

Physicochemical Characterization of Spray-Dried *Morinda citrifolia* Extract Microcapsules Using an Alginate-Chitosan-STPP System

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Abstract

Scopoletin, the primary bioactive marker of *Morinda citrifolia* L. with antihyperlipidemic activity, is susceptible to thermal and oxidative degradation during processing and storage. Despite the established role of microencapsulation in protecting herbal bioactives, studies on spray-dried polyelectrolyte wall systems for *Morinda citrifolia* extract remain limited, particularly regarding the influence of chitosan concentration on microcapsule formation and encapsulation performance. This study investigated the effect of chitosan concentration on the physicochemical properties and scopoletin encapsulation efficiency of spray-dried alginate-chitosan-sodium tripolyphosphate (STPP) microcapsules. *Morinda citrifolia* fruit extract was prepared by maceration in 70% ethanol and characterized prior to microencapsulation. Two formulations differing in chitosan concentration were spray-dried: F1 (1% w/v) and F2 (2% w/v), with sodium alginate (1% w/v), STPP (0.1% w/v), and extract concentration held constant. Microcapsules were characterized by FTIR spectroscopy, particle size analysis (PSA), differential scanning calorimetry (DSC), moisture content determination, scanning electron microscopy (SEM), and scopoletin quantification via validated TLC–densitometry. FTIR confirmed extract integration into the polymer matrix through hydrogen bonding, electrostatic interactions, and ionic crosslinking, with more pronounced polyelectrolyte interactions in F1. F1 also exhibited a narrower particle size distribution, greater thermal stability, lower moisture content ($3.38 \pm 0.20\%$), and higher encapsulation efficiency (88.37%) than F2 (moisture: $4.89 \pm 0.16\%$; EE: 72.09%; $p = 0.043$). SEM revealed greater particle discreteness in F1, whereas F2 exhibited denser aggregation. These results demonstrate that chitosan concentration critically governs polyelectrolyte complex formation and encapsulation performance, with F1 showing optimal physicochemical characteristics as a stable, standardized *Morinda citrifolia*-based oral herbal formulation.

Keywords

Morinda citrifolia, Spray-Drying, Microencapsulation, Polyelectrolyte Complex, Encapsulation Efficiency

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1. INTRODUCTION

Noni (*Morinda citrifolia* L.) is classified in the family Rubiaceae and possesses antimicrobial, anti-inflammatory, antioxidant, and metabolic regulatory activities (Hou et al., 2025). Among its phytochemical constituents, scopoletin is a major bioactive marker associated with the antihyperlipidemic properties of noni by modulating 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR) and cholesterol 7 α -hydroxylase (CYP7A1), important enzymes involved in cholesterol homeostasis (Purwaningsari et al., 2021). Because of its pharmacological significance and high analytical reproducibility, scopoletin is utilized as the standard chemical marker for the quality control of noni-based phytopharmaceuticals (Forcepta et al., 2021; Dewi et al., 2022). Despite its significant therapeutic po-

tential, developing stable and standardized noni formulations remains challenging because scopoletin and other bioactive compounds are susceptible to environmental stressors, including heat, oxidation, and light, which can lead to physicochemical degradation during processing and storage (Widjastuti et al., 2023). The hygroscopic nature of dried noni extracts further complicates handling and formulation into oral dosage forms. Therefore, strategies are needed to improve the stability and handling properties while preserving the integrity of bioactive compounds.

Microencapsulation has been widely explored as an effective strategy for protecting sensitive phytochemicals from environmental degradation and improving their physicochemical stability (Widjastuti et al., 2023; Díaz-Montes, 2023; Estevinho and López-Rubio, 2024). Sodium alginate and chitosan are com-

monly employed as wall materials because of their biocompatibility, biodegradability, and their ability to form polyelectrolyte complexes through ionic interactions, which may enhance the structural stability of encapsulated compounds (Mardiyanto et al., 2020; Asita et al., 2023). In alginate-chitosan systems, chitosan concentration is an important formulation variable that may influence solution viscosity, atomization behavior during spray drying, microparticle morphology, and encapsulation efficiency (Villate et al., 2023). Ionic crosslinking of chitosan with sodium tripolyphosphate (STPP) further reinforces particle integrity through electrostatic interactions between chitosan amino groups and STPP phosphate groups, thereby improving encapsulation performance (Ifitah et al., 2025; Mardiyanto et al., 2020).

Spray drying is widely used in herbal microencapsulation because it rapidly removes solvents and produces free-flowing microparticles suitable for oral dosage formulations (Nguyen et al., 2024). Compared with freeze-drying, spray drying offers continuous processing, lower operational costs, and greater scalability for industrial production (Díaz-Montes, 2023; Mardani et al., 2024). Despite atomization involving thermal exposure, the short residence time of droplets within the drying chamber minimizes thermal degradation of thermosensitive compounds, such as scopoletin, particularly under controlled inlet temperatures (Mardani et al., 2024). Complex coacervation, despite yielding thicker and more well-defined core-shell structures, requires precise pH control and multistep processing, which may compromise reproducibility and scalability when applied to herbal extract systems with complex phytochemical matrices (Mehta et al., 2022).

Previous studies on noni extract microencapsulation have primarily utilized maltodextrin and gum arabic-based wall systems, focusing more on antioxidant and biological activities than on detailed physicochemical characterization of standardized marker compounds (Widjastuti et al., 2023; Zhang et al., 2020a; Nguyen et al., 2024). Although maltodextrin provides excellent film-forming properties and solubility, it lacks the electrostatic crosslinking mechanism characteristic of alginate-chitosan polyelectrolyte systems, which may limit the formation of structured polymer networks and reduce responsive matrix behavior relevant to oral delivery (Halahlah et al., 2023; Díaz-Montes, 2023). Existing reports on alginate-chitosan systems applied to herbal extracts in the Indonesian context have predominantly employed ionic gelation or submicron particle methods rather than spray drying combined with a ternary alginate-chitosan-STPP wall system (Mardiyanto et al., 2020).

Alginate-chitosan systems crosslinked with STPP have been investigated for bioactive encapsulation; most studies have used ionic gelation or complex coacervation techniques, which may limit process scalability and reproducibility when applied to phytochemical-rich herbal extracts (Mehta et al., 2022; Bhatia et al., 2024). Despite the growing interest in alginate-chitosan-based delivery systems, their application as spray-dried ternary alginate-chitosan-STPP matrices for *Morinda citrifolia* extract remains underexplored. Furthermore, the effect of chitosan

concentration on microcapsule formation, physicochemical characteristics, and encapsulation performance, evaluated using scopoletin as a quantitative marker, has not been reported. Given the antihyperlipidemic properties of scopoletin and its relevance to oral herbal formulations, the evaluation of physicochemical attributes, including particle size distribution, moisture content, thermal stability, and encapsulation efficiency, is essential because these parameters critically determine the processing performance, storage stability, and bioactive compound retention in the resulting microcapsules.

Therefore, this study aimed to evaluate the influence of chitosan concentration on the physicochemical characteristics of spray-dried alginate-chitosan-STPP microencapsulated noni extract using scopoletin as a quantitative chemical marker. This study extends current knowledge of *Morinda citrifolia* microencapsulation by applying a spray-dried ternary alginate-chitosan-STPP wall system and quantitatively evaluating encapsulation performance using scopoletin as a chemical marker. It was hypothesized that variations in chitosan concentration would modulate the density of the polyelectrolyte complex network formed through electrostatic interactions between alginate and chitosan, reinforced by ionic crosslinking with STPP, thereby influencing the physicochemical characteristics and encapsulation performance of the resulting microparticles. These findings are expected to support the rational design of stable and standardized noni-based oral herbal formulations.

2. EXPERIMENTAL SECTION

2.1 Materials

The materials used in this study were dried *Morinda citrifolia* L. fruits (noni), ethanol (Merck, Germany), chitosan (N&R Industries, Indonesia), sodium alginate (Shandong Jiejing Group, China), sodium tripolyphosphate (STPP; Sigma-Aldrich, USA), acetic acid, Tween 80, demineralized water, and scopoletin standard (Merck, Germany).

Table 1. Moisture Content of *Morinda citrifolia* Fruit Extract

Replication	Moisture content (%)
1	7.59
2	7.58
3	7.58
Mean ± SD	7.58 ± 0.007

Requirement according to Indonesian Herbal Pharmacopoeia: ≤10%

2.2 Instrumentation

The instruments used in this study were a rotary evaporator and spray dryer (Büchi Labortechnik AG, Switzerland), a thin-layer chromatography (TLC)-densitometric (CAMAG, Switzerland), an IRTracer-100 Fourier-transform infrared (FTIR) spectrometer (Shimadzu, Japan), an Analysette 22 NeXT particle size analyzer (Fritsch GmbH, Germany), an Apreo 2 scanning electron microscope (SEM; Thermo Fisher Scientific, USA), and

a DSC 4000 differential scanning calorimeter (PerkinElmer Inc., USA).

2.3 Methods

2.3.1 Preparation of *Morinda citrifolia* Extract

Dried *M. citrifolia* fruit was pulverized into a fine powder. Maceration was selected as the extraction method to minimize the thermal degradation of heat-sensitive phytochemicals, including scopoletin (Undale et al., 2026). The powder was extracted using 70% ethanol at a material-to-solvent ratio of 1:10 (w/v) for three days, with three maceration cycles. The selection of 70% ethanol was based on the physicochemical properties of scopoletin, a coumarin derivative with moderate polarity arising from its hydroxyl and methoxy substituents. The ethanol–water (70:30, v/v) solvent system provides an intermediate polarity compatible with the physicochemical characteristics of scopoletin, enabling efficient solvation of both the aromatic ring and polar substituents, thereby enhancing the extraction of scopoletin from the plant matrix into the solvent phase (Sulistiawanti et al., 2025).

