Sustainable protein extraction from co-products using an integrated bio-inspired process

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Abstract. Agricultural co-products such as olive pomace, date pits and oilseed meals contain valuable proteins that are difficult to recover with solvent-intensive, high-energy methods. We present a bio-inspired, modular workflow that emulates digestive logic under mild conditions to improve selectivity and preserve functionality. We present a bio-inspired, integrated process that emulates natural digestion through six coordinated stages: (1) gentle physical pretreatment; (2) selective enzymatic de-phenolization; (3) progressive enzymatic hydrolysis; (4) synergistic microbial fermentation; (5) membrane ultrafiltration; and (6) final stabilization by drying or encapsulation. Operated under mild, solvent-free conditions, this sequence increases protein yields, preserves bioactivity and functionality, and adapts across diverse biomasses within a low-impact, circular-bioeconomy framework. The resulting protein fractions are suitable for human food and animal feed, as well as cosmetic, pharmaceutical, and biodegradable-materials applications. A digital twin is envisioned to simulate and optimize kinetics and transport, enabling adaptive control, improved reproducibility, and efficient scale-up. This work has been preprinted on ChemRxiv [1] to accelerate feedback and technology transfer. Overall, the approach offers a modular, data-guided route to convert agri-food residues into high-value protein ingredients while significantly reducing waste, chemicals, and energy demand along the processing chain.

1 Introduction

In the current global landscape shaped by population expansion, climate variability, resource depletion, and intensifying strain on agri-food systems the development of sustainable and locally sourced protein alternatives has become a strategic necessity. A wide array of co-products, including olive pomace, date pits, oilseed meals, cereal hulls, fruit peels, and plant stalks, represents an underexploited reservoir of biomolecules. These materials, commonly diverted to animal feed, compost, or waste, actually contain high-

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value constituents such as proteins, peptides, fibers, polyphenols, and lipids suitable for applications in food, nutraceuticals, biomaterials, cosmetics, and pharmaceuticals [1-6]. Protein recovery from these residues therefore fulfills a dual objective: reducing waste along the agri-food chain while building regional supply chains for plant-based and alternative proteins [4-6]. Extracted proteins often feature a balanced amino-acid composition and favorable techno-functional properties solubility, emulsification, foaming, and gelation but their extraction remains difficult due to the complex structure of lignocellulosic or oleaginous matrices and the formation of protein–phenolic complexes that restrict solubilization and purity [7-9]. To overcome these barriers, numerous "green" extraction strategies have emerged, combining enzymatic hydrolysis with assisted techniques such as ultrasound, high pressure, pulsed electric fields, and eco-compatible solvents. These mild processes provide selective and less denaturing alternatives to classical acid or alkali extraction routes, which are often energy-demanding and detrimental to protein functionality [8-13]. In parallel, bio-inspired methodologies that replicate digestive mechanisms combining gentle pretreatment, selective de-phenolization, sequential hydrolysis, cooperative fermentation, and low-pressure membrane separation offer promising routes for recovering functional protein fractions under moderate conditions, consistent with the environmental and economic constraints of modern agri-food sectors [6], [14-15].

Several representative examples highlight both the opportunities and limitations associated with protein recovery from agri-food residues. Olive pomace, one of the main by-product streams in Mediterranean regions, contains low-molecular-weight peptides with demonstrated antihypertensive effects and can deliver high yields of soluble extracts when subjected to optimized treatments such as Alcalase hydrolysis, ultrasound, or deep eutectic solvents, illustrating the complementarity between enzymatic and emerging technologies [16-17]. Date pits, widely available across North Africa and the Middle East, form a highly lignified matrix with growing interest as a protein source, yet require matrix-opening and mechano-enzymatic approaches precisely adjusted to their structure [18]. *Rapeseed* and *sunflower meals* rank among the most protein-dense co-products, providing globulins and albumins of notable techno-functional value, provided that enzymatic, fermentative, or green-solvent steps are implemented to reduce inhibitors and phenolic bindings [7, 11]. Other residues, such as tannin-rich grape marc, necessitate combined and mild treatments to release functional protein fractions without degradation [19]. Beyond the food sector, demonstration projects already confirm the partial replacement of animal proteins in feed formulations, emphasizing the contribution of these streams to territorial biorefinery development and circular-economy strategies [20-21].

The aim of the present study is therefore to establish the feasibility of exploiting Mediterranean co-products as protein reservoirs and to describe a bio-inspired extraction and fractionation framework integrating gentle pretreatment, enzymatic de-phenolization, sequential hydrolysis, cooperative fermentation, and low-pressure ultrafiltration. The study also defines the corresponding operating windows and performance indicators (yield, degree of hydrolysis, purity, nitrogen-solubility index, environmental footprint) and discusses scale-up perspectives based on digital-twin modelling and predictive-control strategies to support pilot- and industrial-scale deployment [1-21].

