Process simulation for recombinant human lysozyme production from transgenic rice: Impact of intensification strategies.

by

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Abstract

Traditional batch bioprocessing dominates biologics manufacturing but faces limitations in equipment utilization, high costs, and scalability, particularly for downstream purification. This report evaluates the economic and technical impacts of process intensification on the downstream production of recombinant human lysozyme (rHLZ) from transgenic rice seed. Using SuperPro Designer (Intelligen, Inc.), five distinct purification train configurations were modeled to meet a projected annual market demand of 1,000 kg/yr. This production target was established through a demographic-based analysis of children with egg-white allergies who require safe, plant-based alternatives to traditional animal-derived lysozyme. The configurations examined in this study range from traditional methods to highly integrated systems. The baseline batch model serves as the industry standard, utilizing sequential processing and significant intermediate storage. The pool-less strategy attempts to reduce capital costs by eliminating non-essential hold tanks, while the high-capacity resin model explores the use of a modern chromatography resin which reduces column size and buffer consumption and enhances binding capacity and life span of the resin. More advanced strategies include the semi-continuous model, which employs cyclic extraction and a rotary vacuum drum filter to maximize equipment utilization, and the combined intensification model, which integrates the most effective elements from all previous configurations. The results of the simulation analysis reveal a significant disparity in performance between traditional and intensified designs. The baseline batch configuration proved to be the most resource-intensive, requiring a capital expenditure of $26 million and resulting in a cost of goods of $12.2/g. In striking contrast, the combined intensification model emerged as the most efficient, reducing capital expenditure by 60% and lowering the cost of goods 40% while simultaneously improving volumetric productivity. Ultimately, this research demonstrates that the strategic implementation of semi-continuous operations and process intensification strategies using high-capacity resins can substantially lower the economic barriers to producing biologics within plant-based seed expression systems.

Table of Contents

Abbreviations

CAGR	Compound Annual Growth Rate
rHLZ	Recombinant Human Lysozyme
MCC	Multi-Column Chromatography
SPD	SuperPro Designer
PMI	Process Mass Intensity
M	Million
GP	Grana Padano
HEWL	Hen Egg White Lysozyme
CAPEX	Capital Expenditure
OPEX	Operating Expenditure
CoGs	Cost of Goods
Filter-Press	Plate-and-Frame Filter
CEX	Cation Exchange
UF/DF	Ultrafiltration/Diafiltration
NaCl	Sodium Chloride
NaOH	Sodium Hydroxide
PES	Polyethersulfone
CIP	Clean in Place
BV	Bed Volume
DBC	Dynamic Binding Capacity
RVDF	Rotary Vacuum Drum Filter
NPV	Net Present Value
MWCO	Molecular Weight Cut-Off
PAT	Process Analytical Technology
QbD	Quality-by-Design

List of Figures

List of Tables

Introduction

The global biologics market was valued at approximately $400 billion in 2024, with a projected compound annual growth rate (CAGR) of 8.5% through 2030 (Grand View Research, n.d.), which includes a growing number of therapeutic proteins, vaccines, and industrial enzymes. For example, the Food and Drug Administration (FDA) approved 50 new biologics in 2024, which includes 32 small molecules and peptides and 16 proteins (10 monoclonal antibodies, 3 bispecifics, 2 fusion proteins, and 1 protein-toxin molecule) (Mullard, 2025). This growth has been accompanied by concerns about manufacturing costs, scalability, and robustness as biomanufacturers strive to meet annual production demands.

While biomanufacturing capacity is growing, the unpredictable nature of future demand and the introduction of more complex and specialized products and therapies complicate forecasting physical production capacity and ensuring the capacity is suitable for a given specialized product. Consequently, the industry is transitioning from traditional batch manufacturing toward continuous manufacturing for both recombinant drugs and biosimilars. This shift is supported by regulatory frameworks, such as the International Council for Harmonization (ICH) Q13 guidance, and is further incentivized by the significant economic advantages of intensified processing (Niazi, 2025).

Building on these industry trends toward more efficient manufacturing, the following sections provide essential context for understanding the specific strategies evaluated in this study. First, an overview of biomanufacturing processes outlines the upstream and downstream phases critical to recombinant protein production. This is followed by outlining process modalities: batch, intensified, and continuous, and the key drivers pushing adoption of intensified bioprocessing to overcome traditional limitations. Finally, production platforms are examined, with emphasis on plant-based expression systems like transgenic rice seeds, which offer unique advantages in stability and scalability for recombinant human lysozyme (rHLZ) purification.

Overview of Biomanufacturing Processes

Biomanufacturing encompasses both upstream processing (cell culture or plant growth) and downstream processing (purification and recovery). Upstream processing is the initial phase where the biological “factory,” such as a plant, bacteria, or mammalian cell, is selected, engineered (if needed), and grown under suitable conditions to allow for target protein

accumulation. This involves preparing a specific environment like a bioreactor or planting within a field (for terrestrial plants) and allowing the organisms to grow until a peak density/biomass and/or target protein yield is reached. This stage is focused on yield optimization, ensuring the living cells have the right nutrients and conditions to maximize the amount of protein or target product produced before harvesting begins. When possible, cell growth conditions such as temperature, pH, and agitation are optimized and scaled for manufacturing.

Once the cells or biomass is grown, the focus shifts to downstream processing, which is the series of physical and chemical steps required to isolate and purify the product. Downstream processing includes assembling unit operations to take a complex biological mixture and refine it to a smaller volume of purified product. The complex mixture contains the target protein along with unwanted cell impurities such as debris, native proteins, and other host cell impurities along with product-, and process-derived impurities. The goals for downstream processing are to reduce process volumes quickly, stabilize the product, and then remove impurities, focusing on critical impurities first (Harrison et al., 2015). Unit operations are selected and designed to discriminate between the product and impurities using various physico-chemical properties such as charge, hydrophobicity, solubility, size, etc.

Downstream processing uses a variety of unit operations to differentiate between impurities and the product. First, the cells/biomass must be harvested from the depleted media or field. Cells are recovered from spent media using solid-liquid separations such as centrifugation, sedimentation, or microfiltration while terrestrial plants are harvested using traditional agricultural methods and processed for particle size reduction. For intracellular products, cell lysis methods are required and include various mechanical and chemical methods. For plant-based products, the product must be solubilized from the plant material using an extraction method. The goal of extraction is to selectively solubilize the target product while minimizing the co-extraction of impurities, particularly critical impurities (Dixon et al., 2018). Extraction is controlled through modification of the solid-to-liquid ratio, buffer composition (components, pH, and ionic strength), and time. Following, the residual biomass or cell debris must be removed using solid-liquid separations such as dead-end filtration, microfiltration, and/or centrifugation. The resultant clarified liquid is then moved through the capture and purification steps. Typically, one or more chromatography steps are used to capture the target protein, resulting in significant concentration and purification. Ion exchange, affinity, hydrophobic, and size exclusion methods

are commonly used with ion exchange operations as the most popular method for purification of proteins due to its versatility at different pHs (Tosoh Bioscience, n.d.). Chromatography may be operated as a single column in batch format or more continuously, using multiple columns in series or parallel, termed multi-column chromatography. Tangential flow filtration is another common unit operation applied to concentrate the product, exchange processing buffers for compatibility, separate impurities from the product based on molecular weight, or formulate the final product in a suitable solution.

The selected steps and combination of operations and conditions used depends on the unique characteristics of the target product combined with the selected host system properties and required product specifications and quality attributes. Sequential processing steps are designed until the product is suited for its given application such as medical, research, diagnostic, pharmaceutical, nutraceutical and/or industrial use. Designing suitable downstream processing methods is critical as they contribute significantly to the overall manufacturing cost and are critical to product safety and efficacy. Process development is key to designing effective and economical processes and is challenged by the expansive design space due to the wide variety of methods and process conditions. In addition, there is often extreme pressure for rapid process development. Process modeling and simulation using software such as SuperPro Designer (SPD) by Intelligen, Inc. can facilitate process development through the identification of process bottlenecks which impact economics or productivity, and evaluation of process scale-up impacts and scheduling challenges early in the development process.

Another critical element for consideration during process development is process modality. Upstream and downstream processes can be accomplished through various process modalities (batch, semi-continuous, or continuous processes). Each modality presents opportunities and challenges.

Process Modalities and Drivers for Intensified Bioprocessing

Batch Processing

For several decades, industrial protein production has relied primarily on batch bioprocessing (Dream, 2023). In this framework, unit operations are executed sequentially and are defined by discrete production cycles, often utilizing intermediate hold tanks to provide scheduling flexibility. While this structure simplifies material traceability and process validation,

it is inherently limited by the "bottleneck": the slowest operating piece of equipment that dictates the overall material flowrate. Common bottlenecks in batch processing often include long cleaning cycles or underutilized equipment during hold steps. In the past, upstream production was the focus of biologics manufacturing, yet true bottlenecks often arise during downstream processing. Common bottlenecks in the downstream processes include chromatography scale-up issues such as reduction in separation performance and binding capacity (Bestchrom, 2026). In addition, dead-end and tangential flow filtration often present challenges including fouling, increased pressure requirements, and batch-to-batch variability.

In general, processes are limited by insufficient throughput for upstream production, yield losses with additional purification steps, and lack of flexibility for multi-product facilities. Furthermore, as production scales increase, capital and operational expenditures associated with batch processing rise significantly. To address these limitations, the industry is adopting process intensification strategies and continuous or semi-continuous operations. These approaches aim to improve throughput and reduce costs by downsizing equipment and improving utilization through monitoring and control strategies.

Table 1 summarizes the key differences between batch, intensified, and continuous processing frameworks. While batch operations offer traceability and regulatory familiarity, their stop-and-go structure introduces idle equipment time, long cleaning cycles, and limited throughput. These inefficiencies are increasingly incompatible with the demand for flexible, high-yield manufacturing environments. Since downstream processing consumes 50 to 80% of the production costs, intensification in this phase of production is particularly impactful (Kumar et al., 2020). Intensification strategies can achieve faster processing through advanced technology and/or materials utilization, elimination of time consuming or expensive operations, or staggering of operations to streamline the process.

To address limitations of traditional batch processes, various strategies for process intensification and adoption of continuous and semi-continuous processing methods have been investigated. A continuous process is a manufacturing approach where inputs are added to the system while products are continuously removed with long periods of uninterrupted operation. Semi-continuous processes attempt to reach steady state while remaining in a fundamentally batch modality. Continuous and semi-continuous processes can be complex, particularly with scale-up and require precise control and equipment synchronization (Dreams, 2023).

Table 1. Comparison of Batch, Intensified, and Continuous Biologics Manufacturing (Adapted from Dream, 2023)

	Batch	Intensified	Continuous
Modality Definition	Multiple discrete steps, hold times between phases	Shorter, segmented run times integrated with semi-continuous elements	Continuous flow with no routine shutdown or startup
Validation	Batch-by-batch validation	Combines batch and continuous validation strategies	Continuous validation and monitoring
Testing/ Monitoring	After critical operations and at the end of the batch	After critical operations and in-line testing and control of integrated semi-continuous elements	In-line real-time testing and control
Labor and Equipment Cost (Long-term)	Higher	Moderate	Lower
Scale Suitability	Small-scale or early-stage production	Pilot or mid-scale production	Large-scale, commercial production
Scale-up Complexity	Easier but costly due to large equipment	Cost-effective scale-up with control strategies	Complex, requires precise control and synchronization
Startup/ Shutdown Time	Longer startup and shutdown	Shorter startup, more frequent segmentation	Minimal once at steady state
Operational Risk	Easier to isolate failures, more human intervention	Balanced risk control with automation	Failures can propagate, less human intervention
Product Variability	Higher due to batch-to-batch differences	Moderate	Lower when at steady state
Inventory and Supply Chain	Simpler	Moderate	More intense inventory and supply requirements

Continuous bioprocesses are typically implemented by incrementally modifying existing batch operations rather than replacing the entire flowsheet at once. In practice, batch campaigns may first adopt tighter scheduling and reduce intermediate pooling (e.g., pool-less or segmented campaigns) to create longer, more uniform processing windows, then introduce cyclic or overlapping unit operations (e.g., staggered extractions or filtration cycles) that begin to approximate semi-continuous flow. As control strategies, in-line monitoring, and automation mature, these semi-continuous elements are further integrated using equipment such as perfusion bioreactors, multi-column chromatography systems, and continuously operated filtration steps, gradually transforming a sequence of discrete batches into a coordinated, steady-state continuous operation. Throughout this progression, the underlying unit operations generally remain the same with changes in mode of operation, synchronization, and the use of intermediate storage and hold steps.