A total of 750 g of powdered material was macerated at room temperature for 24 h with intermittent stirring. The mixture was then filtered through filter paper to separate the filtrate. The remaining residue was remacerated twice using fresh solvent for 24 h each to ensure exhaustive extraction of the plant material. The combined filtrates were concentrated under reduced pressure using a rotary evaporator at 40 °C and subsequently dried in an oven at 45 °C until a constant weight was achieved. The resulting viscous extract was stored in an airtight glass container at 4 °C until further analysis. The extract was weighed, and the extraction yield was calculated using Equation (1).

$$\% \text{ Extraction yield} = \frac{W_{\text{extract}}}{W_{\text{rawmaterial}}} \times 100\% \quad (1)$$

2.3.2 Determination of Moisture Content of The Extract

The moisture content of *M. citrifolia* fruit extract was determined using the gravimetric method adapted from the Indonesian Herbal Pharmacopeia (Indonesian Ministry of Health, 2017). A porcelain crucible was cleaned, dried in an oven at 105 °C for 1 h, cooled in a desiccator, and weighed to obtain the weight of the empty crucible (W_1). Approximately 10 g of the extract was placed in the crucible and weighed to obtain the combined weight of the crucible and sample (W_2). The sample was dried in an oven at 105–110 °C until a constant weight was achieved. During the drying process, the crucible was periodically removed, cooled in a desiccator, and reweighed until the difference between two consecutive weighings did not exceed 0.005 g. The final weight after drying was recorded as W_3 . The moisture content was calculated and expressed as a percentage (% w/w) using Equation (2). All measurements were performed in triplicate.

$$\%MC = \frac{W_2 - W_3}{W_2 - W_1} \times 100\% \quad (2)$$

Note:

W_1 = weight of the empty crucible (g)

W_2 = weight of crucible with sample before drying (g)

W_3 = weight of crucible with sample after drying (g)

2.3.3 Identification and Quantification of Scopoletin

Identification and quantification of scopoletin in *Morinda citrifolia* extract were performed using thin-layer chromatography (TLC)-densitometry. Chromatographic separation was carried out on silica gel 60 F254 TLC plates (Merck KGaA, Germany) utilizing a binary mobile phase of toluene and ethyl acetate (65:35, v/v). Standard and sample solutions were applied as 8 mm bands using a Linomat 5 applicator (CAMAG, Switzerland). Plate development was performed in a chamber previously equilibrated with mobile phase vapor, and the developed plates were air-dried prior to analysis. The plates were observed under UV light at 254 and 366 nm using a TLC Visualizer (CAMAG, Switzerland). Scopoletin identification was confirmed by comparing the R_f value and UV absorption profile of the sample with those of the reference standard. Densitometric scanning was subsequently performed at 345 nm using a TLC Scanner 4 controlled by WinCATS software.

2.3.3.1 Calibration Curve

A calibration curve was constructed by applying scopoletin standards at concentrations ranging from 10 to 80 ng/band under identical chromatographic conditions. Peak areas were plotted against the corresponding amounts to obtain the calibration curve, which showed good linearity ($r \geq 0.99$).

2.3.3.2 Method Validation

The TLC–densitometric method was validated in accordance with ICH Q2(R2) guidelines for linearity, precision, accuracy, limit of detection (LOD), and limit of quantification (LOQ).

Linearity was evaluated using scopoletin standard solutions within the concentration range of 10–80 ng/spot, and the calibration curve was constructed by plotting peak area against scopoletin concentration. The linearity of the method was assessed using the correlation coefficient (r) and the coefficient of determination (R^2).

Precision was evaluated as intra-day repeatability using hexaplicate injections ($n = 6$) of a homogeneous analyte at identical operational parameters, and the results were expressed as relative standard deviation (RSD%).

Accuracy was determined using the standard addition method at three concentration levels: 80%, 100%, and 120% of the target concentration. Each level was analyzed in triplicate, and the results were expressed as percentage recovery.

LOD and LOQ were calculated based on the standard deviation of the response (σ) and the slope of the calibration curve

(S) using Equations (3) and (4), respectively.

$$LOD = \frac{3.3\sigma}{S} \quad (3)$$

$$LOQ = \frac{10\sigma}{S} \quad (4)$$

2.3.3.3 Determination of Scopoletin in the Sample

Approximately 100 mg of *Morinda citrifolia* extract was accurately weighed, dissolved in methanol, and diluted to a final volume of 10 mL in a volumetric flask. The sample solution was applied to the TLC plate at 5.5 μ L per spot. After chromatographic development, densitometric scanning was performed at 345 nm, and the scopoletin content was quantified using the external standard method based on an established calibration curve. All analyses were performed in six replicates each.

2.3.4 Preparation of Alginate-Chitosan Microencapsulated Extract

Microencapsulated extract of *Morinda citrifolia* was prepared using an alginate-chitosan biopolymeric system. In this formulation, alginate served as the primary gel-forming polymer, whereas chitosan, as a cationic secondary polymer, contributed to increased capsule rigidity through electrostatic interactions. Tween 80 was used as a nonionic emulsifier to facilitate homogeneous dispersion of the extract, and STPP was incorporated as an ionic crosslinking agent. Two formulations, designated F1 and F2, were prepared to evaluate the effect of chitosan concentration on the physicochemical characteristics of the resulting microcapsules.

Chitosan concentrations of 1% and 2% (w/v) were chosen based on preliminary orientation studies and prior research, which indicate that chitosan concentration is crucial for the formation of alginate-chitosan polyelectrolyte complexes. This concentration significantly affects the encapsulation efficiency, particle characteristics, and processability of spray-drying (Jayanudin and Rochmadi, 2017; Pudziuelytė et al., 2022; Villate et al., 2023). These concentrations were considered suitable for evaluating the influence of chitosan content while maintaining stable emulsion characteristics and acceptable spray-drying performance. Therefore, 1% and 2% (w/v) chitosan solutions were selected to represent two different polymer content levels for the systematic evaluation of encapsulation performance and physicochemical properties.

Sodium alginate (1 g) was dissolved in 100 mL of demineralized water. Chitosan was dissolved in 100 mL of 1% acetic acid at concentrations of 1 g (F1) and 2 g (F2). To prepare the emulsion, 5 g of *M. citrifolia* extract was dissolved in ethanol at a 1:4 (w/v) ratio and subsequently added to the alginate solution under continuous mechanical stirring (IKA RW 20). The chitosan solution was gradually incorporated, followed by the addition of Tween 80 (1% v/v) and STPP (0.1% w/v). The

resulting emulsion was spray-dried using a Büchi Mini Spray Dryer B-300 (0.5 mm nozzle) under the following optimized conditions: inlet temperature of 120–125 °C, outlet temperature of 60–70 °C, feed rate of 5 mL/min, and air aspiration rate of 100%. These parameters were selected through preliminary orientation studies to ensure efficient solvent evaporation and stable atomization while minimizing the thermal degradation of scopoletin and other thermolabile constituents (Nguyen et al., 2024). The obtained microencapsulated powder was collected and stored in a desiccator until further characterization.

2.3.5 Characterization of Spray-Dried Microcapsules

Physicochemical characterization was performed to evaluate the suitability of the spray-dried microcapsules for solid oral formulations. Encapsulation efficiency values above 70% were indicated as satisfactory retention of the bioactive marker during spray-drying (Fahrudin et al., 2025; Nemickaite et al., 2025), whereas a residual moisture content below 5% ensured storage stability and handling properties (Mardani et al., 2024; Pudziuelytė et al., 2025). Particle size uniformity was quantified using the coefficient of variation (CV), with lower CV values indicating a more homogeneous particle population (Mardani et al., 2024). Thermal behavior was assessed using DSC thermograms, with higher transition enthalpy and improved thermal characteristics indicating stronger polymer-extract interactions and structural stability (Wu et al., 2025). Additionally, morphological evaluation determined particle integrity, surface characteristics, and the degree of aggregation, as these factors may affect powder flowability and physical stability. Complementing these analyses, Fourier-transform infrared spectroscopy (FTIR) and particle size analysis (PSA) were performed alongside evaluations of moisture content and encapsulation efficiency to comprehensively compare the quality attributes of the different formulations. FTIR, DSC, and SEM were employed as qualitative characterization techniques. Representative spectra and thermograms were obtained for each sample, and SEM observations were conducted on multiple fields of view, and representative micrographs are presented.

Fourier Transform Infrared Spectroscopy (FTIR): FTIR spectra of the extract, wall materials, and microcapsules were recorded over the wavenumber range of 4000–400 cm^{-1} at a resolution of 4 cm^{-1} using the KBr pellet method. Peak shifts and intensity changes were analyzed to evaluate the possible intermolecular interactions between the polymers and the encapsulated extract.

Particle Size Analysis: The particle size distribution of the microcapsules was analyzed by laser diffraction, with demineralized water as the dispersant. Cumulative volume percentiles (D10, D50, and D90), representing the particle diameters at 10%, 50%, and 90% of the cumulative distribution, were recorded to profile each sample. All measurements were performed in triplicate.

Thermal Analysis: The thermal behavior of the extract, wall materials, and microcapsules was analyzed using Differential

Table 2. Summary of Validation Parameters for TLC-Densitometric Determination of Scopoletin

Parameter	Result	Acceptance Criteria
Linearity range	10-80 ng/spot	-
Regression equation	$y = 31.44x + 10.58$	-
Correlation coefficient (r) / R ²	0.9994 / 0.9989	≥ 0.999
Precision - mean ± SD	0.043 ± 0.001 % (w/w)	-
Precision - %RSD	2.63 %	≤ 5 %
Recovery range	96.41-105.75 %	90-107 %
LOD	1.11 ng/spot	-
LOQ	3.37 ng/spot	Within linear range

Scanning Calorimetry (DSC). Approximately 5-8 mg of each sample was weighed into an aluminum crimped pan and heated from 30 to 400 °C at a heating rate of 10 °C/min under a nitrogen purge flow of 20 mL/min. The resulting thermograms were evaluated to assess polymer-extract compatibility and thermal stability of the encapsulated extract.

Moisture Content Analysis: The moisture content of the spray-dried microcapsules was determined using a moisture analyzer. Approximately 1-2 g of sample was analyzed at 105 °C until a constant weight was achieved. All measurements were performed in triplicate.

Encapsulation Efficiency Analysis: The encapsulation efficiency (EE) was determined by directly quantifying scopoletin in the spray-dried microcapsules and comparing it with the scopoletin content previously determined in the extract before encapsulation (Villate et al., 2023). This parameter reflects the proportion of scopoletin retained within the polymeric matrix after spray drying, with losses attributable to thermal exposure, surface deposition, and processing-related factors. An accurately weighed 100 mg portion of spray-dried microcapsules was dispersed in methanol and diluted to volume in a 10 mL volumetric flask. The suspension was sonicated twice for 5 min each to disrupt the polymeric matrix and facilitate the release of scopoletin into the extraction medium. The resulting suspension was then centrifuged at 8,000 rpm for 5 min. The resulting supernatant was collected and analyzed using the previously validated TLC–densitometric method. The scopoletin content was quantified using an external standard calibration curve, expressed as µg of scopoletin per mg of sample (µg/mg), and was used to calculate encapsulation efficiency. All analyses were performed in triplicate. The encapsulation efficiency was calculated using Equation (5):

$$\%EE = \frac{(\text{Scopoletin content in microcapsules})}{(\text{Initial scopoletin content in extract})} \times 100\% \quad (5)$$

where the scopoletin content in microcapsules refers to the amount of scopoletin recovered from the spray-dried microcapsules after extraction, whereas the initial scopoletin content in the extract refers to the scopoletin content determined prior to the microencapsulation process. Both values were expressed as µg of scopoletin per mg of sample and were determined

using the validated TLC–densitometric method.

