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Protein Recovery from Ethanol By-Products: A Comprehensive Review of Zein Extraction, Processing Technologies, and Industrial Integration Across Dry-Mill Streams

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Abstract
The U.S. ethanol industry generates more than 40 million metric tons of by-products annually, including whole stillage, thin stillage, wet distillers' grains, syrup, and distillers dried grains with soluble (DDGS). These materials contain substantial quantities of protein. Especially the hydrophobic prolamin zein yet remain underutilized as low-value feed. This review synthesizes the biochemical composition of major ethanol by-product streams, evaluates classical and emerging extraction technologies, examines industrial integration and sustainability advantages, and analyzes market potential. Protein valorization enables ethanol plants to evolve into diversified biorefineries supplying renewable materials and functional proteins while reducing carbon intensity.

Keywords: Zein, Ethanol By-Products, Distillers' Grains, Protein Extraction, Biorefinery, Thin Stillage, DDGS, Sustainability

Introduction
The Dry-mill ethanol production is a cornerstone of the U.S. biofuel industry, converting more than five billion bushels of corn annually into renewable fuel. However, the bioprocess generates large coproduct streams that contain significant quantities of recoverable protein. Traditionally, these streams are blended into DDGS, a valuable livestock feed ingredient, but this approach does not maximize the biochemical potential of corn proteins—especially zein, the hydrophobic prolamin that dominates the endosperm. Zein possesses unique functional properties including film formation, hydrophobicity, and biodegradability, making it highly attractive for food coatings, pharmaceuticals, and bioplastics (Shukla & Cheryan, 2001). Shifting from bulk feed utilization to targeted protein fractionation aligns with modern biorefinery principles: producing high-value materials, improving process efficiency, and reducing carbon intensity [1].

Despite decades of research on zein extraction from wet-milling streams, limited attention has been given to dry-mill coproducts such as thin stillage, syrup, WDG, and DDGS—even though these streams represent the largest untapped protein reservoir in global agriculture. Recent advances in ultrasound-assisted extraction, membrane filtration, enzymatic pretreatment, and deep eutectic solvents (DES) have created new opportunities for practical protein recovery at scale. This review

consolidates biochemical, engineering, economic, and sustainability perspectives to support commercialization of protein extraction in ethanol plants.

Ethanol By-Product Composition

Ethanol by-products originate from whole stillage, the material remaining after distillation removes ethanol. Whole stillage contains suspended fiber, protein, yeast biomass, lipids, organic acids, and solubles. Centrifugation separates whole stillage into wet distillers' grains (WDG) and thin stillage. Thin stillage is evaporated to produce syrup (condensed solubles), which is recombined with WDG and dried to produce DDGS.

Whole stillage typically contains 7–12% solids, with protein levels between 18–22% of dry matter [2]. Thin stillage contains 4–8% solids—primarily soluble protein fragments, peptides, glycerol, lactic acid, and yeast metabolites. Syrup contains 25–35% solids and is enriched in soluble protein, heat-damaged protein aggregates, and fermentation by-products. DDGS, produced after high-temperature drying, contains 27–34% crude protein but exhibits significant variability, with heat-induced chemical modifications that reduce protein solubility [3].

Composition differences across streams strongly influence extraction strategies: thin stillage favors membrane-based recovery, whole stillage favors enzymatic and ultrasound-assisted extraction, WDG supports solvent-assisted pretreatment, and DDGS requires more aggressive processing such as alkaline solubilization.

Zein Structure, Properties, and Commercial Value

Zein is the predominant storage protein of corn endosperm and represents one of the most commercially valuable protein fractions recoverable from ethanol by-products. As a hydrophobic prolamin, zein displays unique solubility behavior—insoluble in water but soluble in aqueous ethanol and acetone—making it distinct from other plant proteins (Shukla & Cheryan, 2001). Structurally, zein is composed of α-, β-, γ-, and δ-subfractions, dominated by α-zein, which comprises up to 85% of total zein. Its high hydrophobicity, driven by abundance of leucine, proline, and alanine, gives rise to coiled-coil α-helical domains that exhibit film-forming, thermoplastic, and barrier properties (Argos et al., 1982).

Zein's functional utility is diverse: edible coatings, pharmaceutical excipients, biodegradable plastics, fibers, adhesives, and controlled-release systems [4]. Market prices range from \$20–\$40/kg for food-grade material, far exceeding the \$0.20–\$0.30/kg value of DDGS. This economic disparity motivates extraction of zein from currently underutilized by-product streams such as thin stillage, WDG, syrup, and DDGS.

Zein purity is influenced by thermal degradation during ethanol processing. DDGS-derived zein exhibits increased crosslinking and reduced solubility, whereas zein in WDG and whole stillage is less damaged. Understanding zein's molecular structure is crucial for selecting extraction methods that preserve its functionality for high-value markets.