Relative to traditional batch processes, continuous and highly intensified operations offer several important benefits. First, they can achieve higher volumetric productivity by reducing non-productive time and bottlenecks (Cytiva, 2025). This is especially evident in capture and polishing steps, translating into more product per unit volume and per unit time. Second, equipment can be downsized and used more efficiently, lowering capital expenditure and reducing the facility footprint because vessels and columns no longer need to be sized for large, infrequent batches (Hawdon, 2025). Third, continuous operation supports real-time monitoring and control, which can lower product variability and improve consistency (Challener, 2024). This relies on the process reaching steady state; during start-up and shutdown, the systems may experience some variability in product quality. Finally, by smoothing material and buffer flows, continuous processes can reduce inventory swings compared to large inventories for batch operations (Niazi, 2025). On a commercial scale, these benefits substantially decrease cost of goods compared to the conventional batch baseline.

However, these advantages come with notable disadvantages and implementation challenges. Continuous systems are more complex to design and operate, requiring precise equipment synchronization, advanced automation, and robust process analytical technologies to maintain control over long operating periods. Disturbances or failures can propagate through multiple unit operations before they are detected, increasing operational risk and complicating deviation management compared to self-contained batches. From a regulatory and quality

standpoint, defining lots, establishing cleaning and hold—time validation, and demonstrating state of control over extended campaigns are more demanding than for traditional batch release paradigms. In addition, the need for continuous raw-material supply, higher operator and engineering oversight, and specialized control infrastructure can offset some cost savings and make initial adoption difficult, especially in multiproduct or early-stage facilities that prioritize flexibility and simplicity.

Intensified Processing

Intensified processing is a strategy focused on maximizing productivity through improvement of biomanufacturing processes at unit, operational, or functional levels. It seeks to overcome traditional batch constraints by integrating semi-continuous elements into established workflows rather than complete replacement. In an intensified downstream process, unit-operations are integrated or streamlined by shortening or eliminating hold steps, adopting higher-capacity or higher-throughput technologies (e.g., high-capacity resins, membrane adsorbers, or MCC systems), and using advanced monitoring and control to sustain performance at higher utilization levels (Crowley et al., 2024).

Intensifying the capture chromatography step is a primary focus, as the purification of a biomolecule by chromatography has a high process mass intensity (PMI), an indicator of downstream operating expenses. This is the amount of solution (equilibration, wash, elution, regeneration) used to produce a certain amount of product (e.g., kg solution/kg product). For antibody manufacturing, processes can have a PMI as high as 7000 kg/kg product (Jungbauer et al., 2024). The volumes required depend on bed volumes so developing improved chromatography resins that offer high resin capacity, enhanced flow rates, and increased resin lifetime can reduce PMI, process times, column size, and consumable costs. Additional examples of process intensification include use of membrane adsorbers in a bind/elute mode rather than traditional packed bed chromatography, which alleviates diffusion limitations, enables higher flow rates, shortens cycle times, and reduces buffer usage (Qu et al., 2024). Multi-column chromatography, using small columns connected in series that collect flowthrough during loading of the prior column, enhance resin utilization, increase volumetric productivity, reduce PMI, and reduce resin volume requirements (Najera, 2016).

Intensified systems enable greater efficiency without fully departing from established batch principles. By downsizing equipment and connecting critical operations more tightly,

throughput can be increased while maintaining control over product quality. This hybrid approach not only reduces the footprint and cost associated with scale-up but also prepares facilities for eventual transitions to fully continuous operations. In many ways, process intensification represents the natural evolution of biomanufacturing, bridging regulatory comfort with the efficiency and agility demanded by modern biologics manufacturing frameworks.

Process intensification strategies outlined above provide a flexible framework applicable across the biomanufacturing industry, but their impact is particularly pronounced when paired with expression systems that are inherently decoupled between upstream production and downstream processing. Plant-based systems, especially seed expression in transgenic rice, offer unique compatibility with intensified downstream trains by enabling stable, on-demand biomass storage, and product generation. The following sections detail how these advantages position transgenic rice as an optimal host for recombinant human lysozyme (rHLZ) production under both conventional batch and intensified processing schemes.

Production Platforms and Plant-based Expression Systems

The biomanufacturing of biologics relies heavily on a few common expression platforms which includes mammalian and microbial cell lines (Dixon et al., 2018). Target proteins are typically either secreted into a liquid medium or contained within suspended cells. In the case of extracellular secretion, cells are separated from the liquid broth containing the target protein through centrifugation or depth filtration, and for intracellular production, cell disruption is required to release the target protein into the liquid broth. This is followed by “clarification” to remove smaller debris or impurities that complicate downstream operations. While effective, these platforms involve high operational and capital costs due to expensive growth media and the need for complex stainless steel or single-use bioreactor systems. They also carry the risk of introducing and propagating human pathogens. Using plants as bioreactors offers a safer and potentially more cost-effective alternative.

General processing strategies for plant systems and traditional bioreactor systems (both plant, mammalian, and microbial cells) are shown in Figure 1. Plant-based platforms generally follow one of two paths including plant cell culture or whole-plant systems (seeds and/or leafy tissue). Plant cells can be cultured in bioreactors similarly to mammalian and microbial cells, providing higher batch-to-batch consistency (Xu et al., 2012, Dixon et al., 2018). However, this consistency comes at the cost of cell line stability, which can degrade over time and lead to lower

recombinant protein concentrations (Dixon et al., 2018). While stability issues can be mitigated by using C-terminal sorting signals to target storage vacuoles, whole-plant systems often provide more straightforward scaling solutions (Shaaltiel et al., 2007). In addition, plant-based cell culture has similar disadvantages of more conventional platforms including the high operational and capital costs for upstream processes.

In whole-plant systems, or terrestrial systems, proteins are expressed in either leafy tissue (e.g., tobacco) or seeds (e.g., rice or corn), each with distinct downstream processing frameworks (Dixon et al., 2018). Figure 1 illustrates general downstream processing frameworks for leafy and seed-based systems as compared to bioreactor-based systems. The production systems share common general processing steps including pre-processing, extraction (intracellular products only), clarification, pretreatment and conditioning, capture, and polishing. Selected unit operations may deviate between production systems particularly in the pre-processing and extraction phases.

Terrestrial systems allow for simple scale-up by expanding acreage rather than increasing bioreactor volume. Leafy tissue systems are well understood and offer high biomass and quick growth resulting in higher yields than seeds and bioreactor systems (Dixon et al., 2018). However, leafy tissue is prone to genetic instability and requires immediate freezing or desiccation after harvest to maintain biological activity or addition of buffer additives during extraction to prevent degradation of the target protein (Dixon et al., 2018). Seed systems serve as natural, desiccant storage containers with enhanced product stability, allowing for the decoupling of harvest and processing methods. Seeds have naturally high protein accumulation and genetic stability (Dixon et al., 2018). Therefore, recombinant proteins can be expressed at relatively high levels (10 g/kg) and have been stable in rice grain for 3-5 years under ideal temperature and moisture conditions (Huang, 2004) and at least ten months at room temperature within protein bodies (Wakasa et al., 2015), providing a distinct advantage over more conventional production systems. Additionally, unlike leafy tissue, seeds have limited enzymatic activity and lower amounts of phenolics as compared to leafy tissue systems, which improves product stability and provides more flexibility with downstream processing methods. Furthermore, efficient extraction, clarification, and capture strategies have been developed, resulting in 95-98% purity in a single chromatography step (Wilken and Nikolov, 2006; 2010).

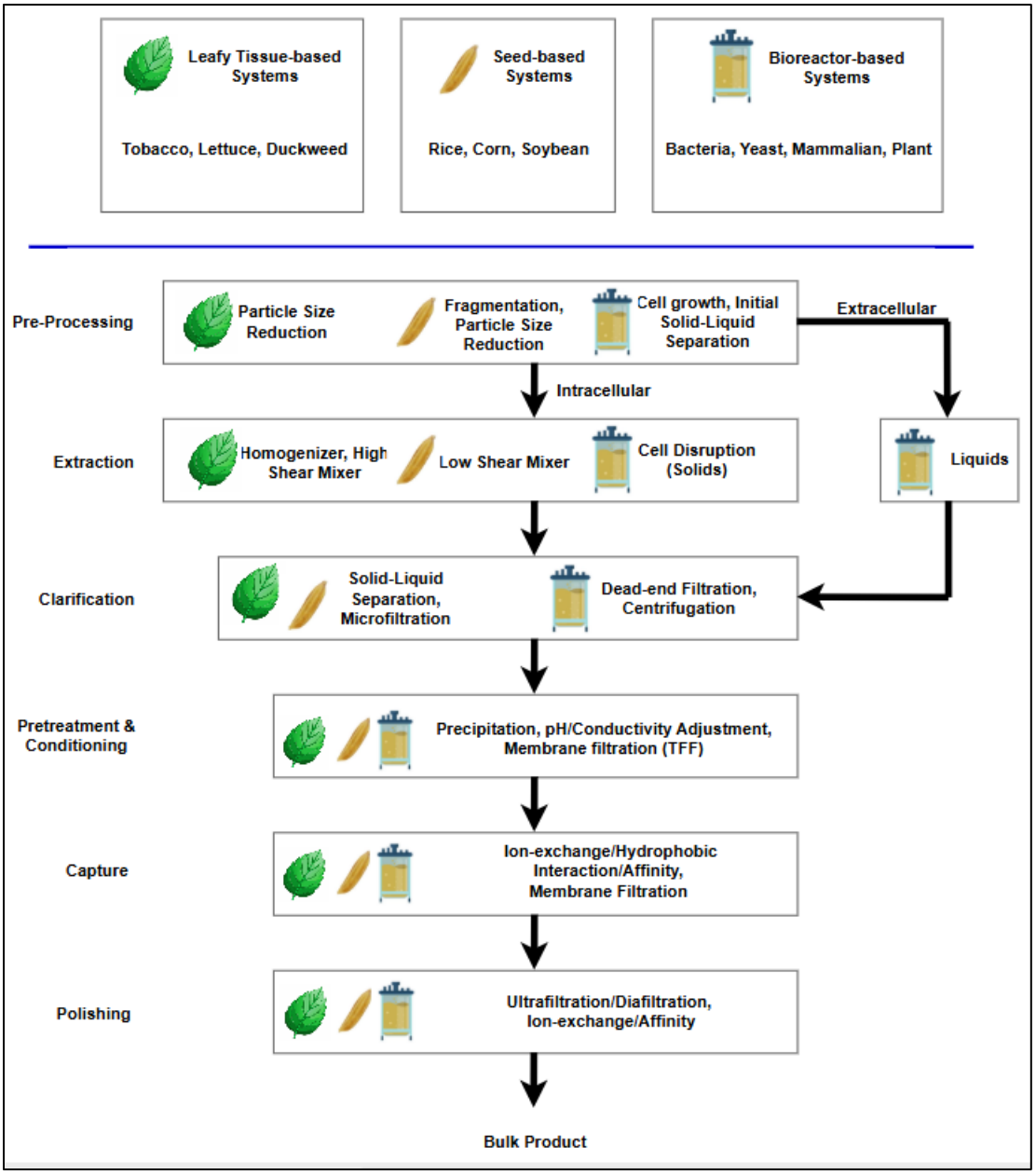

Figure 1. Downstream Processing of Recombinant Proteins from Leafy Tissue, Seed, and Bioreactor Systems (Adapted from Dixon et al., 2018)

When considering these expression platforms, each have distinct benefits depending on the desired product and product quality. For this study, a seed expression system will be utilized for its expression levels, genetic stability, established agricultural infrastructure and harvesting, high accumulation levels of recombinant proteins within seeds, and availability of published literature for extraction and purification, respectively.

