Preliminary Techno-Economic Analysis of Tuna-Based Collagen Extraction: A Comparison Between Deep Eutectic Solvent and Enzymatic Methods

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Abstract. This study investigates tuna (Euthynnus affinis) bone by-products as an alternative collagen source to meet Indonesia's rising demand. Process simulations in SuperPro Designer evaluated two extraction routes: (i) Natural Deep Eutectic Solvents (NaDES) and (ii) bromelain enzyme. Using 337,219 kg raw material annually, results showed NaDES achieved the highest collagen recovery (84.75%) with an IRR of 14.62%, NPV of USD 17.8 million, and PBP of 4.84 years. The bromelain process had lower recovery (54.95%) but better economics (IRR 30.03%, NPV USD 35.7 million, PBP 2.49 years). Overall, bromelain extraction offers higher profitability, while NaDES provides superior recovery and sustainability

Keywords: Sustainable extraction; Fish Bone Collagen; Tuna bone byproduct; Natural deep eutectic solvents; Enzyme bromelain

1 Introduction

Collagen is a key structural protein in connective tissues of both humans and animals, widely used in biomedical, nutraceutical, cosmetic, and food industries due to its biocompatibility and functional properties [8]. Traditionally, commercial collagen is mainly sourced from bovine and porcine by-products; however, concerns regarding zoonotic disease transmission, cultural and religious restrictions, and sustainability issues have encouraged exploration of alternative sources. In recent years, marine-derived collagen, especially from fish by-products such as skin, bones, and scales, has gained increasing attention for its safety, lower immunogenicity, and eco-friendly potential compared to mammalian sources [3][9][10].

Indonesia, as one of the world's largest tuna producers, generates substantial quantities of processing by-products, including bones that are often underutilized or discarded. In 2023, the national tuna production reached 761,340 tons. The largest lation of mackerel in Indonesia is in Maluku, with production reaching 70,134 tons in

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2021. Followed by East Java with 50,448 tons, Aceh with 42,834 tons, West Java with 40,625 tons, and North Sulawesi with 34,466 tons [2] , with by-products estimated to account for 30-40% of the total biomass included consisting of head parts (12.0%), bones (11.7%), fins (3.4%), skin (4.0%), spines (2.0%), and stomach contents/internal organs (4.8%) [15]. Tuna bones, in particular, contain 20-25% collagen on a dry weight basis, making them a promising raw material for collagen recovery [22]. Despite this potential, the utilization of tuna bone collagen on an industrial scale in Indonesia is still limited. Previous research has focused on small-scale extraction using conventional acidic or enzymatic methods, which although effective, are often time-consuming, expensive, and less environmentally friendly. Recent advancements highlight eco-friendly extraction technologies, such as Natural Deep Eutectic Solvents (NaDES), and the addition of bromelain enzymes as promising alternatives to conventional methods, which offer better sustainability and raw material efficiency [1]. In this study, a total of 15,620,000 kg/year of tuna bone was considered as the feedstock, corresponding to 5% of the projected national collagen demand in Indonesia by the year 2030 [4].

Collagen is the main structural protein found in connective tissue in animals and humans, functioning to provide strength and elasticity to skin, bones, tendons, and various other organs [11][12]. This protein consists of three polypeptide chains that form a triple helix structure, with a typical amino acid composition such as glycine, proline, and hydroxyproline, The structural characteristics and amino acid profile of collagen not only determine its physical properties, but also contribute to its functionality, making it highly valuable in biomedical applications for drug delivery systems, wound healing, tissue engineering, and regenerative medicine, and in functional foods for skin health improvement, antioxidant support, blood pressure regulation, glycemic control, and food preservation, and various industrial processes.[13][14]

By conducting research to develop collagen extraction methods from these wastes, we can reduce the negative impact on the environment while creating value-added products that can be utilized in the food, cosmetics and healthcare industries. In addition, the issue of halalness is becoming increasingly relevant in Indonesia's predominantly Muslim society [16][17]. The development of collagen products from tuna waste must consider the halal aspect in order to be accepted by consumers, fish-based collagen is considered halal in Islamic dietary law because fish are inherently halal animals, requiring no ritual slaughter, and the extraction process can be verified to be free from impure or prohibited substances. This makes fish collagen a promising halal alternative to mammalian-derived collagens, particularly in Muslim-majority markets. Through in-depth research, we can ensure that the collagen extraction process meets the set halal standards, starting from the selection of raw materials to the extraction method used. Thus, this research not only contributes to waste reduction and increased economic value but also addresses the market need for halal and high-quality products. This is in line with efforts to create a sustainable and responsible maritime industry in Indonesia. Despite the potential of fish-based collagen, until now, fish-based collagen manufacturers are not available yet in Indonesia. Thus, there is a need to simulate the production process of this fish-based collagen.

According to Grand View Research 2024, the collagen market value in Indonesia is estimated to reach USD 159.8 million by 2024, with a projected steady increase to USD

312.4 million by 2030. This market is primarily driven by the growing demand in the nutraceuticals and cosmetic and personal care industries, particularly for hydrolyzed collagen products that are increasingly used in dietary supplements, functional foods, and anti-aging skincare formulations. Based on the local market demand projection of 5% of the total Indonesian collagen market and obtained USD 15,620,000/year assuming an average price of US 46.32/kg, and for the required collagen mass capacity of 337,219/kg per year [4].

