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# Pectin-encapsulated amorphous naringenin with enhanced aqueous solubility prepared by simple coacervation and precipitation

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This study presents a sustainable approach for enhancing the solubility and processability of naringenin, a poorly water-soluble bioactive flavonoid, through fruit peel-inspired encapsulation. Amorphous naringenin nanoparticles were first prepared by anti-solvent precipitation using polyvinylpyrrolidone (PVP) as a crystallization inhibitor. Subsequently, ethanol-induced coacervation with pectin, a biopolymer abundant in citrus peel, was employed to encapsulate and recover the nanoparticles under ambient conditions, avoiding energy-intensive drying methods. The process yielded microcapsules with significantly reduced crystallinity, as confirmed by X-ray diffraction, and improved dissolution behavior in both deionized water and simulated intestinal fluid compared to pristine naringenin. High-methoxy pectin demonstrated superior encapsulation efficiency (up to 76%) and produced more compact and spherical microcapsules than low-methoxy pectin, highlighting the influence of pectin structure on encapsulation performance. This simple, low-energy, and scalable technique not only improves the functional properties of naringenin but also valorizes pectin from fruit by-products, providing a sustainable strategy for the development of functional food ingredients.

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## Sustainability spotlight

Poor water solubility limits the bioavailability and functional application of many natural flavonoids such as naringenin. This work presents an environmentally friendly strategy to overcome this challenge by employing pectin-based encapsulation derived from fruit peel by-products. The process uses a mild, solvent-driven coacervation and precipitation method under ambient conditions, avoiding energy-intensive drying and synthetic polymers. This green and scalable approach not only improves ingredient performance but also valorizes agricultural residues and reduces chemical waste. The work supports the principles of resource efficiency and circular economy, aligning with the United Nations Sustainable Development Goals (SDG 9: Industry, Innovation and Infrastructure; SDG 12: Responsible Consumption and Production; SDG 13: Climate Action).

## 1. Introduction

Fruit peels are widely available agricultural by-products generated in large quantities during fruit processing, accounting for 20–50% of the total fruit weight depending on the type. While often discarded, they are rich in bioactive compounds such as flavonoids, pectin, and phenolic acids, with flavonoid levels frequently higher than in the pulp or juice, making them a valuable but underutilized source for food and health-related applications.<sup>1,2</sup>

Among these phytochemicals, flavonoids like naringin and its aglycone, naringenin, are notable for their antioxidant, anti-

inflammatory, and cardioprotective effects.<sup>3,4</sup> However, despite their pharmacological potential, their application is often limited by poor oral bioavailability. Naringin is poorly absorbed through the gastrointestinal tract and is typically hydrolyzed to naringenin, which itself has low solubility and limited absorption, with only about 15% being bioavailable.<sup>4,5</sup>

Various strategies have been developed to improve flavonoid solubility and bioavailability, including nanocrystallization to reduce particle size and solid dispersion to lower crystallinity. Encapsulation further enhances solubility and protects flavonoids from environmental stressors, while enabling controlled release.<sup>6</sup> A variety of microencapsulation methods such as spray drying, freeze drying, coacervation, liposomes, ionic gelation, and emulsions have been employed, each with distinct advantages and limitations.<sup>6–11</sup>

A previous study explored a simple method for enhancing naringenin solubility, with solid dispersion selected for its ability to reduce crystallinity, a major barrier to dissolution,

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while requiring fewer excipients and simpler preparation compared to other techniques.<sup>12</sup> The work demonstrated that dissolving a polymer–flavonoid mixture (PVP/naringenin–hesperetin, 80/20 w/w) in ethanol followed by solvent evaporation yielded a solid dispersion with significantly reduced crystallinity and markedly improved solubility in phosphate buffer (pH 6.8) containing 2% Tween 20.<sup>12</sup> In this context, solvent evaporation plays a critical role in obtaining the solid product, with techniques such as spray drying or freeze-drying commonly employed. However, these methods are energy-intensive, requiring either high heat or reduced pressure. Alternative approaches such as precipitation or coagulation may be considered to remove excess solvent prior to drying. In this study, we explore the potential of pectin, a naturally abundant polymer found in fruit peels as a low-cost, functional encapsulation material.

Pectin offers multiple functional benefits, including emulsification, gelling, and binding, and has been used in encapsulation *via* techniques like spray drying and complex coacervation<sup>13–18</sup> With citrus peel containing up to 30% pectin on a dry basis,<sup>19–21</sup> extracted pectin aligns well with circular economy strategies. As an encapsulation matrix, it can enhance the stability and expand the applicability of sensitive bioactive compounds.<sup>6,22</sup>

In this work, naringenin was prepared as an amorphous nanoparticle using PVP as a crystallization inhibitor. The process involved dissolving naringenin and PVP in ethanol, followed by introducing the solution into water as a non-solvent. Pectin was added to the aqueous phase, resulting in the spontaneous formation of encapsulated naringenin. The encapsulation efficiency of pectin and its ability to coagulate naringenin nanoparticles were evaluated to facilitate their separation prior to drying. The final product was characterized in terms of particle size, crystallinity, and morphology, while its dissolution behavior was assessed in both deionized water and simulated intestinal fluid.