Whole-plant systems contain the target protein intracellularly, requiring particle size reduction before downstream purification. For leafy tissue, the process begins with the mechanical grinding and pressing of the leaves, rupturing the cells, and releasing the intracellular proteins. This creates a protein-rich green juice with high moisture and fibrous pulp (Nynäs et al., 2024). Robust clarification steps are required before the protein can be captured. Seed systems follow a similar initial path of mechanical grinding, like leafy tissues, but benefit from the seed's lower moisture content. Whole or fractionated seeds are milled into dry flour which is then mixed with a buffer to produce a crude protein extract. While this still requires a clarification step to separate the spent grain from the protein extract, seed extracts generally contain fewer complicating impurities like proteases and phenolic compounds (Dixon et al., 2018). Once clarified, the extracts from both seed and leafy tissue converge with the bioreactor stream at the pretreatment and conditioning stage, where the solutions pH or conductivity may be adjusted to be compatible with the subsequent capture step.

While seed systems generally offer significant advantages in stability and storage, transgenic rice specifically has emerged as a robust host for the large-scale production of high-value and industrial recombinant proteins. This study focuses on rHLZ as a model protein to demonstrate how the inherent benefits of the rice seed platform can be further enhanced through downstream process intensification. By moving beyond traditional batch methods, the following analysis explores how the purification of rHLZ can be optimized to meet specific market demands while improving overall economic and technical performance.

The lysozyme market is substantial, with a total value of $654 million (M) as of 2026 and a projected CAGR of 7.2% through 2034 (Precedence Research, n.d.). Hen egg-white lysozyme (HEWL) is widely used as an industrial enzyme for commercial processing of cheeses. It acts as a preservative and functional ingredient in foods such as Grana Padano (GP) cheese. However, up to 2.5% of children exhibit egg-white allergies according to Nolting et al. (2024), which limit their ability to consume products containing HEWL and create an incentive for alternative lysozyme sources. Transgenic rice provides a plant-based expression system that can produce rHLZ, while avoiding some safety and allergy concerns associated with animal-derived materials.

To establish a realistic production target for rHLZ, a market-based calculation was performed using demographic data on children in the United States, estimated egg white allergy

prevalence, recommended cheese consumption, milk-to-cheese conversion ratios, and HEWL usage per unit of milk. These calculations result in a projected mass of HEWL used in GP cheese that would be inaccessible to children with egg white allergy. Conversion from mass to activity units and adjustment for the higher specific activity of rHLZ result in an annual rHLZ demand of 1,000 kg/yr, which was used as the design basis for the production and purification models. The unit cost was decided as a function of HEWL unit cost adjusted for high activity. Calculations are listed in the equation section of the appendix. By holding final product throughput constant, differences in key economic and technical metrics can be attributed to process configuration rather than scale.

Objective

This study evaluates how downstream process intensification strategies improve the economics and performance of a base case using rHLZ extraction and purification from transgenic rice. A process simulation tool, SuperPro Designer (Intelligen, Inc.), was used to construct and compare multiple configurations of a downstream purification train, including traditional batch and intensified variations. The goal of the study was to quantify the economic and technical benefits of downstream process intensification for rHLZ production from transgenic rice seed, using SPD simulations to compare five configurations against a traditional batch baseline while targeting an output of 1,000 kg/yr. Specific objectives include evaluating reductions in capital expenditure (CAPEX), operating expenditure (OPEX), and cost of goods (CoGs), alongside improvements in volumetric productivity, equipment utilization, and facility footprint. Ultimately, the analysis identifies optimal intensification strategies, such as pool-less operations, high-capacity resins, and semi-continuous processing that lower economic barriers for plant-based biologics manufacturing, while maintaining the same fundamental unit operations and product specifications. A visualization of the intensification strategy is presented below (Figure 2). The five processing configurations are: batch [1], pool-less [2], high-capacity resin [3], semi-continuous [4], and combined intensification [5].

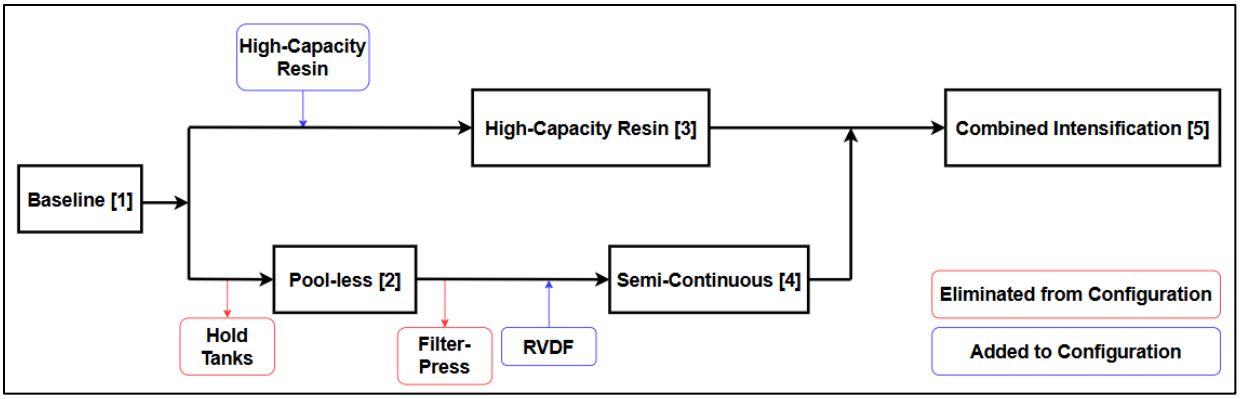

Figure 2. Progression of rHLZ Processing Trains with Increasing Intensification. Changes to the baseline model are indicated in the flow diagram by equipment eliminated (red) or added (blue) and the resulting model name and [number].

Methodology

To analyze the intensification strategies, SuperPro Designer (Intelligen), version 15, was used to simulate the extraction and purification of 1000 kg rHLZ annually. Chemical prices, disposal costs, and consumable costs were based on the 2026 vendor quotes. The fixed capital investment (equipment cost, engineering, construction, installation, electrical) and facility dependent costs (maintenance, insurance, depreciation) were determined by the SuperPro Designer software's built-in cost analysis. Operating labor and quality control were also determined from the software's built-in cost analysis. Labor costs were estimated by assuming a $30/h rate with 2 labor hours per equipment operation time, and quality analysis costs assumed $37/h rate with the same labor hour usage. The total operating cost was determined by summing the chemical, consumable, labor, utility, and waste disposal costs.

The batch process, with no explicit intensification strategies, serves as the baseline configuration. It comprises six major procedures: extraction, solid-liquid separation, clarification, cation exchange (CEX) chromatography, ultrafiltration/diafiltration (UF/DF), and final filtration. The campaign is operated in batch mode over one business quarter representing a realistic business operation for on-demand supply. Operating on an annual revenue stream from a single business quarter opens doorways for future products within the same production platform. Figure 3 illustrates the operational flow of the baseline batch process. Equipment sizes, process times, cleaning schedules, and material consumption were extracted from the simulations to compute CAPEX, OPEX, CoGs, volumetric productivity, and other performance metric.

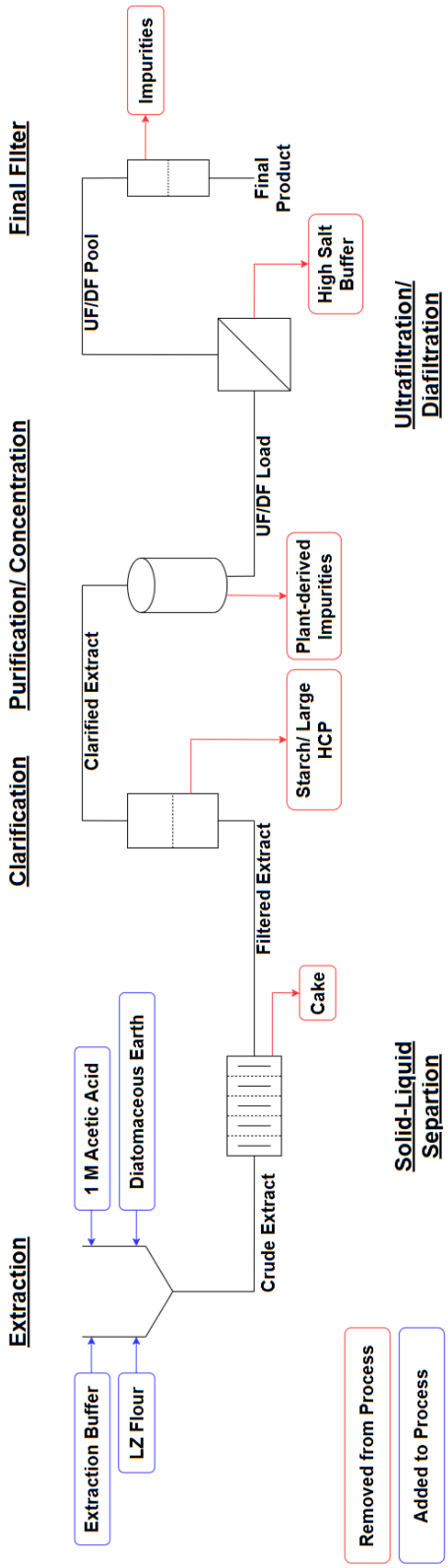

Figure 3. Flow Diagram for Baseline Batch Model of rHLZ Extraction and Purification. Process waste and impurities are removed (red) and materials and reagents are added (blue).

The processing train and operating conditions were derived from literature (Wilken, 2009; Wilken and Nikolov, 2006; 2010). The process starts with an extraction carried out at a 1 to 5 rice flour-to-buffer ratio using an extraction buffer (pH 4.5, 50 mM sodium acetate with 50 mM sodium chloride, NaCl). The crude extract has a rHLZ yield of 4 g/kg flour, a slight increase from 3.3 g/kg reported by Wilken (2009) which reflects enhancements of the expression level required for commercial scale. To maintain a pH of 4.5, 0.75% v/v 1 M acetic acid is added to maintain pH and limit the solubility of native proteins while efficiently extraction rHLZ. Following extraction, 0.8% m/v diatomaceous earth is incorporated as a filter aid. This was based on general industry guidance to use 0.5-2.0% m/v of filter aid for a 20% solids solution. The slurry is then transferred to a solid-liquid separation operation using a plate and frame filter (filter-press). After separation, a 20% v/v wash (pH 6), 50 mM sodium acetate buffer with, 50 mM NaCl and 64 mM TRIS base) is used to recover residual rHLZ within the filter cake. TRIS base is added to raise the pH of the solution to 6 and improve the buffering capacity during the subsequent chromatography step. The resultant liquid suspension is clarified using single-use 20 in, 0.2 µm absolute polyethersulfone (PES) depth filters, after which the clarified extract is transferred to a hold tank dedicated as the chromatography column feed.