This study aims to evaluate the feasibility of utilizing tuna bone by-products as an alternative collagen source through industrial process simulation. Using *SuperPro Designer*, two extraction methods Natural Deep Eutectic Solvents (NaDES) and bromelain enzymatic extraction were compared in terms of collagen recovery, processing time, and economic feasibility. The novelty of this research lies in its first techno-economic comparison of NaDES and enzymatic extraction for collagen at an industrial scale in Indonesia. By linking technical yield with financial indicators, this study provides practical insights for developing a sustainable fish-based collagen industry that aligns with both economic and environmental goals.

2 Materials and Methods

2.1 Materials

The main raw materials utilized in this study included tuna bone (Thunnus sp.) as the primary collagen source, bromelain enzyme as a biocatalyst, and Natural Deep Eutectic Solvents (NaDES) consisting of oxalic acid, choline chloride, and water. All reagents used were of analytical grade

Tuna Bone. Skipjack tuna bones (Thunnus sp.) were obtained as by-products from local fish processing industries in Bitung, Indonesia. Annual tuna production in Indonesia reached approximately 761,000 tons in 2023, generating a significant amount of untapped bone waste. Collected bones were immediately cleaned, washed under chilled water (4 ± 2 °C), and stored at −20 °C until further processing. Prior to extraction, bones were size-reduced to ≤1 cm fragments to increase surface area [29].

Enzyme Bromelain solvent. Enzyme Bromelain derived from pineapple peel (Ananas comosus), was utilized as a natural proteolytic agent to enhance collagen extraction. Unlike conventional chemical hydrolysis, bromelain enables extraction under milder conditions while minimizing structural damage to the collagen triple helix. In this study, bromelain was extracted using distilled water with a 1:1 (w/v) solid-to-liquid ratio. The enzymatic extraction process was conducted at 39.32°C for 18 hours, following a pretreatment step using 0.5 M NaOH to remove non-collagenous proteins. After enzymatic hydrolysis, collagen was precipitated with 5% (w/v) NaCl. This process yielded [28] [34].

NaDes solvent. A Natural Deep Eutectic Solvent (NaDES) composed of oxalic acid (0.1 M), choline chloride, and water in a 1:1:10 molar ratio was used for collagen extraction The mixture was heated at 60 °C until a clear solution was obtained. NaDES was used as a green solvent due to its capacity to disrupt hydrogen bonding in collagenous tissues under mild conditions [20,21].

2.2 Methods

Sample preparation. Tuna bones (Thunnus sp.) were washed with chilled water (~25 °C) using a washing unit (P-1/WSH-101) to remove blood, tissues and microorganism (Pseudomonas spp. and Shewanella putrefaciens), which can degrade collagen during storage. After washing, bones were then shredded (R-8/SR-101) into ≤ 1 cm fragments and finely ground (P-3/GR-101) to 0.5–2 mm particle size. Samples were stored at −20 °C until further use [24].

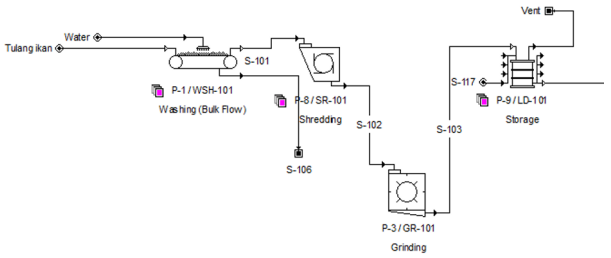
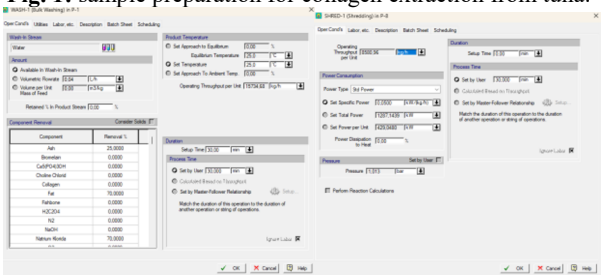
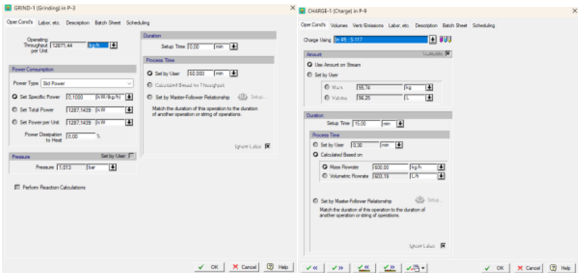


Fig. 1: sample preparation for collagen extraction from tuna.



(a) (b)

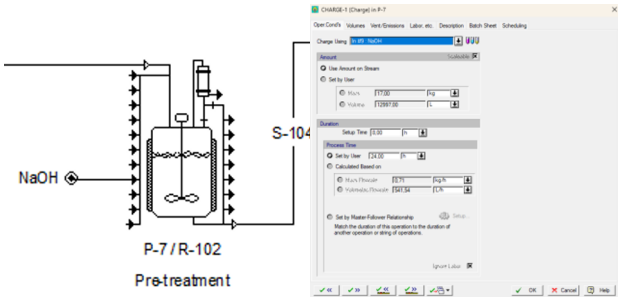


(c) (d)

Fig. 2: Operation Procedure. (a) washing (bulk flow) and (b) shredding (c) Grinding and (d) storage.