2 Proposal for an innovative bio-inspired process

2.1 Background and originality of the approach

Agricultural co-products such as olive pomace, date pits, and sunflower or rapeseed oilcake constitute protein- and bioactive compound–rich biomass. However, their valorization remains limited by the structural rigidity of lignocellulosic matrices and the high phenolic content that interferes with extraction and purification. Conventional extraction routes whether chemical or mechanical present several drawbacks: poor selectivity, substantial energy demand, reliance on solvents or additives, and frequent loss of protein functionality.
To overcome these limitations, we designed a bio-inspired extraction strategy that combines the physiological mechanisms of digestion observed in ruminant and omnivorous systems with the adaptive metabolic responses of intestinal microbiota. This hybrid concept enables gradual, selective, and multifunctional recovery of plant proteins from heterogeneous co-products. The process follows a modular and reconfigurable design, coupling enzymatic, fermentative, and membrane operations in a coordinated sequence.
The distinctiveness of this approach lies in three main aspects:

- **Dynamic integration of processing stages** based on each biomass profile (particle size, polyphenolic content, and protein composition).
- **An operational cascade** inspired by biological digestion: pre-shredding → enzymatic pre-digestion → microbial conversion → membrane absorption → stabilization.
- **Cross-biomass flexibility**, achieved without toxic solvents or high-energy operations, ensuring both environmental compatibility and preservation of functional properties.

2.2 Detailed process steps

2.2.1 Bio-mimetic physical pre-treatment

This initial phase aims to loosen the cellular architecture of co-products while maintaining the native conformation of proteins. Raw materials are first gently dehydrated at 40–50 °C to safeguard thermolabile compounds, then milled under controlled conditions to reach an optimal particle size—below 500 µm for date pits and under 300 µm for oilseed cakes enhancing the surface area available for subsequent reactions. Sieving and homogenization finalize the preparation, ensuring uniform texture and reactivity. The operation mirrors the mastication and salivation mechanisms of ruminants, where gradual mechanical and enzymatic actions soften plant tissues before enzymatic digestion.

2.2.2 Targeted Enzymatic Detoxification

This stage removes anti-nutritional factors such as polyphenols and tannins that form complexes with proteins and impede enzymatic hydrolysis. The treatment involves a mild enzymatic pre-hydrolysis using natural tannase or peroxidase often produced by *Aspergillus spp.* to degrade or neutralize phenolic structures. Conditions remain close to physiological values (pH 5.8–6.0; 40–45 °C; 1–2 h incubation). A microfiltration step may follow to eliminate phenol–lignin aggregates and clarify the medium. Typically, this reduces soluble polyphenols by 60–80 %, substantially improving protein accessibility. The concept echoes the salivary enzymatic detoxification observed in certain herbivores, which initiates digestion by neutralizing plant metabolites prior to intestinal processing.

2.2.3 Progressive enzymatic hydrolysis

Here, the polysaccharide framework of plant material is degraded to liberate protein fractions tightly associated with fibres. The reaction employs a tailored blend of non-GMO enzymes cellulase, hemicellulase, pectinase, alcalase, papain chosen according to biomass composition. Acting synergistically, they cleave glycosidic bonds and detach proteins under mild physiological conditions (pH ≈ 6.0, 50 °C, 3–4 h). Spectrophotometric monitoring at 280 nm tracks peptide release, enabling real-time dosage adjustment. The process yields over 70 % soluble hydrolysates rich in low-molecular-weight peptides (1–10 kDa) exhibiting strong emulsifying capacity. This stage reflects the sequential enzymatic secretions in gastric and intestinal phases of animal digestion, ensuring controlled and efficient macromolecule breakdown.

2.2.4 Synergistic microbial fermentation

Fermentation continues protein hydrolysis, generates bioactive peptides, and neutralizes residual inhibitors. The procedure can be conducted under solid-state or semi-liquid conditions using GRAS strains such as Lactobacillus plantarum, Bacillus subtilis, and Aspergillus oryzae, renowned for their proteolytic potential and affinity for plant substrates. It proceeds at near-neutral pH and 30–37 °C for 24–48 h. Process dynamics are followed through measurements of total protease activity and degree of hydrolysis, which guide the adjustment of fermentation parameters. The outcome is a peptide-enriched hydrolysate (< 3 kDa) with diminished enzyme inhibitors and pleasant natural aromas. This step is inspired by the microbial symbiosis of intestinal ecosystems, where cooperative metabolisms finalize nutrient degradation.

