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Sustainable bromelain extraction from pineapple waste: ATPS purification, freeze-drying, and cosmetic safety evaluation

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ABSTRACT

Background: Pineapple processing generates substantial waste; peels represent a rich source of bromelain, a proteolytic enzyme with dermatological applications.

Objective: To develop sustainable bromelain extraction from pineapple peels using aqueous two-phase system (ATPS), evaluate freeze-dried extract, and assess safety for topical cosmetic use.

Methods: Bromelain was extracted using PEG-4000/$MgSO_4$ ATPS and freeze-dried. Proteolytic activity was quantified by tyrosine release and gelatin digestion assays. Purified bromelain was incorporated into gel cleanser and serum formulations (2-5%, pH 4.5-7.0) and evaluated for physicochemical properties, microbial safety, and skin compatibility through human repeat insult patch testing (HRIPT).

Results: The 10% PEG-4000/30% $MgSO_4$ system achieved optimal balance (37.64% yield, 2.80 U/mL activity). Freeze-drying produced free-flowing powder. Formulations met ASEAN microbial limits (<1,000 cfu/g) and demonstrated non-irritating properties with low hypersensitivity risk in HRIPT.

Conclusion: ATPS extraction combined with freeze-drying produces stable, bioactive bromelain suitable for safe topical applications, demonstrating successful valorization of agricultural waste into high-value cosmetic ingredients.

Keywords: aqueous two-phase system, bromelain, freeze-drying, pineapple waste valorization, topical cosmetics

Introduction

Agricultural byproducts represent critical resources in the circular economy framework, yet most are managed through basic strategies such as composting, animal feed production, and biogas generation [1]. The environmental and economic costs of handling these residues necessitate value-added approaches that reintroduce agri-food byproducts into production cycles as functional ingredients with substantial health benefits and market value [2,3].

Pineapple (*Ananas comosus*), the third most produced tropical fruit globally after banana and citrus [4], generates significant processing waste. The Philippines, as the world's second-largest pineapple exporter with approximately 680,000 metric tons exported in 2024 [5], faces substantial waste management challenges. Only 20% of the pineapple plant is utilized as product, while the remaining 80%—consisting of crown, peel, leaves, core, and stems—is discarded during processing [6]. High disposal costs and greenhouse gas emissions from these wastes make valorization strategies increasingly urgent [7].

Pineapple peels are rich in bioactive compounds, particularly bromelain, a mixture of proteolytic enzymes with thiol-containing active sites [8]. Bromelain has attracted considerable interest across pharmaceutical, culinary, and cosmetic industries due to its proteolytic activity and associated health benefits [9]. In skincare applications, bromelain functions as a gentle enzymatic exfoliant, selectively hydrolyzing keratinized proteins in the stratum corneum to remove dead skin cells without the mechanical irritation of physical exfoliants or pH-dependent keratolysis of chemical alternatives [10]. This natural mechanism, combined with anti-inflammatory and anti-aging properties, positions bromelain as a valuable ingredient for dermocosmetic formulations.

Despite growing demand, bromelain production faces challenges including complex extraction procedures, multiple purification steps, and often low yields [11]. Aqueous two-phase system (ATPS) extraction has emerged as a promising solution for primary protein recovery operations. ATPS employs environmentally compatible, water-soluble polymers (such as polyethylene glycol) and salts that create two immiscible aqueous phases, enabling selective biomolecule partitioning based on hydrophobicity, molecular weight, and surface charge [12,13]. This technique offers advantages over conventional methods: mild non-denaturing conditions, low cost, scalability potential, and effective removal of interfering compounds such as polysaccharides, pigments, and polyphenols that reduce enzyme activity [13,14]. Previous studies demonstrated successful bromelain recovery from pineapple peels using PEG-based ATPS with good yield and reasonable purity [13,15].

However, extracted bromelain requires stabilization for commercial applications. Lyophilization (freeze-drying) represents the preferred encapsulation technique for thermolabile bioactive compounds, operating through sublimation at low temperatures that preserve structural and functional integrity [16,17]. Freeze-drying avoids the high temperatures of spray-drying and other thermal processes, minimizing denaturation and activity loss—particularly critical for enzyme preservation [18,19].

While several studies have addressed bromelain extraction or cosmetic enzyme applications separately, an integrated approach combining sustainable extraction, and comprehensive safety evaluation for topical use remains underexplored. This study addresses this gap by: (1) optimizing ATPS-based bromelain extraction from pineapple peel waste using PEG-4000/$MgSO_4$ systems; (2) evaluating freeze-drying for powder stabilization; (3) incorporating purified bromelain into cosmetic formulations (gel cleanser and serum); and (4) conducting comprehensive safety assessment including heavy metal analysis, microbial profiling, and human repeat insult patch testing (HRIPT). By repurposing industrial pineapple peels as a sustainable bromelain source, this research demonstrates the valorization potential of agricultural byproducts while contributing to the development of naturally derived cosmetic ingredients.