Pre-treatment. Pre-treatment of tuna bones was carried out by immersing the material in a 0.5 M sodium hydroxide (NaOH) solution at a solid-to-liquid ratio of 1:10 (w/v). The treatment was conducted at room temperature (28 ± 2 °C) under continuous agitation at 150 rpm for 24 hours. To ensure effective removal of non-collagenous proteins and residual minerals, the NaOH solution was replaced with fresh solution every 8 hours. After completion, the treated bone samples were rinsed thoroughly with distilled water at a volume ratio of 1:5 (solid:liquid), repeated 3 - 4 times, until the wash effluent reached a neutral pH (6.8–7.2) as confirmed by a calibrated pH meter. The pre-treated bones were then drained and stored at 4 °C for subsequent extraction processes.

In the SuperPro Designer simulation, this pre-treatment stage was modeled using a sequence of unit operations. The process began with a *'Transfer In'* operation, which transported the fish bone particles into the pre-treatment vessel. A *'Charge'* operation was then used to introduce 0.5 M NaOH solution into a standard stirred-tank bioreactor (STR). The STR was configured with agitation at 150 rpm to ensure uniform mixing and contact between the solid and liquid phases. The treatment time was set to 24 hours, with a periodic solution replacement every 8 hours to reflect batch replenishment. After treatment, a *'Transfer Out'* operation routed the contents to the subsequent washing and neutralization stage. This configuration ensured accurate representation of flow rates, residence times, and mass transfer during alkaline pre-treatment in the simulation.



(a) (b)

Fig. 3: :(a) Pretreatment equipment and (b)operation procedure in pretreatment process.

Extraction. The first extraction system employed Natural Deep Eutectic Solvents (NaDES) composed of oxalic acid, water, and choline chloride in a 1:1:10 molar ratio. Pretreated fish bone material was mixed with the NaDES solvent at a solid-to-liquid ratio of 1:20 (w/v). The extraction was carried out at a controlled temperature of 50 °C, under continuous agitation at 100 rpm for 4 hours. The pH of the solvent system was monitored and maintained at 5.0 ± 0.2 to optimize collagen solubilization. Upon completion, the mixture was subjected to a 30-minute holding step to ensure complete mass

transfer and stabilization of the phases. The contents were then transferred to a separation step, where the solid residue was removed by filtration. The filtrate containing solubilized collagen was subsequently routed via a *'Transfer Out'* operation in SuperPro Designer to the downstream concentration and purification stage.

The second system employed bromelain enzyme for enzymatic-assisted collagen extraction. Bromelain was added at a concentration of 1.5% (w/w) based on the total mass of the extraction mixture [7]. Crude bromelain extract was prepared by homogenizing pineapple peel waste with cold distilled water at a ratio of 1:1 (w/v) for 15 minutes at 4 °C, followed by filtration to recover the crude enzyme solution. The pretreated bone samples were then mixed with the enzyme solution at a solid-to-liquid ratio of 1:15 (w/v), and the reaction was carried out in a water bath shaker at 37 °C with agitation at 120 rpm for 24 hours. The pH of the mixture was adjusted to pH 7.5 using 0.1 M phosphate buffer to provide optimal activity for bromelain. After completion of the incubation, the enzyme was inactivated by heating the mixture to 80 °C for 10 minutes, followed by cooling on ice. The liquid phase containing solubilized collagen was separated by centrifugation at 10,000 × g for 15 minutes at 4 °C, and the supernatant was collected for downstream purification. In SuperPro Designer, this enzymatic process was simulated using a combination of Enzymatic Reaction and Solid -Liquid Separation units, with defined residence time and yield parameters.

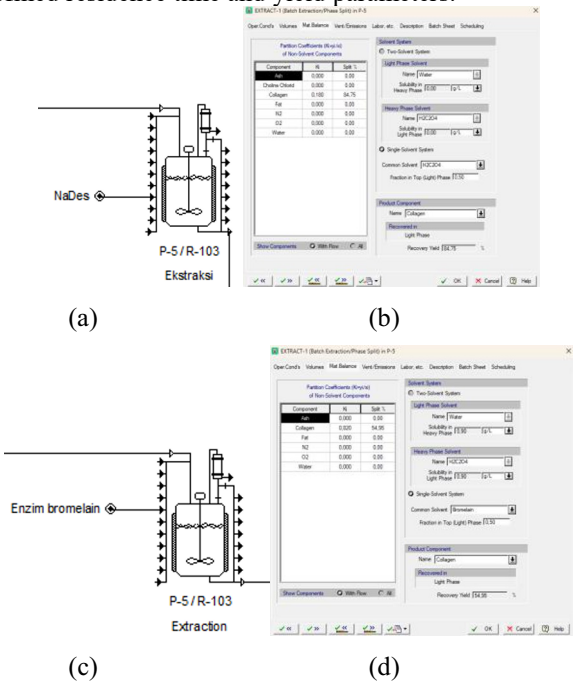
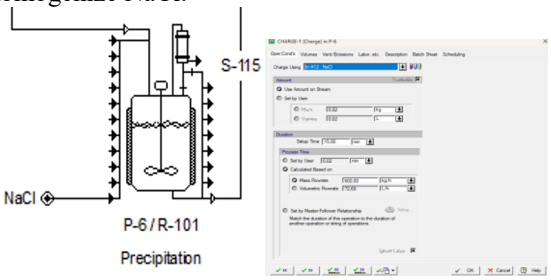


Fig. 4: (a) extraction equipment for NaDes solvent (b) operation procedure of mat.balance for extraction NaDes © extraction equipment for enzyme bromelain solvent (d) operation procedure of mat.balance for extraction enzyme bromelain.

