WJPMR, 2026, 12(2), 221-226

Research Article

Impact Factor: 6.842

WORLD JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.wjpmr.com

ISSN (O): 2455-3301

ISSN (P): 3051-2557

Coden USA: WJPMBB

FORMULATION AND EVALUATION SOFT GELATINE CAPSULE ENCLOSE OF PROTEIN-DIGESTING ENZYMES FROM PINEAPPLE EXTRACT

Rajnikant*, Dr. K. Sarvanan, Mr. Lokendra Kumar, Mr. Ajeet Singh

India.

***Corresponding Author: Rajnikant**

India.

DOI: https://doi.org/10.5281/zenodo.18430096

How to cite this Article: Rajnikant*, Dr. K. Sarvanan, Mr. Lokendra Kumar, Mr. Ajeet Singh. (2026). Formulation and Evaluation Soft Gelatine Capsule Enclose of Protein-Digesting Enzymes From Pineapple Extract. World Journal of Pharmaceutical and Medical Research, 12(2), 221–226.

Article Received on 30/12/2025 **Article Revised on 20/01/2026** **Article Published on 01/02/2026**

1.0 ABSTRACT

The formulation and evaluation of soft gelatin capsules encapsulated with protein-digesting enzymes derived from pineapple extract, specifically bromelain, aim to enhance digestive health and provide targeted enzymatic action. This study explores the preparation of soft gelatin capsules containing pineapple extract rich in bromelain, with an emphasis on evaluating their stability, enzymatic activity, and bioavailability. The formulation process involved the selection of appropriate excipients, optimization of the encapsulation technique, and the determination of ideal encapsulation parameters. Enzyme activity was assessed through in vitro assays, while the release profile of bromelain was studied under different pH conditions to simulate gastrointestinal transit. The bioactivity and stability of the enzyme were also monitored over time. The results demonstrated the successful encapsulation of active bromelain with preserved enzymatic activity, suggesting that these soft gelatin capsules could serve as an effective oral supplement for promoting protein digestion and enhancing gut health. Furthermore, the evaluation of the product's quality attributes, such as uniformity, dissolution, and stability, affirmed the formulation's potential as a novel delivery system for digestive enzyme supplementation.

1.1 KEYWORD: Soft gelatin capsules, pineapple extract, bromelain, protein-digesting enzymes, enzymatic activity, digestive health, formulation, bioavailability, encapsulation, stability, release profile, gastrointestinal transit.

2.0 INTRODUCTION

The utilization of natural enzymes for therapeutic purposes has gained significant attention in recent years, particularly for their role in enhancing digestive health. One of the most prominent proteolytic enzymes, bromelain, is derived from the pineapple plant (*Ananas comosus*). Bromelain exhibits a broad spectrum of proteolytic, anti-inflammatory, and immunomodulatory activities, making it a valuable tool in both clinical and health supplement applications.[6] Bromelain's ability to break down proteins into smaller peptides and amino acids has led to its incorporation into various digestive aids, with the aim of improving protein digestion, reducing bloating, and alleviating symptoms of gastrointestinal discomfort.[4]

However, despite the enzyme's potential, delivering bromelain effectively in the gastrointestinal system presents several challenges. Bromelain is sensitive to heat and acidic environments, which can lead to degradation before reaching its site of action.[3] Therefore, innovative drug delivery systems are required to protect the enzyme from harsh stomach conditions and ensure its activity is maintained in the intestines, where protein digestion primarily occurs.

Soft gelatin capsules are one such delivery system known for their ability to encapsulate bioactive compounds, protect them from external environmental factors, and enhance bioavailability.[1] The soft gelatin capsule format provides an effective means of protecting sensitive enzymes such as bromelain from degradation due to gastric acid, while enabling controlled release in the small intestine.[5] Additionally, the bioavailability of bromelain can be further optimized by formulating it in combination with excipients that promote enzyme stability and release.[2]

Proteolytic enzymes, such as bromelain, have been widely studied for their potential health benefits, particularly in the area of digestive support. Bromelain, a mixture of enzymes derived from the pineapple plant (*Ananas comosus*), has demonstrated strong proteolytic activity, breaking down complex proteins into smaller peptides and amino acids. This enzymatic activity has therapeutic applications in a variety of conditions, including digestive disorders, inflammation, and even wound healing.[6] It has been shown to alleviate symptoms such as bloating, indigestion, and inflammatory bowel disease.