Capture chromatography is performed at pH 6 using CEX chromatography (SP-Sepharose^{TM} FF) to capture rHLZ from the clarified extract (Wilken, 2009); with an isoelectric point (pI) of 10.2, lysozyme is highly protonated at pH 6, giving a net positive charge that binds strongly to the negatively charged ligands attached to the resin. First, the column is equilibrated with 8 bed volumes (BVs) of equilibration buffer (pH 6, 50 mM sodium acetate buffer with, 50 mM NaCl and 64 mM TRIS base) at a linear velocity of 300 cm/h. Clarified extract is loaded at 200 cm/h onto SP-Sepharose^{TM} FF CEX resin. After loading, the column is washed with 10 BVs of equilibration buffer at 300 cm/h to remove the remaining interstitial volume with any unbound impurities. The rHLZ is then eluted from the CEX resin via a linear salt gradient starting with equilibration buffer (with 0.05 M NaCl) and increasing up to 1 M NaCl over 10 BVs at 150 cm/h. The rHLZ peak is captured in the first 2.5 BVs of the elution process. The rHLZ is collected in a second dedicated hold tank used to feed the subsequent (UF/DF) step. UF/DF using a 10 kDa molecular weight cut-off (MWCO) PES membranes further concentrates the solution 9-fold, to a target rHLZ concentration of 50 g/L and performs a buffer exchange, replacing the high salt elution buffer with the final formulation buffer (pH 4.7, 60 mM sodium

acetate). The concentrated solution is sterile filtered using single-use 20 in, 0.2 μm absolute PES depth final filters. This step ensures final sterility before final filling and packaging.

Clean in place (CIP) cycles are applied to all reusable equipment using a dedicated cleaning solution, 1 M (4% w/v) sodium hydroxide. This solution breaks down fats, proteins, starches and oils (Lu, 2026). The extraction tank and both hold tanks undergo 1 h CIP cycles using 0.5 L of cleaning solution per liter of equipment volume (L/L) followed by 15 min purified water washes using 0.2 L/L. The filter-press and UF/DF system are cleaned using 1 h CIP cycles and 20 L of cleaning solution per square meter of filtration area (L/m^2) which is follow by a 15 min purified water flush at 100 L/m^2. The single-use depth filters are discarded after each batch. Column regeneration is performed via 6 BVs of 1 M NaOH at a linear velocity of 100 cm/h followed by buffer re-equilibration. Operating times, equipment utilization, and cleaning requirements for this baseline configuration provide the reference against which intensified models are evaluated.

Configuration Design

The intensified configurations differ from the baseline configuration through use of a pooling strategy, more efficient resin choice with higher binding capacity and extended lifecycle, equipment capacity, and mode of operation. These variations are explained in detail outlining key differences that result in significant economic and performance gains.

Baseline Batch Model [1]

The batch model was developed as the baseline, with extractions performed in batch-wise cycles and solid-liquid separation using a filter-press. Hold tanks are used to simulate a basic process with limited automation that relies on surge capacity rather than advanced scheduling and controls. Chromatography uses SP-Sepharose^{TM} FF CEX resin, with a dynamic binding capacity (DBC) of 25 g/L and a lifespan of 100 cycles. Additional simulation parameters and conditions are listed below in Table 2.

Table 2. Simulation Process Parameters and Conditions for rHLZ Batch Purification from Transgenic Rice Extract

Unit Operation	Parameter Assumption	rHLZ Conc. (g/L)	Overall Yield (%)	Purity (%)
Rice Flour	4 g rHLZ per kg flour	N/A	N/A	N/A
Extraction	1:5 flour-to-buffer ratio; pH 4.5; 30 min	0.68	100%	54%
Solid-Liquid Separation / Clarification	Plate and frame (50 LMH) + 0.2 µm absolute filtration (600 LMH); cake wash	0.69	94%	63%
Capture (CEX)	pH 6; 25 g/L dynamic binding capacity (DBC) 200 cm/H linear load rate; gradient NaCl elution (150 cm/h); concentration (6 g/L)	6.55	85%	86%
Concentration and Formulation	10 kDa MWCO membrane; final concentration (50 g/L); final formulation (pH 4.7); UF/DF (38 LMH); final filtration (84 LMH)	49.03	69%	98%

Pool-less Model [2]

The pool-less process model was developed as a reductionist intensification strategy aimed at eliminating non-essential hold steps between downstream unit operations. In this configuration, the overall process flow and unit operations are identical to the baseline batch process, but intermediate storage tanks are removed wherever material can be transferred directly to the subsequent operation without compromising controllability or product quality. Specifically, the hold tanks following clarification and chromatography are removed. Clarified extracts from depth filtration are transferred directly to the chromatography column, and the elution pool is directed immediately to the UF/DF system. This approach reduces the number of large stainless-steel vessels in the process and simplifies transfer steps, while maintaining batch-wise operation and the same fundamental equipment (filter-press, CEX chromatography, UF/DF, and final filtration).

The pool-less configuration relies on more precise scheduling and coordination of unit operations to ensure that downstream equipment is available when upstream steps are complete. It requires more stringent control of flow rates and transfer timing to avoid interruptions or

unintended pooling. CIP strategies remain similar to the batch case, but fewer tanks require CIP cycles, and transfers are planned so that equipment utilization is improved without introducing overlaps that would compromise cleaning requirements. Quantitative impacts on capital, operating costs, and processing times are reported in the Results.

High-Capacity Resin Model [3]

The high-capacity resin model was designed to evaluate the impact of replacing the baseline CEX resin with a higher DBC medium as a process intensification strategy while preserving the general batch processing framework. In this configuration, the SP-Sepharose™ FF resin used for the rHLZ capture is replaced with a strong cation exchange resin. Capto™ S ImpAct resin has a more rigid base matrix, functionalized surface extenders, and optimized pores (Cytiva, n.d.), providing substantially higher flowrates, rHLZ DBC (80 g/L), and lifespan (200 cycles) while maintaining the same chemical compatibility. The upstream and intermediate steps, including extraction, solid-liquid separation, clarification, UF/DF, and final filtration, follow the same sequence and operating mode as in the batch process. The column is sized based on its higher DBC, allowing the same mass of rHLZ to be captured with a reduced resin volume.

Capto™ S ImpAct resin has improved capture affinity and more rigid structure through the addition of polymer-grafted ligands with sulfonate and pyrrolidone building blocks, ultimately lowering volume requirements and increasing linear flowrates (Cytiva, n.d.). The equilibration and wash cycles operate at 400 cm/h using 5 BVs of extraction buffer. Loading operates at 400 cm/h, doubling the linear flowrate, compared to the batch configuration. Only 5 BVs of elution buffer at 200 cm/h are used to elute rHLZ because of the tighter peak formation from improving the binding capacity; the rHLZ peak ends at 1.25 BVs. Regeneration still operates at 100 cm/h; however, only 3 BVs of 1 M NaOH are used because the base matrix has a higher resolution. Because of the improved binding capacity and lower buffer requirements, the column pool is nearly 6% more concentrated. This change in concentration further reduces the processing time of the UF/DF step that concentrates the material to a final concentration of 50 g/L. The resulting changes in equipment sizing, processing times, and economics associated with this resin substitution are quantified in the results.

Semi-Continuous Model [4]

The semi-continuous model was created to represent an intensified configuration in which the overall process operates on an extended batch basis, with several unit procedures

operating in a more continuous or cyclic manner. The flowsheet retains the same major procedures as the batch model: extraction, solid-liquid separation, clarification, CEX chromatography, UF/DF, and final filtration, but modifies the mode of operation and equipment selection to increase equipment utilization and reduce the size of key vessels. In this configuration, extraction is carried out in multiple smaller cycles rather than in a single large batch.

Solid-liquid separation is performed using a rotary vacuum drum filter (RVDF) instead of a filter-press, followed by clarification using only two single-use 20 in, 0.2 μm absolute PES depth filters. The RVDF, directly feeding the clarification filter step, is operated for an extended period to continuously remove solids and generate a clarified stream in alignment with the extraction output. The combination of cyclic extraction and continuous filtration and clarification maximize equipment utilization by reducing upstream equipment size and scheduled cleanings. The clarified extracts from several cycles are pooled in a dedicated hold vessel to achieve the desired batch-equivalent volume for chromatography and subsequent steps.

CEX chromatography and UF/DF in the semi-continuous model are operated with the same basic methods and operating conditions as in the batch configuration. The semi-continuous model coordinates cleaning cycles to minimize downtime while preserving adequate regeneration and sanitization between production campaigns. Quantitative improvements in equipment utilization, processing time, and cost metrics arising from this semi-continuous strategy are presented in the results section.

Combined Intensification Model [5]

This configuration combines the intensification elements from all of the configurations into a single downstream processing scheme. The objective of this model is to assess the combined effect of operating the process in a semi-continuous manner while also leveraging the increased DBC and extended lifetime of Capto™ S ImpAct resin. As in the semi-continuous model, extraction is implemented as multiple smaller cycles feeding directly to solid-liquid separation carried out by a RVDF flowing into the depth filtration step and being pooled into a common hold vessel. These operations provide a more continuous and uniform feed to the capture column. The chromatography step employs Capto™ S ImpAct resin, and the column is sized according to its enhanced DBC. The column operates with the same operational and conditional profile as the high-capacity resin model.

The UF/DF and final filtration steps follow the same operational and conditional profile to the high-capacity resin configuration. CIP protocols are maintained in alignment with the high-capacity resin configuration, with cleaning cycles balancing resin longevity, column performance, and overall campaign duration. This configuration combines smaller, more frequently cycled upstream equipment with a higher-capacity chromatography step to maximize equipment utilization and minimize non-productive time. The resulting impact on capital requirements, operating costs, and overall productivity is analyzed in the results section.

Because all five downstream configurations share the same core sequence of unit operations, the process flow diagrams were consolidated into three representative figures. Figure 4 shows the baseline batch and high-capacity resin models using a single common diagram. Since both follow the same extraction, solid-liquid separation, clarification, capture chromatography, UF/DF, and final filtration sequence; only the chromatography resin selection and associated operating parameters differ between these two cases. The pool-less configuration is illustrated separately, in Figure 5, highlighting the elimination of intermediate hold tanks while preserving the same underlying operations and overall process logic. Finally, the semi-continuous and combined intensification models are represented by a shared diagram, in Figure 6; it captures their common use of cyclic extraction coupled to continuous solid-liquid separation and clarification, followed by the same downstream capture and polishing train. Within this framework, each configuration is distinguished by its specific operating conditions, resin properties, and scheduling, rather than by changes in the fundamental unit operations themselves.