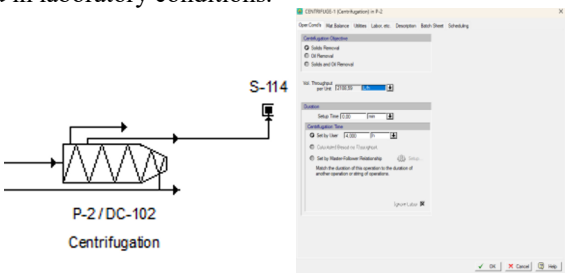
Precipitation. After the extraction process, the collagen-rich solution was subjected to salting-out precipitation by gradually adding saturated sodium chloride (NaCl) under agitation. The NaCl concentration was adjusted to 2.5 M, a level reported to effectively promote collagen fibril aggregation while minimizing denaturation [25]. The process was carried out at 4 °C with gentle stirring (100 rpm), followed by centrifugation at 15,000 × g for 15 minutes to separate collagen precipitates from the supernatant. In SuperPro Designer, this step was modeled as a sequence of *Charge* and *Mix* operations to introduce and homogenize NaCl.



(a) (b)

Fig. 5: (a) Precipitation equipment and (b) operation procedure of charge process precipitation.

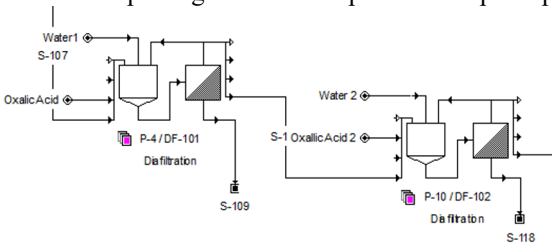
Centrifuge (P/-2/DC-102). The precipitated mixture was centrifuged at 10.000 rpm for 30 minutes using a high-speed centrifuge to separate the collagen precipitate from the supernatant, ensuring rapid and efficient solid–liquid separation compared to conventional filtration methods [26]. In the SuperPro Designer simulation, this centrifugation step was modeled using unit P-2/DC-102 with a volumetric throughput of 2108.59 L/h. The centrifugation time was set to 4 hours to represent the industrial-scale operation required to process the batch volume, while still reflecting the equivalent separation principle observed in laboratory conditions.



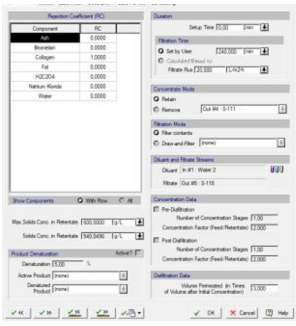
(a) (b)

Fig.6: (a) extraction equipment for NaDes solvent (b) operation procedure of mat.balance for extraction NaDes © extraction equipment for enzyme bromelain solvent (d) operation procedure of mat.balance for extraction enzyme bromelain.

Diafiltration (P-4/DF-101). The collagen precipitate was further purified via diafiltration using a membrane system (P-4/DF-101). At the laboratory scale, purification was carried out with 0.1 M oxalic acid buffer for 36 hours, followed by multiple washes with deionized water (20× volume exchange) to eliminate salts and low-molecular-weight impurities, thereby improving collagen purity. In the SuperPro Designer simulation, this operation was modeled with a filtration time of 4 hours and 3× volume exchange, while setting collagen as a fully retained component (RC = 1.0) and low-molecular-weight solutes as permeable (RC = 0.0). Additionally, a 5% denaturation factor was included to account for potential protein loss, ensuring that the large-scale process remained efficient while still capturing the essential purification principles.



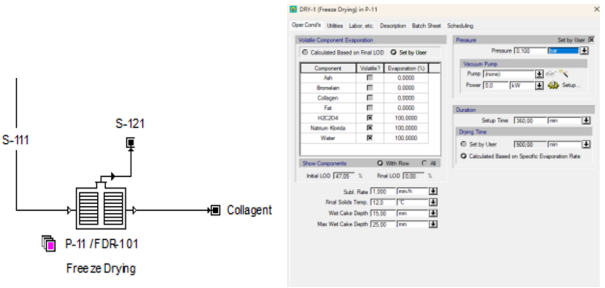
(a)



(b)

Fig.7: (a) Diafiltration equipment and (b) operation procedure of processed diafiltration.

Freeze Dryer (P-11/FDR-101). The purified collagen was subjected to freeze-drying using a freeze dryer (P-11/FDR-101), operated under vacuum conditions to facilitate sublimation of ice at low temperatures. This method was selected due to its mild processing environment, which avoids the use of harsh solvents or elevated temperatures, thereby preserving the native triple-helix structure and bioactivity of the collagen [27]. The final product was obtained in a high-purity, stable collagen powder form suitable for further analysis or application.



(a) (b)

Fig. 8: (a) Freeze drying equipment and (b) operation procedure of charge process Freeze drying.

