R. (2026). Upcycled coconut protein as a sustainable ingredient for a novel hybrid plant-based milk alternative with chickpea protein. *International Journal of Food Science and Technology*, vvag174. https://doi.org/10.1093/ijfood/vvag174
61. Walle, H., & Moges, D. (2017). Optimization of cereal-legume blend ratio to enhance the nutritional quality and functional property of complementary food. *Ethiopian Journal of Science and Technology*, *10*(2), 109–122. https://doi.org/10.4314/ejst.v10i2.3
62. Wang, Y., et al. (2022). Effects of combined treatment with ultrasound and pH shifting on foaming properties of chickpea protein isolate. *Food Hydrocolloids*, *124*, 107351. https://doi.org/10.1016/j.foodhyd.2021.107351
63. Yang, Y., et al. (2012). Myofibrillar protein synthesis following ingestion of soy protein isolate at rest and after resistance exercise in elderly men. *Nutrition & Metabolism*, *9*(1), 57. https://doi.org/10.1186/1743-7075-9-57
64. Yang, Y., et al. (2016). Secondary Structure and Subunit Composition of Soy Protein In Vitro Digested by Pepsin and Its Relation with Digestibility. *BioMed Research International*, *2016*(1), 5498639. https://doi.org/10.1155/2016/5498639

Figures

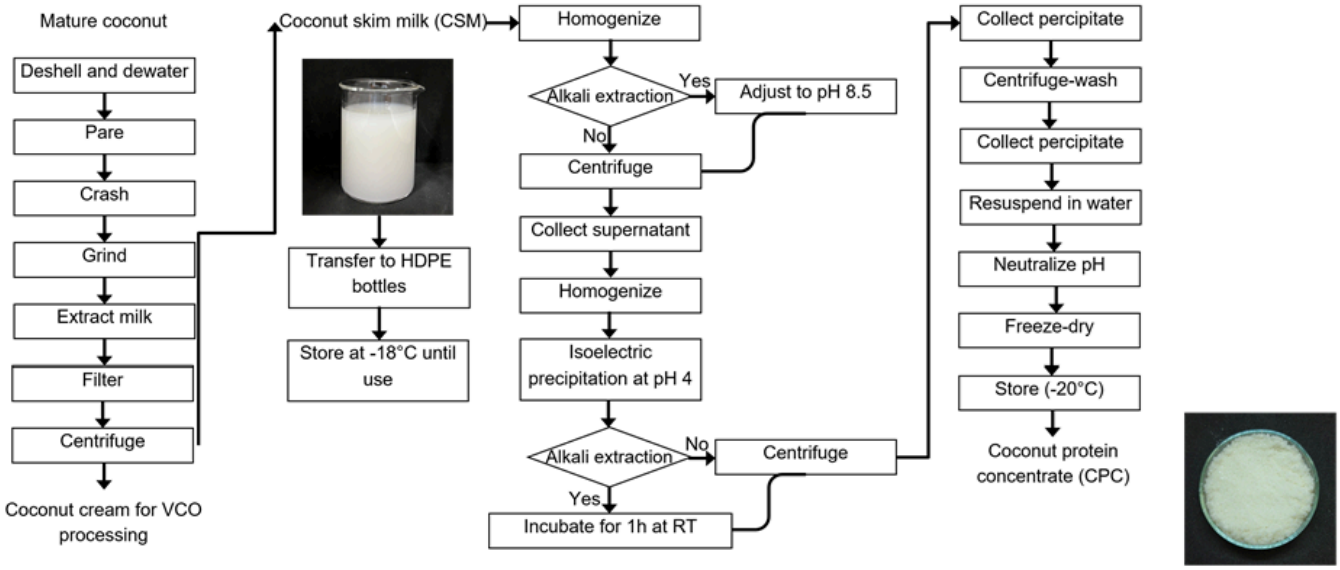


Figure 1

Schematic diagram of coconut skim milk collection and the coconut protein isolation following the alkaline-isoelectric point precipitation and representative image of the freeze-dried coconut protein concentrate recovered. VCO: virgin coconut oil; HDPE: high-density polyethylene; RT: room temperature