## 2. Materials and methods

### 2.1 Materials

Naringenin (90%), pectin (high ester, DE 65–68%, from apple), pectin (low ester, DE 30–35%, from apple), and bile salt from pig were purchased from Myskinrecipes, Thailand. Ethanol (95%) was purchased from RCI Labscan, Thailand. KCl, KH<sub>2</sub>PO<sub>4</sub>, NaHCO<sub>3</sub>, MgCl<sub>2</sub> and (NH<sub>4</sub>)<sub>2</sub>CO<sub>3</sub>, NaCl, CaCl<sub>2</sub> were purchased from Carlo Erba, Italy.

### 2.2 Solubility test

Naringenin solubility was evaluated in 95% ethanol and deionized water. Initially, 10 mg of naringenin was added to 5 mL of each solvent in separate vials, followed by stirring for 2 hours at room temperature. Additional naringenin was incrementally added until visible precipitation occurred, indicating saturation. The suspensions were then filtered through a 0.22 μm nylon syringe filter to remove undissolved particles. The resulting clear solutions were dried in an oven at 70 °C

overnight to evaporate the solvent. The dried residue was weighed, and solubility was calculated and reported in mg mL<sup>-1</sup>.

### 2.3 Preparation of naringenin reprecipitate and amorphous naringenin nanoparticle

To reprecipitate naringenin, ethanolic solution of naringenin was prepared by dissolving 100 mg of naringenin in 1 mL of warm 95% ethanol. The naringenin ethanolic solution was instantly dropped into 19 mL DI water. To prepare amorphous naringenin nanoparticle (NGPVP), 10 mg naringenin and 10 mg PVP was dissolved in 1 mL of 95% ethanol. The mixture was then added dropwise into 19 mL DI water. The morphology of the obtained products was characterized using SEM (Hitachi, SU5000). SEM specimens were prepared by dropping the obtained sample onto aluminium foil and then drying it in an oven overnight. The samples were coated using Au sputter coater (Quorum, Q15R S, USA) at sputter current 15 mA, sputter time 80 s. SEM images were acquired at an accelerating voltage of 5 kV with a spot intensity of 15.

### 2.4 Pectin encapsulated naringenin particle preparation

A stock of 2% w/v pectin solution was prepared by dissolving pectin in DI water. The pectin solution was heated at 100 °C and stirred for 2 hours or until a clear solution was obtained, then the solution was cooled down to room temperature. Pectin solutions at concentrations of 0.005–0.2% w/v were prepared, diluted from stock solution. In a separated container, a mixture of 1% w/v naringenin and 1% w/v PVP in 95% ethanol was prepared. Then, 5 mL of ethanolic mixture was dropped into 95 mL of a stirring pectin solution at each concentration. The naringenin core/pectin shell was formed spontaneously and precipitated after the solution was left stand still for 10 min. The supernatant was taken to the naringenin concentration evaluation. The supernatant was diluted using ethanol for complete dissolution of naringenin. The encapsulation efficiency was calculated using an equation below, where NG<sub>initial</sub> is the initially added amount of naringenin and NG<sub>left</sub> is the amount of naringenin remaining in the supernatant.

$$\text{Encapsulation efficiency (\%)} = \frac{\text{NG}_{\text{init}}(\text{mg}) - \text{NG}_{\text{left}}(\text{mg})}{\text{NG}_{\text{init}}(\text{mg})} \times 100$$

The encapsulated products were analyzed the particle size, polydispersity index (PDI), using Zetasizer (Nano ZS ZEN3600, Malvern Panalytical). The powder samples were prepared by decanting the supernatant, followed by freeze-drying. The resulting encapsulated products were designated as NGPVP-HM and NGPVP-LM, where HM and LM indicate the use of high-methoxy pectin and low-methoxy pectin, respectively, during the encapsulation process. The powder products were then subjected to X-ray diffractometry and FTIR spectroscopy. The crystallinity was evaluated using X-ray diffractometer (D8 Advance, Bruker, Germany). Powder-XRD measurements were performed using Cu K $\alpha$  radiation and operating at 2000 W with 30 mA and 40 kV. Samples were measured in the 2 $\theta$  range of 5–



45° at a step interval of 0.02° at ambient temperature. The chemical functionality was evaluated using Attenuated Total Reflectance Fourier-Transform Infrared (ATR-FTIR) spectroscopy, operated at room temperature in the range between 450 and 4000  $\text{cm}^{-1}$ .

### 2.5 Determination of soluble naringenin content

The sample was filtered using a syringe filter with 0.2  $\mu\text{m}$  pore size, then diluted by ethanol and DI water to the appropriate dilution. The final concentration of ethanol was fixed to 50% v/v to prevent further precipitation of naringenin during the measurement. The sample was analyzed using UV-vis spectrophotometer and a quartz cuvette, measuring at 288 nm. The amount of naringenin was calculated from a standard curve prepared from known concentration of naringenin.