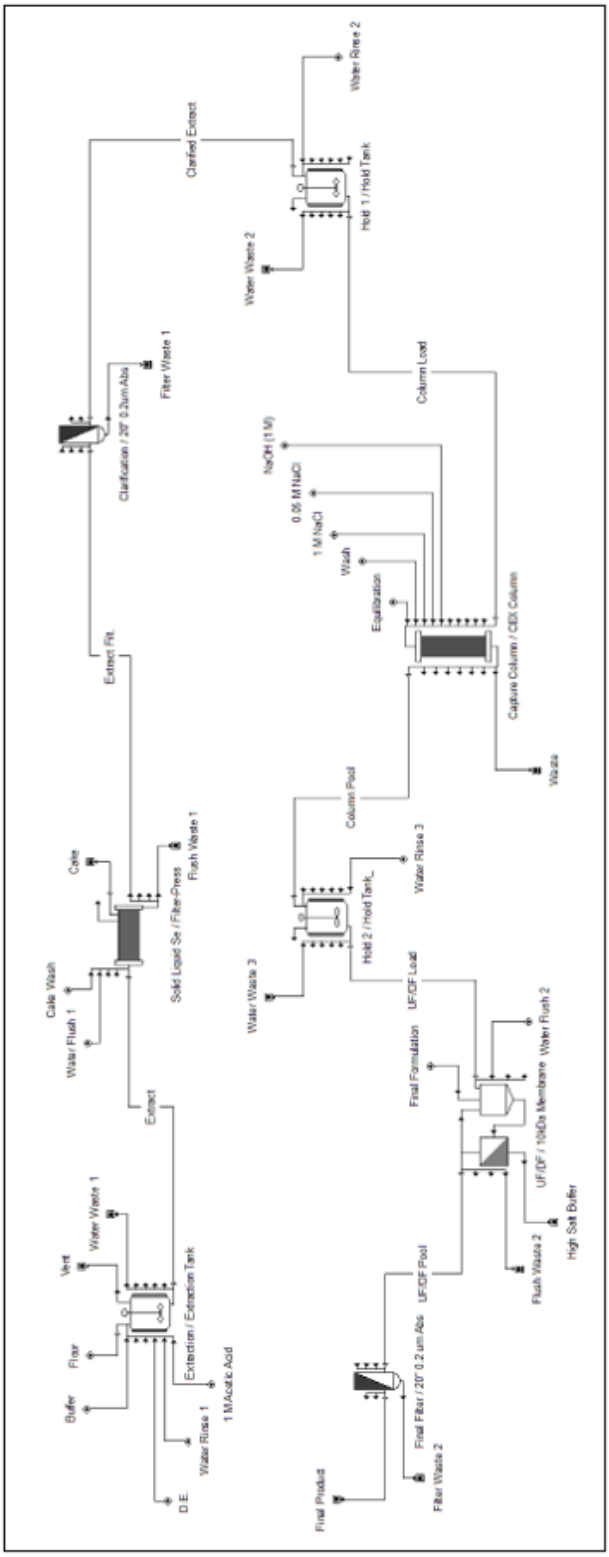

Figure 4. Process Flow Diagram (Baseline Batch and High-Capacity Resin Models)

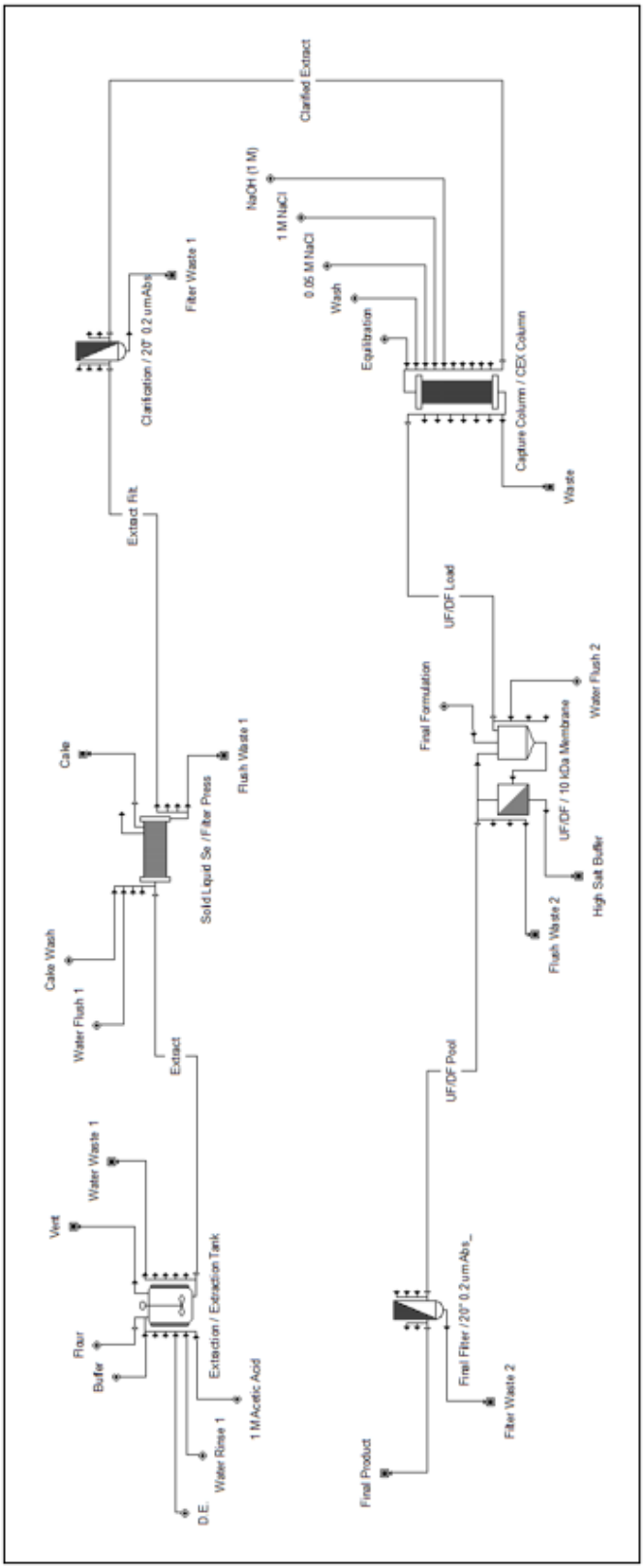

Figure 5. Process Flow Diagram (Pool-less Model)

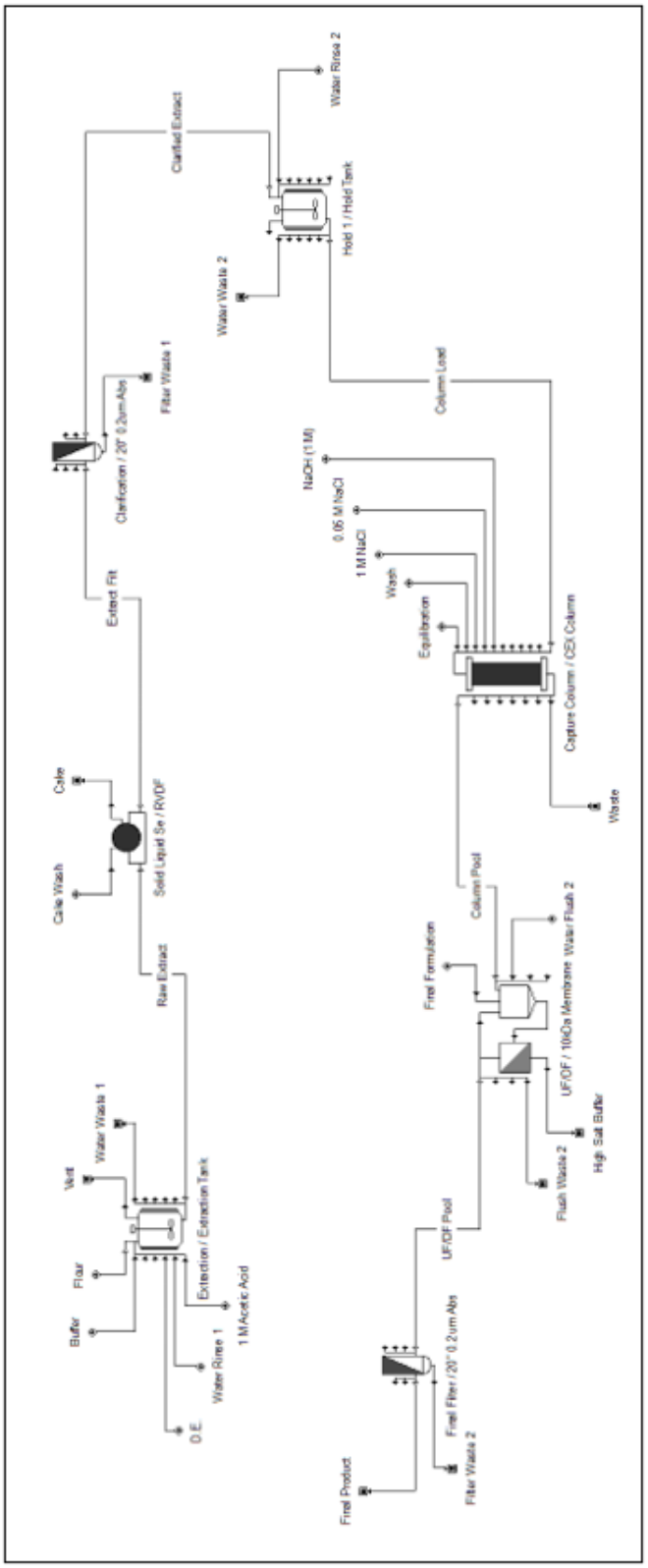

Figure 6. Process Flow Diagram (Semi-Continuous and Combined Intensification)

Results

Summary of Key Performance Metrics

Five downstream processing configurations were modeled to produce approximately 1,000 kg/yr of food-grade rHLZ from transgenic rice. These configurations were: a baseline batch process, a pool-less process, a high-capacity resin process, a semi-continuous process, and combined intensification strategy process. Each model used the same basic process flow: extraction, solid-liquid separation, clarification, capture chromatography, UF/DF, and final filtration; they differed in pooling strategy, resin selection, and mode of operation.

All configurations were scaled to meet the same annual rHLZ production target so that differences in performance reflect process configuration and scheduling rather than scale. For each configuration, CAPEX, equipment costs, consumable costs, OPEX, CoGs, gross margin, net present value (NPV), volumetric productivity, and labor costs were evaluated and are listed in Table 3. The batch model exhibited the highest CAPEX, OPEX, and CoGs, providing a baseline against which the intensified processes were compared. Across these five models, equipment reduction, resin selection, and semi-continuous operation improved the quality attributes of all configurations, relative to the baseline batch process.

Table 3. Summary of Key Economic and Technical Metrics for Batch and Intensified rHLZ Production Configurations

Model	Productivity (g/L*Day)	CAPEX ($M)	Equipment ($M)	Consumables ($k/batch)	OPEX ($M/yr)	CoGs ($/g)	Gross Margin (%)	NPV ($M)	Labor ($M/yr)
1	0.92	26	3.0	5.4	12	12.2	79	240	0.71
2	0.96	24	2.7	5.4	11	11.4	80	240	0.62
3	1.21	24	2.8	5.4	10	10.4	82	250	0.60
4	0.93	12	1.4	3.6	7.5	7.5	87	270	1.6
5	1.20	10	1.2	3.6	7.2	7.2	88	270	1.6

Economic Performance: CAPEX, OPEX, and CoGs

The total capital investment for the batch configuration is $26M. This configuration has the highest main process equipment costs; a large extraction tank, a filter-press, two hold tanks, a conventional CEX column, a UF/DF membrane system, and two filtration systems, combined to cost $3M. Therefore, this configuration represents the highest investment model, with capital costs characteristic of traditional batch downstream processing relying on substantial intermediate storage capacity. The batch configuration also has the highest operating costs ($12M/yr) and cost of goods ($12.2/g). Consumables including a total of eleven 20 in 0.2 µm absolute PES depth filters, 339 L of SP-Sepharose™ FF resin, and 8.6 m^2 of 10 kDa MWCO PES membranes had a combined cost of $5.4k/batch. This model has a relatively high gross margin and NPV at 79% and $240M, respectively. Labor costs were also considered; the batch model dedicates $0.71M on labor costs annually.