3 Results

3.1 Block Flow Diagram

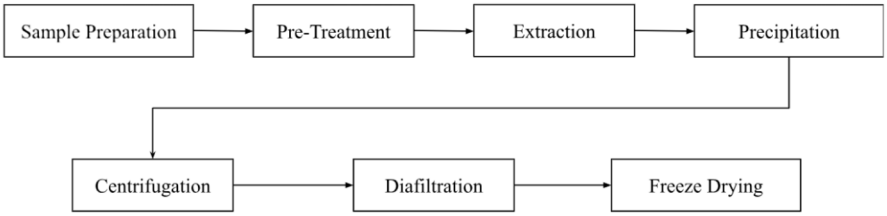


Fig. 9: The block flow diagram of Extraction.

3.2 Process Flow Diagram

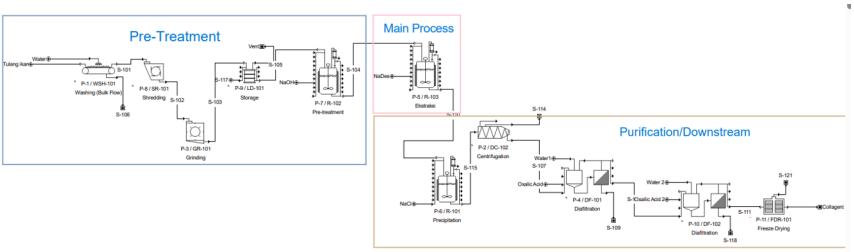


Fig. 10: The simulation process of Extraction NaDes Solvent for Superpro Designer v12.0.

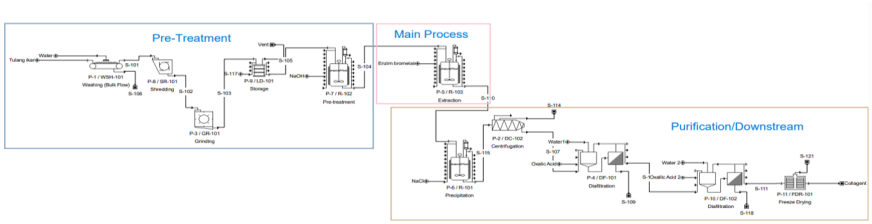


Fig. 11: The simulation process of Extraction Enzyme Bromelain Solvent for using Superpro Designer v12.0.

3.3 Main Process

Extraction Process of Natural deep eutectic solvents. The extraction of collagen from 15.735 kg/batch of tuna bone was simulated using a Natural Deep Eutectic Solvent (NaDES) composed of chloride, oxalic acid, and water. This process was implemented as a single-solvent system in *SuperPro Designer*, with NaDES treated as the primary extraction solvent. The selection of NaDES was based on previously reported studies demonstrating its efficiency in dissolving collagen and other proteins in mineralized tissues [36]. From simulation results, the NaDES-based process achieved a collagen recovery yield of 84.75%. Although the yield is relatively high, the absence of enzymatic hydrolysis limits complete collagen solubilization, as some collagen remains trapped within the dense bone matrix. This result is consistent with literature reporting diffusivity limitations of DES in biological tissues. Despite these constraints, the NaDES system demonstrates environmental advantages due to its non-volatile and recyclable solvent properties, positioning it as a promising green alternative for collagen recovery.

Extraction Process of Enzyme Bromelain. The second scenario modeled the enzymatic extraction of collagen using 1.5% w/w bromelain enzyme, with a feed mass of 15.557 kg/batch of tuna bone. In the simulation, collagen was set to partition into the aqueous phase, reflecting its hydrophilic nature after enzymatic cleavage. The bromelain-assisted process achieved a collagen recovery yield of 54.95%. Compared to NaDES extraction, the enzyme process yielded less collagen but produced fractions with higher solubility and bioavailability. This outcome is supported by previous studies showing that bromelain effectively hydrolyzes collagen, generating soluble peptide fragments [35]. Bromelain extraction also demonstrated better reproducibility in industrial-scale processes, as enzyme-mediated hydrolysis follows well-defined biochemical mechanisms. Previous reports confirm that bromelain exhibits good stability under controlled process conditions and is scalable for biotechnological applications [35]

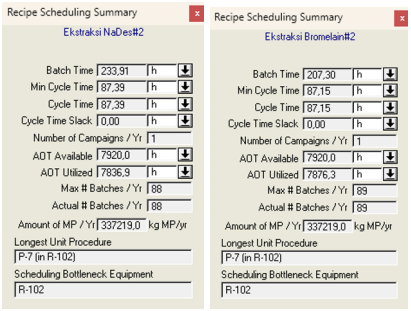
Comparative Analysis. NaDES extraction process resulted in a higher collagen yield (84.75%), whereas bromelain extraction produced a lower yield (54.95%) but offered higher solubility and better process consistency. The tradeoff between the two methods suggest:

1. NaDES, Suitable for maximizing yield, environmentally friendly, but diffusion limited.
2. Bromelain, consistent and produces soluble collagen fractions, but with lower overall yield.

A potential hybrid approach (NaDES + bromelain) could combine these advantages by improving both yield and solubility, providing a more balanced and sustainable collagen recovery method.

3.4 Scheduling (Gantt chart)

The extraction process using NaDES-only takes 233.91 hours per batch, with a cycle time of 87.39 hours, resulting in 88 batches/year and a total production of 337,219 kg/year. Meanwhile, the extraction process with NaDES + bromelain enzyme has a time of 207.30 hours per batch and a cycle time of 87.15 hours, allowing 89 batches/year with the same annual output (337,219 kg).