### 2.6 Dissolution profile evaluation

The dissolution profiles of the powder samples were evaluated using a dialysis membrane technique. The samples were loaded into dialysis bags with a molecular weight cutoff of 6–8 kDa. The amount of powder introduced into each dialysis bag was adjusted to be equivalent to 5 mg of naringenin. For the encapsulated samples (NGPVP-HM and NGPVP-LM), the actual naringenin content was calculated based on their respective encapsulation efficiencies. In the case of NGPVP, the naringenin content was determined by redissolving the sample in 50% (v/v) ethanol, followed by spectrometric analysis as described in Section 2.5. The dialysis bag was immersed in a 5 mL stirred medium including DI water or simulated intestinal fluid (SIF). The concentration of naringenin outside the dialysis bag was measured at time intervals and plotted as dissolution profile. The simulated intestinal fluid (SIF) was prepared as follows, the stock salt solution was prepared by mixing 6.8 mL of 37.3 g per L KCl, 0.8 mL of 68 g per L  $\text{KH}_2\text{PO}_4$ , 42.5 mL of 84 g per L  $\text{NaHCO}_3$ , 9.6 mL of 117 g per L NaCl, 1.1 mL of 30.5 g per L  $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ , and 40  $\mu\text{L}$  of 0.3 M  $\text{CaCl}_2$ . Then the SIF was prepared by mixing of 50 mL of stock salt solution and 80 mg of bile salt.

### 2.7 Statistical analysis

All analyses were performed using Minitab statistical software (Version 22.4.0.0, Minitab Ltd, UK). Data were analyzed by one-way analysis of variance (ANOVA). All results were expressed as mean  $\pm$  standard deviation (SD) ( $n = 3$ ). Post hoc multiple comparisons were performed using Tukey's honestly significant difference (HSD) test. Differences were considered statistically significant at  $p < 0.05$ .

## 3. Results and discussion

Pristine naringenin exhibited clear crystallinity, as observed by SEM (Fig. 1a and d). Its aqueous solubility was extremely low, at less than 5  $\mu\text{g mL}^{-1}$ . In contrast, naringenin was highly soluble in 95% ethanol, with a solubility of  $58.8 \pm 1.7 \text{ mg mL}^{-1}$ . Consistent with the literature, the water solubility of naringenin has been reported to be similarly low, with values of 2.523  $\mu\text{g mL}^{-1}$

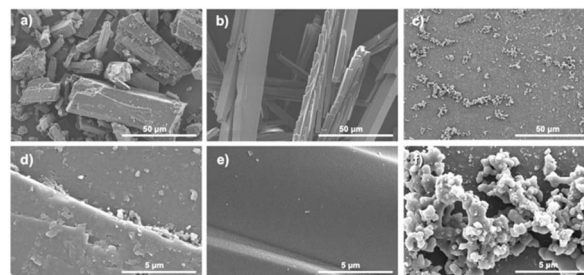


Fig. 1 SEM images of (a and d) pristine naringenin, (b and e) naringenin precipitate, (c and f) NGPVP precipitate.

0.062  $\mu\text{g mL}^{-1}$  reported by Stasiłowicz-Krzemień *et al.*<sup>23</sup> and 1.94  $\mu\text{g mL}^{-1}$  reported by Nesalin *et al.*<sup>24</sup>

Although naringenin solubility is pH-dependent, the extent of improvement remains limited. For example, in buffered solutions at pH 1.2, 6.8, and 7.4, the solubility values were reported to be 2.69, 3.08, and 5.55  $\mu\text{g mL}^{-1}$ , respectively.<sup>24</sup> Such poor aqueous solubility is commonly observed in flavonoids, including both aglycone and glucoside forms. For instance, kaempferol exhibits a low water solubility of 0.113  $\text{mg mL}^{-1}$  at 30 °C.<sup>25</sup> In comparison, flavonoid glucosides generally show improved water solubility due to the presence of polar sugar moieties. For example, naringin exhibits a solubility of up to 500  $\mu\text{g mL}^{-1}$ ,<sup>26</sup> while liquiritin has been reported to reach 980  $\mu\text{g mL}^{-1}$ .<sup>27</sup> However, despite their higher solubility, glucosides must be enzymatically hydrolyzed into their aglycone forms in the intestine prior to absorption. Overall, the extremely low solubility of flavonoids is a well-recognized and inherent limitation of this class of compounds. This challenge persists even as new flavonoids with promising biological potential, such as liquiritin, hesperidin, or plant extracts derived from citrus peels, roselle calyces, green tea leaves, buckwheat seeds, *etc.* continue to be identified and reported.<sup>28,29</sup>

To enhance the solubility of naringenin, a simple and practical approach involving the reduction of crystallinity and particle size was employed. As previously reported, polyvinylpyrrolidone (PVP) has been widely used in the preparation of solid dispersions to improve drug solubility. Kanaze *et al.*<sup>12</sup> demonstrated the successful formation of an amorphous solid dispersion by combining PVP with a flavonoid mixture (naringenin and hesperetin) in ethanol, followed by solvent evaporation. The solubility of the solid-dispersed naringenin–PVP was found to increase by 51.4-fold when dissolved in a buffer medium at pH 6.8. In another study, Kumar and Abraham<sup>30</sup> prepared naringenin nanoparticles using an antisolvent nanoprecipitation method, wherein an ethanolic solution of naringenin was added dropwise into an aqueous PVP solution. This process yielded naringenin nanocrystals. Both studies underscore the effectiveness of PVP in enhancing the solubility of poorly water-soluble compounds.