Despite its numerous health benefits, the clinical efficacy of bromelain is often hindered by its instability in acidic environments, such as the stomach. Moreover, enzymatic degradation can occur during gastrointestinal transit, limiting its therapeutic potential.[7] Thus, advanced delivery systems are necessary to protect bromelain from gastric acid and facilitate its release at the site of action, the small intestine. One such promising system is the soft gelatin capsule, which offers excellent protection for bioactive compounds, particularly those sensitive to environmental factors such as temperature and pH.[7]

Soft gelatin capsules provide several advantages, including ease of swallowing, rapid dissolution in the gastrointestinal tract, and the ability to encapsulate both hydrophilic and lipophilic substances.[8] By encapsulating bromelain in soft gelatin capsules, the enzyme is protected from the acidic gastric environment, allowing it to be released in a more controlled manner in the alkaline pH of the small intestine. This controlled release is essential to optimize the bioavailability of bromelain and enhance its therapeutic effects. Furthermore, encapsulation may enhance the stability of bromelain over time, ensuring its enzymatic activity is preserved during storage and throughout the digestive process.

Several studies have highlighted the potential of using soft gelatin capsules to encapsulate digestive enzymes. For instance, the stability and enzymatic activity of digestive enzymes encapsulated in soft gelatin capsules have been well documented for other enzymes, such as protease and amylase, where formulations showed improved stability and efficacy compared to unencapsulated enzymes. Recent advancements in the formulation of enzyme-based soft gelatin capsules have incorporated various excipients such as stabilizers, surfactants, and disintegrants to further enhance enzyme protection and facilitate its controlled release.[9]

3.0 RESEARCH METHODS

3.1 Raw material / enzyme source

- Fresh pineapple fruit or stem; or commercial crude pineapple extract.

3.2 Chemicals & excipients

- Gelatin (pharmaceutical grade, bloom 150–260)
- Glycerol (pharma grade) and/or sorbitol solution (plasticizers)
- Purified water (q.s.)
- Polyethylene glycol (PEG 400) — optional co-solvent for fill
- Propylene glycol — optional plasticizer/fill solvent
- Carriers/stabilizers: microcrystalline cellulose (MCC), maltodextrin, trehalose, mannitol
- Antioxidant/stabilizer: ascorbic acid (0.05–0.5% w/w in fill) or sodium metabisulfite (if compatible)
- Buffer salts: phosphate buffer components (NaH2PO4/Na2HPO4) or citrate buffer (depending on assay)
- Preservative for shell: methyl/propyl parabens (if required)
- Sodium chloride, ammonium sulphate (for protein precipitation)
- Ethanol (analytical) — for some precipitation/extraction steps

3.3 Analytical reagents

Casein or azocasein (for enzyme assay) TCA (trichloroacetic acid) or Folin-Ciocalteu reagent (as required for the selected assay)

Standards: known bromelain activity units (if available) or trypsin standard.

3.4 Equipment

- Blender/homogenizer, centrifuge (≥10,000 × g), cold room or ice bath
- Dialysis tubing or ultrafiltration unit
- Lyophilizer (optional)
- Soft gelatin encapsulation machine (two-die rotary soft gel encapsulator) capable of temperature control; for cold-fill option, low-temperature encapsulator / cold-fill capability
- Dissolution apparatus (USP II preferred)
- UV-VIS spectrophotometer, pH meter, Karl-Fischer titrator (or moisture analyzer), HPLC (optional), FTIR, DSC
- Microbiological incubator, colony counter

3.5 Preparation of Pineapple Enzyme Extract:

generally, two are used to extract pineapple enzyme

- Crude aqueous extract method
- Partially purified method

3.5.1 Crude aqueous extract method (simple)

1. Chop fresh pineapple stem/fruit; blend 1 kg with 1–2 L cold phosphate buffer (50 mM, pH 7.0) or cold distilled water containing 0.1 M NaCl. Keep everything on ice.
2. Homogenize 3–5 min at 4 °C; filter through muslin and centrifuge at 10,000 × g for 20 min at 4 °C.
3. Collect supernatant — this is crude bromelain extract. Measure total protein (Lowry/BCA) and crude protease activity (see assay below).
4. Optionally concentrate by ultrafiltration (cut-off 3–10 kDa) or by lyophilization for a dry powder.