Methods

Collection of raw materials

Pineapple (*Ananas comosus*) peels were obtained from Hi-Las Marketing Corporation (Taguig City, Metro Manila, Philippines), a Halal-certified fruit processing facility producing fresh-cut pineapple products. The facility processes only pineapple flesh, leaving peels as unutilized byproducts. After mechanical peeling, the peels were separated from other waste fractions and immediately stored at -20°C until further use to preserve enzymatic activity and prevent microbial degradation. Figure 1 illustrates the complete process flow diagram.

Analysis of trace contaminants in raw materials

To ensure safety and compliance with food-grade processing standards, pineapple peel samples were analyzed for heavy metal contamination at the Chemistry Laboratory, Standards and Testing Division, Industrial Technology Development Institute (DOST-ITDI, Taguig City, Philippines), an ISO/IEC 17025-accredited testing laboratory. Heavy metal testing is essential as agricultural products can accumulate toxic elements (lead, cadmium, arsenic, mercury) from contaminated soil, water, or fertilizers, posing significant health

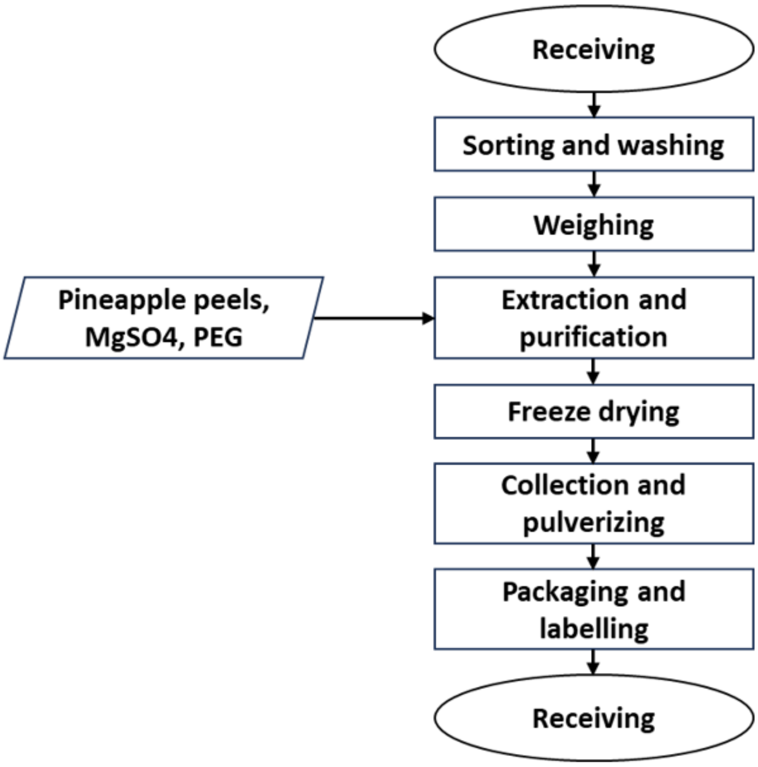

Figure 1. Process flow diagram for the collection, extraction, and freeze-dryingof bromelain from pineapple peels. The workflow shows sequential steps from raw material procurement through juice extraction, ATPS-based partial purification, freeze-drying, and final powder production.

risks including neurotoxicity, nephrotoxicity, and carcinogenic effects [20].

Lead (Pb) and cadmium (Cd) were determined using a Shimadzu AA-6800 Flame Atomic Absorption Spectrophotometer (FAAS) following AOAC Official Method 986.15 (Modified). Arsenic (As) was analyzed using a Shimadzu Hydride Vapour Graphite (HVG) system coupled to a Shimadzu AA-6800 Atomic Absorption Spectrophotometer (AAS), also following AOAC 986.15 (Modified). Mercury (Hg) was quantified using a Milestone DMA 80 Direct Mercury Analyzer according to US EPA Method 7473. Results were compared against WHO/FAO acceptable levels for heavy metals in fruits and ASEAN Guidelines on Limits of Contaminants for Cosmetics (version 2.0).

Extraction and partial purification of bromelain

Frozen pineapple peels were thawed, cut into 1-2 cm pieces, and juiced using a tabletop electric juicer (Kyowa KW-4210). The juice was filtered through cheesecloth and stainless-steel strainer, then centrifuged at 10,000 × g for 20 min at 4°C using a DYNAMICA Velocity 18R Pro centrifuge. The supernatant was collected as crude bromelain extract.

Partial purification was performed using aqueous two-phase system (ATPS) with polyethylene glycol 4000 (PEG-4000) and magnesium sulfate ($MgSO_4$). Four formulations were evaluated: (a) 5% PEG-4000, 30% $MgSO_4$, 15% distilled water; (b) 10% PEG-4000, 40% $MgSO_4$; (c) 10% PEG-4000, 30% $MgSO_4$, 10% distilled water; and (d) 20% PEG-4000, 30% $MgSO_4$. All formulations contained 50% (w/w) crude extract.