In the present work, we combined the concepts of solid dispersion and nanoprecipitation. A solution of naringenin and PVP in ethanol was prepared and then introduced into water, serving as a non-solvent. This induced rapid precipitation,



while the presence of PVP effectively inhibited crystal growth, leading to the formation of nano-sized amorphous naringenin particles (NGPVP). Fig. 1 shows the morphology of the obtained particles with the Fig. 1c and f clearly exhibits round particle without the edge.

Although the amorphous naringenin/PVP nanoparticles (NGPVP) were successfully obtained, they remained as a fine dispersion in the aqueous medium (Fig. 2b). The particle size is too small to be separated by coarse sieve filtration or sedimentation. In addition, conventional drying techniques, such as spray drying or freeze-drying, are energy intensive and may potentially degrade flavonoid compounds. Therefore, an alternative approach to recover the amorphous particles without the use of excessive heat or vacuum was considered.

To facilitate the separation, a pectin-assisted encapsulation strategy was employed. Pectin, a naturally derived polysaccharide with amphiphilic and gel-forming properties, was introduced into the aqueous phase prior to nanoprecipitation. When the ethanolic solution of NGPVP was added into the pectin solution, spontaneous precipitation was observed (Fig. 2c and d). This behavior is attributed to coacervation, a phase separation phenomenon where macromolecules such as pectin aggregate and encapsulate solutes in a dense, polymer-rich phase.<sup>31</sup> The interaction is likely enhanced by ethanol-induced dehydration of pectin chains, promoting the formation of a pectin shell around the hydrophobic NGPVP core. This process is categorized as simple coacervation where only one polymer is involved. It is simpler, suitable for a hydrophobic core, and cost effective compared to complex coacervation where another polymer like protein is introduced to generate ionic interaction. In either coacervation system, pectin plays a crucial role as a wall-forming material due to its natural origin, excellent biocompatibility, and ability to form a protective polymeric matrix.<sup>32–35</sup>

To optimize encapsulation conditions, the effect of pectin concentration and methoxyl content on encapsulation behavior was investigated. High methoxy (HM) and low methoxy (LM) pectin were tested at varying concentrations (0.1–1% w/v). SEM images (Fig. 3) revealed that both types of pectin were able to

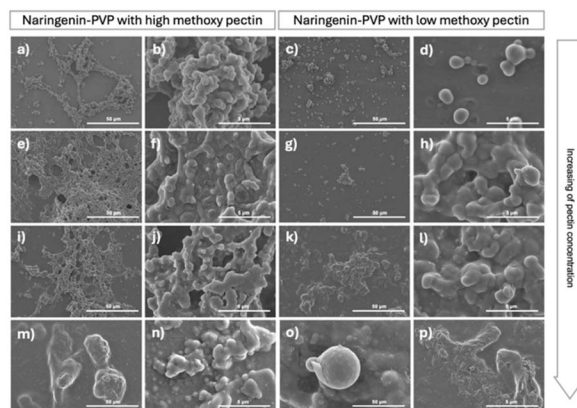


Fig. 3 SEM images of pectin encapsulated naringenin using HM pectin and LM pectin at various concentrations (a–d) 0.1% w/v pectin, (e–h) 0.2% w/v pectin, (i–l) 0.4% w/v pectin, (m–p) 1% w/v pectin.

induce coalescence and encapsulation of NGPVP particles, with increasing pectin concentration resulting in more defined and compact microcapsules. Notably, high methoxy pectin tended to produce more discrete, spherical particles, whereas low methoxy pectin led to a smeared or aggregated morphology, especially at lower concentrations.

This morphological difference may be attributed to the higher degree of methyl esterification in HM pectin, which renders the polymer more hydrophobic. The increased number of methyl groups could enhance hydrophobic interactions with the naringenin-rich core, promoting more efficient surface adsorption and coalescence into compact particles. In contrast, LM pectin contains more free carboxyl groups, which increase its hydrophilicity and may reduce its affinity toward hydrophobic surfaces, thereby resulting in less defined encapsulation. This behavior aligns with previous studies that reported stronger interactions between hydrophobic bioactive compounds and HM pectin matrices.<sup>13</sup> Thus, the degree of methoxylation plays a critical role not only in gelation properties but also in driving interfacial interactions during coacervation-