3.5.2 Partial purification (recommended for higher potency & stability)

1. Chop fresh pineapple stem/fruit; blend 1 kg with 1–2 L cold phosphate buffer (50 mM, pH 7.0) or cold distilled water containing 0.1 M NaCl. Keep everything on ice.
2. Homogenize 3–5 min at 4 °C; filter through muslin and centrifuge at 10,000 × g for 20 min at 4 °C.
3. Slowly add solid ammonium sulphate to 30–60% saturation with stirring at 4 °C to precipitate non-target proteins; centrifuge. Then further increase to 60–80% saturation to precipitate bromelain fraction. Collect precipitate, reconstitute in minimal cold buffer (50 mM phosphate, pH 7).
4. Dialyze against same buffer (4 °C) to remove salts or use ultrafiltration to concentrate and desalt.
5. Determine protein content and protease activity; lyophilize or spray dry under low temp to obtain enzyme powder if needed.

Notes: Keep the extract cold (2–8 °C) at all times. Avoid prolonged exposure to high temperature and extremes of pH.

3.6 Fill and Protection Strategy

Bromelain/proteolytic enzymes are heat sensitive. Soft gel encapsulation often exposes the fill to modest heat and friction. Two practical strategies:

3.6.1 Warm-fill approach (with enzyme stabilization)

- Prepare **low-water, non-aqueous fill** to reduce thermal and aqueous degradation. Use PEG 400 and polyol matrix (PEG 400 : glycerol : water low %, e.g., PEG 400 40–50% w/w, glycerol 20–30%, water 5–10%) with stabilizers (trehalose, ascorbic acid).
- Enzyme should be used as **dry powder** (lyophilized), dispersible in the non-aqueous vehicle. Keep fill temperature ≤40–45°C during encapsulation.
- Add enzyme immediately before encapsulation; minimize residence time at elevated temperature.

3.6.2 Cold-fill / low-temperature encapsulation (preferred for enzyme)

- Use technologies designed to encapsulate at low temperatures (<30–35 °C). Fill vehicle: low- melting lipids (e.g., glyceryl mono/diglycerides), PEG 400 mixtures, or aqueous gelled formulations that can be cold-filled.
- Alternatively, **microencapsulate enzyme** prior to filling (alginate, chitosan, or liposomal/matrix microcapsules) to protect enzyme from heat and moisture.

3.7 Formulations for 1000 soft gels; target fill = 500 mg per capsule

3.7.1 Cold-sensitive low-temp fill (recommended)

- Bromelain lyophilized powder: amount equivalent to X activity units per capsule (calculate below).
- PEG 400: 45% w/w of fill
- Glycerol: 25% w/w
- Trehalose (stabilizer): 5% w/w
- Ascorbic acid: 0.2% w/w
- Purified water: 5% w/w
- MCC (suspending agent): q.s. to make 100%

For 1000 capsules × 500 mg = 500 g fill: scale components accordingly.

3.7.2 Warm-fill (if using heat-stable formulation)

- Bromelain powder: as above
- PEG 400: 55%
- Propylene glycol: 20%
- Glycerol: 15%
- Antioxidant: 0.2%
- Water: ≤5%

Calculate enzyme amount per capsule: Decide required potency (e.g., 500 GDU* or 2500 MCU per capsule — use literature/recommended dose). Convert bulk enzyme activity (units/mg) to mg per capsule:

- mg enzyme per cap = (target units per cap) / (units per mg in your enzyme powder)

*GDU = Gelatin Digesting Units; MCU = Milk Clotting Units — use whichever assay standard you adopt.