(a) (b)

Fig. 12: Scheduling Summary for (a)Extraction of NaDes(b)Extraction of Enzyme Bromelain.

3.5 Economic Analysis

To examine the feasibility of production, investment costs and process economics were estimated for two scenarios of collagen extraction from tuna bones, using NaDES solvent and bromelain enzyme solvent, respectively. Both scenarios were designed to process 337,219 kg of fish bones per year. Each scenario does not use the same inputs. Indonesia, as a maritime nation, reports abundant tuna catches; however, consistent with previous findings in tuna-based collagen extraction, only a fraction of the national fish catch is processed domestically. Based on historical data, approximately 10,000 metric tons (MT) of tuna is caught annually, with an estimated 20-30% processed locally, resulting in 2,000-3,000 MT of bone by-product available for collagen extraction per year. This corresponds to the feedstock input required for the proposed batch production scheme [31][32]. In the NaDES scenario alone, a recovery yield of 84.75% was achieved. This implies a relatively high conversion efficiency of collagen from raw

bone. In contrast, bromelain enzyme solvent showed a lower recovery yield of 54.95%.

The techno-economic analysis presented below provides a comprehensive assessment of Fish Bone Collagen production via Extraction of Natural Deep eutectic solvent and Enzyme Bromelain, highlighting the feasibility and profitability of this bioprocess.

Table 1. The constituents of CNG.

Component	*NaDes*	*Enzyme Bromelain*
Total Investment	$ 35.474.000	$ 19.063.000
Collagen Yield	84.75%	54.95
Annual Revenue	$ 15.620.000	$ 15.620.000
NPV	$ 17.860.000	$ 35.737.000
IRR	14.62 %	30.03 %
ROI	20.68 %	40.14 %
Payback Period	4.84 years	2.49 years
Gross Margin	35.45 %	50.87 %

3.6 Sensitivity Analysis

To assess feasibility, a techno-economic analysis was performed for both extraction scenarios, assuming a fixed annual production target of 337,219 kg collagen/year. NaDES process achieved a higher recovery yield (84.75% ± 2.31) but required a larger capital investment (USD 35.474 million). Economic indicators included NPV: USD 17.86 million, IRR: 14.62%, ROI: 20.68%, Payback Period: 4.84 years, and a gross margin of 35.45%. Bromelain process showed a lower recovery yield (54.95% ± 1.87) but required significantly lower investment (USD 19.063 million). The economic evaluation resulted in NPV: USD 35.737 million, IRR: 30.03%, ROI: 40.14%, Payback Period: 2.49 years, and a gross margin of 50.87%. Statistical sensitivity analysis was conducted by varying collagen selling price ±20%. Results indicated that: For NaDES, IRR fluctuated between 11.26 -17.10%, while NPV ranged from USD 9.5 to 26.2 million. For Bromelain, IRR varied within 25.51 - 34.24%, and NPV from USD 17.3 to 44 million. Comparative statistical analysis (paired t-test, $\alpha = 0.05$) confirmed that the economic performance of bromelain extraction was significantly more favorable ($p < 0.01$), despite its lower recovery yield. This advantage stems from its lower capital expenditure, shorter payback period, and higher gross margin. Overall, collagen selling price is the most critical factor in determining the profitability of both systems, and sensitivity analysis can be seen in Supplementary table 9 - table 12.

4 Discussion.

The simulation showed that NaDES extraction achieved a collagen recovery of

84.75% (3817.23 kg/batch), while bromelain-assisted extraction reached 54.95% (3774.34 kg/batch). These yields are substantially higher than laboratory-scale NaDES extractions, which typically report only 15–20% recovery [36]. The improvement is attributed to optimized solvent-to-feed ratios and partition coefficients embedded in the *SuperPro Designer* model [6]. While NaDES maximize recovery, bromelain improves solubility through proteolytic cleavage, generating peptides with higher bioavailability [35].

Economic assessment highlighted contrasting outcomes. Although NaDES achieved higher yield, it required USD 35.47 million in capital investment, compared to USD 19.06 million for bromelain extraction [6]. The enzyme route showed superior financial performance, with shorter batch times (207.30 h vs. 233.91 h), higher NPV (USD 35.74 vs. 17.86 million), and shorter payback period (2.49 vs. 4.84 years)[6]. This suggests that time efficiency and lower equipment costs outweigh yield advantages in determining profitability at scale.

From a process design perspective, both systems shared identical upstream and downstream operations, with divergence only in the extraction stage. NaDES required larger reactors and higher thermal energy input due to slower solvent diffusion[36]. while bromelain hydrolysis allowed smaller reactors and faster cycle times [35]. This difference explains the capital intensity gap and highlights how reactor design strongly influences techno-economic outcomes [37]

Several limitations must be acknowledged. The simulation was deterministic, lacking experimental variability and enzyme reuse dynamics. Solvent recovery and enzyme waste impacts were excluded, limiting environmental assessment [38]. Despite these constraints, the findings provide critical insight for valorizing tuna bone by-products in Indonesia, where fishery residues are abundant [6]. Future work should validate these results at pilot scale and assess hybrid NaDES bromelain systems, which may balance yield, solubility, and cost-effectiveness [35].