Morphological Observation: The surface morphology, particle shape, and structural integrity of the microcapsules were examined using scanning electron microscopy (SEM) at accelerating voltages of 10 kV and magnifications of 5000× and 10000×. Prior to imaging, samples were sputter-coated with gold to enhance surface conductivity. Observations focused on the particle shape, surface texture, and structural integrity of the microcapsules.

Statistical Analysis: Quantitative data are expressed as mean ± standard deviation (SD). Statistical analyses were performed using IBM SPSS Statistics (IBM Corp., Armonk, NY, USA). Data normality was assessed using the Shapiro-Wilk test. For normally distributed data, Differences between Formula 1 (F1) and Formula 2 (F2) were evaluated using an independent-samples t-test, or the Mann–Whitney U test as a non-parametric alternative for non-normally distributed data. A value of $p < 0.05$ was considered statistically significant.

3. RESULTS AND DISCUSSIONS

3.1 Physicochemical Characteristics of *Morinda citrifolia* Extract

The extraction process yielded 234 g of dry extract from 750 g of fruit powder, corresponding to an extraction yield of 31.2%. This value exceeded the minimum requirement specified in the Indonesian Herbal Pharmacopeia (Farmakope Herbal Indonesia) for thick noni fruit extract (≥10.1%) (Indonesian Ministry of Health, 2017), confirming the effectiveness of the extraction process in recovering phytochemical constituents from the *M. citrifolia* fruit. The yield obtained was higher than the 15.31%-23.33% yields reported by Dewi et al. (2022) after 70% ethanol maceration of *M. citrifolia* fruits from three different Indonesian locations. The elevated yield in this study is likely attributed to the optimal polarity of 70% ethanol, which efficiently dissolves a wide range of polar and semipolar phytochemicals, including phenolic compounds and scopoletin. Additionally, repeated solvent replacement during remaceration sustained the concentration gradient between the plant matrix and the extraction medium, enhancing mass transfer and improving constituent recovery (Jha and Sit, 2022).

Following extraction, physicochemical characterization was conducted to evaluate extract quality and determine its suit-

Table 3. Accuracy of TLC-Densitometric Method at Three Concentration Levels

Scopoletin Added (ng/spot)	Scopoletin Found (ng/spot)	Recovery (%)
40	38.56	96.41
50	52.88	105.75
60	58.56	97.60

Table 4. Determination of Scopoletin Content in *Morinda citrifolia* Extract

Replication	Scopoletin Concentration (%)
1	0.043
2	0.041
3	0.043
4	0.044
5	0.041
6	0.043
Mean ± SD	0.043 ± 0.001
CV	2.63 %

Notes: Scopoletin content (%) was calculated from the peak area using the calibration equation ($y = 31.44x + 10.58$), accounting for sample dilution (5.5 µL spot from 10 mL solution)

Table 5. Comparative Composition of Formulation I and II for Extract Microencapsulation

Component	Formula 1	Formula 2
Noni Extract	5 g	5 g
Alginate	1% (w/v)	1% (w/v)
Chitosan	1% (w/v)	2% (w/v)
	(1% acetic acid)	(1% acetic acid)
STPP	0.1% (w/v)	0.1% (w/v)
Tween 80	1 % (v/v)	1%

ability for microencapsulation, including moisture content and scopoletin content, with scopoletin serving as the quantitative marker. These parameters were used to establish a pre-encapsulation baseline to assess marker compound retention following spray drying (Chemat et al., 2012; Porras-Yepes et al., 2020). The moisture content of the extract was 7.58%, within the recommended ≤10% limit for herbal extract quality and was considered appropriate for formulation (Länger et al., 2018). Moisture content is a critical physicochemical parameter because it affects extract stability during storage and ensures the precision of extract weight during feed solution preparation. Maintaining an appropriate moisture level supports the reproducibility of the feed concentration, which directly affects spray-drying efficiency and the physicochemical properties of the resulting microcapsules (Chendjou et al., 2025). Adequate moisture control preserves the stability of bioactive compounds, particularly scopoletin, as excessive thermal exposure or improper storage accelerates degradation and reduces marker retention prior to microencapsulation (Saqueti et al.,

2021). Complete moisture content data are presented in Table 1. Before quantitative determination, scopoletin was identified qualitatively via thin-layer chromatography (TLC) on silica gel 60 F₂₅₄ plates using toluene-ethyl acetate (65:35, v/v) as the mobile phase, comparing the extract against a reference standard under identical chromatographic conditions, as previously reported (Kumar et al., 2024). Under UV light at 366 nm, the scopoletin standard produced a characteristic fluorescent spot with an R_f value of approximately 0.28, while the extract samples showed R_f values of 0.27-0.28. The close agreement in R_f values and UV absorption profiles confirmed the presence of scopoletin in the extract. Densitometric scanning was subsequently performed at 345 nm, corresponding to the absorption maximum of scopoletin under the applied conditions.

Analytical method validation was conducted in accordance with ICH Q2(R2) guidelines (International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use, 2023). The validation confirmed that the TLC-densitometric method provided reliable and sufficiently sensitive quantification of scopoletin as a marker compound for quality control and retention assessment. The evaluated parameters included linearity, precision, accuracy, and sensitivity. Linearity was assessed over a concentration range of 10-80 ng/spot. The calibration curve showed excellent linearity, yielding the following regression equation:

$y = 31.44x + 10.58$

The correlation coefficient was $r = 0.9994$, and the coefficient of determination was $R^2 = 0.9989$, indicating a strong linear relationship between scopoletin concentration and peak area response across the validated range.

Precision was evaluated by repeatability testing using six replicates under identical conditions. The mean scopoletin content was $0.043 \pm 0.001\%$ (w/w), with an RSD of 2.63% (44.382 ± 1.167 ng/spot), demonstrating that the method showed good repeatability and acceptable analytical precision for TLC-densitometric phytochemical analysis.

Accuracy was determined using the standard addition method at three concentration levels (40, 50, and 60 ng/spot), yielding recovery values of 96.41%, 105.75%, and 97.60%, respectively, with an overall range of 96.41–105.75%. These values adhered to the AOAC-recommended limits of 90–107% for analyte concentrations at the ppm level (AOAC International, 2016), confirming acceptable accuracy without significant matrix interference or systematic error. Furthermore, these results are comparable to the 99.88% scopoletin recovery reported by Tasfiyati

et al. (2022) for *Morinda citrifolia* using HPLC-DAD, further supporting the applicability of the present TLC–densitometric method.

Sensitivity was assessed by calculating the LOD and LOQ based on the standard deviation of the response (σ) and calibration slope (S) according to the ICH Q2(R2) equations. The LOD and LOQ were 1.11 and 3.37 ng/spot, respectively, both below the lowest calibration concentration (10 ng/spot), confirming adequate sensitivity for detecting and quantifying low scopoletin levels in *M. citrifolia* extract.

Overall, the TLC-densitometric method demonstrated acceptable analytical performance across all validated parameters and was considered fit for purpose. Subsequently, it was used to quantitatively determine scopoletin in *M. citrifolia* extract and to evaluate marker retention following microencapsulation. The detailed validation results are presented in Table 2, and the recovery data used for the accuracy evaluation are presented in Table 3.

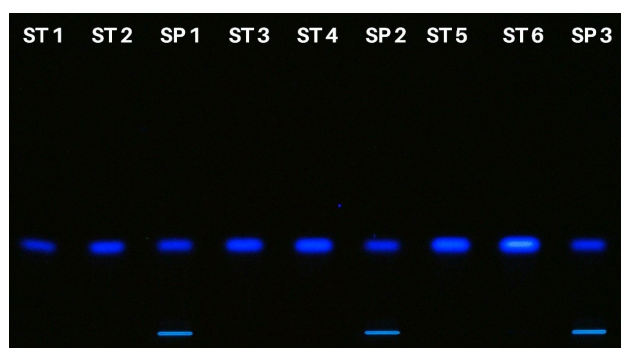


Figure 1. The TLC Profile of Scopoletin in *Morinda citrifolia* Extract was Analyzed on Silica Gel 60 F254 Using Toluene-ethyl Acetate (65:35, v/v) as the Mobile Phase and Visualized under UV Light at 366 nm. ST1–ST6 Represent Scopoletin Standard Solutions, whereas SP1–SP3 Represent *Morinda citrifolia* Extract Samples

The scopoletin content in the pre-encapsulation extract was $0.043 \pm 0.001\%$ (w/w), with a CV of 2.63% ($n = 6$), indicating acceptable analytical repeatability for TLC-densitometric quantification. The results are summarized in Table 4 and illustrated in Figure 1. This value served as a quantitative reference point for calculating the encapsulation efficiency and assessing the marker retention following spray drying. The content obtained was approximately 10-fold lower than that reported by Wigati and Anwar (2017) ($0.46 \pm 0.05\%$) and did not meet the minimum pharmacopoeial requirement of $\geq 0.38\%$ (Indonesian Ministry of Health, 2017).

The reduced scopoletin content may reflect differences in raw material quality, geographical origin, fruit maturity, extraction conditions, and post-extraction processing, all of which can influence scopoletin recovery and stability (Jha and Sit, 2022). Thermal degradation during rotary evaporation may have also contributed, as scopoletin (7-hydroxy-6-methoxycoumarin)

is susceptible to oxidative and thermal degradation under prolonged heating, particularly above 60 °C (Joshi et al., 2021). Although this content fell below pharmacopoeial specifications, the marker compound remained quantitatively detectable at a well-defined concentration, providing a measurable baseline for determining the encapsulation efficiency. The sub-pharmacopoeial level further supports the rationale for microencapsulation as a protective strategy, given that encapsulation within alginate-chitosan matrices provides physical protection against thermal and oxidative stressors, thereby enhancing the stability of thermosensitive phenolic and coumarin-type compounds (Eghbal and Choudhary, 2018).

3.2 Microencapsulation Performance

Following physicochemical characterization of the *Morinda citrifolia* extract, it was integrated into a spray-dried microencapsulation system using sodium alginate, chitosan, and sodium tripolyphosphate (STPP) as the primary wall-forming agents. Tween 80 was incorporated as an emulsifying agent to facilitate homogeneous dispersion of the extract within the feed solution prior to atomization. Alginate and chitosan are naturally derived polysaccharides with established biocompatibility, biodegradability, mucoadhesive properties, and gel-forming capacity, which are important for forming a stable polymeric encapsulating matrix (Conzatti et al., 2017; Atma et al., 2025). The combination of these oppositely charged polymers was selected for their ability to form polyelectrolyte complexes through electrostatic interactions between anionic alginate and cationic chitosan, thereby enhancing matrix integrity, improving encapsulation stability, and protecting thermosensitive phytochemical constituents, such as scopoletin, during thermal processing (Eghbal and Choudhary, 2018).

Compared with other encapsulation techniques, such as complex coacervation and emulsification–internal gelation, spray drying offers practical advantages, including continuous processing, scalability, and efficient production of dry powder formulations suitable for pharmaceutical applications (Díaz-Montes, 2023). These advantages support its use for microencapsulating *M. citrifolia* extract.