Reagents were added sequentially (PEG-4000, $MgSO_4$, distilled water when applicable) and homogenized at 1200 rpm for 2 min after each addition using a multi-mixer (Golden Bat, Far East, Inc.), minimizing bubble formation. After final addition, mixtures were homogenized for 10 min to ensure complete dissolution and phase equilibration. The homogenized mixture was transferred to a separatory funnel and refrigerated at 8°C for 20 h to facilitate complete phase separation. The upper polymer-rich phase containing bromelain was collected after the lower salt-rich phase was discarded.

Determination of proteolytic activity

Proteolytic activity was quantified using a modified Murachi method [21] with casein as substrate and L-tyrosine as standard. A 0.5% (w/v) casein solution in 0.05 M phosphate buffer (pH 7.0) was prepared. Ten milliliters of substrate solution was combined with 1.0 mL activating buffer (0.03 M cysteine and 0.006 M tetrasodium EDTA in 0.05 M phosphate buffer, pH 7.0) and pre-incubated at 37°C for 10 min.

The enzymatic reaction was initiated by adding 0.5 mL of crude or partially purified extract, followed by incubation at 37°C for 15 min. The reaction was terminated with 15.0 mL of 5% (w/v) trichloroacetic acid (TCA) [22], allowed to stand for 20 min, then centrifuged at 10,000 × g for 10 min at 4°C. Blank samples were prepared identically except TCA was added before enzyme addition.

Soluble peptide concentration in supernatants was measured spectrophotometrically at 280 nm using a Hitachi U-2900 UV-Vis Spectrophotometer (1-cm pathlength quartz cuvette). Proteolytic activity was expressed as units (U), defined as the amount of enzyme liberating 1 μg tyrosine equivalents per milliliter per minute under assay conditions. A calibration curve was prepared using L-tyrosine standards (25.0-100.0 μg/mL) following Enzyme Development Corporation protocol [23]. All measurements were performed in duplicate (N=2).

Freeze-drying and encapsulation

Partially purified bromelain was lyophilized to enhance stability and enable long-term storage. Bromelain was homogenized to ensure uniform dispersion, frozen at -20°C for at least 24 h, then lyophilized using a Büchi Lyovapor L-200 freeze dryer (chamber pressure: 0.1 mbar; shelf temperature: -50°C). Sublimation continued until complete water removal, indicated by stable pressure readings and constant sample weight. Freeze-dried powders were collected, weighed to calculate yield, and stored in airtight containers under desiccated conditions at room temperature.

Gelatin digestion unit (GDU) analysis

To validate enzymatic activity under standardized conditions, GDU analysis was performed on two bromelain preparations: (1) freeze-dried bromelain purified using 10% PEG-4000/30% $MgSO_4$, and (2) freeze-dried bromelain purified using 5% PEG-4000/30% $MgSO_4$. GDU quantifies the amount of enzyme required to digest standardized gelatin substrate under defined conditions [10]. Analysis was conducted at the Chemistry Laboratory, Dole Philippines, Inc. (Polomolok, Southern Cotabato, Philippines), a pineapple processing company with in-house enzyme activity characterization facility.

Formulation development and characterization

Extracted, partially purified, and freeze-dried bromelain was incorporated into two cosmetic formulations: a gel cleanser and a serum. Formulations were developed at the Halal Cosmetics Laboratory, Standards and Testing Division (DOST-ITDI). Bromelain was incorporated at 2-5% (w/w) with pH adjusted to 4.5-7.0 to maintain enzyme stability and activity while ensuring skin compatibility.

Physicochemical characterization: Solubility, color, odor, pH, cleansing properties, lathering ability, and foam height were evaluated. Testing was performed at the Standards and Testing Division laboratories (DOST-ITDI), an ISO/IEC 17025-accredited facility. Results were compared against reference values from commercial products.

Microbial profiling: Aerobic plate count, molds and yeast count, and *Staphylococcus aureus* detection were performed at the Microbiology Section, DOST-ITDI, following ASEAN Guidelines on Limits of Contaminants for Cosmetics (version 2.0).

Table 1. Results of heavy metals in raw pineapple peels

Heavy Metals (mg/kg)	WHO/FAO Limits on Heavy Metals[a]	ASEAN Limits on Heavy Metals[b]	Pineapple Peels
Lead (Pb)	0.3	NMT 1 mg/kg	Not Detected*
Arsenic (As)	0.5	NMT 20 mg/kg	0.0426 mg/kg
Cadmium (Cd)	0.2	NMT 5 mg/kg	Not Detected**
Mercury (Hg)	0.03	NMT 5 mg/kg	0.000706 mg/kg

[a]WHO/FAO Acceptable Levels for Heavy Metals in Fruits (based on maximum tolerable daily intake); [b]ASEAN Guidelines on Limits of Contaminants for Cosmetics, ver 2.0; *Detection Limit, Pb = 0.051 mg/L; **Detection Limit, Cd = 0.016 mg/L; NMT = Not More Than.