Extraction Technologies for Proteins from Ethanol By-Products

Extraction of zein and non-zein proteins from ethanol by-products requires adaptation to stream composition. Classical aqueous ethanol extraction (60–95%) remains the dominant method, exploiting zein's solubility profile (Shukla & Cheryan, 2001). Ethanol concentration determines selectivity: lower concentrations extract more impurities; higher concentrations favor α-zein.

Alkaline extraction (pH 10–12) solubilizes glutelin and partially denatures zein and heat-damaged proteins, improving overall yield but reducing specificity. Enzymatic pretreatment—using proteases, cellulases, or hemicellulases—enhances mass transfer and protein exposure by disrupting fiber–protein complexes.

Ultrasound-assisted extraction (UAE) significantly improves protein release via cavitation, enhancing solvent penetration and reducing extraction time (Xie et al., 2020). UAE is particularly effective for WDG and whole stillage. Microwave-assisted extraction and thermal–mechanical disruption offer additional opportunities but require further industrial validation.

Membrane filtration (UF/NF) is well suited for thin stillage, producing concentrated protein streams with minimal solvent use. Deep eutectic solvents (DES) show strong specificity for zein and offer greener alternatives but face challenges in viscosity and recovery.

Extraction strategies must be tailored to stream characteristics: thin stillage favors membranes; WDG favors UAE + ethanol; syrup and DDGS require aggressive pretreatments.

Purification Strategies for Zein and Non-Zein Proteins

Purifying protein fractions from ethanol by-products requires separating zein from oils, pigments, heat-damaged proteins, and solubles. Anti-solvent precipitation is a primary method: zein is first extracted in 70–90% ethanol, then precipitated when ethanol concentration is reduced below ~40% (Shukla & Cheryan, 2001). This simple, scalable step provides strong selectivity for α-zein.

Defatting improves zein purity by removing co-extracted oils. Hexane is traditional, but ethanol, isopropanol, and supercritical CO_2 are now preferred for safety and environmental compliance [5]. Lipid reduction enhances membrane stability and reduces fouling.

Isoelectric precipitation is used for non-zein protein recovery. Adjusting pH to ~4.5 precipitates glutelin and albumin fractions while zein remains insoluble in water. This approach is useful after alkaline extraction.

Ultrafiltration (UF) and nanofiltration (NF) enable membrane-based concentration and polishing of protein extracts. Thin stillage UF can concentrate soluble proteins 3–10×, while NF removes small molecules such as salts, glycerol, and organic acids [6]. Membrane fouling remains a challenge but can be mitigated with defatting, enzymatic pretreatment, and high-shear mixing.

Chromatography—particularly reverse-phase chromatography—is effective for high-purity zein, though limited to specialty markets due to cost [7].

Deep eutectic solvents (DES) offer selective solubilization of zein and improved purity over aqueous ethanol (Xie et al., 2020). DES recovery is an active research area.

Industrial purification typically requires multi-stage systems such as defatting → ethanol extraction → anti-solvent precipitation → membrane polishing. This integrated approach yields higher purity and more consistent product properties for commercial use.

Integration of Protein Extraction into Dry-Mill Ethanol Plants

Dry-mill ethanol plants offer several insertion points for protein extraction with minimal disruption. Whole stillage, collected directly after distillation, offers the highest-quality protein, as thermal

degradation has not yet occurred. Ultrasound-assisted extraction (UAE), enzymatic hydrolysis, and moderate ethanol extraction are well suited for this stream.

Thin stillage is ideal for membrane-based recovery. UF and NF remove soluble proteins before evaporation, reducing evaporator solids load, lowering fouling, and improving backset quality. Reducing solubles in backset improves fermentation stability by decreasing lactic acid, glycerol, and yeast metabolites.

Wet distillers' grains (WDG) provide a high-protein, high-moisture substrate suitable for UAE or enzymatic pretreatment. Extracting protein prior to drying decreases dryer load, reducing natural gas use and greenhouse gas emissions.

DDGS extraction is feasible but less efficient due to thermal protein crosslinking. Alkaline extraction, DES, and multi-stage purification systems are necessary for effective recovery.

Energy integration is essential: ethanol extraction steps can use low-pressure steam from distillation columns, and solvent recovery can be integrated into existing distillation systems. Reduced dryer load directly decreases energy costs, improving carbon-intensity (CI) scores under LCFS frameworks [8].

Effective integration requires attention to water recycling, solvent management, plant footprint, and regulatory standards. When optimized, protein extraction supports ethanol plant evolution into modern biorefineries with diversified revenue from high-value proteins, specialty materials, and improved CI performance.

Market Analysis, Economic Feasibility, and Global Demand

The global zein market has historically been limited by supply rather than demand, as commercial zein is produced almost exclusively from wet-milled corn gluten meal (CGM). This structural bottleneck has kept prices elevated—typically USD $20–$40 per kg for food- and pharmaceutical-grade material and $10–$20 per kg for industrial-grade zein (Wang et al., 2020). Specialty zein fractions, including electrospun fibers and high-purity α-zein, command significantly higher prices.