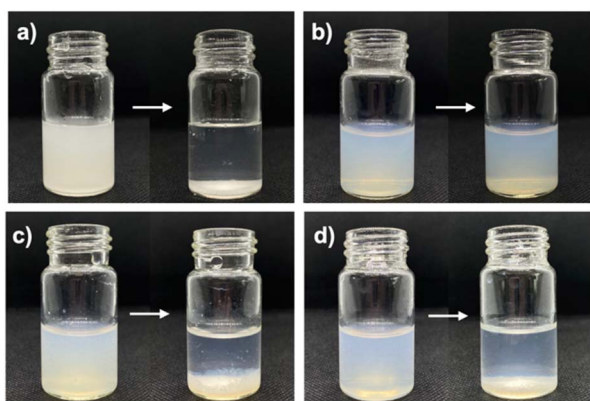


Fig. 2 Appearance for the precipitation process (a) naringenin precipitate, (b) NGPVP precipitate, (c) NGPVP-HM precipitate, (d) NGPVP-LM precipitate.

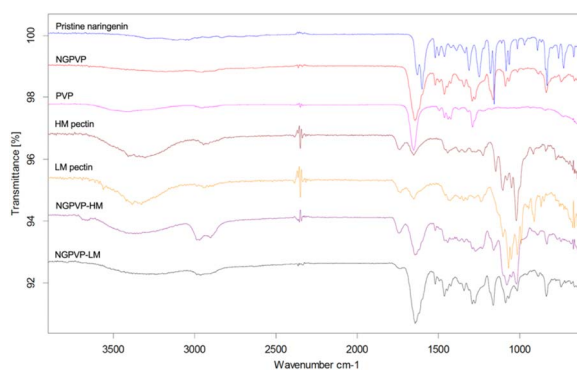


Fig. 4 FTIR spectra of powder samples: pristine naringenin (blue), NGPVP (red), PVP (pink), HM pectin (brown), LM pectin (yellow), NGPVP-HM 0.05% (purple), NGPVP-LM 0.05% (black).



based encapsulation of hydrophobic compounds like naringenin.

From FTIR results (Fig. 4), PVP exhibits a characteristic absorption band at  $1651\text{ cm}^{-1}$ , corresponding to the C=O stretching vibration, along with a band at  $1289\text{ cm}^{-1}$  attributed to C–N stretching. Naringenin shows sharp characteristic peaks at  $1628\text{ cm}^{-1}$  and  $1600\text{ cm}^{-1}$ , which are assigned to the C=O stretching vibration of the conjugated carbonyl group and the C=C stretching vibrations of the aromatic rings, respectively. Additionally, naringenin exhibits characteristic phenolic C–O stretching vibrations in the fingerprint region at  $1156\text{ cm}^{-1}$ , accompanied by several adjacent bands.

For NGPVP, the C=O stretching band is slightly shifted from  $1651\text{ cm}^{-1}$  to a lower wavenumber at  $1644\text{ cm}^{-1}$ , while shoulder peaks around  $1628\text{ cm}^{-1}$  and  $1600\text{ cm}^{-1}$ , characteristic of naringenin, remain observable. This shift indicates the presence of intermolecular interactions, most likely hydrogen bonding between naringenin and PVP, whereas the continued presence of naringenin-related bands suggests that its molecular structure remains intact. Similar shifts arising from naringenin–PVP interactions have been reported in PVP–naringenin nanoparticles by Kumar *et al.*<sup>30</sup> and in PVP-based solid dispersions by Kanaze *et al.*<sup>12</sup>

Pectin exhibited characteristic absorption bands at  $1739$ ,  $1652$ , and  $1020\text{ cm}^{-1}$ , corresponding to the C=O stretching of ester groups, the C=O stretching of carboxylate groups, and the C–O stretching of the pectin backbone, respectively, in good agreement with the literature.<sup>36</sup> For pectin-encapsulated NGPVP, the FTIR spectra reflected the superposition of the spectral features of each component. It should be noted that the relative peak intensities were influenced by the amount of each component in the formulation. In the encapsulated samples, 50 mg of naringenin and 50 mg of PVP were used, while the amount of pectin was calculated to be 4.7, 9.5, 19, 47.5, 95, and 190 mg for the process pectin concentrations of 0.005%, 0.01%, 0.02%, 0.05%, 0.1% and 0.2% w/v, respectively.

For the FTIR results of encapsulated naringenin (0.05% pectin), the characteristic band of NGPVP at  $1643\text{ cm}^{-1}$  was still observed at the same position, indicating that the addition of pectin did not disrupt the interaction between NG and PVP. However, upon the addition of pectin, for example, in the NGPVP-HM sample at a pectin concentration of 0.05%, this band split into two peaks, with a new band appearing at  $1634\text{ cm}^{-1}$ . This newly emerged band can be attributed to the carboxylate groups of pectin. Interestingly, the shift of this band to a slightly lower wavenumber ( $1634\text{ cm}^{-1}$ ) suggests that pectin in the encapsulation system experiences a different chemical environment compared to pectin alone, likely due to interactions with NGPVP and possible formation of a pectin coating layer.