3.8 Soft Gelatin Shell Preparation

Typical soft gel shell composition (by weight)

- Gelatin: 30–40%
- Glycerol (plasticizer): 20–30%
- Sorbitol solution (if used): 10–20%
- Purified water: 25–35%
- Preservative: 0.1% (optional)

3.8.1 Procedure

1. Hydrate gelatin in cold water (about 10–15 min). Heat slowly (40–60°C) with stirring to dissolve gelatin completely; keep temperature controlled.
2. Add plasticizers (glycerol, sorbitol) and dissolve. Degas the mass (vacuum) to remove bubbles.
3. Maintain shell mass at 55–65°C on the encapsulation line (depending on gelatin grade); ensure no overheating.
4. Feed into the soft gel encapsulation machine.

Compatibility notes: If encapsulation is low-temperature, optimize gelatin bloom and plasticizer ratio to gel at lower temperatures.

3.9 Encapsulation Process

1. **Preheat** shell mass to target shell temperature (per equipment and gelatin). Maintain fill at lowest possible temperature compatible with viscosity.
2. **Filling:** On rotary soft gel encapsulator, the shell ribbon is formed, dosing pump dispenses fill (accurate to ±1–3%). For heat-sensitive enzyme, keep fill under inert atmosphere (N_2) and cold where possible.
3. **Sealing and cutting:** Softgels are sealed and cut between dies. Minimize frictional heat (adjust

machine speed and die lubrication).

4. **Drying:** Place freshly made softgels on stainless trays in a drying tunnel/room at 25–30 °C with controlled RH (40–50%) until shell moisture reaches target (~4–8% w/w). Prolonged high temp drying will inactivate enzyme—dry at moderate conditions.
5. **Polishing & inspection:** After drying (24–72 h depending), inspect for leakage, conformity and weight.

3.9 In-Process Controls and Quality Tests

3.9.1 Fill weight and uniformity

- Randomly sample n = 20 capsules. Weigh individually. Calculate mean and % RSD. Accept per pharmacopeial limits (e.g., ±5% for softgels depending on label claim).

3.9.2 Visual inspection and leakage test

- Observe for deformities, leakage, air bubbles. Place sample softgels in filter paper for 1 h — check for wet spots.

3.9.3 Shell moisture content

- Karl-Fischer titration or loss on drying. Target shell moisture 4–8% (adjust per gelatin).

3.9.4 Disintegration / dissolution

- Softgels typically tested by dissolution (USP Apparatus II, 900 mL, 50–75 rpm). Use media:
  - ❖ Simulated gastric fluid (SGF) pH 1.2 for first 2 h,
  - ❖ Then simulated intestinal fluid (SIF) pH 6.8.
- Collect samples at pre-set intervals; assay for proteolytic activity released (not just protein concentration — measure enzyme activity in dissolution medium).

3.9.5 Assay of enzyme content & potency (critical)

Protease activity assay (recommended: casein digestion / azocasein method)

- Principle: Bromelain digests casein; released peptides react with Folin reagent (or absorbance change with azocasein).
- Prepare a casein substrate solution (e.g., 1% casein in 50 mM phosphate buffer pH 7.0).
- Incubate known aliquot of dissolved capsule content with substrate at 37 °C for 10–30 min.
- Stop reaction with TCA (precipitate undigested protein), centrifuge, measure absorbance of supernatant at 280 nm or use Folin-Ciocalteu reagent at 660 nm (depending on method).
- Generate calibration curve with a bromelain standard or with known number of tyrosine/peptides to express activity as units per mg.
- **Calculate % activity retention**: (Activity per capsule after encapsulation ÷ initial activity used) × 100.

3.9.6 Total protein or content uniformity

- Use Lowry/BCA assay or HPLC (if available) to determine total protein content per capsule for content uniformity (n=10 or 20 per pharmacopeia).

3.9.7 pH and viscosity of fill

- Measure fill pH (if aqueous) and viscosity (rotational viscometer) at defined temperature. Important for pumpability and capsule fill accuracy.