Human repeat insult patch testing (HRIPT): Safety evaluation was conducted at VMV Skin Research Center + Clinics (Legazpi Village, Makati, Philippines), a third-party dermatological testing facility. HRIPT assessed potential for skin irritation and allergic contact dermatitis among normal adult subjects following established protocols [24]. Testing evaluated reactions to 109 top allergens referenced by the North American Contact Dermatitis Group and European Surveillance System on Contact Allergies. The study protocol included an Induction Phase (9 visits over 2 weeks), a 14-day Rest Period, and a Challenge Phase (3 visits), with continuous dermatological evaluation throughout.

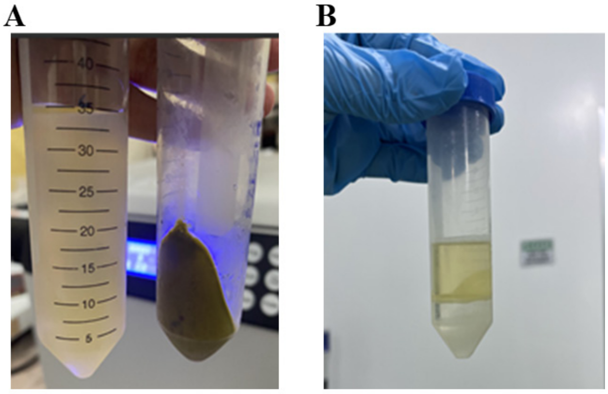

Figure 2. Phase separation in ATPS. (A) Homogenized mixture of PEG-4000, $MgSO_4$, and crude bromelain extract. (B) Clear phase separation after 20 h equilibration at 8°C, showing upper PEG-rich phase containing bromelain and lower salt-rich phase containing contaminants.

Results

Heavy metal analysis of pineapple peels

Heavy metal analysis confirmed that pineapple peels met safety standards for both food and cosmetic applications (Table 1). Lead and cadmium were not detected (detection limits: 0.051 mg/L and 0.016 mg/L, respectively). Arsenic (0.0426 mg/kg) and mercury (0.000706 mg/kg) were well below WHO/FAO acceptable levels and ASEAN cosmetic limits, confirming the raw material is safe for bromelain extraction and topical applications.

Optimization of ATPS extraction

Bromelain was successfully extracted and partially purified from pineapple peels using ATPS with PEG-4000 and $MgSO_4$. Visual inspection confirmed effective phase separation, with bromelain partitioning into the upper PEG-rich phase while contaminants (polysaccharides, pigments) remained in the lower salt-rich phase (Figure 2).

Table 2 presents the comparative performance of four ATPS formulations. The 10% PEG-4000/30% $MgSO_4$ system achieved optimal balance with 37.64% yield and 2.80-2.82 U/mL activity at moderate production cost (49.2 PhP/100g, approximately 0.86 USD/100g). Although the 5% PEG-4000/30% $MgSO_4$ formulation exhibited highest specific activity (3.78-3.85 U/mL), its low yield (16.78%) limits scalability. Conversely, the 20% PEG-4000/30% $MgSO_4$ system produced highest yield (78.74%) but showed activity equivalent to crude extract (2.30-2.32 U/mL), indicating insufficient purification.

All ATPS formulations showed good reproducibility between duplicates, with relative standard deviation below 5% for enzymatic activity measurements.

Table 2. Proteolytic activity and recovery efficiency of different ATPS formulations

Sample (N = 2)	Tyrosine released (μg/mL)	Enzyme activity (U)	Yield (wt. %)	Average product cost (PhP/100g)[a]	Performance
Crude extract	122.68	2.30			Baseline
5% PEG 30% $MgSO_4$ (T1)	201.24	3.78	16.78%	34.2 PhP	Highest activity, lower yield, lowest cost
5% PEG 30% $MgSO_4$ (T2)	204.96	3.85			
10% PEG, 40% $MgSO_4$ (T1)	174.44	3.27	13.18%	55.6 PhP	Higher activity, lowest yield, low cost
10% PEG, 40% $MgSO_4$ (T2)	184.61	3.46			
10% PEG, 30% $MgSO_4$ (T1)	149.13	2.80	37.64%	49.2 PhP	High activity, higher yield, lower cost
10% PEG, 30% $MgSO_4$ (T2)	150.33	2.82			
20% PEG, 30% $MgSO_4$ (T1)	122.46	2.30	78.74%	79.2 PhP	Low activity, highest yield high cost
20% PEG, 30% $MgSO_4$ (T2)	123.39	2.32			

[a] Production cost based on reagent consumption; 1 USD ≈ 57 PhP (February 2026 exchange rate).