The pool-less configuration reduced CAPEX by 10% via the elimination of intermediate hold tanks after clarification and chromatography. By directing clarified extract to the column and the column elution pool directly to UF/DF, the number of large stainless-steel vessels was reduced and associated installation costs were avoided. As a result, the total main equipment investment decreased by 9% relative to the batch case. All other hardware remained essentially unchanged, indicating that capital savings were driven primarily by tank elimination. The OPEX and CoGs both decreased 7% because of lower cleaning solutions and purified water consumption, and ultimately less labor requirements associated with fewer tanks and CIP operations. There is no change in consumable costs while gross margin and NPV increased 2% and labor costs decreased 13%.

In the high-capacity resin configuration, CAPEX decreased 8% relative to the batch case; this is because of the reduced extraction scale and the modified column hardware associated with the Capto™ S ImpAct resin. This advanced resin has a 650% higher cost per liter than SP-Sepharose™ FF; counteractively, the resin volume decreased 73%, resulting in only a marginal increase in consumable costs. As resin volume decreased, the chromatography system size subsequently decreased as well by 26%, ultimately reducing total main equipment costs by 8% illustrating how resin performance affects process scale. The OPEX and CoGs both decreased 15% because of the higher capture efficiency. The gross margin and NPV rose 4% and labor costs were reduced 14%.

The semi-continuous configuration showed a dramatic decrease in CAPEX of 50% because the extraction vessel was downsized relative to the batch model because of the use of a cyclic extraction strategy. In addition, RVDF was used in place of the filter-press for solid-liquid separation, reducing the number of clarification filters and ultimately reducing equipment cost by 50%. The number of filters was reduced because of the extended time allocated for clarification, resulting in the use of only two, 20 in 0.2 μm absolute depth filters per batch. This, coupled with a moderate increase in resin cost per batch contributed to a 34% reduction in total consumable costs. OPEX and CoGs decreased 39%, and the gross margin and NPV both increased 10% and 12%, respectively. Unlike the other configurations, labor costs increased significantly (130%) as more operator support is required for the cyclic extraction step.

The combined intensification configuration integrated all the strategies from the other configurations including cyclic extraction, continuous filtration using RVDF, and advanced CEX resin. By improving the efficiency of the extraction and solid-liquid separation, and the adoption of the Capto^{TM} S ImpAct resin ultimately reducing CAPEX 60%, and OPEX and CoGs decreased 40%. This model significantly reduced CAPEX, OPEX, and CoGs due to equipment downsizing and enhanced utilization and improved resin performance. These contributing factors resulted in a 60% reduction in equipment costs, the most of any configuration. Similar to the semi-continuous model, the consumable costs decreased 34% for the same reason as the semi-continuous configuration: a reduction in filter usage and moderate increase in resin costs per batch. The gross margin and NPV increased 11% and 13%, respectively, and labor costs increased 124%.

Taken together, the results show that the highest CAPEX, OPEX, and CoGs were associated with the batch baseline model, while the combined intensification configuration achieved the highest performance metrics. All equipment costs are shown in Table 4, and a breakdown of consumable cost per batch is listed in Table 5. A bar graph showing the equipment cost distribution is shown in Figure 7.

Table 4. Main Equipment Pricing of rHLZ Production Train Configurations

Model	Extraction Tank ($k)	Filter-Press /RVDF ($M)	Clarification Filter System ($k)	Holding Tank 1 ($k)	Chromatography System ($k)	Holding Tank 2 ($k)	UF/DF System ($k)	Final Filter System ($k)	Total Cost ($M)
1	199	1.63	7.1	195	729	8.9	5.7	3.6	3
2	199	1.63	7.1	N/A	729	N/A	5.7	3.6	2.7
3	188	1.63	7.1	184	542	6.8	5.7	3.6	2.8
4	112	0.21	3.8	204	762	N/A	5.9	3.8	1.4
5	107	0.20	3.8	193	565	N/A	5.9	3.8	1.2

Table 5. Consumable Cost of rHLZ Production Train Configurations ($/batch)

Model	Clarification Filter (20" 0.2 µm absolute PES depth filter)	Chromatography Resin (SP Sepharose FF or Capto S ImpAct)	UF/DF (10 kDa MCWO PES Membrane)	Final Filter (20" 0.2 µm absolute PES depth filter)	Total
1	2430	2388	58	540	5416
2	2430	2388	58	540	5416
3	2430	2405	58	540	5433
4	540	2420	58	540	3558
5	540	2436	58	540	3574

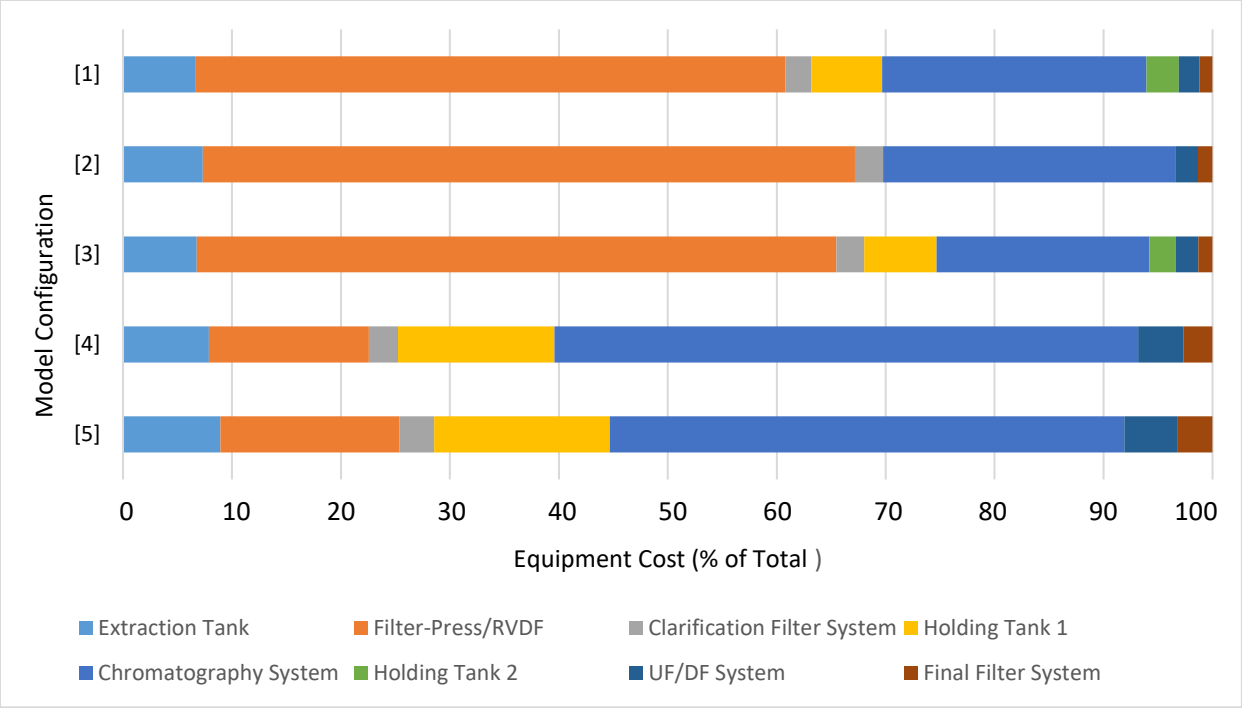

Figure 7. Comparison of Main Equipment Pricing Distribution

Technical Performance: Productivity and Time

Technical performance for the models was assessed in terms of volumetric productivity and operational and total processing time per batch. Operational process times are shown below in Table 6 and volumetric productivity is found in Table 3. Illustrations for equipment occupancy are shown in Figures 8-12.

In the batch configuration, a single large extraction step was followed by sequential downstream operations with intermediate hold steps. The large extraction volume and conventional chromatography cycle created relatively long batch times (17.6 h). The chromatography step was the primary bottleneck taking 7.1 h, consuming 40% of the total batch processing time, limiting the number of batches completed per year. This configuration has the lowest volumetric productivity with 0.92 grams rHLZ per liter of extract per day(g/L/day).

The pool-less configuration improved effective productivity by eliminating post-clarification and post-chromatography hold steps. While the underlying extraction, filtration, chromatography, UF/DF, and filtration operation times remained essentially the same as in the baseline batch case, the removal of intermediate storage slightly shortened the total cycle time 4%, from 17.6 h to 17 h. This allowed for the same annual throughput with a more streamlined

operation. As a result, volumetric productivity increased to 0.95 g/L/day, improved modestly without major changes in the core unit operations.

The high-capacity resin configuration significantly altered the performance of the chromatography operation. The Capto™ S ImpAct resin increased the operating flow rates and higher DBC due to its more rigid structure. This increased the length of the capture step, while the solid-liquid separation time is reduced because of lower extract volume; together this effectively doesn't change the total processing time. However, the new resin captures tighter peaks enabling less BVs of elution, increasing the rHLZ concentration in the column pool by over 560%, enabling a much shorter UF/DF step; this reduces the total batch time 24% from 17.6 h to 13.4 h. The volumetric productivity rose to 1.21 g/L/day, a 32% increase from the baseline batch configuration.

The semi-continuous configuration used smaller extraction cycles with purified water rinses before subsequent extractions. Starting after the first extraction, raw extract was passed through the RVDF and clarification filters before pooling in a large hold tank dedicated to column load material. This fundamental alteration increased the extraction step by 360%; however, because the column load material collects throughout extraction step, loading onto the column begins much earlier, effectively maintaining the same total processing time as the baseline batch configuration. This more efficient upstream framework increases the volumetric productivity rise marginally to 0.93 g/L/day.

The combined intensification configuration uses the same cyclic extraction framework as the semi-continuous configuration, and the Capto™ S ImpAct resin. The increased extraction time and quicker loading start time are consistent with the semi-continuous process, but the concentration of the column pool and the UF/DF operating time aligned with the high-capacity resin configuration. This reduced the overall processing time 23% from 17.6 h to 13.5 h and increased the volumetric productivity to 1.2 g/L/day.

Table 6. Equipment Utilization: Operational and Transfer Time (in hours)

Model	Extraction	Solid-Liquid Sep.	Clarification	Holding 1	Chromatography	Holding 2	UF/DF	Final Filter	Total
1	1.08	1.18	1.18	0.33	7.11	0.33	4.98	0.39	17.63
2	1.08	1.18	1.18	N/A	7.11	N/A	4.98	0.39	16.96
3	1.08	1.02	1.02	0.33	7.70	0.33	0.46	0.37	13.38
4	5.0	6.65	6.65	6.82	7.11	N/A	5.05	0.39	17.51
5	5.0	6.65	6.65	6.82	7.71	N/A	0.47	0.38	13.51

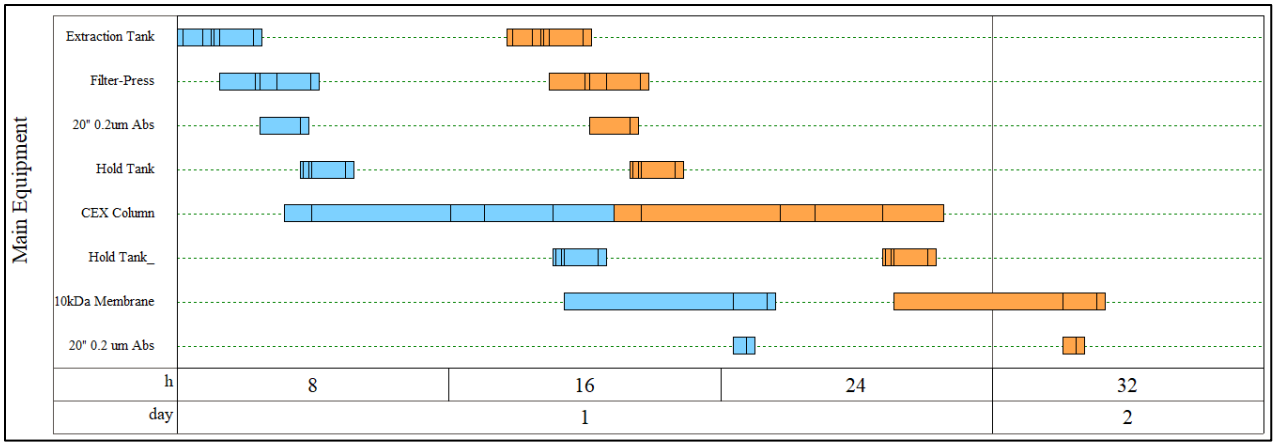

Figure 8. Equipment Utilization Chart for the Baseline Batch Model [1]. The figure shows the occupation time and scheduling of operations through the process and equipment and between batches with batch 1 (blue) and batch 2 (orange).