2.2.5 Adaptive membrane separation

The final purification relies on tangential ultrafiltration (UF) to fractionate peptides by molecular weight and concentrate bioactive fractions while eliminating fibrous residues. A cascade of 10 kDa and 3 kDa cut-off membranes enables recovery of peptides in the 3–10 kDa range or lower, depending on the target functionality. UF proceeds until approximately four-fold concentration, after which the permeate is rapidly cooled to preserve structural integrity. The process delivers protein extracts of 75–85 % purity with consistent peptide profiles suitable for functional or nutraceutical applications. The principle parallels selective filtration in intestinal and hepatic systems, where molecules are sorted and concentrated for metabolic routing.

2.2.6 Drying or encapsulation

The final stage ensures product stabilization for storage and formulation. Two mild routes are favored: lyophilization, which retains peptide bioactivity by avoiding thermal stress, and low-temperature drying (< 60 °C) as a cost-effective alternative limiting denaturation. Optionally, encapsulation within natural carriers such as gum arabic or modified starch enhances oxidation resistance, masks off-flavours, and allows controlled release in end-use products. The stabilized protein ingredients can serve as functional agents in food, cosmetic, or pharmaceutical applications, as bio-additives in biodegradable packaging, or as texturizing and nutritional supplements. This step anchors the process within a sustainable, versatile valorization pathway for plant-based co-products.

2.3 Interest and originality of the process

The proposed framework goes beyond a simple combination of existing extraction operations. It follows a genuinely bio-inspired rationale, reproducing in a controlled sequence the key stages of herbivorous digestion. This original logic enables dynamic adjustment to the unique composition of each feedstock by integrating its phenolic signature and protein density into the process design. Unlike conventional, standardized industrial protocols, this approach coordinates enzymatic and microbial reactions in a progression modeled on natural hydrolytic cascades.

The objective is not limited to yield optimization but extends to the generation of multifunctional protein fractions with high digestibility, bioactivity, and compatibility for food, cosmetic, or biomaterial applications. In doing so, the process provides a coherent response to technological barriers associated with extracting proteins from complex lignocellulosic matrices while remaining consistent with principles of circularity, energy efficiency, and environmental and human safety.

2.4 Reproducibility and adaptability of the proposed bio-inspired process

A major strength of the proposed method lies in its modular adaptability combined with strong reproducibility across diverse agricultural co-products. Each operation—pretreatment, enzymatic detoxification, hydrolysis, fermentation, and membrane fractionation—can be fine-tuned to the physicochemical properties of the target biomass, including lignin content, phenolic load, protein profile, and particle size.

This adaptability is primarily based on:

- **Tailored biocatalyst selection**, where enzyme and microbial strains are chosen according to the plant matrix (fiber-rich meals vs. lipid-rich kernels).
- **Real-time parameter adjustment**, modulating pH, temperature, and residence time based on bio-indicators such as peptide release (A280) and degree of hydrolysis.
- **Cross-biomass compatibility**, validated on substrates like sunflower and rapeseed meals, date pits, and olive pomace, demonstrating inter-biomass transferability without redesigning the flowsheet.

The methodology ensures reproducibility under semi-controlled experimental conditions, anchored in a standardization logic driven by biological response indicators rather than raw-material uniformity. This versatility supports future scale-up to pilot and industrial implementations across Mediterranean agro-ecological contexts.

As a forward perspective, a digital-twin model will be developed to simulate the entire biotransformation sequence. This virtual layer will integrate enzymatic and microbial kinetics dependent on substrate characteristics, enabling predictive optimization of parameters such as temperature, pH, residence time, and moisture content. Coupled with an AI-based supervisory interface, the model will facilitate adaptive control, enhance reproducibility, and accelerate process scalability across diverse biomass profiles.

Conclusion

Transforming agricultural residues into alternative protein sources provides a strategic response to the intertwined challenges of food security, sustainability, and circular bioeconomy. This study emphasized the high protein potential of date pits, olive pomace, and sunflower meals, while underscoring the structural and compositional barriers that limit their exploitation through conventional methods.

To address these challenges, we developed a bio-inspired process that emulates natural digestive mechanisms—mechanical, enzymatic, and microbial—to achieve selective and functional extraction of proteins under mild, eco-compatible conditions. The system integrates real-time adaptation to substrate variability, synergistic coordination between enzymatic and fermentative stages, and fine molecular fractionation to deliver high-quality extracts exhibiting nutritional and bioactive functionalities.

By aligning biological inspiration with process engineering and sustainability principles, this framework offers a scalable pathway for the efficient valorization of plant-based co-products, contributing directly to the development of sustainable protein industries within circular, resource-conscious economies.

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