Figure 2. Physical Morphology of Microcapsules Prepared Using Different Formulations

Two formulations were prepared with identical compositions, differing only in chitosan concentration. Formula 1

Table 6. Particle Size Distribution Parameters of Noni Fruit Microcapsules Prepared with Formula 1 and Formula 2

Particle Size Distribution Percentile	Formula 1 (Mean ± SD, μm)	Formula 2 (Mean ± SD, μm)	p-value
D10	7.65 ± 0.05	10.75 ± 2.55	0.014
D50	16.73 ± 0.23	33.04 ± 2.60	< 0.001
D90	32.99 ± 0.94	275.20 ± 23.14	< 0.001

Notes: Values are presented as mean ± SD (n = 3). Independent samples t-test; statistically significant at p < 0.05. D10 was analyzed using unequal variance assumption

Table 7. Moisture Content of Noni Fruit Microcapsules

Formula	Moisture Content (%)
F1	3.38 ± 0.20
F2	4.89 ± 0.16

Notes: Values are presented as mean ± SD (n = 3). Independent samples t-test showed a statistically significant difference between formulations (p < 0.001)

contained 1% (w/v) chitosan, whereas Formula 2 contained 2% (w/v) chitosan. The amount of *M. citrifolia* extract was maintained at 5 g for both formulations (Table 5). This single-variable design was adopted to directly evaluate the effect of chitosan concentration on the physicochemical characteristics of the resulting microcapsules. Both formulations were processed under identical spray drying conditions, with an inlet temperature of 120–130 °C, an outlet temperature of 60–70 °C, and a nozzle diameter of 0.5 mm. The resulting microencapsulated powders are shown in Figure 2.

The microencapsulation performance was evaluated using six complementary physicochemical parameters: Fourier-transform infrared spectroscopy (FTIR), particle size analysis (PSA), differential scanning calorimetry (DSC), moisture content (MC), encapsulation efficiency (EE), and scanning electron microscopy (SEM). These analyses assessed intermolecular interactions, formulation homogeneity, thermal behavior, residual moisture characteristics, scopoletin retention during spray drying, and particle morphology. Unlike several previous studies on alginate-chitosan microencapsulation that primarily focused on morphological observations, the present study systematically evaluated the effect of chitosan concentration on multiple physicochemical characteristics of spray-dried *M. citrifolia* microcapsules (Widjastuti et al., 2023).

Visual inspection of the spray-dried powders (Figure 2) revealed distinct differences between the formulations. Formula 2 showed greater particle aggregation and larger particle clusters than Formula 1, likely due to the higher chitosan concentration. Higher chitosan levels may increase feed viscosity, reduce atomization efficiency at a fixed nozzle diameter, resulting in larger droplets and dried particles. Stronger electrostatic interactions between chitosan and alginate at higher polymer concentrations may contribute to greater interparticle association during drying (Chen et al., 2020; Safitri et al., 2022). These aggregation characteristics may affect powder flowability and scopoletin retention. Quantitative FTIR, PSA, DSC, MC,

EE, and SEM results are presented in the following sections.

3.3 Evaluation of Microencapsulation Parameters

Fourier-transform infrared (FTIR) spectroscopy was employed to evaluate the intermolecular interactions during microcapsule formation and to confirm the incorporation of *Morinda citrifolia* extract within the alginate-chitosan polymer matrix. The individual components were first characterized to identify their characteristic absorption bands as references for spectral interpretation (Figure 3). Key bands included the O–H/N–H stretching region (3200–3600 cm⁻¹) shared by both alginate and chitosan, the COO⁻ stretching bands of alginate (1600–1620 cm⁻¹ asymmetric; 1410–1450 cm⁻¹ symmetric), the phosphate group vibrations of STPP (P=O at 1250–1280 cm⁻¹; P–O–P at 980–1050 cm⁻¹), and the aromatic C=C stretching of *M. citrifolia* extract (1510–1620 cm⁻¹), the latter being consistent with its phenolic phytochemical profile, including scopoletin (Li et al., 2025). These bands served as the primary reference points for evaluating intermolecular interactions in the microcapsule formulations.

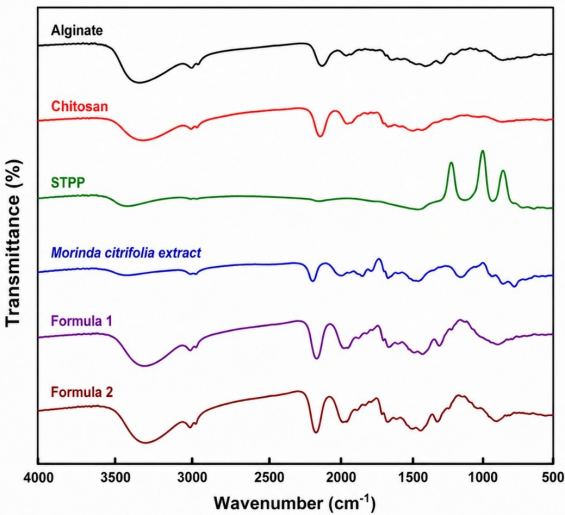


Figure 3. FTIR Spectra of Alginate, Chitosan, STPP, *Morinda citrifolia* Extract, Formula 1, and Formula 2 Microcapsules

In both formulations, the O–H/N–H stretching band appeared in the range of 3385–3435 cm⁻¹, exhibiting broadening and a shift toward lower wavenumbers relative to the individual component spectra, with a greater downward shift observed

Table 8. Encapsulation Efficiency of Scopoletin in Noni Fruit Microcapsules

Formula	Retained Scopoletin Content (%)	Encapsulation Efficiency (%)
F1	0.038 ± 0.001	88.37 ± 1.53
F2	0.031 ± 0.001	72.09 ± 1.88

Notes: Values are presented as mean ± SD (n = 3). Mann-Whitney U test showed a statistically significant difference between formulations (p = 0.043)

in Formula 2. These changes are commonly associated with the formation of intermolecular hydrogen bonds involving the hydroxyl and amino groups of chitosan, the carboxylate groups of alginate, and the phenolic hydroxyl groups present in the extract (Atma et al., 2025). In the carboxylate region, COO⁻ asymmetric stretching was more prominent in Formula 2 at approximately 1598-1600 cm⁻¹, with spectral overlap from the aromatic C=C vibrations of the extract. The COO⁻ symmetric stretching in both formulations appeared in the range of 1411-1416 cm⁻¹, slightly shifted from the characteristic alginate band at 1410-1450 cm⁻¹. These spectral changes suggest electrostatic interactions between negatively charged alginate carboxylate groups and protonated amino groups (-NH₃⁺) of chitosan, supporting the formation of an alginate-chitosan polyelectrolyte complex (PEC) (He et al., 2024). The presence of aromatic C=C bands associated with the extract further supports the incorporation of extract-derived phenolic constituents within the polymer matrix following microencapsulation.

Spectral modifications in the phosphate region further support the role of STPP in the crosslinking process. The P=O stretching band, originally observed at 1250-1280 cm⁻¹ in STPP, appeared in the range of 1249-1252 cm⁻¹ in both formulations, accompanied by a reduction in band intensity in Formula 2. The C-O/P-O-P stretching band shifted upward from 980-1050 cm⁻¹ to approximately 1080-1102 cm⁻¹ in the microcapsule formulations, with a greater shift magnitude in Formula 2. These shifts are consistent with enhanced ionic interactions between the phosphate groups of STPP and the protonated amino groups of chitosan (Song et al., 2025). The greater spectral perturbations observed in Formula 2 are consistent with the higher chitosan concentration, which likely increased the availability of positively charged -NH₃⁺ groups for interaction with both alginate carboxylate and STPP phosphate groups, suggesting the formation of a denser polyelectrolyte network. This interpretation is supported by SEM results showing greater particle aggregation and a more compact surface morphology in Formula 2.

No additional absorption bands were detected in either formulation, indicating that microcapsule formation predominantly involved physical interactions, including hydrogen bonding, electrostatic attraction, and ionic crosslinking, rather than the formation of new covalent bonds. The characteristic absorption bands of M. citrifolia extract remained detectable in both formulations, including C-H stretching in the range of 2920-2930 cm⁻¹, supporting the successful incorporation of the extract within the polymer matrix while preserving its prin-

cipal functional groups. Collectively, the systematic spectral changes observed across the hydroxyl, amine, carboxylate, and phosphate regions provide strong evidence for the successful integration of the extract into the alginate-chitosan-STPP matrix and support the formation of a well-organized PEC network. The more pronounced spectral perturbations observed in Formula 2 suggest stronger intermolecular interactions, which may contribute to differences in physicochemical characteristics. The structural implications of these findings were further evaluated through complementary analyses, including particle size analysis (PSA), differential scanning calorimetry (DSC), moisture content (MC), encapsulation efficiency (EE), and scanning electron microscopy (SEM).

Particle size distribution is a critical physicochemical parameter in microencapsulation, as it directly influences powder flowability, dispersion uniformity, encapsulation efficiency, and the release behavior of bioactive compounds (Fahrudin et al., 2025). The particle size analysis (PSA) revealed clear differences between Formula 1 (F1) and Formula 2 (F2) in terms of particle size range, distribution pattern, and inter-replicate reproducibility, with important implications for processing performance and functional delivery.

F1 had a narrow and reproducible particle size distribution, with D10, D50, and D90 values of 7.65, 16.73, and 32.99 μm, respectively, and low inter-replicate coefficients of variation (0.64-2.84%), indicating uniform droplet formation and consistent drying behavior. The relatively small and homogeneous particles may improve powder flowability, dispersion, and dissolution during pharmaceutical processing. The narrow size distribution may also support encapsulation efficiency and storage stability by promoting more uniform wall formation, reducing surface exposure of encapsulated compounds, and minimizing particle aggregation.

In contrast, F2 showed a broader and more heterogeneous particle size distribution, with D10, D50, and D90 values of 10.75, 33.04, and 275.20 μm, respectively. The higher inter-replicate coefficients of variation (4.96-8.41%) relative to F1 indicate lower particle homogeneity and less uniform particle formation during spray drying.

The observed differences are likely attributable to the higher chitosan concentration in F2. Higher chitosan levels may increase feed viscosity and reduce atomization efficiency, resulting in larger, more heterogeneous droplets during spray drying (Chen et al., 2022). Additionally, stronger electrostatic interactions between the protonated chitosan amino groups and the alginate carboxylate groups at higher polymer concentrations

may promote premature particle incorporation prior to atomization. These findings align with the SEM observations, which showed increased aggregation and denser particle clustering in F2, confirming the impact of chitosan concentration on particle formation and microcapsule structural organization (Chen et al., 2022; Villate et al., 2023).