Particle size analysis (Fig. 5a) showed that the bare NGPVP particles exhibited an average hydrodynamic diameter of approximately 200 nm. Upon encapsulation with pectin, the measured particle size increased, supporting the formation of a polymeric shell around the nanoparticle core. However, caution must be taken when interpreting DLS data in this context, particularly under conditions where aggregation or

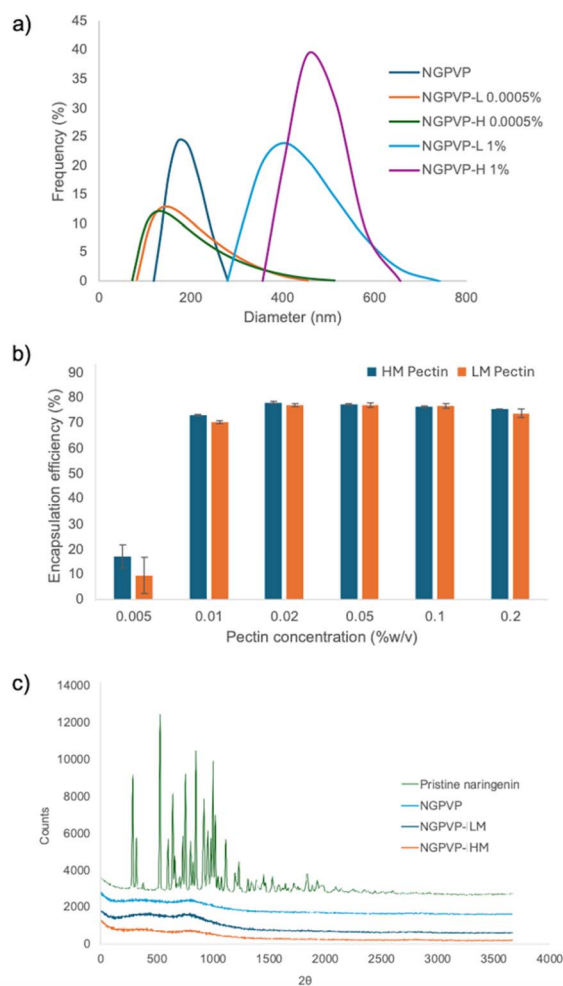


Fig. 5 (a) Particle size distribution of NGPVP and pectin encapsulated naringenin, (b) encapsulation efficiency of pectin encapsulated naringenin, (c) XRD patterns of naringenins and pectin encapsulated naringenins.

precipitation occurs. At high pectin concentrations (*e.g.*, 1% w/v), rapid precipitation was observed shortly after the addition of the ethanolic NGPVP solution into the pectin-containing aqueous phase. In such cases, the particles tend to sediment quickly, and the DLS measurements predominantly capture the fraction of smaller, suspended particles that remain in solution. Therefore, the reported particle sizes may not represent the full population or average size of the encapsulated material under these conditions. The true size of the larger coacervates or microcapsules formed during precipitation is likely to exceed the detection range of DLS or be lost from measurement due to sedimentation. Interestingly, when a very low concentration of pectin (0.0005% w/v) was used, insufficient to induce visible precipitation, the DLS profile showed a broadened distribution with a distinct tail extending toward larger particle sizes. This suggests that even at sub-gelling concentrations, pectin molecules may adsorb onto the surface of NGPVP particles and increase the hydrodynamic diameter. The gradual size increase observed under these dilute conditions provides indirect



evidence of surface interaction between pectin and the naringenin/PVP complex. This may be attributed to weak hydrophobic interactions or hydrogen bonding between the polymer and the flavonoid.

The encapsulation efficiency (Fig. 5b) was found to be strongly dependent on the concentration. At the lowest tested concentration (0.005% w/v), encapsulation was minimal, with HM pectin yielding approximately 20% efficiency and LM pectin only about 10%. This suggests that the amount of pectin available at this level was insufficient to fully coat or aggregate with the dispersed NGPVP particles, resulting in significant loss of naringenin to the supernatant.

A steep increase in encapsulation efficiency was observed as the pectin concentration was increased from 0.005% to 0.01% w/v, suggesting a critical threshold above which sufficient polymer is available to initiate effective coacervation or interfacial complexation. Beyond this threshold, encapsulation efficiency plateaued, reaching values of approximately 76% for both HM and LM pectin in the range of 0.02–0.2% w/v. This plateau indicates that, beyond a certain pectin-to-particle ratio, additional pectin does not contribute significantly to further encapsulation, either due to surface saturation or aggregation-induced steric hindrance that limits further coverage. The plateau values of 78% for NGPVP-HM and 77% for NGPVP-LM are relatively high when compared with the broad range of encapsulation efficiencies reported in the literature (30–90%).<sup>37–39</sup> These values are comparable to those obtained from more complex encapsulation techniques, suggesting that the pectin-assisted approach employed in this study provides efficient encapsulation while maintaining a relatively simple and mild processing strategy.

Statistical analysis supported these observations. One-way ANOVA revealed that the encapsulation efficiency increased sharply when the pectin concentration increased from 0.005 to 0.01% ( $p < 0.05$ ), while no statistically significant improvement was observed at higher concentrations ( $p > 0.05$ ), indicating saturation of the encapsulation process. One-way ANOVA further confirmed a significant effect of pectin concentration ( $p < 0.05$ ). However, no statistically significant difference was observed between HM and LM pectin at concentrations  $\geq 0.02\%$  ( $p > 0.05$ ).