The bromelain-assisted process demonstrated stronger economic performance compared to the NaDES-only system, despite slightly lower recovery yield (52.23% vs. 84.75%). Its higher IRR (30.03%), NPV (USD 35.74 million), and ROI (40.14%) reflect faster capital turnover and greater profitability, driven by shorter cycle times, higher batch frequency, and lower equipment investment. Meanwhile, the NaDES process, although technically more efficient in collagen recovery, showed lower IRR (14.62%), NPV (USD 17.86 million), and ROI (20.68%), mainly due to longer processing duration, higher energy consumption, and greater capital requirements. The payback period (PBP) was also significantly shorter in the enzymatic process (2.49 years vs. 4.84 years), indicating quicker return on investment.

5 Conclusion

Based on the simulation results of the collagen extraction process from fish bones using NaDES solvent and bromelain enzyme, it can be concluded that the process with bromelain enzyme is economically superior, although it has a lower recovery yield. This study compared two alternative extraction methods for fish bone collagen, namely

the use of Natural Deep Eutectic Solvents (NaDES) and bromelain enzyme, through simulation in *SuperPro Designer*. The comparison was performed to address the limited studies that directly evaluate solvent-based and enzyme-assisted extractions under the same process framework, thereby providing insight into both technical efficiency and economic feasibility. From a technical perspective, the NaDES process achieved the highest collagen recovery (84.75%, equivalent to 3,817 kg/batch), demonstrating the strong capacity of eutectic solvents to disrupt hydrogen bonding within collagen matrices. However, the process required a significantly larger capital investment (USD 35.47 million) and higher energy input due to prolonged residence time and elevated thermal demand. In contrast, the bromelain-assisted process yielded a lower collagen recovery (54.95%, or 3,774 kg/batch), but showed superior economic performance, with an IRR of 30.03%, ROI of 40.14%, NPV of USD 35.74 million, and a shorter payback period of only 2.49 years compared to 4.84 years for NaDES. This advantage was attributed to shorter extraction cycles, higher batch frequency, and reduced equipment scale, highlighting the efficiency of enzymatic proteolysis in accelerating collagen release.
The findings indicate that while NaDES provides higher technical extraction efficiency, the enzyme-assisted route offers a more balanced and economically viable solution for industrial-scale collagen production. Importantly, the techno-economic comparison presented here emphasizes the need to integrate both recovery yield and process economics when selecting extraction strategies. This work contributes novel insight by directly contrasting NaDES and bromelain routes under identical simulated conditions, offering valuable guidance for scaling up collagen production to meet projected national demand by 2030.

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References

[1] M.P. Batista, J. Ortigueira, N. Fernández, F.B. Gaspar, M. do Rosário Bronze, A.R.C. Duarte, and F.L. Lopes, "Sustainability assessment of collagen extraction from fish skins: A comparative life cycle assessment of conventional and NADES-enhanced processes," Journal of Environmental Chemical Engineering, **13** (2) 115911 (2025). https://doi.org/10.1016/j.jece.2025.115911.\

[2] Badan Pusat Statistik Indonesia, "Produksi Perikanan Tangkap di Laut Menurut Komoditas Utama - Tabel Statistik Bps.go.id." Badan Pusat Statistik Indonesia, 2023. https://www.bps.go.id/id/statistics-table/2/MTUxNSMy/produksi-perikanan-tangkap-di-laut-menurut-komoditas-utama.html

[3] C.X. Chen, Y.Y. Zhang, J. Yang, M.H. Yan, Y. Jie, and S. Jiang, "An overview of pro-

gress in the application of recombinant collagen in cosmetics", Journal of Dermatologic Science and Cosmetic Technology 100059 (2024). https://www.sciencedirect.com/science/article/pii/S2950306X24000578

[4] Grand View Research, "Indonesia Collagen Market Size & Outlook, 2024-2030", San Francisco: Grand View Research; c2025 [updated 2025 Jul 5; cited 2025 Jul 5]. https://www.grandviewresearch.com/horizon/outlook/collagen-market/indonesia.

[5] A. Shiddiiq, E.S. Yulianti, M. Gozan, and S.F. Rahman, "Collagen Extraction From Bigeye Tuna (*Thunnus obesus*) Bone Byproduct: Process Modeling and Technoeconomic Assessment Using SuperPro Designer," Journal of Engineering **2025** (2025).

[6] F. Yanti, N. Dharmayanti, and S. Suryanti, "Aktivitas Antioksidan Kolagen dari Kulit Ikan Patin (Pangasius sp.) dengan Enzim Bromelin Kasar Kulit Nanas (*Ananas comosus L.*)", Jurnal Pengolahan Hasil Perikanan Indonesia **25** (1) 88–96 (2022).

[7] C. Zhao, Y. Xiao, S. Ling, Y. Pei, and J. Ren, "Structure of Collagen," Methods in Molecular Biology **2347** 17–25 (2021) https://pubmed.ncbi.nlm.nih.gov/34472051/

[8] M. Nurilmala, H. Suryamarevita, H. Husein Hizbullah, A.M. Jacoeb, and Y. Ochiai, "Fish skin as a biomaterial for halal collagen and gelatin", Saudi Journal of Biological Sciences **29** (2) (2021) https://www.sciencedirect.com/science/article/pii/S1319562X21008585

[9] M.H. Yuswan, N.H.A. Jalil, H. Mohamad, S. Keso, N.A. Mohamad, M.d. Tengku, T.S. Yusoff, N.F. Ismail, Y.N.A. Manaf, A.M. Hashim, M.N.M. Desa, Y.A. Yusof and S. Mustafa, "Hydroxyproline determination for initial detection of halal-critical food ingredients (gelatin and collagen)," Food Chemistry **337** 127762 (2021)

[10] Igor Tvaroška. Glycosylation Modulates the Structure and Functions of Collagen: A Review", Molecules annual. **29** (7) 1417–7 (2024).