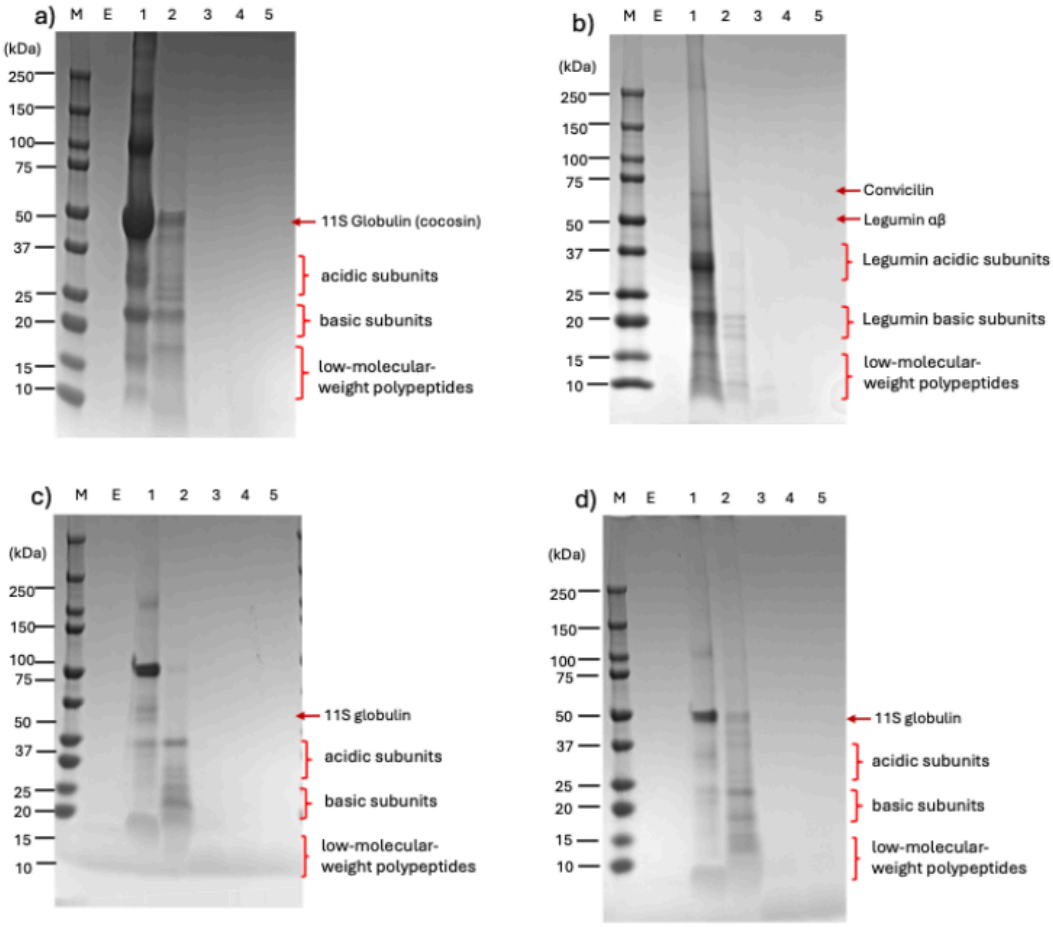


Figure 2

SDS-PAGE analysis of protein and their breakdown during in vitro digestion of the PBMA based on a) 100% CPC, b) 100% CHP, c) 50:50 CPC:CHP and d) 34:66 CPC:CHP. Lane M- protein standard marker, lane E - enzyme control, lane 1 - undigested (control), lane 2 - gastric digestate, lanes 3-5 - intestinal digestates at 30, 60, and 120 min.