Despite the lack of statistical significance between HM and LM at higher pectin concentrations, a consistent visual trend was observed, in which HM pectin exhibited slightly higher encapsulation efficiency than LM pectin across all concentrations. Mechanistically, this trend may be attributed to the higher degree of methyl esterification in HM pectin, which enhances hydrophobic interactions with the poorly soluble naringenin core. In contrast, the higher content of free carboxyl groups in LM pectin increases solubility and electrostatic repulsion, potentially limiting the formation of condensed interfacial layers around the hydrophobic particle surface. To validate this hypothesis, further studies employing pectin samples with systematically varied degrees of methoxylation would be required.

At the highest concentration tested (0.2% w/v), a slight decline in encapsulation efficiency was observed. This may be

attributed to excess pectin molecules forming non-productive aggregates in solution, or to competitive self-association of pectin chains that reduces their availability for particle surface interaction. Similar observations have been reported in polysaccharide-based encapsulation systems, where high polymer concentrations can lead to macroscopic phase separation or entanglement, thus reducing encapsulation efficiency.<sup>8,13</sup>

The XRD patterns (Fig. 5c) clearly indicate the crystalline nature of pristine naringenin, as evidenced by the presence of numerous sharp and intense diffraction peaks. This crystallinity is significantly reduced upon incorporation with PVP, as shown by the broad and diminished peaks in the NGPVP. The disappearance of sharp diffraction signals suggests that PVP effectively disrupts the crystalline lattice of naringenin, promoting its transformation into an amorphous state. Moreover, samples subjected to further encapsulation, including NGPVP-HM and NGPVP-LM, maintain their amorphous nature, as no sharp peaks reappear in their diffraction profiles. This observation confirms that the encapsulation process, involving a pectin matrix, does not induce recrystallization of naringenin.

Overall, the results can be mechanistically explained as follows. NG and PVP interact to form an amorphous core, while pectin chains subsequently capture these amorphous particles, promoting their agglomeration and precipitation. In the first step, a 1 : 1 NG : PVP ratio is effective in preventing crystallization. For the subsequent encapsulation step, only a small amount of pectin is required, with an NG : pectin weight ratio of 4 : 1 being sufficient.

The dissolution profiles of the various naringenin formulations at 37 °C are shown in Fig. 6. Pristine naringenin exhibited poor solubility especially in DI water, consistent with its known low aqueous solubility. The incorporation of PVP significantly enhanced naringenin solubility, as seen in the NGPVP

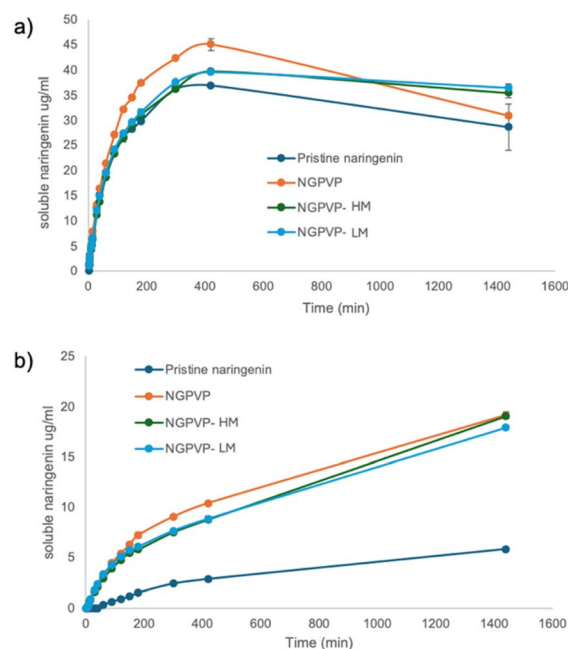


Fig. 6 Dissolution profile at 37 °C (a) in SIF, and (b) in DI water.



formulation, which achieved the highest maximum soluble concentration in both media. This improvement is attributed to the amorphous state induced by PVP. Interestingly, after further encapsulation with pectin, the NGPVP-HM and NGPVP-LM samples demonstrated slightly reduced initial solubility compared to NGPVP yet still showed substantial improvement over pristine naringenin. This suggests that the pectin layer does not significantly hinder the dissolution capacity of the amorphous naringenin–PVP complex.

In DI water (Fig. 6b), the impact of PVP and pectin on solubility was more pronounced than in SIF (Fig. 5a), possibly because the intestinal fluid itself enhances the solubilization of naringenin to some extent, minimizing the relative difference between formulations.