Table 3. Protease activity of freeze-dried bromelain compared to liquid extract

Sample (10% PEG 4000, 30% $MgSO_4$)	Tyrosine released (μg/mL)	Enzyme Activity
Partially purified bromelain	149.73	2.81 U/mL
Freeze-dried Bromelain	82.02	3.07 U/g

Based on the balanced performance considering activity, yield, and cost-effectiveness, the 10% PEG-4000/30% $MgSO_4$ formulation was selected for subsequent freeze-drying experiments.

Freeze-drying of purified bromelain

The partially purified bromelain (10% PEG-4000/30% $MgSO_4$) was successfully freeze-dried, yielding a porous powder (Figure 3). Comparison between liquid and freeze-dried forms revealed that lyophilization concentrated the enzyme, resulting in higher mass-specific activity (Table 3).

The reduced tyrosine release in freeze-dried samples (82.02 versus 149.73 μg/mL) reflects transient rehydration kinetics rather than permanent activity loss, as lyophilized proteins typically form microaggregates requiring time for complete dissolution. The higher mass-normalized activity (3.07 U/g versus 2.81 U/mL) confirms effective enzyme concentration through water removal, demonstrating that freeze-drying preserved catalytic functionality while enabling stable powder formulation.

Physical properties of freeze-dried bromelain

Freeze-dried bromelain exhibited a free-flowing, non-caking, and non-sticky powder form although slightly hygroscopic.

GDU analysis quantified functional proteolytic activity across different preparation methods

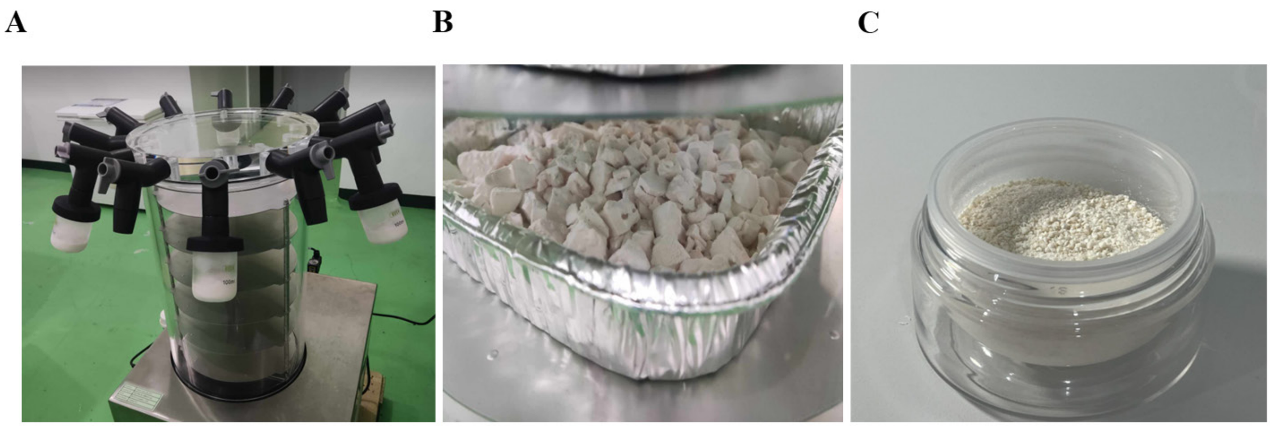

Figure 3. Freeze-drying process and product. (A) Büchi Lyovapor L-200 freeze dryer used for lyophilization. (B) Freeze-dried bromelain showing characteristic porous structure after sublimation. (C) Pulverized freeze-dried bromelain as fine powder.

Table 4. Gelatin Digestion Unit (GDU) Activity of Bromelain Preparations

No.	Sample	Bromelain activity (GDU/g)*
1	FDB1: Freeze-dried bromelain powder (10% PEG, 30% $MgSO_4$)	371.43
2	FDB2: Freeze-dried bromelain powder (5% PEG, 30% $MgSO_4$)	385.22

*GDU measurements were conducted at Dole Philippines, Inc.

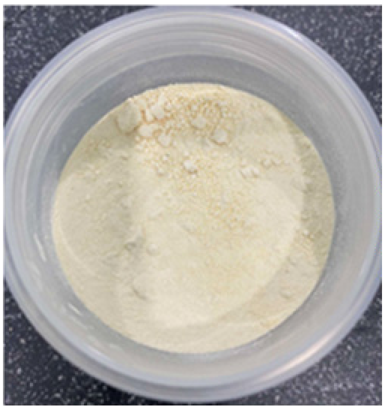

Figure 4. Visual presentation of bromelain powder

(Table 4). The 5% PEG-4000 formulation (FDB 2) showed higher activity (385.22 GDU/g) than the 10% PEG-4000 formulation (FDB 1: 371.43 GDU/g), consistent with tyrosine release assay results (Table 2).

