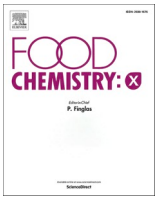
Food Chemistry: X 23 (2024) 101548

Contents lists available at ScienceDirect

Food Chemistry: X

journal homepage: www.sciencedirect.com/journal/food-chemistry-x



Microencapsulated granaticins from *Streptomyces vilmorinianum* YP1: Optimization, physiochemical characterization and storage stability

Xuechen Si [a,1], Zuoyun Yuan [b,1], Huilin Li [a], Yunping Zhu [a,c,*], Yawen Zhou [d], Jia Liu [e], Zhichao Wu [e]

[a] Key Laboratory of Green Manufacturing and Biosynthesis of Food Bioactive Substances, China General Chamber of Commerce, School of Food and Health, Beijing Technology and Business University, Beijing 100048, China
[b] COFCO Nutrition and Health Research Institute, Beijing 102200, China
[c] Beijing Engineering and Technology Research Center of Food Additives, Beijing Technology & Business University, Beijing 100048, China
[d] School of light industry science and engineering, Beijing Technology and Business university, China
[e] Internal Trade Food Science Research Institue Co., Ltd, Beijing, 102200, China

ARTICLE INFO

Keywords:
Natural pigments
Granaticins
Encapsulation efficiency
Microcapsules
Response surface methodology
Storage stability

ABSTRACT

Granaticins are natural pigments derived from microorganisms with promising bioactivity. However, their practical applications have been restricted due to inherent instability. To improve the stability of granaticins from the novel strain *Streptomyces vilmorinianum* YP1, microcapsules were prepared using gum Arabic (GA) by a freeze-drying method. The optimal parameters for microencapsulation were determined using response surface methodology. Under the optimal conditions (GA 9.2% (*v*/v), a wall/−core ratio 4.8 (*w*/w), encapsulating temperature 29 °C), the maximum encapsulation efficiency achieved was 93.64%. The microcapsules were irregular single crystals with an average particle size of 206.37 ± 2.51 nm. Stability testing indicated improved stability of the microencapsulated granaticins. Notably, granaticnic B retention increased by 17.0% and 6.6% after exposure to sunlight and storage at 4 °C, respectively. These finding suggest that GA as a well material significantly enhances the stability of granaticins from *S. vilmorinianum* YP1, facilitating their potential applications.

1. Introduction

Granaticins and its derivatives, a category of secondary metabolites found in microorganisms, appear blue in alkaline environments (Zheng et al., 2023). As a member of benzoisochromanequinones (BIQs), which are a class of important compounds for the study of polyketone synthase, secondary metabolism and regulation. Until now, the chemical structure of more than ten granaticin derivative have been reported, including granaticin A/B, granaticinic acid, granaticinic derivative MM44785 and so on. Furthermore, granaticins and their derivatives showcase a diverse array of bioactivities, including anticancer, antibacterial and anti-biofilm, making them intriguing subjects for pharmaceutical and food industry applications (Frattini, Djaballah, & Kelly, 2011; Iwasaki & Omura, 2007; Ogilvie, Wiebauer, & Kersten, 1975a; Ogilvie, Wiebauer, & Kersten, 1975b; Ricicová & Podojil, 1965). However, due to the sensitivity of sunlight and temperature, the applications of granaticins and its derivative have been significantly limited (Barcza, Brufani, Keller-Schierlein, & Zähner, 1966). Therefore, effective measures are required to further study the function and enhance the application potential of granaticins by improving their stability during processing and storage.

Microencapsulation, the process of encapsulating natural or polymeric compounds in polymeric shells, is an efficient method for improving the stability and bioavailability of bioactive compounds (Dhakal & He, 2020; Van, Nguyen, Nguyen, & Ach, 2024), and has been widely employed in the food industry. During microencapsulation, the appropriate wall material and embedding parameters have a significant effect on the stability and encapsulation efficiency of the microcapsules. Several wall materials, such as proteins (soybean protein, whey protein, etc.), carbohydrates (maltodextrin, chitosan, etc.), and hydrophilic colloids (gum Arabic, pectin, etc.), have been reported to exhibit excellent performance due to their ability to encapsulate core materials.