From a functional perspective, the broader size distribution and higher variability of F2 may adversely affect powder handling, dosage uniformity, and solvent interactions with encapsulated compounds. Both formulations fell within the commonly reported size range for spray-dried microcapsules (1-1000 μm) (Estevinho and López-Rubio, 2024). The size parameters of F1 were consistent with those documented for spray-dried alginate and chitosan-based microcapsules. Villate et al. (2023) reported particle sizes of approximately 5-50 μm for chitosan-coated alginate microcapsules, whereas Lemmadi et al. (2025) and Fahrudin et al. (2025) reported particle sizes of approximately 10-20 μm for spray-dried herbal microcapsules. The lower D90 value and narrower particle size distribution of F1 suggest more controlled particle formation during spray drying. Conversely, the substantially higher D90 value of F2 (275.20 μm) suggests greater particle aggregation, supporting the hypothesis that elevated chitosan concentration reduces atomization efficiency and promotes particle association during drying. Overall, F1 exhibited greater particle homogeneity and reproducibility under the conditions studied. Complete PSA data are presented in Table 6.

Differential scanning calorimetry (DSC) was used to assess thermal transitions and elucidate the intermolecular interactions within the alginate-chitosan microencapsulation system. Thermal characterization provides insights into polymer matrix restructuring, molecular chain mobility, and polyelectrolyte complex (PEC) formation, thereby reflecting the extent of network integration achieved in the microcapsules. Thermograms of the individual components were used to establish reference baselines for comparative interpretation. Sodium alginate showed a broad endothermic transition in the 80-90 $^{\circ}\text{C}$ range, associated with the dehydration of its hydrophilic carboxylate-rich backbone. Chitosan showed a characteristic endothermic peak at approximately 66 $^{\circ}\text{C}$, corresponding to the release of bound moisture, followed by higher-temperature transitions related to polysaccharide decomposition. STPP showed an endothermic transition at approximately 114 $^{\circ}\text{C}$, attributable to dehydration and structural rearrangement of the phosphate network. These thermal profiles align with previous reports on chitosan-STPP systems characterized by ionic gelation and serve as references for interpreting thermal changes following microcapsule formation (Alehosseini et al., 2022).

The most distinct thermal differences were observed between F1 and F2. F1 showed a principal endothermic transition at approximately 143 $^{\circ}\text{C}$, with a substantially higher enthalpy than that of F2 ($\delta H = 2735.61 \text{ J/g}$ vs. 462.77 J/g). This elevated transition temperature, relative to those of the individual polymer components, reflects the reduced polymer chain mobility and a more thermally integrated polyelectrolyte

complex (PEC) network in F1. The higher enthalpy of F1 is due to the cooperative contribution of multiple intermolecular interactions. The electrostatic attraction between protonated chitosan amino groups ($-\text{NH}_3^+$) and alginate carboxylate groups ($-\text{COO}^-$) is the primary driving force for PEC formation, while additional ionic crosslinking mediated by STPP phosphate groups further enhances network connectivity and restricts segmental chain motion (Sreekumar et al., 2018). Consequently, greater thermal energy was required to disrupt the polymer network. This interpretation is supported by FTIR results showing band broadening and wavenumber shifts in the O-H/N-H and $-\text{COO}^-$ stretching regions, indicative of extensive intermolecular interactions within the PEC matrix (Chen et al., 2024).

In contrast, F2 showed an endothermic transition in the lower-temperature region of approximately 60-70 $^{\circ}\text{C}$, with a markedly reduced enthalpy, remaining closer to the thermal profile of pure chitosan. These findings indicate weaker network integration and greater polymer chain mobility than in F1. A higher chitosan concentration may disturb the charge equilibrium essential for proper PEC formation (Atma et al., 2025). Under such conditions, a proportion of protonated amino groups may remain unpaired because of the limited availability of alginate carboxylate groups, resulting in less uniform electrostatic complexation and reduced crosslink density. Consequently, polymer chains retain greater conformational freedom and require less thermal energy for structural transitions, reflected in the substantially lower enthalpy of F2. A higher chitosan concentration may further promote the formation of localized polymer-rich domains rather than a uniformly interconnected network, an interpretation consistent with PSA results showing a broader particle-size distribution and greater variability in F2, as well as SEM observations indicating increased aggregation and reduced particle uniformity (Fahrudin et al., 2025).

Both formulations showed additional thermal transitions in the higher-temperature region ($\sim 295\text{--}300 \text{ }^{\circ}\text{C}$), corresponding to overlapping polysaccharide backbone decomposition events. In F1, this transition appeared broader and more diffuse, suggesting gradual decomposition within an integrated matrix. F2 showed a continued thermal transition up to approximately 390 $^{\circ}\text{C}$, reflecting differences in decomposition behavior that may indicate a less homogeneous matrix structure at higher chitosan concentrations.

Overall, the DSC results indicated that F1 had a more thermally integrated polymeric structure than F2, as evidenced by a higher transition temperature and substantially greater enthalpy. These findings suggest that the alginate-to-chitosan ratio in F1 promoted more effective PEC formation and stronger intermolecular interactions. The DSC results were consistent with those obtained from FTIR, PSA, and SEM analyses, collectively supporting the formation of a more homogeneous and structurally integrated polymer network in F1. From a functional perspective, the enhanced thermal integration of F1 may improve structural stability during spray drying and help retain

thermosensitive constituents, including scopoletin, within the polymer matrix throughout thermal processing. DSC thermograms of the individual components and both formulations are presented in Figure 4.

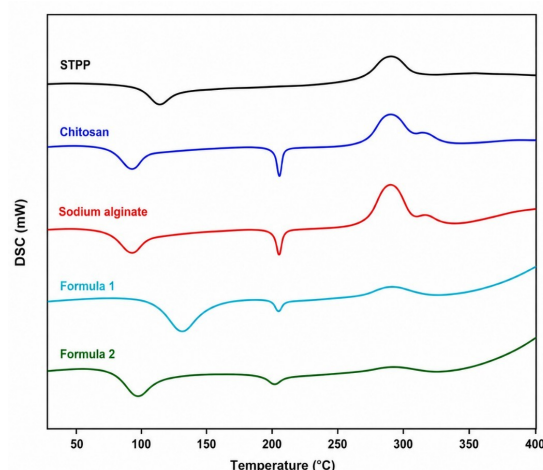


Figure 4. Differential Scanning Calorimetry (DSC) Thermograms of STPP, Chitosan, Sodium Alginate, Formula 1, and Formula 2 Microcapsules

Moisture content is a critical physicochemical parameter in spray-dried microcapsules, as it directly affects powder stability, flowability, storage resistance, and preservation of encapsulated bioactive compounds. The moisture content values for both formulations are listed in Table 7. F1 had a lower moisture content ($3.38 \pm 0.20\%$) than F2 ($4.89 \pm 0.16\%$). Both values remained below the commonly accepted threshold of 5% for spray-dried powders; however, the comparatively higher residual moisture content of F2 may increase its susceptibility to degradation during storage. An independent samples t-test confirmed a significant difference between the formulations ($p < 0.001$), indicating that chitosan concentration significantly affected residual moisture content after spray drying.

The lower moisture content of F1 indicates more effective moisture removal during spray drying and the formation of a stable and homogeneous powder matrix. This finding aligns with the PSA and SEM results, which showed that F1 had smaller, more homogeneous particles with well-defined boundaries. A narrower particle size distribution enhances the surface area-to-volume ratio, which, together with factors such as feed viscosity and particle porosity, can facilitate moisture diffusion from the particle core to the surface during spray drying, thereby minimizing residual water retention in the final powder (Laureanti et al., 2023). Conversely, the broader particle size distribution and greater aggregation observed in F2 likely hindered moisture evaporation during drying and promoted the retention of bound moisture within the polymer matrix.

The difference in moisture content may also reflect the higher chitosan concentration in F2. Chitosan contains hy-

drophilic amino ($-\text{NH}_2$) and hydroxyl ($-\text{OH}$) functional groups that can interact with water molecules through hydrogen bonding. Previous studies have shown that these groups contribute to water absorption and hygroscopicity in chitosan-based systems (Facchi et al., 2023). At higher concentrations, these interactions may increase water retention within the polymer network. This interpretation is further supported by the FTIR findings, which confirm the intermolecular interactions between the hydroxyl and amino groups in the matrix.

From a thermal perspective, the lower moisture content of F1 is consistent with the DSC results, which show a higher transition temperature and substantially greater enthalpy than in F2, reflecting a more integrated and thermally stable polymer matrix. These findings are more appropriately interpreted as co-occurring outcomes of the denser polymer network in F1 rather than as a direct causal relationship. Reduced residual moisture and improved thermal stability may arise simultaneously from the same structural characteristics, namely reduced plasticization effects and limited polymer chain mobility, as reported in polysaccharide-based microencapsulation systems (Halahlah et al., 2023).

Overall, F1 exhibited lower residual moisture content and improved moisture regulation compared with F2 under the applied spray-drying conditions. The moisture content of F1 ($3.38 \pm 0.20\%$) was lower than the values reported for comparable spray-dried herbal microcapsule systems, including *Boesenbergia rotunda* microcapsules prepared using maltodextrin-gum arabic (8.69%) (Fahrudin et al., 2025) and *Artemisia herba-alba* phenolic microcapsules (approximately 4.34%) (Lem-madi et al., 2025). These comparisons suggest that the alginate-chitosan-STPP system effectively reduced residual moisture while maintaining acceptable powder quality. Collectively, these findings indicate that the polymer composition used in F1 resulted in lower residual moisture content and a powder with more favorable physicochemical characteristics following spray drying.

Following physicochemical characterization using Fourier-transform infrared spectroscopy (FTIR), particle size analysis (PSA), differential scanning calorimetry (DSC), and moisture content analysis, the microencapsulation system was quantitatively evaluated based on its encapsulation efficiency (EE) for scopoletin retention. Scopoletin was used as the marker compound for EE determination, providing a direct quantitative indicator of the proportion of the bioactive marker successfully retained within the alginate-chitosan polymer matrix following spray drying. Unlike particle-size or structural characterization, EE provides direct evidence of marker-compound preservation and therefore represents a key performance parameter for evaluating the effectiveness of microencapsulation.