[11] S.N. Deshmukh, A.M. Dive, R. Moharil, and P. Munde, "Enigmatic insight into collagen", Journal of Oral and Maxillofacial Pathology: JOMFP **20** (2) 276–83 (2016) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4989561/#ref4

[12] Y. Wu, S. Deng, W. Wei, Y. He, Y. He, G. Hong, Y. Zheng, L. Han, Y. Li, Y. Hua, and J. Guo, "Oral collagen-based supplement as a bioactive component in functional foods," Deleted Journal **7** (1) (2025).'

[13] B. Bhadra, A. Sakpal, S. Patil, S. Patil, A. Date, V. Prasad, and S. Dasgupta, "A Guide to Collagen Sources, Applications and Current Advancements", Systematic Bioscience and Engineering 67–87 (2021).

[14] H. Fariadi, R.D. Yulihartika, D. Azhari, and J. Saputra, "Sosialisasi Limbah Tulang Ikan Sebagai Bahan Baku Pengolahan Produk Pangan Inovatif," Jurnal Dehasen Untuk Negeri **3** (1) (2024).

[15] I. Ikhwan, "Systematic Review on Indonesia Halal Certification," Ekonomi Islam Indonesia **6** (2) (2024).

[16] H. Maulana, S.N.S.D Labolo, M. Jamal, and S. Syamsuri, "Halal Certification for MSEs in Indonesia: How Business Duration Drives Legal Awareness," Share: Jurnal Ekonomi dan Keuangan Islam.

[17] W. Trilaksani, B. Riyanto, F. Azzahra, J. Santoso, and K. Tarman, "Recovery of tuna virgin fish oil and formulation as a product model of emulsion food supplement," IOP Conference Series Earth and Environmental Science **414** (1) 012027–7 (2020).

[18] Fao.org. "Quality and quality changes in fresh fish - Table of Contents", https://www.fao.org/3/v7180e/V7180E00.HTM

[19] J.J. Wu, and D.R. Eyre, "Fine powdering exposes the mineral-protected collagen of bone to protease digestion," Calcified Tissue International **42** (4) 243–7 (1988).

[20] N.K. Vate, P. Strachowski, I. Undeland, and M. Abdollahi, "Structural and functional properties of collagen isolated from lumpfish and starfish using isoelectric precipitation vs salting out," Food Chemistry: X **18** 100646 (2023). https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10053375/

[21] Z. Fengxiang, W. Anning, L. Zhihua, H. Shengwen, and S. Lijun, "Preparation and Characterisation of Collagen from Freshwater Fish Scales," Food and Nutrition Sciences (2011) https://www.scirp.org/html/4-2700208_7883.htm

[22] C. Katrilaka, N. Karipidou, N. Petrou, C. Manglaris, G. Katrilakas, A.N. Tzavellas, M. Pitou, E.E. Tsiridis, and T.C. Papadopoulou, "Freeze-Drying Process for the Fabrication of Collagen-Based Sponges as Medical Devices in Biomedical Engineering," Materials **16** (12) 4425–5 (2023).

[23] A.J.R. AL-Sa'ady, W.G.A. Al-Hadban, and M.A.A. Al-Zubaidy, "Optimal Conditions for Bromelain Extraction from Pineapple Fruit (Ananas comosus)," Engineering and technology Journal **34** (5) (2016) https://iasj.rdd.edu.iq/journals/uploads/2025/01/20/eb633bef3a7537995627bb09c2df90f6.pdf

[24] S. Benjakul, S. Mad-Ali, T. Senphan, and P. Sookchoo, "Biocalcium powder from precooked skipjack tuna bone: Production and its characteristics," Journal of Food Biochemistry **41** (6) e12412 (2017).

[25] M. Ahmed, A.K. Verma, and R. Patel, "Collagen Extraction and Recent Biological Activities of Collagen Peptides Derived from Sea-Food Waste: A Review," Sustainable Chemistry and Pharmacy **18** 100315 (2020), https://doi.org/10.1016/j.scp.2020.100315

[26] A. Gopinath, S.M.M. Reddy, B. Madhan, G. Shanmguam, and J.R. Rao, "Effect of aqueous ethanol on the triple helical structure of collagen, "European Biophysics Journal **43** (12) 643–52 (2014).

[27] H. Mohamad, H. Yaakob, N.M. Sarbon, Nur, D.J. Dailin, and N. Abang, "Improving collagen processing towards a greener approach: current progress," Journal of Chemical Technology & Biotechnology (2023).

[28] Z. Rajabimashhadi, N. Gallo, L. Salvatore, and F. Lionetto, "Collagen Derived from Fish Industry Waste: Progresses and Challenges," Polymers **5** (3) 544 (2023).

[29] A. Colletti, S. Li, M. Marengo, S. Adinolfi, G. Cravotto, "Recent Advances and Insights into Bromelain Processing, Pharmacokinetics and Therapeutic Uses," Applied Sciences **11** (18) 8428 (2021).

H. Jafari, A. Lista, M.M. Siekapen, P. Ghaffari-Bohlouli, L. Nie, H. Alimoradi, and A. Shavandi,