* Corresponding author at: Key Laboratory of Green Manufacturing and Biosynthesis of Food Bioactive Substances, China General Chamber of Commerce, School of Food and Health, Beijing Technology and Business University, Beijing 100048, China.
E-mail address: zhuyp@th.btbu.edu.cn (Y. Zhu).
[1] These authors equally contributed to the article.

https://doi.org/10.1016/j.fochx.2024.101548
Received 5 May 2024; Received in revised form 1 June 2024; Accepted 8 June 2024
Available online 9 June 2024

Among them, gum Arabic (GA) is a natural heteropolysaccharide that has been widely used as an emulsifier and wall material due to its high encapsulation efficiency and good emulsibility (Ozturk, Argin, Ozilgen, & McClements, 2015). Several previous studies have reported the formation of microcapsules with GA as the wall material. For example, GA-modified starch modified starch complexes were used to produce fennel oleoresin products with improved redispersibility and stability (Chranioti & Tzia, 2014). GA and maltodextrin (mixed at a ratio of 2:1 with a concentration of 20% (*w*/w)) stabilized emulsions were utilized to encapsulate noni fruit extract, successfully improving the robust stability across diverse storage conditions (Nguyen, Nguyen Di, Phan, Kha, & Nguyen, 2024). Nevertheless, there are still no reports on the physicochemical and stability of natural granaticins by microencapsulation.

In our previous work, a novel granaticins producer, *Streptomyces vilmorinianum* YP1 (CGMCC No. 23383, hereinafter referred to as *S. vilmorinianum* YP1), was isolated, and its secondary metabolites were analyzed. Granaticin A, granaticin B, granaticinic acid and several other derivatives (Fig. S1) were purified and identified. The content of granaticin B isolated from *S. vilmorinianum* YP1 reached 227 mg/L, which was the highest level of granaticin B reported in wild bacteria and almost two times that of *S. vilmorinianum* GIMV4 0.0001 (Deng, Li, & Li, 2019). However, the application of granaticins is limited due to their high instability in the presence of high temperature, light, and humidity leading to a reduced economic value. Therefore, the objective of this study was to optimize the preparation of granaticins microcapsules encapsulated with GA by freeze-drying in order to improve granaticins stability and broaden its range of applications. Furthermore, the physicochemical properties of the microcapsules were evaluated by scanning electron microscopy (SEM), Zeta potential analysis and thermo gravimetric analyzer (TGA). The conversion of granaticins was determined based on the changes in the content of granaticins during storage under sunlight and different temperature conditions.

2. Materials and methods

2.1. Materials

S. vilmorinianum YP1 is isolated from a soil sample in China and kept in the China General Microbiology Culture Collection Center (strain number GCMCC 23383). The original strain is stored in the laboratory of Beijing Technology and Business University. Granaticin A, granaticin B, granaticinic acid and granaticinic derivative MM44785 (90–95% purity) were obtained by laboratory isolation and purification in the early stage. Absolute methanol, ethanol, Dimethyl sulfoxide (DMSO), and ethyl acetate of analytical grade; were purchased from Beijing MREDA Technology Co., Ltd. (Beijing, China). Lactose, tryptone, yeast powder, and GA were purchased from Shanghai Aladdin Biochemical Technology Co., Ltd. (Shanghai, China). Maltodextrin and chitosan were purchased from Beijing Solarbio Science and Technology Co., Ltd. (Beijing, China).

2.2. Extraction of granaticins from S. vilmorinianum YP1

S. vilmorinianum YP1 was activated on optimized LB plates (10 g/L yeast extract powder, 5 g/L tryptone, 10 g/L lactose, 4 g/L NaCl, 20 g/L agar in solid medium). A single colony was selected and inoculated into 50 mL of liquid medium, and incubated at 28 °C for 36 h (Zheng et al., 2023). Subsequently, the colony was inoculated and fermented at a volume ratio of 1% (*v*/v) for 48 h. The granaticins in the fermentation broth were significantly enriched when the culture was dark blue.

Secondary metabolites were extracted according to the modified method of (Kordjazi, Mariniello, Giosafatto, Porta, & Restaino, 2024). The bacteria were removed by centrifugation and the culture filtrate was adjusted to pH 3–4 with HCl, and then immediately extracted 3 times with 3 volumes of ethyl acetate (Walter, 1974). A rotary evaporator under vacuum (37 °C) was used to concentrate the extract. Approximately 30 g of granaticin extract was obtained from 50 L of fermentation broth.

2.3. Preparation of microcapsules by freeze-drying

Granaticins microcapsule powder were prepared according to the modified method (Zhao, 2015). A stock solution of GA (10% *v*/v) was prepared by dissolving the powder in deionized water with magnetic stirring (700 rpm) at 30 °C for 1 h. Then, granaticins were added to the solution, and the previous step was repeated. Subsequently, the microcapsules were freeze-dried (BICOOL Instrument, Beijing, China) at −50 °C for 1–2 d to harvest the microcapsules, after which the samples were stored in sealed conical tubes at −18 °C for further analysis.