Both formulations retained measurable amounts of scopoletin after spray drying; however, a clear difference in retention capacity was observed between them. Formula 1 (F1) showed a retained scopoletin content of $0.038 \pm 0.001\%$ (w/w), corresponding to an EE of $88.37 \pm 1.53\%$, whereas Formula 2 (F2) showed a retained scopoletin content of $0.031 \pm 0.001\%$ (w/w)

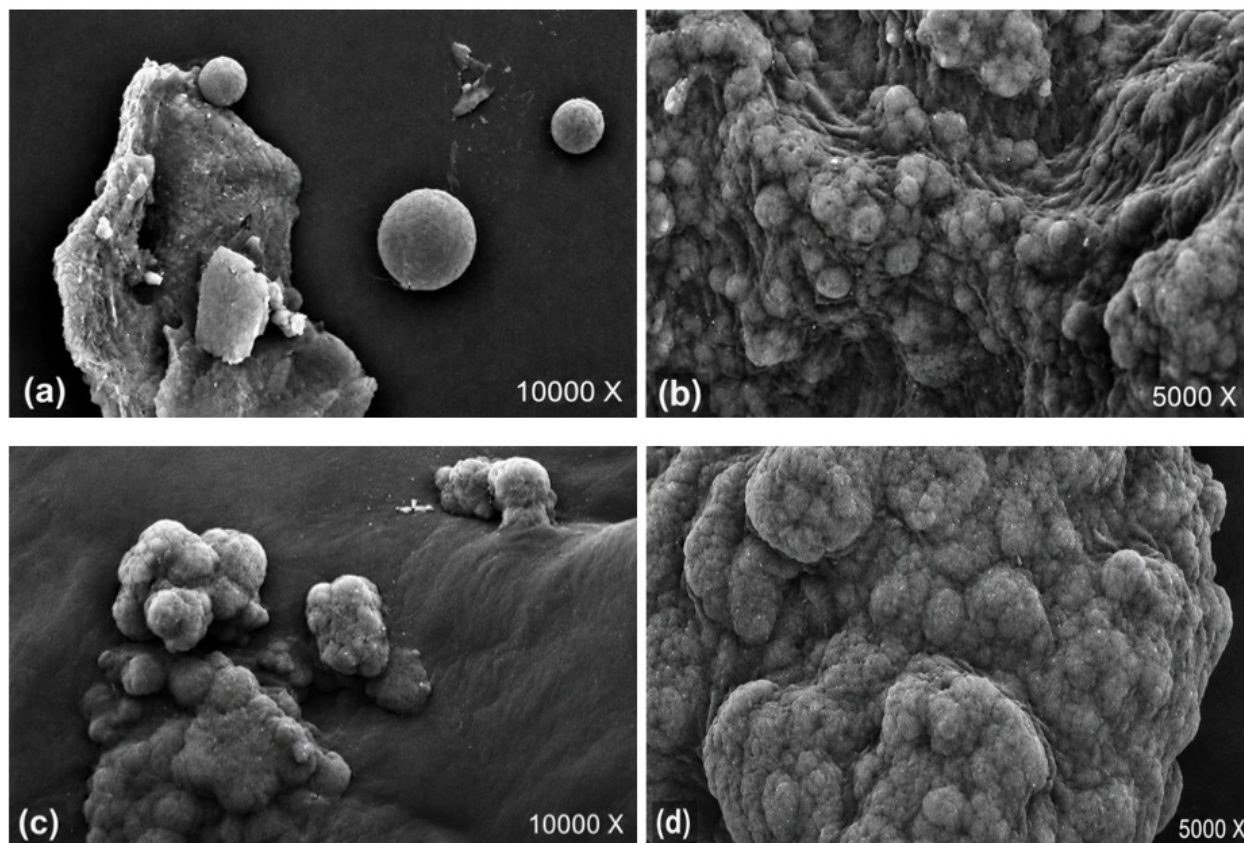


Figure 5. Scanning Electron Microscopy (SEM) Images of *Morinda citrifolia* L. Fruit Microcapsules: Formula 1 (Alginate-Chitosan 1:1, F1) at 10000 $\times$ (a) and 5000 $\times$ (b); Formula 2 (Alginate-Chitosan 1:2, F2) at 10000 $\times$ (c) and 5000 $\times$ (d)

and an EE of $72.09 \pm 1.88\%$ (Table 8). The Mann-Whitney U test confirmed a statistically significant difference between the formulations ($p = 0.043$), indicating that the polymer composition significantly affected scopoletin retention during spray drying. The low coefficients of variation for both F1 (1.53%) and F2 (1.88%) confirmed good analytical repeatability and consistent quantification across replicates.

The markedly higher EE observed in F1 indicates that the alginate-chitosan matrix containing a lower chitosan concentration provided more favorable conditions for scopoletin retention during spray-drying. Lower polymer concentrations reduce feed viscosity, thereby improving atomization efficiency and droplet formation, which subsequently produces smaller and more uniform particles (García-Jiménez et al., 2024). This interpretation is consistent with the PSA findings, which showed that F1 had a narrower particle-size distribution and greater homogeneity than F2, indicating more controlled atomization and a more uniform matrix development. FTIR and DSC analyses further support this mechanism, as F1 showed stronger intermolecular interactions and a more thermally integrated polymer network, both of which are conducive to efficient scopoletin entrapment. Furthermore, the lower moisture content of F1 suggests a denser, more compact

matrix structure that hinders scopoletin diffusion during and after spray drying.

The lower EE observed in F2 may be mechanistically attributed to the higher chitosan concentration, which could disturb the stoichiometric balance between protonated amino groups ($-\text{NH}_3^+$) and alginate carboxylate groups ($-\text{COO}^-$) during PEC formation. Since PEC assembly depends strongly on the charge ratio between the two polymers, deviations from the optimal balance may impair matrix uniformity (Atma et al., 2025). An increased chitosan concentration can increase the viscosity of the precursor solution, which may hinder atomization efficiency and matrix formation during drying. This, in turn, may lead to a wider particle size distribution and diminished compound retention (Busto et al., 2022; García-Jiménez et al., 2024). These interpretations are further supported by SEM observations, which revealed greater aggregation and lower structural uniformity in F2, as well as its higher moisture content, which may reflect a less compact matrix with greater susceptibility to compound loss during processing.

The EE achieved by F1 (88.37%) compares favorably with values reported for other spray-dried herbal microencapsulation systems. Safitri et al. (2022) reported an EE of 51.80% for *Ruellia tuberosa* extract encapsulated in a chitosan-STPP sys-

tem, whereas Fahrudin et al. (2025) reported an EE of 75.06% for *Boesenbergia rotunda* extract microcapsules prepared using maltodextrin-gum arabic as wall materials. Although direct comparisons should be interpreted with caution due to differences in extract composition, polymer systems, and analytical methods, the present findings demonstrate effective retention of scopoletin within the alginate-chitosan-STPP matrix during spray drying.

Overall, the EE values obtained for both formulations confirmed that the alginate-chitosan system effectively retained scopoletin during spray drying, with F1 showing significantly higher marker retention than F2. These findings highlight the influence of polymer concentration on microencapsulation performance (Mardani et al., 2024) and provide quantitative evidence that chitosan concentration is a key formulation variable that governs particle formation, matrix organization, and scopoletin retention. The detailed EE data are presented in Table 8.

Scanning electron microscopy (SEM) was used to evaluate the surface morphology, structural integrity, and interparticle organization of the spray-dried microcapsules. SEM images of Formula 1 (F1) and Formula 2 (F2) at magnifications of 10000 $\times$ (Panels a and c) and 5000 $\times$ (Panels b and d) are shown in Figure 5. F1 showed a heterogeneous morphology comprising spherical and subspherical particles with generally smooth and intact surfaces (Panel a), accompanied by mild interparticle adhesion and relatively homogeneous particle dispersion at lower magnification (Panel b). Surface wrinkling and partial collapse were also observed, consistent with those reported for spray-dried polysaccharide systems (Fahrudin et al., 2025). These features result from rapid solvent evaporation during spray drying, which promotes early shell formation before the residual internal moisture fully diffuses outward. Insufficient mechanical strength in the developing shell leads to particle shrinkage and surface indentation, producing irregular rather than perfectly spherical particles. Despite these surface irregularities, individual particle boundaries remained largely distinguishable, indicating relatively good particle separation and controlled particle formation during spray drying.

F2 showed a more aggregated morphology, with clusters of subspherical particles fused at multiple contact points and less distinct interparticle boundaries at higher magnification (Panel c), resulting in larger, more compact aggregates at lower magnification (Panel d). The higher chitosan concentration in F2 likely increased the feed viscosity, reduced the atomization efficiency, and produced larger droplets during spray drying (Zhang et al., 2020a). The larger droplet size may have contributed to non-uniform moisture removal, promoting shell contraction and increased interparticle contact. Additionally, a higher chitosan concentration may have reduced the efficiency of electrostatic complexation by limiting the relative availability of alginate carboxylate groups, thereby favoring the formation of localized polymer-rich domains and increasing particle cohesion during solvent evaporation (Zhang et al., 2020b).

The SEM observations were consistent with the findings

of the complementary analyses. The increased aggregation and reduced structural discreteness of F2 were associated with a wider particle-size distribution in PSA, a lower transition enthalpy in DSC, higher moisture content, and reduced encapsulation efficiency (72.09% vs. 88.37% for F1). Collectively, these findings indicate that the lower chitosan concentration in F1 promoted a more uniform polyelectrolyte complex (PEC) network, thereby improving scopoletin retention. From a practical standpoint, the greater morphological discreteness of F1 may also support better powder flowability and redispersibility during downstream processing.

The observed morphological characteristics can be explained by the proposed interaction mechanism within the microencapsulation system, as illustrated in Figure 6. The figure presents a schematic representation of polyelectrolyte complex (PEC) formation involving alginate, chitosan, and sodium tripolyphosphate (STPP) during *Morinda citrifolia* extract encapsulation. The formation of this system is primarily driven by electrostatic interactions between the protonated amine groups ($-\text{NH}_3^+$) of chitosan and the negatively charged carboxylate groups ($-\text{COO}^-$) of alginate, which form the primary structure of the polymeric network. As depicted in Figure 6, the phosphate groups (PO_4^{3-}) of STPP may interact with the alginate carboxylate groups and protonated amino groups of chitosan, forming multivalent ionic bridges that enhance the crosslinking density and contribute to the development of a more integrated three-dimensional polymer matrix (Gierszewska et al., 2018; Bhatia et al., 2024). The ionic bridging role of STPP is considered particularly important in Formula 1, where the ratio of alginate, chitosan, and STPP may have promoted more balanced polymer interactions and greater matrix integration, thereby contributing to the improved encapsulation efficiency observed in this formulation (Bhatia et al., 2024).

As illustrated in Figure 6, the *Morinda citrifolia* extract is physically entrapped within this interconnected polymeric network. The retained extract may be further stabilized by secondary interactions, including hydrogen bonding between the hydroxyl and carbonyl groups of the polymer chains and the bioactive constituents of the extract. This interpretation is consistent with the FTIR analysis, which demonstrated characteristic shifts in the $-\text{OH}$, $-\text{NH}$, and $\text{C}=\text{O}$ absorption regions, supporting the occurrence of intermolecular interactions and their contribution in stabilizing the polymer matrix (Gierszewska et al., 2018).