Notably, after prolonged dissolution (1440 min) in SIF, the NGPVP formulation exhibited a marked decline in solubility, which may be due to reprecipitation or recrystallization of naringenin at high supersaturation levels. In contrast, both NGPVP-HM and NGPVP-LM formulations maintained a more stable solubility over time. One-way ANOVA revealed that at 1440 min (in SIF) the solubility of NGPVP was not different from NG, but the NGPVP-LM, NGPVP-HM were significantly higher than NG group ( $p < 0.05$ ). This stabilizing effect is likely due to the presence of pectin, which may act as a barrier around naringenin particles, inhibiting recrystallization or aggregation during the dissolution process.

In deionized water (DI), the solubilities of NGPVP, NGPVP-LM, and NGPVP-HM were 19.15, 19.05, and 17.91  $\mu\text{g mL}^{-1}$ , respectively, which are approximately 3–4-fold higher than that of NG (5.83  $\mu\text{g mL}^{-1}$ ). At 1440 min, no statistically significant differences were observed among NGPVP, NGPVP-HM, and NGPVP-LM, as determined by one-way ANOVA ( $p > 0.05$ ). Although this level of enhancement is modest compared to values reported in the literature, substantially higher solubilities have been achieved using other strategies. For example, a phospholipid complex increased the aqueous solubility from  $43.83 \pm 0.039$  to  $79.31 \pm 0.718 \mu\text{g mL}^{-1}$ ,<sup>40</sup> while complexation with hydroxypropyl- $\beta$ -cyclodextrin (HP- $\beta$ -CD) enhanced the solubility of naringenin from 4.38  $\mu\text{g mL}^{-1}$  to as high as 1272.31  $\mu\text{g mL}^{-1}$ .<sup>41</sup> In another study, the solubility of naringenin (2.523  $\pm 0.062 \mu\text{g mL}^{-1}$ ) was improved by nearly 210-fold in a 1:3 Naringenin:HP- $\beta$ -CD system.<sup>23</sup> A variety of formulation approaches, including liposomes, nanoparticles, self-nanoemulsifying drug delivery systems, nanosuspensions, solid dispersions, and inclusion complexes, have also been extensively reviewed.<sup>42</sup>

Nevertheless, the performance of a formulation should not be judged solely by the magnitude of solubility enhancement. From an energy saving and process efficiency perspective, the present approach offers a practical advantage, as the product is obtained directly as a precipitate that can be readily recovered and processed into a powder, an aspect that has been comparatively less explored. In practical terms, as demonstrated in this study, out of 100 mL of water in the system, approximately 95 mL can be removed simply by decantation, leaving only about 5 mL to be removed by thermal drying. This implies that roughly 95% of the drying energy can be saved.

In addition, the choice and amount of excipients are critical for real applications; minimizing additive content is generally preferable, especially when food or pharmaceutical grade materials are required. In our system, PVP was used at a 1:1 ratio relative to naringenin, while the amount of pectin was four times lower than that of naringenin. Thus, the total additive content in our process is substantially lower than in typical inclusion based approaches, such as cyclodextrin complexation, which usually require at least a 1:1 molar ratio, corresponding to approximately five times the mass of naringenin. Nevertheless, further improvement in solubility will still be necessary for our system to better compete with existing techniques. The pectin induced precipitation strategy could also be combined with other solubility enhancement agents, such as surfactants, phospholipids, or other crystallization inhibitors, which will be the subject of future studies.

From an application perspective, the use of this system in functional food formulations will require consideration of additional ingredients and subsequent processing steps to obtain a final product with acceptable stability, sensory properties, and performance. Additionally, the formulation could be evaluated *in vivo* to determine whether the improvements observed *in vitro* translate into enhanced bioavailability and biological efficacy, as the behavior in complex biological systems may differ from that observed in the present *in vitro* experiments. Overall, this study offers a practical and scalable platform for formulating poorly water-soluble flavonoids using food-grade materials, while underscoring the need for further optimization and validation before translation to functional food or pharmaceutical products.

## 4. Conclusion

This study successfully demonstrated a simple and scalable method for encapsulating naringenin using pectin, inspired by the natural composition of citrus fruit peels. The integration of PVP facilitated the formation of amorphous naringenin nanoparticles with significantly improved solubility compared to pristine crystalline naringenin. The subsequent encapsulation with pectin, particularly high methoxy pectin, effectively promoted the separation of these nanoparticles from the aqueous medium through coacervation, offering an alternative to energy-intensive drying methods. The pectin layer not only aided in the recovery of the encapsulated material but also contributed to maintaining the amorphous state of naringenin and stabilizing its solubility over time. This fruit peel-inspired approach provides a cost-effective and efficient strategy for enhancing the bioavailability and applicability of naringenin and potentially other hydrophobic bioactive compounds, aligning well with circular economy principles by utilizing agricultural by-products.

## Author contributions

Weerapha Panatdasirisuk: writing – original draft, methodology, validation, data curation, formal analysis, conceptualization, visualization, validation. Weerachai Chantana:



methodology, formal analysis, data curation, writing – review & editing. Adisak Hawan: data curation, formal analysis. Phimonwan Wongjeen: data curation, formal analysis. Verawat Champreda: supervision, conceptualization, writing – review & editing, project administration, validation, funding acquisition.

## Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

The data supporting the findings of this study are fully available within the article.

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