Dry-mill ethanol plants represent the largest untapped reservoir of zein globally. U.S. ethanol plants generate more than 20 million metric tons of DDGS annually, containing approximately six million tons of protein [2]. Even recovering 5% of this protein as zein would exceed current global production several-fold. Expanded supply could reduce zein cost barriers, enabling adoption in broader applications such as biodegradable packaging and sustainable coatings.

Zein's hydrophobicity and ability to form cohesive, oxygen-barrier films make it attractive for edible coatings, pharmaceutical encapsulation, and bioplastic materials [9]. Its biodegradability positions it favorably against petroleum-based plastics amid global restrictions on single-use plastics and expanded sustainability initiatives in the EU and Asia-Pacific.

Non-zein protein fractions—glutelin, globulin, albumin, and yeast-derived peptides—offer parallel opportunities in functional foods, pet nutrition, emulsifiers, and nutraceuticals. Bioactive peptides from corn proteins have demonstrated antioxidant, antihypertensive, and antimicrobial properties [10].

Technoeconomic analyses indicate protein extraction profitability when [11]:

- zein yield ≥ 3–5% of DDGS mass
- protein purity ≥ 70%
- solvent recovery ≥ 90%
- energy integration reduces dryer load by ≥ 10–20%

Dry-mill plants already possess fermentation tanks, distillation columns, and solvent handling systems, providing a strong infrastructure foundation for new bioproduct streams. Integration of protein extraction supports biorefinery evolution, diversifying revenue and reducing exposure to DDGS market fluctuations.

Environmental, Sustainability, and Life-Cycle Considerations

Protein extraction supports the environmental objectives of modern ethanol biorefineries by improving efficiency, reducing waste, and lowering carbon intensity (CI). DDGS drying is one of the highest energy-consuming operations in ethanol plants; removing proteins upstream reduces dryer load, decreasing natural gas consumption and greenhouse gas emissions.

Thin stillage extraction decreases soluble organic loading in backset—reducing lactic acid, glycerol, and yeast metabolites. This stabilizes fermentation, lessening the need for antibiotics and reducing contamination risk. Lower organic content also improves evaporator performance and reduces fouling frequency.

Zein-based materials offer major sustainability benefits. As biodegradable polymers, zein films and fibers can displace petroleum-derived plastics, contributing to lower environmental persistence and reduced microplastic pollution. Life-cycle assessments (Rosenboom et al., 2018) confirm substantial GHG reductions when renewable biopolymers replace synthetic polymers.

Extraction from WDG or whole stillage reduces formation of Maillard reaction products—heat-induced compounds that decrease protein digestibility in DDGS [3]. Cleaner, lower-temperature coproducts can improve nitrogen-use efficiency in animal diets and reduce environmental nitrogen losses.

Integration of protein extraction enhances circular bioeconomy performance by upcycling underutilized biomass into specialty ingredients, nutraceuticals, and renewable materials. Improved coproduct valorization enhances ethanol plant sustainability metrics under LCFS, RFS, and RED II frameworks, providing potential financial incentives.

Future Directions, Research Gaps, and Conclusions

Future development of protein extraction systems in ethanol plants requires addressing several unresolved challenges. First, detailed biochemical mapping of whole stillage, thin stillage, syrup, WDG, and DDGS is needed to quantify temporal variability caused by changes in corn quality, fermentation conditions, and backset composition. Advanced proteomic and metabolomic profiling would improve extraction predictability and process control.

Second, scale-up studies remain limited for ultrasound-assisted extraction (UAE), microwave-assisted extraction, and deep eutectic solvents (DES). Pilot-scale demonstrations should evaluate energy use, mass-transfer behavior, solvent recovery, and membrane fouling under real industrial conditions.

Third, multi-stage purification systems require optimization. Integrated workflows—such as enzymatic pretreatment → ethanol extraction → anti-solvent precipitation → UF/NF polishing—offer strong potential but must be adapted to high-throughput industrial environments.

Fourth, high-value applications of non-zein proteins remain underexplored. Corn-derived peptides exhibit antioxidant, antimicrobial, and antihypertensive properties, representing emerging opportunities in nutraceuticals and functional foods.

Fifth, regulatory pathways for food-grade and pharmaceutical-grade corn proteins must be clarified, including solvent residue limits, allergenicity assessments, and microbial safety guidelines.

Finally, life-cycle assessments (LCA) should be expanded to quantify energy savings, GHG reductions, waste minimization, and material substitution benefits associated with protein recovery.

Declarations

Ethics Approval and Consent to Participate: This study did not involve human participants or animal subjects. As such, ethical approval and consent to participate were not required.

Consent for Publication: Not applicable. This manuscript does not contain any individual person's data in any form.

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