The proposed interaction mechanism is also consistent with the SEM observations, which show a relatively integrated particle morphology and moderate aggregation, indicating the successful formation of a dense and interconnected polymer matrix. Furthermore, the enhanced thermal stability observed in the DSC analysis supports the interpretation that reduced molecular mobility within the crosslinked network contributed to the structural integrity of microcapsules (Bhatia et al., 2024). These interactions may also explain the higher encapsulation efficiency observed in Formula 1, suggesting that a more organized polymer matrix promoted more effective retention of

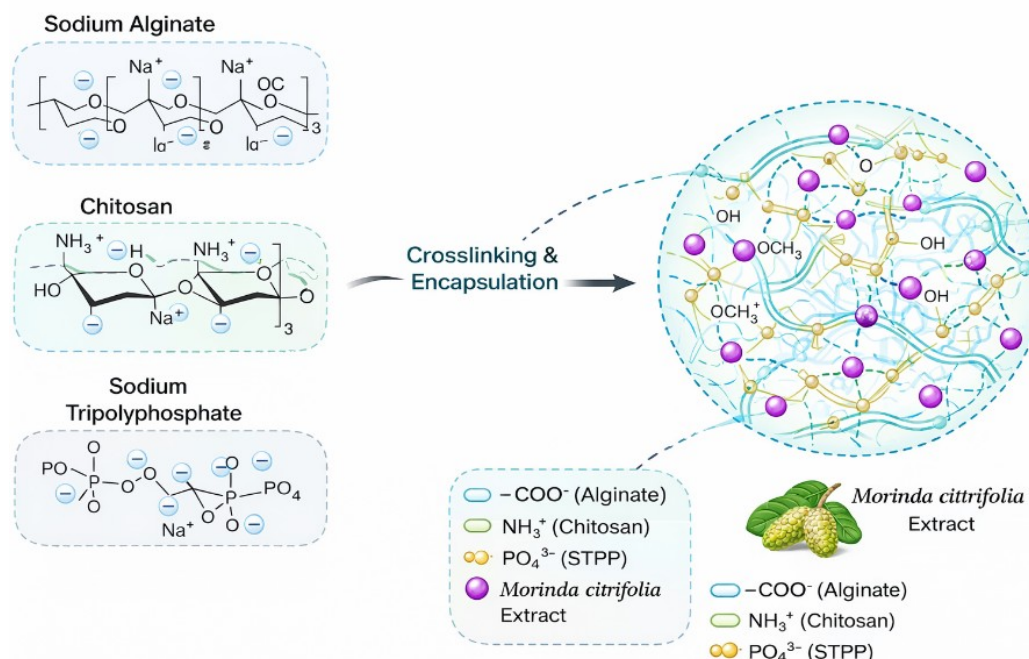


Figure 6. Schematic Illustration of the Proposed Interaction Mechanism Between Chitosan, Alginate, and Sodium Tripolyphosphate (STPP) During Microcapsule Formation and Encapsulation of *Morinda citrifolia* Extract

scopoletin during spray drying.

Collectively, the electrostatic, ionic bridging, and hydrogen bonding interactions described above provide a molecular-level explanation for the formation and physicochemical performance of the alginate-chitosan-STPP microcapsule system, with the crosslinking density potentially contributing to the matrix compactness, structural integrity, and compound retention within the polymer network (Gierszewska et al., 2018). These properties may support powder handling, storage stability, and formulation reproducibility in spray-dried oral herbal formulations. However, several limitations of this study should be acknowledged. The physicochemical characteristics reported in this study were evaluated immediately after preparation; therefore, their stability during long-term storage remains unclear. In addition, the *in vitro* release behavior and post-encapsulation antihyperlipidemic activity were not assessed. Future studies should address these aspects further to establish the practical applicability of the developed microencapsulation system.

4. CONCLUSIONS

The alginate-chitosan-STPP polyelectrolyte complex matrix effectively encapsulated *Morinda citrifolia* extract through electrostatic interactions and hydrogen bonding. Chitosan concentration significantly influenced microcapsule characteristics, with Formula 1 (1% w/v chitosan) outperforming Formula 2 (2% w/v) in encapsulation efficiency (88.37% vs. 72.09%, $p = 0.043$), moisture content (3.38% vs. 4.89%), particle size distribution (D90: 32.99 vs. 275.20 μm), particle morphol-

ogy, and thermal characteristics. These findings indicate that optimizing the polymer charge balance, rather than increasing the chitosan concentration, is essential for developing efficient spray-dried alginate-chitosan microcapsules. Thus, Formula 1 represents a promising carrier system for developing stable and standardized *Morinda citrifolia*-based oral formulations. As functional performance parameters such as loading capacity, release kinetics, and storage stability were not evaluated in this study, future work should address these aspects alongside *in vivo* biological performance to further establish the applicability of this delivery system.

5. ACKNOWLEDGEMENT

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REFERENCES

Alehosseini, E., H. Shahiri Tabarestani, M. S. Kharazmi, and S. M. Jafari (2022). Physicochemical, Thermal, and Morphological Properties of Chitosan Nanoparticles Produced by Ionic Gelation. *Foods*, **11**(23); 3841

- AOAC International (2016). *Official Methods of Analysis of AOAC International*. AOAC International, Gaithersburg, MD, 20 edition
- Asita, N., M. S. Zubair, and Y. Syukri (2023). Formulasi Self-Nanoemulsifying Drug Delivery System (SNEDDS) yang Memanfaatkan Tanaman Obat: Narrative Review. *Jurnal Sains Farmasi & Klinis*, **10**(2); 184–196. (in Indonesia)
- Atma, Y., A. Sadeghpour, B. S. Murray, and F. M. Goycoolea (2025). Chitosan-Alginate Polyelectrolyte Complexes for Encapsulation of Low Molecular Weight Fish Bioactive Peptides. *Food Hydrocolloids*, **160**; 110789
- Bhatia, S., Y. A. Shah, A. Al-Harrasi, M. Jawad, T. S. Khan, E. Koca, and L. Y. Aydemir (2024). Tuning the Structure and Physiochemical Properties of Sodium Alginate and Chitosan Composite Films Through Sodium Tripolyphosphate (STPP) Crosslinking. *International Journal of Biological Macromolecules*, **264**(Part 2); 130463
- Busto, M. D., Y. González-Temiño, S. M. Albillos, S. Ramos-Gómez, M. C. Pilar-Izquierdo, D. Palacios, and N. Ortega (2022). Microencapsulation of a Commercial Food-Grade Protease by Spray Drying in Cross-Linked Chitosan Particles. *Foods*, **11**; 2077
- Chemat, F., M. A. Vian, and G. Cravotto (2012). Green Extraction of Natural Products: Concept and Principles. *International Journal of Molecular Sciences*, **13**(7); 8615–8627
- Chen, B., C. Ai, Y. He, Y. Zheng, L. Chen, and H. Teng (2024). Preparation and Structural Characterization of Chitosan–Sodium Alginate Nanocapsules and Their Effects on the Stability and Antioxidant Activity of Blueberry Anthocyanins. *Food Chemistry: X*, **23**; 101744
- Chen, G.-L., H.-Y. Cai, J.-P. Chen, R. Li, S.-Y. Zhong, X.-J. Jia, X.-F. Liu, and B.-B. Song (2022). Chitosan/Alginate Nanoparticles for the Enhanced Oral Antithrombotic Activity of Clam Heparinoid from the Clam *Coelomactra antiquata*. *Marine Drugs*, **20**(2); 136
- Chen, P., F. Xie, F. Tang, and T. McNally (2020). Unexpected Plasticization Effects on the Structure and Properties of Polyelectrolyte Complexed Chitosan/Alginate Materials. *ACS Applied Polymer Materials*, **2**(7); 2957–2966
- Chendjou, S. M. S., R. K. T. Tsatsop, T. A. S. Zome, N. Amba, and S. B. Kenmogne (2025). A Screening Study of the Spray Drying Process for the Microencapsulation of Phenolic Compounds from *Sarcocephalus latifolius* (Sm.) Bruce Root Extracts Using a Two-Level Factorial Design. *International Research Journal of Pure and Applied Chemistry*, **26**(3); 82–94
- Conzatti, G., D. Faucon, M. Castel, F. Ayadi, S. Cavalie, and A. Tournette (2017). Alginate/Chitosan Polyelectrolyte Complexes: A Comparative Study of the Influence of the Drying Step on Physicochemical Properties. *Carbohydrate Polymers*, **172**; 142–151
- Dewi, R. T., G. Primahana, A. W. Septama, M. Angelina, L. Meilawati, S. Fajriah, and G. F. Swandiny (2022). Quality Control Standardization of Indonesian Noni Fruit (*Morinda citrifolia*) Extract and Evaluation of Their Angiotensin-Converting Enzyme Inhibitory Activity. *Pharmacia*, **69**(3); 709–717
- Díaz-Montes, E. (2023). Wall Materials for Encapsulating Bioactive Compounds via Spray-Drying: A Review. *Polymers*, **15**(12); 2659
- Eghbal, N. and R. Choudhary (2018). Complex Coacervation: Encapsulation and Controlled Release of Active Agents in Food Systems. *LWT–Food Science and Technology*, **90**; 254–264
- Estevinho, B. N. and A. López-Rubio (2024). Recent Advances in Encapsulation for Food Applications. *Foods*, **13**(4); 579
- Facchi, D. P., A. P. Gerola, D. B. Scariot, L. C. Figueiredo, H. D. M. Follmann, A. C. de Oliveira, D. Lazarin-Bidóia, C. V. Nakamura, E. G. Bonafé, and A. F. Martins (2023). Chitosan, Chitosan Derivatives, and Chitosan-Based Nanocomposites: Eco-Friendly Materials for Advanced Applications. *Frontiers in Chemistry*, **11**; 1327426
- Fahrudin, F. I., S. Phongthai, and P. Intipunya (2025). Enhancing Stability of *Boesenbergia rotunda* Bioactive Compounds: Microencapsulation via Spray-Drying and Its Physicochemical Evaluation. *Foods*, **14**(15); 2699
- Forcepta, C., K. Nisa, and D. I. Anggraini (2021). Manfaat Buah Mengkudu (*Morinda citrifolia*) dalam Menurunkan Kadar Kolesterol Total dan LDL pada Penyakit Dislipidemia. *Medula*, **11**(3); 253–258. (in Indonesia)
- García-Jiménez, J. R., M. L. Luna-Guevara, J. J. Luna-Guevara, L. A. Conde-Hernández, M. E. Ramos-Cassellis, and H. Hernández-Cocoletzi (2024). Microencapsulation of *Tecoma stans* Extracts: Bioactive Properties Preservation and Physical Characterization Analysis. *Foods*, **13**(7); 1001
- Gierszewska, M., J. Ostrowska-Czubenko, and E. Chrzanowska (2018). pH-Responsive Chitosan/Alginate Polyelectrolyte Complex Membranes Reinforced by Tripolyphosphate. *European Polymer Journal*, **101**; 282–290
- Halalah, A., V. Piironen, K. S. Mikkonen, and T. M. Ho (2023). Polysaccharides as Wall Materials in Spray-Dried Microencapsulation of Bioactive Compounds: Physicochemical Properties and Characterization. *Critical Reviews in Food Science and Nutrition*, **63**(24); 6983–7015
- He, M., Y. Zheng, J. Shen, J. Shi, Y. Zhang, Y. Xiao, Y. Bai, Y. Zhang, X. Zhao, and Y. Qin (2024). Chitosan/Sodium Alginate Multilayer pH-Sensitive Films Based on Layer-by-Layer Self-Assembly for Intelligent Packaging. *Journal of Renewable Materials*, **12**(2); 215–233
- Hou, S., D. Ma, S. Wu, Q. Hui, and Z. Hao (2025). *Morinda citrifolia* L.: A Comprehensive Review on Phytochemistry, Pharmacological Effects, and Antioxidant Potential. *Antioxidants*, **14**(3); 295
- Ifitah, E. D., A. N. Putri, and A. K. Soesantyo (2025). Edible Coating of Cherry Tomatoes (*Solanum lycopersicum*) Based on Chitosan Nanoparticles (NPCh) and Mint (*Mentha piperita*) Essential Oil with Addition of Aloe Vera Gel. *Science and Technology Indonesia*, **10**(2); 336–349
- Indonesian Ministry of Health (2017). *Indonesian Herbal Pharmacopoeia*. Ministry of Health Republic of Indonesia, Jakarta,