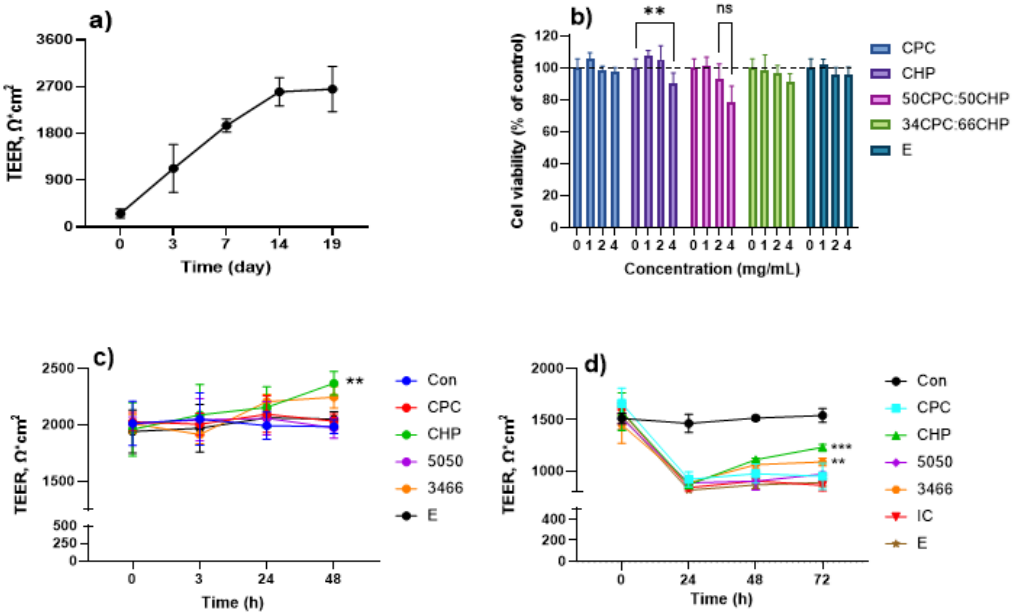


Figure 3

Effect of the 3 kDa fractions on intestinal barrier integrity in a healthy and inflammation-induced leaky gut model. (a) Development of transepithelial electrical resistance (TEER) during Caco-2 monolayer differentiation over 19 days; (b) Cell viability following exposure to digested coconut protein concentrate (CPC), chickpea protein (CHP), 50:50 CPC:CHP, 34:66 CPC:CHP and enzyme control (E) at concentrations of 0-4 mg/mL; (c) Changes in TEER over 48 h following treatment of intact Caco-2 monolayers with the digested protein samples. (d) Changes in TEER over 72 h following treatment of inflammation-compromised Caco-2 monolayers with the digested protein samples. Con, untreated control; IC, inflammatory cocktail; E, enzyme control. Data are presented as mean ± [SD/SEM]. **p < 0.01 and ***p < 0.001; ns: no significant difference.

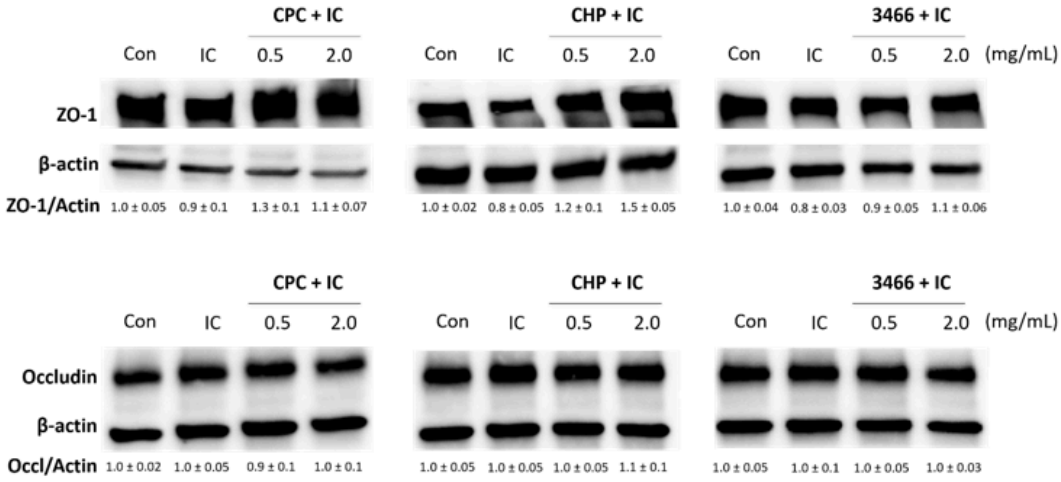


Figure 4

Effect of 3 kDa fractions on selected tight junction proteins in inflammation-induced leaky gut model using Caco-2 cells. CPC: Coconut protein concentrate; CHP: chickpea protein; IC: inflammatory cocktail.

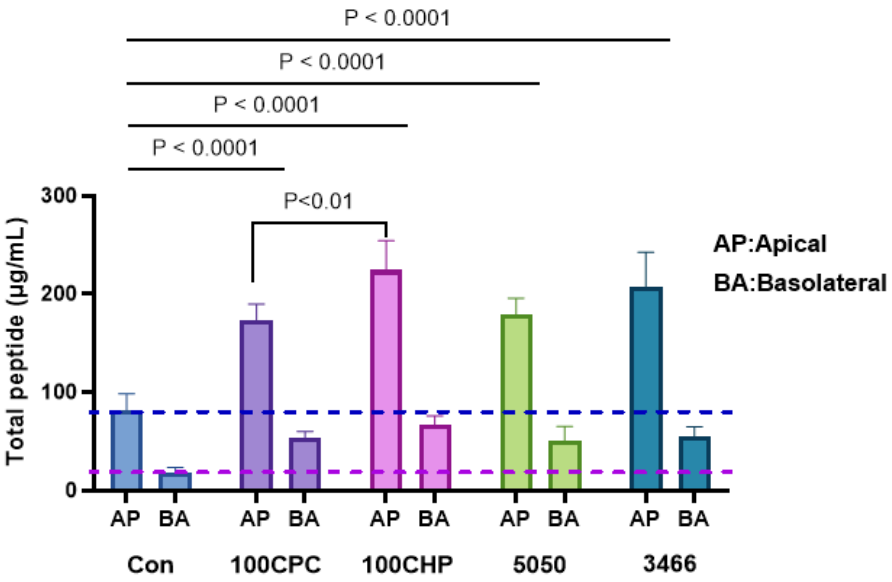


Figure 5

Total peptide transport across Caco-2 cell monolayer.