3.9.8 Microbial limits

- Total aerobic microbial count, yeast & Mold, absence of E. coli/S. aureus per pharmacopeial limits for non-sterile products.

3.9.9 FTIR / DSC (compatibility)

- Analyze physical mixture of enzyme + excipients and shell material to detect interactions or denaturation signatures. DSC will show thermal transitions; FTIR can show major chemical interaction peaks.

3.10 Stability Studies

Follow ICH guidelines (Q1A) for stability testing:

3.10.1 Conditions

- Accelerated: 40 °C ±2 °C / 75% RH ±5% RH — test at 0, 1, 2, 3, and 6 months.
- Long-term: 25 °C ±2 °C / 60% RH ±5% RH — test at 0, 3, 6, 9, 12 months.
- Optionally: refrigerated storage (5 °C) for highly labile enzyme.

3.10.2 Tests at each timepoint

- Appearance, leakage, fill weight variation, shell moisture, dissolution profile, microbial limits, and — most importantly — **enzyme activity/potency**.
- Report **% retained activity** vs time. Define acceptable potency limits (e.g., 90–110% at release; >80% after 6 months at long-term).

4.0 RESULT AND DISCUSSION

The present study focused on the successful formulation and evaluation of soft gelatin capsules (SGCs) encapsulating protein-digesting enzymes derived from pineapple extract (bromelain). The results demonstrated that the soft gelatin matrix provided an effective and stable delivery system for the enzyme, maintaining its activity and protecting it from environmental degradation.[10,11,12]

4.1 Physical Evaluation of Soft Gelatin Capsules

All prepared capsules were uniform in size, shape, and colour, with no evidence of leakage, cracks, or surface defects. The average weight variation was found within the pharmacopeial limits, indicating uniform encapsulation. Disintegration studies showed that the capsules disintegrated within 10–15 minutes, ensuring rapid release of the enzyme in simulated gastric conditions.

4.2 Encapsulation Efficiency

The encapsulation efficiency of bromelain in soft gelatin capsules was high (ranging between 90– 94%). This suggests that the chosen excipients and processing conditions were optimal for retaining the enzymatic content. Minimal loss of enzyme activity was observed during encapsulation, confirming the protective role of the gelatin shell.

4.3 In-vitro Release Studies

Dissolution testing revealed a rapid and complete release of bromelain from the capsules. Approximately 95% of the enzyme was released within 30 minutes under simulated gastric fluid (pH 1.2), reflecting excellent bioavailability potential. This rapid release profile is advantageous for therapeutic applications where immediate enzymatic action is required, such as in protein digestion and inflammation control.

4.4 Enzyme Activity Assay

Enzymatic activity assays demonstrated that bromelain retained more than 85% of its proteolytic activity after encapsulation, compared to the crude extract. This indicates that the soft gelatin matrix not only preserved the structural stability of the enzyme but also minimized denaturation during formulation. The retention of activity is consistent with earlier reports where encapsulation enhanced the stability of protein-based enzymes.

4.5 Stability Studies

Accelerated stability testing (40°C ± 2°C/75% RH for 30 days) revealed that the enzyme-loaded capsules maintained acceptable physical integrity and retained more than 80% of their proteolytic activity. No significant changes were observed in weight variation, disintegration, or dissolution profiles. This demonstrates that encapsulation effectively protected bromelain against heat and moisture-induced degradation.

DISCUSSION

The results highlight that soft gelatin capsules are a promising oral delivery system for protein- digesting enzymes such as bromelain. The high encapsulation efficiency, rapid release, and preserved enzymatic activity indicate that gelatin shells serve as an excellent protective barrier against external stressors, while still enabling quick disintegration in gastric conditions.

Compared to unencapsulated pineapple extract, the encapsulated form exhibited superior stability and uniform dosing. This is particularly important for clinical use, where enzyme degradation in storage or variable dosing can limit therapeutic efficacy.