Physicochemical properties of topical formulations

Freeze-dried bromelain was successfully incorporated into gel cleanser and serum formulations at 2-5% (w/w) concentration without compromising product stability or performance (Table 5). Bromelain demonstrated complete water solubility, indicating preserved hydrophilicity after lyophilization. Product color and odor remained consistent with formulation ingredients, showing no signs of enzymatic degradation or oxidation.

Both formulations maintained pH values suitable for enzyme stability and skin compatibility. The gel cleanser (pH 6.5-7.0) preserved effective cleansing performance (+++), moderate lathering (++), and acceptable foam height (15.5 cm), indicating bromelain incorporation did not interfere with surfactant functionality. The serum formulation (pH 4.5) provided optimal conditions for bromelain activity while remaining within the acceptable range for topical skin applications.

Microbial safety profile

Microbial analysis demonstrated that both formulations met ASEAN cosmetic safety standards (Table 6). Aerobic plate counts were well below the 1,000 cfu/g limit (<250 cfu/g for cleanser; 470 cfu/g for serum). Molds and yeasts were minimal (<10 cfu/g in both formulations), and

Table 5. Physicochemical characteristics of bromelain-containing topical products

Parameters	Exfoliating Gel Cleanser		Brightening Enzyme Exfoliating Serum	
	Results	Reference Value*	Results	Reference Value*
Solubility of bromelain in water	Soluble	N/A	Soluble	N/A
Color	White	Depending on ingredients	Yellowish	Depending on ingredients
Odor	Fruity, floral, citrusy notes	Depending on ingredients	Earthy notes from coffee extract with sweet, floral undertones	Depending on ingredients
pH	6.5-7.0	5.0-7.0	4.5	2.5-7.0
Cleansing property	+++	+++	Not applicable	N/A
Lathering ability	++	+ to ++	Not applicable	N/A
Foam height (cm)	15.5	8-18	Not applicable	N/A

*Reference values derived from commercial products

Table 6. Microbial profile of bromelain-containing topical products

Microbial Parameters	ASEAN Microbial Limits[a]	Exfoliating Gel Cleanser	Brightening Enzyme Exfoliating Serum
Aerobic Plate Count, cfu/g	< 1,000	<250	470
Molds and Yeast Count, cfu/g	< 1,000	<10	<10
Staphylococcus aureus	absent in 0.1 g sample	<10	<10

[a]ASEAN Guidelines on Limits of Contaminants for Cosmetics, ver 2.0

Staphylococcus aureus was not detected in either product.

These results confirm that bromelain incorporation does not promote microbial proliferation when combined with appropriate preservation systems, validating microbiological safety for topical cosmetic applications.

Human repeat insult patch test (HRIPT)

HRIPT evaluation following standardized protocols assessed skin irritation and allergic contact dermatitis potential among normal adult subjects (Figure 5). The study design included screening/enrollment, Induction Phase (9 visits over 2 weeks), 14-day Rest Period, and Challenge Phase (3 visits), with continuous dermatological monitoring throughout.

Both products demonstrated no irritant reactions during the study period, qualifying them as "non-irritating" and generally safe for normal skin. Due to the presence of known allergens in formulation ingredients, products cannot be labeled "hypoallergenic"; however, the overall risk profile for hypersensitivity reactions was low, supporting the suitability of bromelain-containing formulations for routine topical use on healthy skin.

Discussion

This study demonstrates successful valorization of pineapple peels into functionally active bromelain suitable for topical cosmetic applications through an integrated approach combining ATPS extraction, freeze-drying, and comprehensive safety evaluation. The work addresses critical gaps in sustainable bioprocessing by establishing a complete pathway from agricultural waste to market-ready cosmetic ingredient.

The 10% PEG-4000/30% $MgSO_4$ system achieved optimal balance between activity (2.80