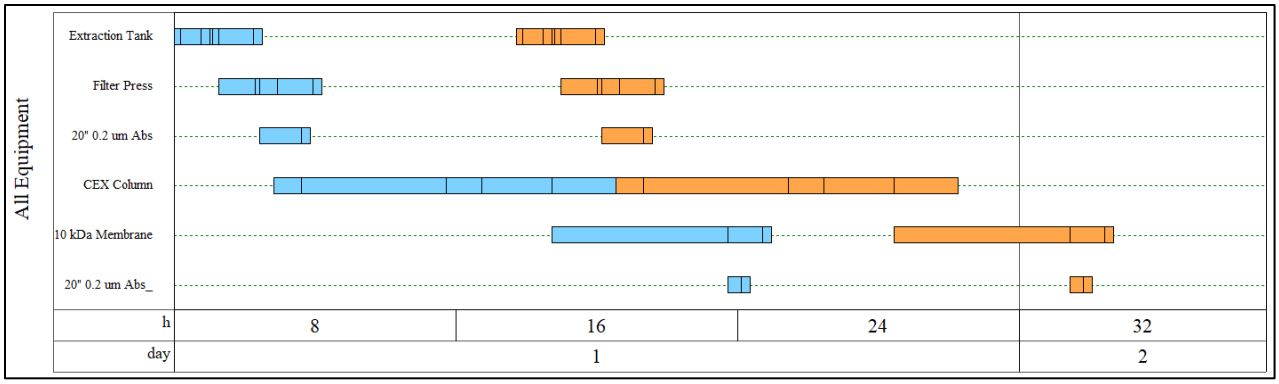

Figure 9. Equipment Utilization Chart for the Pool-less Model [2]. The figure shows the occupation time and scheduling of operations through the process and equipment and between batches with batch 1 (blue) and batch 2 (orange).

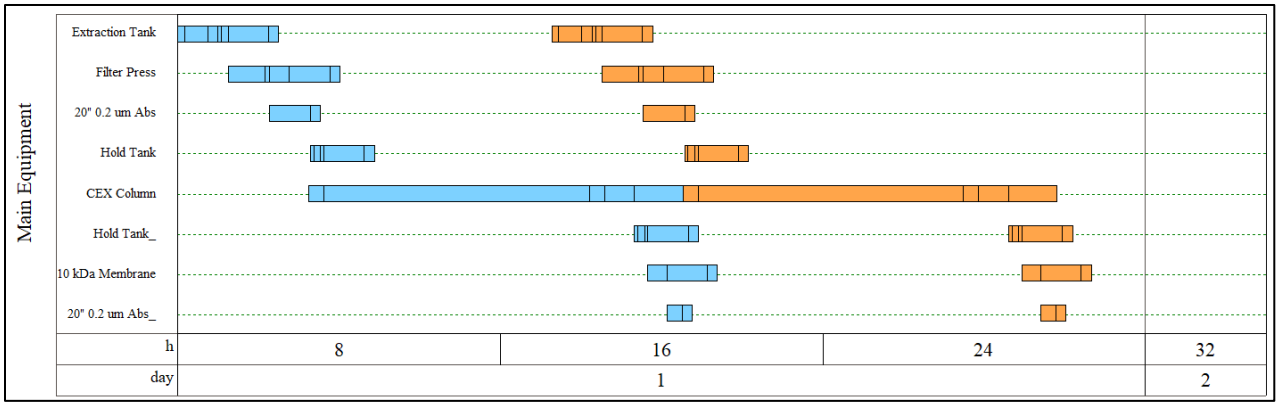

Figure 10. Equipment Utilization Chart for the High-Capacity Resin Model [3]. The figure shows the occupation time and scheduling of operations through the process and equipment and between batches with batch 1 (blue) and batch 2 (orange).

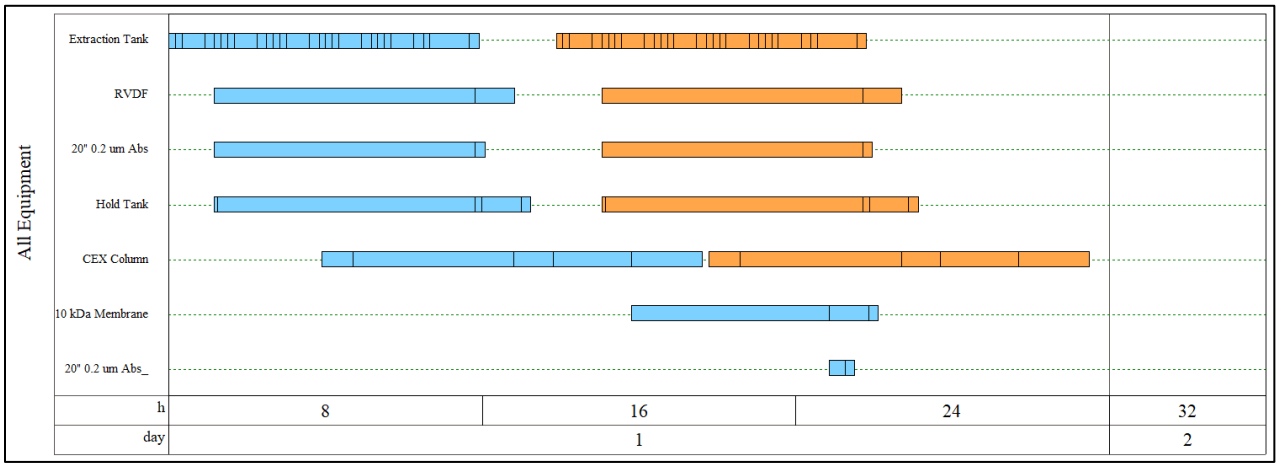

Figure 11. Equipment Utilization Chart for the Semi-Continuous Model [4]. The figure shows the occupation time and scheduling of operations through the process and equipment using cyclic extractions within batches. Flow between batches is defined using batch 1 (blue) and batch 2 (orange).

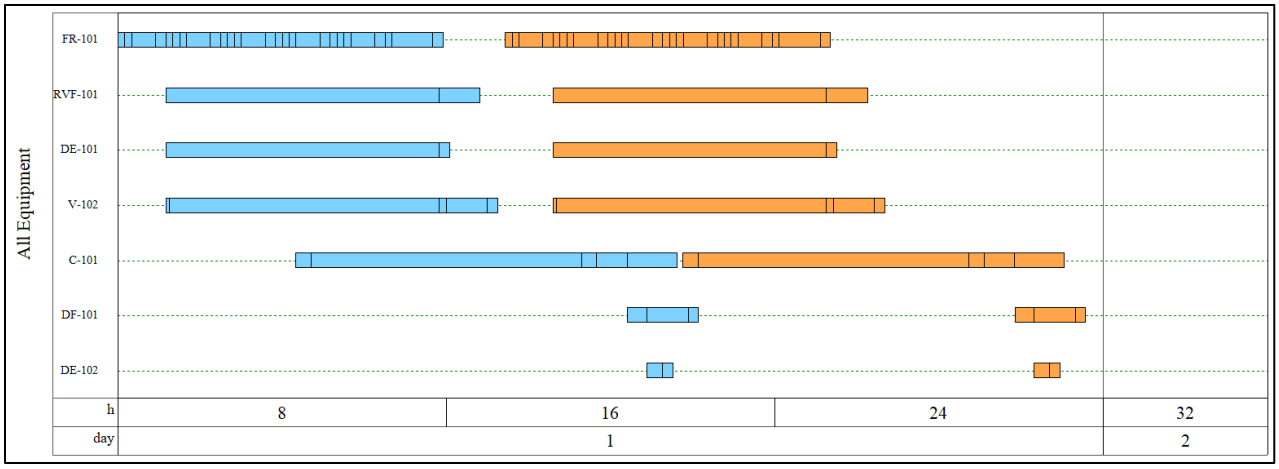

Figure 12. Equipment Utilization Chart for the Combined Intensification Model [5]. The figure shows the occupation time and scheduling of operations through the process and equipment using cyclic extractions within batches. Flow between batches is defined using batch 1 (blue) and batch 2 (orange).

Spatial and Practical Considerations

Spatial and practical considerations were also examined for the models. Table 7 outlines the floor area and membrane area requirements for each model configuration. This highlights floor plan requirements and gives insights into cleaning requirements.

In the batch configuration, the floor area and membrane area combine to produce a relatively large equipment footprint (181 m^2). This configuration is familiar and straightforward to operate but demands substantial floor area and extensive utility and CIP infrastructure. The multiple hold vessels increase the number of surfaces that must be cleaned, validated, and maintained, adding complexity to facility operations.

The pool-less configuration reduced the number of large stainless-steel tanks, leading to a more compact set of equipment. With intermediate storage removed between clarification and column loading and between chromatography and UF/DF, the floor area decreased 44% and the overall equipment footprint decreased 4%. However, this configuration requires tighter scheduling and more sophisticated control to synchronize flows between unit operations, therefore complicating the process from a controls and automations perspective.

The high-capacity resin configuration floor area decreased 26% because of the smaller column due to the improved resin utilization and subsequently the lower extraction volume. This

decreased the total equipment footprint by 3%. Like the baseline batch configuration, the hold tanks add surfaces that need cleaned and maintained.

In the semi-continuous configuration, equipment volumes were reduced for the extraction tank and the RVDF, trading a larger static filter-press for a cheaper, rotating unit with continuous cake removal. The extraction tank floor area was reduced by 65% and the RVDF substitution reduced solid-liquid separation floor area by 78%. Because the clarification step was elongated, only two 20 in 0.2 μm PES depth filters were used to maintain the same filtration flux. These alterations reduced the total equipment footprint by 69%.

The combined intensification configuration combines the compact upstream equipment of the semi-continuous design with the reduced resin inventory of the high-capacity resin configuration. As a result, it offers a similar membrane area to the semi-continuous configuration, while the floor area decreased 50% and the membrane area decreased 73%, respectively, compared to the batch model. There are many implementation challenges associated with intensification, including the need for advanced automation, careful scheduling, and robust cleaning and validation.

Table 7. Equipment Area Requirements Summary Table

Model	Floor Area (m^2)	Membrane Area (m^2)	Total Area (m^2)
1	18	163	181
2	10	163	173
3	14	163	177
4	13	44	57
5	9	40	49

Integrated Assessment and Preferred Designs

Taken together, the five configurations outline a clear progression from conventional batch operation toward increasingly intensified downstream processing for plant-based rHLZ. The baseline batch model serves as a familiar reference point but consistently performs worst across key metrics, exhibiting the highest CAPEX, OPEX, and CoGs, the lowest volumetric productivity, and the largest equipment footprint. It remains most defensible only in contexts

where regulatory familiarity, simple scheduling, and limited automation are prioritized over long-term cost and space efficiency.