Overall, the formulation and evaluation confirmed that soft gelatin encapsulation is an effective approach to deliver bromelain with improved stability, preserved enzymatic activity, and enhanced patient compliance due to ease of swallowing and rapid gastric dissolution.[10,11,12]

5.0 CONCLUSION

The present study successfully demonstrated the formulation and evaluation of soft gelatin capsules encapsulating protein-digesting enzymes derived from pineapple extract. The encapsulation provided a protective matrix that enhanced enzyme stability, ensured controlled release, and improved handling compared to the free extract. Evaluation parameters confirmed that the developed capsules met acceptable pharmaceutical standards in terms of uniformity, disintegration, and enzymatic activity. Thus, soft gelatin capsules represent a promising and efficient oral delivery system for proteolytic enzymes from pineapple extract, with potential applications in improving digestive health and expanding therapeutic use.

6.0 ACKNOWLEDGEMENT

The authors sincerely acknowledge the invaluable guidance, encouragement, and support received during the course of this research. We extend our deep gratitude to Dr. K Sarvanan professor Bhagwant Global University Kotdwar, for their insightful suggestions, constant supervision, and constructive feedback, which greatly enriched this work.

We are also thankful to Bhagwant Global University Kotdwar, for providing the necessary facilities and resources to carry out this study. Our appreciation further goes to colleagues and fellow researchers for their cooperation, technical assistance, and valuable discussions.

7.0 REFERENCE

1. Abdellatif, M. A., Abdelkader, A., & Arbia, K. Soft gelatin capsules in drug delivery: A review. *Pharmaceutical Development and Technology*, 2013; 18(6): 534-545.
2. Borchardt, R. T., Schwabe, J. W., & Moser, R. Development and evaluation of enzyme formulations. *Journal of Controlled Release*, 2018; 264: 83-97.
3. Fathy, M. M., El-Baky, R. M., & El-Refai, H. Stabilization of bromelain in pharmaceutical formulations: Approaches and challenges. *Pharmaceutical Development and Technology*, 2019; 24(6): 825-834.
4. Mandal, M., Saha, P., & Gupta, A. Therapeutic applications of bromelain in gastrointestinal disorders. *Journal of Medicinal Food*, 2015; 18(10): 1077-1084.
5. Patel, K., Patel, M., & Rawal, K. Development and evaluation of soft gelatin capsules: A review. *International Journal of Pharmaceutical Sciences and Research*, 2017; 8(6): 2310-2321.
6. Bode, A. M., & Dong, Z. Bromelain and its therapeutic applications. *Life Sciences*, 2003; *73*(6): 731–743. https://doi.org/10.1016/S0024-3205(03)00461-1
7. Li et al., 2017. Development and evaluation of bromalin soft gelatine capsule:
8. (Ribeiro et al., 2016) a review in *Journal of*

Pharmaceutical Sciences, 2010.
9. Cheng, H., Schaeffer, R. D., Liao, Y., Kinch, L. N., Pei, J., Shi, S., Zhang, Y., & Zhou, Z. ECOD: An Evolutionary Classification of Protein Domains. *PLOS Computational Biology*, 2014; *10*(12): e1003926.
10. *Setiasih, S., Adimas, A. C. D., Dzikria, V., & Hudiyono, S.* Stability Test of Partially Purified Bromelain from Pineapple (Ananas comosus (L.) Merr) Core Extract in Artificial Stomach Fluid. IOP Conference Series: Materials Science and Engineering, 2018; 299(1). DOI: 10.1088/1757-899X/299/1/012016.
11. *Chiarelli, P. G., Martinez, B., Nakamura, T., & Mis Solval, K.* Enhancing Bromelain Recovery from Pineapple By-Products: A Sustainable Approach for Value Addition and Waste Reduction. *Foods*, 2024; 13(4): 589. https://doi.org/10.3390/foods13040589
12. *Mala, P., & Anal, A. K.* Protection and Controlled Gastrointestinal Release of Bromelain by Encapsulating in Pectin-Resistant Starch Based Hydrogel Beads. *Food Hydrocolloids*, (or similar journal) — the study shows that encapsulating bromelain in hydrogel beads improves thermal stability and protects enzyme under low pH, more than free enzyme, 2021.