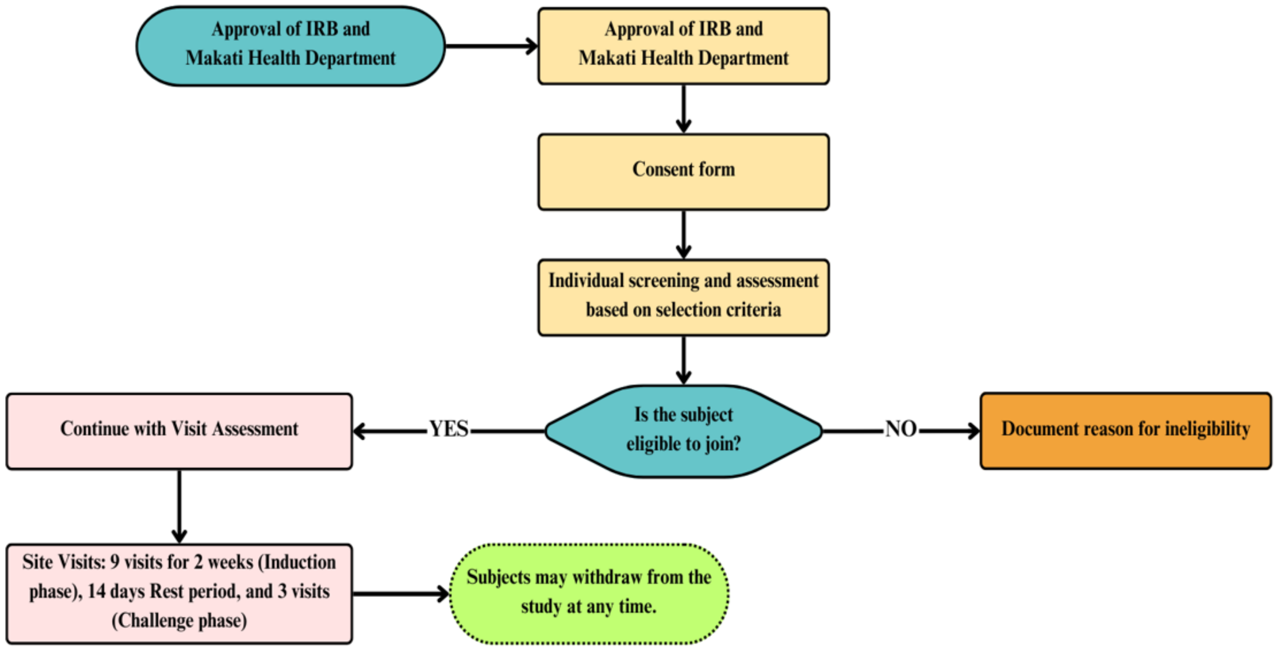

Figure 5. HRIPT study design flowchart. Sequential stages of Human Repeat Insult Patch Testing showing participant screening, Induction Phase, Rest Period, and Challenge Phase with continuous dermatological evaluation. Testing evaluated reactions to 109 allergens referenced by North American Contact Dermatitis Group and European Surveillance System on Contact Allergies.

U/mL), yield (37.64%), and production cost (49.2 PhP/100g), representing a practical compromise for scalable production. While the 5% PEG system exhibited 35% higher specific activity (3.78 U/mL), its substantially lower yield (16.78%) renders it economically unfavorable for industrial applications. This performance trade-off aligns with established ATPS partitioning principles: lower polymer concentrations create steeper chemical potential gradients that enhance selectivity but reduce phase volume ratios, consequently limiting total protein recovery [12,13].

Our results contrast with Ketnawa et al. [13], who reported optimal bromelain recovery at 16% PEG-4000/12.5% $(NH_4)_2SO_4$ with 82.7% yield and 5.3-fold purification. The substantially higher yield in their study likely reflects differences in salt type (ammonium sulfate versus magnesium sulfate) and initial substrate quality, as they used fresh pineapple peels while our material originated from industrial processing waste with potentially higher polysaccharide contamination. However, our 10% PEG system offers practical advantages: simpler formulation, lower reagent consumption, and adequate purification for cosmetic applications where ultra-high purity is not essential.

The enhanced activity observed post-ATPS (2.80 U/mL versus 2.30 U/mL in crude extract) confirms effective removal of enzyme inhibitors—particularly polyphenols and pigments that can cause non-specific protein binding [13]. Notably, the 20% PEG formulation showed no activity improvement despite 78.74% yield, suggesting saturation effects where excessive polymer concentration impairs phase discrimination and traps contaminants in the product phase. This finding provides practical guidance for ATPS optimization: higher PEG concentrations do not necessarily improve outcomes and may compromise purification efficiency while increasing costs.

Lyophilization preserved bromelain functionality, with freeze-dried powder exhibiting 3.07 U/g compared to 2.81 U/mL for liquid extract. The apparent 9.3% increase in mass-specific activity results from water removal concentrating protein content, while the reduced tyrosine release (82.02 versus 149.73 μg/mL) likely reflects delayed rehydration kinetics of lyophilized protein microaggregates rather than permanent denaturation. This interpretation is supported by the high GDU values (371-385 GDU/g) observed after complete rehydration, confirming preservation of catalytic function.

However, all GDU measurements were conducted on freshly prepared samples, limiting conclusions about long-term stability benefits. Quantitative validation requires controlled shelf-life studies under varying temperature and humidity conditions. Previous work on encapsulated plant enzymes suggests 6-12 month stability at room temperature [25], but bromelain-specific data under cosmetic formulation conditions remain needed.