The pool-less configuration represents a low-risk first step toward intensification. By eliminating non-essential hold tanks while preserving batch-wise operation and the same core unit operations, it reduces CAPEX by removing large stainless-steel vessels, lowers OPEX and CoGs through reduced CIP and utilities, and modestly improves volumetric productivity without introducing new equipment. This design is attractive when the objective is to capture tangible savings and streamline the process with minimal disruption to existing operating processes. The high-capacity resin configuration further improves economic performance by leveraging the Capto^{TM} S ImpAct resin to reduce resin volume, column size, and buffer consumption. This lowers OPEX and CoGs while increasing volumetric productivity, even though resin price per liter increases. This option requires higher initial resin costs but makes future gains through lower long-term consumable costs and time savings.

The semi-continuous and combined intensification configurations deliver the strongest overall performance when economic, technical, and spatial criteria are considered together. The semi-continuous design downsizes upstream vessels, replaces the filter-press with a RVDF, reduces filter usage, and compresses the equipment footprint, achieving substantial reductions in CAPEX, OPEX, and CoGs with only modest gains in volumetric productivity. The combined intensification model builds on this framework by coupling cyclic extraction and continuous solid-liquid separation with high-capacity resin, yielding the largest reduction in CAPEX, OPEX, and CoGs, and the highest volumetric productivity among all configurations, while also achieving the most compact footprint. For high-throughput production of plant-based rHLZ, the semi-continuous and combined intensification models emerge as the preferred designs, provided the added demand for automation, scheduling, and validation are met.

Discussion

The downstream processing models developed in this report indicate that modest intensification strategies can generate meaningful improvements in both economic and technical performance for plant-based rHLZ production from transgenic rice. By holding annual output constant at 1,000 kg/yr and varying only pooling strategy, resin selection, equipment intensity, and mode of operation, the analysis isolates the contributions of process configuration to cost,

productivity, and spatial footprint. Across the five configurations, the baseline batch design consistently exhibited the highest CAPEX, OPEX, and CoGs, while the other designs achieved lower capital and operating costs and higher volumetric productivity. These trends are broadly consistent with published economic analyses of intensified and continuous bioprocessing, which report substantial reductions in overall CoGs and improved equipment utilization when downstream processes are intensified or made continuous.

Comparison with Literature

The observation that downstream processing dominates overall manufacturing cost and that chromatography is a major cost driver aligns with prior biomanufacturing economic studies, which estimate that downstream processing can contribute up to 50-80% of total production costs (Kumar et al., 2020). In this study, downstream intensification strategies reduced OPEX and CoGs by 7-40% relative to the baseline batch case, which aligns closely the reported 10-30% CoGs reduction typical for intensified bioprocessing facilities (Mahal et al., 2021). The pool-less configuration, which removed intermediate hold tanks while preserving batch operation, decreased OPEX and CoGs by 7%, mirroring case studies where the elimination of hold tanks and better synchronization between unit operations substantially lowers buffer usage, labor, and cleaning requirements (Jungnelius & Bisschops, 2022).

The high-capacity resin and combined intensification configurations showed large gains in volumetric productivity, with a 30% increase for both cases. This is comparable in magnitude to productivity gains reported for multi-column chromatography systems, which can increase productivity by 19-52% (Xu et al., 2020). Replacing SP-Sepharose™ FF resin with Capto™ S ImpAct resin in the combined intensification model had a negligible effect on consumable costs while reducing CAPEX 7% and OPEX 2%, compared to the semi-continuous model. This mirrors results where advanced capture media shifts costs from operating expenses toward capital investment while improving long-term economics (Yang et al., 2020). Furthermore, the use of transgenic rice as the expression platform is supported by earlier experimental work demonstrating rHLZ expression and extractability (Huang et al., 2002). This work aligns with these findings as the extraction yields 4 g of rHLZ per kg of flour.

Specific Challenges and Solutions in Continuous/Intensified Systems

The results of this study highlight specific challenges in moving from traditional batch processing to intensified configurations for rHLZ. As a central theme, it is important to control upstream conditions to prevent downstream issues, specifically variability of crude extract quality that can affect solids loading rates into the clarification filter and chromatography unit. Because rHLZ adsorption capacity is highly dependent on impurities such as phytic acid and native protein, buffer selection, and operational conditions (Wilken, 2009), tighter control of extraction conditions and pre-clarification treatment is essential for robust operation. Furthermore, pre-fractionation strategies prior to milling can reduce variability by removal of critical impurities such as phytic acid.

The pool-less configuration modeled here eliminates hold tanks and in response, requires tighter controls and automation to maintain proper material flows; however, his brings intense validation requirements. Furthermore, under intensified operation, cleaning validation becomes more demanding. Clearly defined batch processes simplify validation, whereas continuous operations require validated CIP cycles and real-time performance indicators to demonstrate control over long operating periods.

To manage these challenges, intensified rHLZ production requires sophisticated control strategies like process analytical technology (PAT). Using a quality by design (QbD) framework, live monitoring of conductivity, UV absorbance, and turbidity can maintain and adjust a validated operation plan. This highlights the “lights-out” operation goals found in modern intensified facilities, where advanced controls adjust parameters automatically based on validated process conditions and advanced algorithms (Scott et al., 2024).

Future Perspectives and Remaining Challenges

The configurations examined suggest an evolution toward fully continuous manufacturing for plant-based rHLZ. Fully continuous operation involves eventually integrating highly controlled multi-column capture and continuous UF/DF, neither of which were considered in this study. Because transgenic rice allows for extended seed storage, it is uniquely suited for on-demand production.

Regulatory hurdles remain a particular concern, specifically regarding lot definition and cleaning validation for continuous operation. For a food-grade product like rHLZ, cleaning

validation and traceability is less concerning than for pharmaceutical products, but regardless, quality monitoring is essential, despite the more flexible regulatory framework. Looking forward, artificial intelligence (AI) and automation will play a central role in enabling fully continuous rHLZ production. Adaptive controls aid in loading high-capacity resins and predictive maintenance for critical equipment (Scott et al., 2024). Advanced automation technology is a gray area for regulatory agencies. AI is a powerful tool, but it must reinforce the quality, efficacy, and safety requirements that regulators set (FDA and EMA, 2026).

Future work should be conducted looking at the economic feasibility of utilizing the byproducts from the rHLZ processing train as a secondary revenue stream, specifically the spent rice flour generated in the solid-liquid separation step. This spent rice flour is high in proteins and starch, presenting a promising opportunity for further valorization. Rather than being discarded as waste, this material could serve as a starting feedstock for a secondary protein or small molecule product, thereby expanding the overall product portfolio. Implementing such a strategy could increase facility utilization beyond the current rHLZ production campaign, which occupies only one quarter of the operating year. Developing complementary processes to convert this byproduct into value-added products would enable more consistent use of equipment and resources, reduce idle times, and enhance the overall economic sustainability of the manufacturing platform.

In addition to exploring byproduct-derived opportunities, future work should include experimental validation of model assumptions such as resin binding capacities, , impurity clearance, and process yields that were made from literature and vendor product technical documents. Laboratory-scale validation of these aspects would strengthen the accuracy and reliability of the process model. Furthermore, future model development should incorporate advanced capture technologies, such as MCC and membrane adsorbers, to evaluate their potential for process intensification and throughput improvement. Alongside these improvements, the use of software tools like SchedulePro (Intelligen, Inc.), to simulate optimized production scheduling could help assess facility utilization strategies and further demonstrate the benefits of integrating continuous and multi-product operations within the same manufacturing train.

Limitations of the Study

Several limitations in this study must be acknowledged. This model analysis relies on process simulations using assumed equipment and consumable costs, resin lifetimes, operational yields, and filtration fluxes. Batch-to-batch variability is not considered, and a 100% batch success rate was assumed with identical extraction yields between batches. Performance is highly sensitive to production scale; therefore, the magnitude of cost reductions observed here may differ in practice. Furthermore, this work focuses on a single product; changes in demand or raw material quality or availability could dismantle the framework of this study. Furthermore, there is no sensitivity analysis to evaluate major cost drivers and impacts of variability.

Despite these constraints, the modeled configurations outline a plausible roadmap for intensifying rHLZ production. A facility could initially implement pool-less operation and high-capacity resins, then gradually adopt semi-continuous operations and AI-enabled controls. For applications such as the one referenced in this paper, supporting cheese production for children with egg white allergy, this intensified plant-based platform offers a cost-effective, scalable alternative to traditional HEWL.

Conclusion

This study evaluated five downstream processing configurations for producing approximately 1,000 kg/yr of food-grade rHLZ from transgenic rice, demonstrating how process intensification can improve economic and technical performance while maintaining product quality and throughput. By comparing a baseline batch process with pool-less, high-capacity resin, semi-continuous, and combined intensification models, the analysis shows that changes in pooling strategy, resin selection, and operating mode can significantly reshape CAPEX, OPEX, CoGs, volumetric productivity, and equipment footprint without altering the underlying unit operations. Relative to the batch baseline model, all intensified configurations improved economic performance, with the largest improvement observed for the combined intensification model, which also achieved among the highest volumetric productivity and the most compact equipment layout.

At the same time, the results underscore that intensification introduces tradeoffs in labor demands, automation requirements, and validation complexity. Designs that eliminate hold tanks or employ high-capacity resin and semi-continuous operation rely more heavily on precise scheduling, robust CIP strategies, and tighter process control, which may necessitate additional

investment in instrumentation and automation infrastructure. The combined intensification configuration represents the strongest candidate for cost-effective, scalable rHLZ production, offering lower CoGs, reduced capital needs, and higher productivity compared to conventional batch operations. More broadly, the pathway from batch to pool-less, to high-capacity resin, to semi-continuous, and finally to combined intensification provides a flexible roadmap by which plant-based biomanufacturing platforms can incrementally transition toward more efficient, intensified downstream processing to meet future demand for allergen-free lysozyme and related biologic products.

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Appendix

Equations

$$eq.a)\quad 76.5x10^{6} * (2.5\%) = 1.91x10^{6}\ children\ with\ EWA$$

$$eq.b)\quad 30\ \frac{g\ cheese}{day * child}\left(14\frac{kg\ milk}{kg\ cheese}\right)\left(\frac{1\ kg}{1000\ g}\right)\left(\frac{365\ days}{1\ year}\right) = 153.3\frac{kg\ milk}{year * child}$$

$$eq.c)\quad \frac{\frac{25\ g\ LZ}{1000\ L\ milk}\left(\frac{1\ kg}{1000\ g}\right)}{1.0315\ \frac{kg}{L}} = 2.42x10^{-5}\frac{kg\ HEWL}{kg\ milk}$$

$$eq.d)\quad 1.91x10^{6}\ child\ (2.42x10^{-5}\ \frac{kg\ HEWL}{kg\ milk})\left(153.3\frac{kg\ milk}{year * child}\right) = 7.11x10^{3}\frac{kg\ HEWL}{year}$$

$$eq.e)\quad 7.11x10^{3}\ \frac{kg\ HEWL}{year}\left(20{,}000\ \frac{AU}{mg}\right)\left(\frac{1000\ mg}{1\ g}\right)\left(\frac{1000\ g}{1\ kg}\right) = 1.42x10^{14}\ \frac{AU}{year}$$

$$eq.f)\quad \frac{1.42x10^{14}\ \frac{AU}{year}}{150{,}000\ \frac{AU}{mg}(\frac{1000\ mg}{1\ g})(\frac{1000\ g}{1\ kg})} = 947.44\ \frac{kg}{year}$$

$$eq.g)\quad \$378\left(\frac{150{,}000\ \frac{AU}{mg}}{20{,}000\ \frac{AU}{mg}}\right) = \$2{,}835\ /kg$$