- Indonesia, 2 edition
- International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (2023). ICH Harmonised Guideline: Validation of Analytical Procedures Q2(R2). Technical report
- Jayanudin and Rochmadi (2017). Pengaruh Perbedaan Bahan Penyalut terhadap Efisiensi Enkapsulasi Oleoresin Jahe Merah. *ALCHEMY Jurnal Penelitian Kimia*, **13**(2); 275–287. (in Indonesia)
- Jha, A. K. and N. Sit (2022). Extraction of Bioactive Compounds from Plant Materials Using Combination of Various Novel Methods: A Review. *Trends in Food Science & Technology*, **119**; 579–591
- Joshi, H., V. Gajera, and A. Katariya (2021). Review on Scopoletin: A Phenolic Coumarin with Its Medicinal Properties. *International Journal of Pharmaceutical Sciences and Research*, **12**(7); 3567–3580
- Kumar, K. S., N. Kiruthiga, R. Arivukkarasu, S. D. Kumar, and M. Sureka (2024). Isolation and Quantification of Scopoletin from Leaves and Marketed Formulation of *Morinda citrifolia* L. *Asia Pacific Journal of Pharmacotherapy and Toxicology*, **4**; 18–25
- Länger, R., E. Stöger, W. Kubelka, and K. Helliwell (2018). Quality Standards for Herbal Drugs and Herbal Drug Preparations-Appropriate or Improvements Necessary? *Planta Medica*, **84**(6–7); 350–360
- Laureanti, E. J. G., T. S. Paiva, L. M. de Matos Jorge, and R. M. M. Jorge (2023). Microencapsulation of Bioactive Compound Extracts Using Maltodextrin and Gum Arabic by Spray and Freeze-Drying Techniques. *International Journal of Biological Macromolecules*, **253**; 126969
- Lemmadi, S., E. Dumas, F. Adoui, G. Agusti, S. Vessot-Crastes, W. Medfai, and A. Gharsallaoui (2025). Spray-Drying Microencapsulation of *Artemisia herba-alba* Phenolic Extract: Physicochemical Properties, Structural Characterization, and Bioactivity. *Molecules*, **30**(19); 3904
- Li, Q., J. Chai, S. Deng, J. Xu, Y. Feng, R. Yang, and W. Li (2025). The Comparison of the Profile of Phenolic Compounds in Noni (*Morinda citrifolia* L.) Fruit by Different Drying Methods. *Foods*, **14**(8); 1398
- Mardani, M., S. Siahtiri, M. Besati, M. Baghani, M. Baniassadi, and A. Mahdavi Nejad (2024). Microencapsulation of Natural Products Using Spray-Drying: An Overview. *Journal of Microencapsulation*, **41**(7); 649–678
- Mardiyanto, M., I. Solihah, and T. G. Jaya (2020). The Chitosan-Sodium Alginate Submicro Particles Loading Herbal of Ethanolic Extract of Leaves *Senna alata* L. for Curing of Bacterial Infection on Skin. *Science and Technology Indonesia*, **5**(3); 85–89
- Mehta, N., P. Kumar, A. K. Verma, P. Umaraw, Y. Kumar, O. P. Malav, A. Q. Sazili, R. Domínguez, and J. M. Lorenzo (2022). Microencapsulation as a Noble Technique for the Application of Bioactive Compounds in the Food Industry: A Comprehensive Review. *Applied Sciences*, **12**(3); 1424
- Nemickaite, E., U. Zlabiene, A. Mazurkeviciute, M. Marksa, and J. Bernatoniene (2025). Formulation of W/O/W Emulsion-Based Chitosan-Alginate Microcapsules for Encapsulation of Cannabidiol and *A. annua* L. Extract Containing Luteolin and Apigenin: A Response Surface Optimization Approach. *Pharmaceutics*, **17**(3); 309
- Nguyen, C. T., K. Nguyen Di, H. C. Phan, T. C. Kha, and H. C. Nguyen (2024). Microencapsulation of Noni Fruit Extract Using Gum Arabic and Maltodextrin: Optimization, Stability and Efficiency. *International Journal of Biological Macromolecules*, **269**(Part 2); 132217
- Porras-Yepes, M., A. C. Rojas-Arias, and J. Hernández-Torres (2020). Microencapsulation of Bioactive Compounds: Techniques and Applications in Pharmaceutical and Food Industries. *Journal of Food Process Engineering*, **43**(8); e13417
- Pudziuvelytė, L., M. Marksa, V. Jakštas, L. Ivanauskas, A. Laukevičienė, J. Bernatoniene, and D. M. Kopustinskiene (2022). Influence of Technological Factors on the Quality of Chitosan Microcapsules with *Boswellia serrata* L. Essential Oil. *Molecules*, **27**(13); 4217
- Pudziuvelytė, L., E. Petrauskaitė, J. Stabrauskienė, and J. Bernatoniene (2025). Spray-Drying Microencapsulation of Natural Bioactives: Advances in Sustainable Wall Materials. *Pharmaceutics*, **18**(7); 963
- Purwaningsari, D., S. Tehupuring, N. Pranitasari, S. Hayaza, L. Dewi, and D. Ardiana (2021). The Antioxidant Effect of Noni Fruits Extract on HDL and LDL Level of Wistar Rat Induced by High Fat Diet. *Indian Journal of Forensic Medicine & Toxicology*, **15**(2); 2235–2239
- Safitri, A., D. R. T. Sari, and C. Mahdi (2022). Microencapsulation of *Ruellia tuberosa* L. Aqueous Root Extracts Using Chitosan–Sodium Tripolyphosphate and Their In Vitro Biological Activities. *Scientifica*, **2022**; 9522463
- Saqueti, B. H., E. S. Alves, M. C. Castro, P. D. dos Santos, N. Sinosaki, C. E. Senes, J. V. Visentainer, and O. O. Santos (2021). Shelf Life of Bioactive Compounds from Acerola Pulp (*Malpighia* Spp.) through Freeze-Drying and Microencapsulation. *Journal of the Brazilian Chemical Society*, **32**; 2009–2016
- Song, Y., Y. Gu, A. Ren, X. Li, S. Wu, Y. Gong, and Y. Luo (2025). Optimization and Comprehensive Characterization of the Microencapsulation Process for Taro Essence. *Foods*, **14**(5); 754
- Sreekumar, S., F. M. Goycoolea, B. M. Moerschbacher, and G. R. Rivera-Rodriguez (2018). Parameters Influencing the Size of Chitosan-TPP Nano- and Microparticles. *Scientific Reports*, **8**; 4695
- Sulistiawanti, N., H. Hairunnisa, N. Ningrum, and S. Fitriyanti (2025). Review: Perbedaan Penggunaan Pelarut terhadap Nilai Rendemen yang Dihasilkan dengan Berbagai Metode Ekstraksi dalam Proses Ekstraksi. *An-Najat*, **3**(3); 213–231
- Tasfiyati, A. N., L. D. Antika, R. T. Dewi, A. W. Septama, A. Sabarudin, and T. Ernawati (2022). An Experimental Design Approach for the Optimization of Scopoletin Extraction from *Morinda citrifolia* L. Using Accelerated Solvent Extraction. *Talanta*, **238**; 123010

- Undale, V., N. Chaudhari, S. Bharud, and S. M. Firdous (2026). Comparative Analysis of Traditional and Modern Extraction Methods. In *Advances in Extraction Science*. CRC Press, pages 28–33
- Villate, A., M. San Nicolas, M. Olivares, O. Aizpurua-Olaizola, and A. Usobiaga (2023). Chitosan-Coated Alginate Microcapsules of a Full-Spectrum Cannabis Extract: Characterization, Long-Term Stability, and In Vitro Bioaccessibility. *Pharmaceutics*, **15**(3); 859
- Widjastuti, T., L. Nurlaeni, A. Hasbuna, I. Setiawan, I. Yudaas-mara, and W. Tanwiriah (2023). The Microencapsulation of Noni Fruit Extract (*Morinda citrifolia* L.) with Maltodextrin and Its Implementation as Feed Additive on Carcass Quality and Histology of Intestinal Sentul Chicken. *International Journal on Advanced Science, Engineering and Information Technology*, **13**(1); 104–109
- Wigati, D. and K. Anwar (2017). Hypotensive Activity of Ethanolic Extracts of *Morinda citrifolia* L. Leaves and Fruit in Dexamethasone-Induced Hypertensive Rat. *Journal of Evidence-Based Complementary & Alternative Medicine*, **22**(1); 107–111
- Wu, M., W. Zhang, H. Song, Y. Hu, and W. Li (2025). pH-Responsive Chitosan-Alginate Microcapsules as a Delivery System for Food-Medicine Homologous Plant Bioactives in Lung Cancer Therapy. *Food Bioscience*, **71**; 107364
- Zhang, C., X. Chen, J. Zhang, P. A. Kilmartin, and S. Y. Quek (2020a). Fabrication of Spray-Dried Microcapsules Containing Noni Juice Using Blends of Maltodextrin and Gum Acacia: Physicochemical Properties of Powders and Bioaccessibility of Bioactives During In Vitro Digestion. *Foods*, **9**(9); 1316
- Zhang, Y., L. Cui, X. Che, H. Zhang, N. Shi, C. Li, Y. Chen, and W. Kong (2020b). Preparation and Properties of Chitosan-Based Microspheres by Spray Drying. *Food Science & Nutrition*, **8**(3); 1374–1383