Successful incorporation of bromelain into gel cleanser (pH 6.5-7.0) and serum (pH 4.5) demonstrates compatibility with cosmetic matrices. The pH ranges maintained enzyme stability while ensuring skin safety, as bromelain retains activity across pH 5.0-8.0 with optimal performance at pH 6.0-7.0 [8]. Importantly, bromelain addition did not compromise surfactant performance (cleanser maintained +++ cleansing efficacy and 15.5 cm foam height), indicating no significant protein-surfactant interactions that could reduce functionality.

Microbial analysis confirmed both formulations met stringent ASEAN standards, with aerobic counts well below 1,000 cfu/g and no pathogenic contamination detected. This validates that enzyme-containing natural cosmetics can achieve microbiological safety when properly preserved, addressing industry concerns about contamination risks in bio-based products [20].

HRIPT results showing "non-irritating" classification with low hypersensitivity risk support bromelain's suitability for routine topical use. However, the inability to claim "hypoallergenic" status due to known allergens in formulation bases (not bromelain itself) highlights the importance of complete ingredient disclosure. Bromelain's proteolytic mechanism offers advantages over conventional exfoliants: enzymatic cleavage of corneocyte proteins provides gentle exfoliation without the mechanical trauma of scrubs or pH-dependent keratolysis of alpha-hydroxy acids, making it particularly suitable for sensitive skin applications [9,10].

Critical evaluation reveals a limitation: HRIPT was conducted on normal healthy skin only. Clinical efficacy trials comparing bromelain formulations against established exfoliants (glycolic acid, salicylic acid) across diverse skin types, including sensitive and compromised barrier conditions, would strengthen evidence for dermatological applications. The production cost of 49.2 PhP/100g crude extract (~0.86 USD/100g) for optimal ATPS formulation appears economically viable, particularly given the zero-cost raw material (industrial waste). However, this calculation considers only reagent costs and omits labor, equipment depreciation, quality control, and regulatory compliance expenses that substantially impact commercial feasibility. A complete techno-economic analysis incorporating these factors is needed to assess industrial scalability.

PEG-4000 and $MgSO_4$ are recyclable through established protocols [14], potentially reducing operational costs by 30-40% in continuous production systems. Furthermore, the lower salt-rich phase containing polyphenols and polysaccharides could be valorized as antioxidant extracts or animal feed supplements, creating additional revenue streams that improve overall process economics.

This work demonstrates practical waste valorization aligned with circular economy principles. The Philippines generates approximately 544,000 metric tons of pineapple peel waste annually (based on 680,000 MT exports with 80% waste generation) [5,6]. If even 1% were converted to bromelain using this methodology, it could produce ~5,440 metric tons of enzyme powder annually—a substantial supply for cosmetic, pharmaceutical, and food industries while reducing landfill burden and greenhouse gas emissions from decomposing organic waste [7].

However, successful industrial implementation requires addressing practical challenges: establishing reliable peel collection networks from processing facilities, ensuring consistent raw material quality across seasonal variations, developing GMP-compliant production protocols, and navigating regulatory pathways for cosmetic ingredient approval. The ATPS methodology's inherent advantages—aqueous-based processing avoiding organic solvents, ambient temperature operation, and simple equipment requirements—position it favorably for developing country contexts where sustainable bioprocessing infrastructure is emerging [12].

Several limitations warrant acknowledgment. First, the small sample size (N=2) limits statistical power for detecting subtle differences between formulations. Future optimization studies should employ larger sample sizes (N≥5) with formal statistical analysis (ANOVA, response surface methodology) to identify optimal extraction conditions more rigorously. Accelerated stability testing under controlled stress conditions (temperature cycling, light exposure, humidity variations) is ongoing. Stability studies following ICH Q1A guidelines would provide critical data for commercial product development.

Future research should consider: (1) comprehensive shelf-life studies quantifying encapsulation protective effects over 12-24 months; (2) process optimization through design of experiments (DoE) maximizing both yield and activity simultaneously; (3) scale-up validation from laboratory to pilot-scale production; and (4) comparative life cycle assessment (LCA) versus synthetic exfoliants to quantify environmental benefits.

Conclusions

This integrated study establishes technical feasibility for converting pineapple peel waste into high-value bromelain ingredients meeting international cosmetic safety standards. The ATPS-freeze-drying approach preserves enzyme functionality while enabling stable powder formulation suitable for topical applications. Beyond immediate cosmetic applications, this work contributes methodological insights for sustainable bioprocessing of agricultural residues, demonstrating how locally available biomass can be transformed into functional ingredients through environmentally benign extraction technologies.

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Author Contributions

MRVP: Project manajement, Conceptualization, Methodology, Supervision, Writing—review & editing; GABB: Methodology, Data curation, Writing—original draft; CKFJ: Investigation, Analysis, Data curation; JANY: Analysis, Visualization; GNCC: Investigation, Analysis; ANYA: Analysis, Data curation, Writing—draft; RABB: Analysis, Data curation, Writing—draft; PYHM: Investigation, Data curation; JCOA: Methodology, Validation; ACB: Methodology, Analysis, Data curation.

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