2.4. Optimization of microencapsulation conditions

Response surface methodology (RSM) and Box-Behnken design (BBD) were used for the optimization of the microencapsulation parameters of granaticins by Design Expert 13.0. (Stat-Ease. Minneapolis. USA). The GA concentration, wall/−core ratio and temperature were selected as independent variables to evaluate the influence on the encapsulation efficiency. BBD is considered to be slightly more effective than the central composite design and significantly more than the three-level complete factorial designs (Agarry, Wang, Cai, Kan, & Chen, 2022). Therefore, a BBD with three factors and 3 levels (3^3) was used to develop an appropriate model and optimize the conditions for the highest encapsulation efficiency of granaticins (Leylak, Özdemir, Gurakan, & Ogel, 2021). The factors and their levels are shown in Table 1. A total of 17 experiments were conducted and the ANOVA results were used to create a regression equation to forecast the response within a fixed range (Agarry et al., 2022). The BBD data were fitted using the quadratic polynomial model given below:

$$Y = \beta_0 + \beta_1 A + \beta_2 B + \beta_1 C + \beta_{11} A^2 + \beta_{22} B^2 + \beta_{33} C^2 + \beta_{12} AB + \beta_{13} AC + \beta_{23} BC$$

where Y is the predicted response; β_0 is the model intercept coefficient; X_i is the variable; and β_i, β_{ii} and β_{ij} are the linear; and quadratic interaction coefficient terms, respectively. All statistically significant differences were determined at the 95% confidence intervals (Leylak et al., 2021). The regression coefficient (R2), lack of fit, adjusted regression coefficient (Adj-R2), predicted regression coefficient (Pre-R2), predicted residual error sum of squares (PRESS), and adequate precision data were used to assess the compliance of the model and trial point data. (Aslan Türker & Doğan, 2022). The resulting matrix of the BBD experiment for the factors tested is summarized in Table 2.

Table 1
The influence of BBD experiment independent variables on encapsulation EE.

Design samples	Independent variables			Dependent variables	
	A (%)	B (w/w)	C (°C)	Experimental [a]EE (%)	Predicted [a]EE (%)
1	10	3	35	50.39	51.17
2	10	4	30	86.98	87.4
3	10	4	30	91.42	87.4
4	10	5	25	84.49	83.71
5	10	4	30	83.52	87.4
6	5	3	30	37.19	35.73
7	10	3	25	53.73	54.76
8	10	4	30	89.48	87.4
9	15	4	25	43.28	42.6
10	15	5	30	50.17	51.63
11	5	4	25	52.59	53.02
12	10	4	30	85.58	87.4
13	15	4	35	36.79	36.36
14	5	4	35	45.84	46.52
15	15	3	30	35.72	35.37
16	10	5	35	75.6	74.57
17	5	5	30	71.49	71.84

[a] EE is the abbreviation of encapsulation efficiency.

Table 2
ANOVA for response quadratic model for EE.

Source	Sum of Squares	df	Mean Square	F-value	*p*-value	
Model	6957.35	9	773.04	112.44	< 0.0001	significant
A	211.67	1	211.67	30.79	0.0009	
B	1370.78	1	1370.78	199.38	< 0.0001	
C	81.09	1	81.09	11.79	0.0109	
AB	98.51	1	98.51	14.33	0.0068	
AC	0.0169	1	0.02	0.0025	0.9618	
BC	7.7	1	7.70	1.12	0.3251	
A^2	3812.37	1	3812.37	554.51	< 0.0001	
B^2	315.99	1	315.99	45.96	0.0003	
C^2	677.03	1	677.03	98.47	< 0.0001	
Residual	48.13	7	6.88			
Lack of Fit	9.1	3	3.03	0.3108	0.8179	not significant
Pure Error	39.03	4	9.76			
Cor Total	7005.47	16				
R^2	0.9931		Std. Dev	2.62		
Adjusted R^2	0.9843		Mean	63.19		
Predicted R^2	0.705		C.V. %	4.15		
Adequate Precision	25.8697					

2.5. Encapsulation efficiency of GA in the microcapsules

The encapsulation efficiency of GA was determined according to the method of extracting granaticins. As granaticins were red under acidic conditions, encapsulation efficiency was determined colorimetrically. The surface pigment of 10 mg of microcapsules was removed with 10 mL of ethanol, and the mixture was stirred at 3000 rpm for 3 min and then centrifuged at 5000 rpm for 5 min (Hu, Li, Zhang, Kou, & Zhou, 2018); This process was repeated twice, and the precipitate, which was the embedded microcapsule, was collected. Two milliliters of deionized water were added to the precipitate and vortexed at 3000 rpm for 3 min to dissolve the wall material, and 2 mL of ethyl acetate was added to the solution to extract all granaticins. The mixture was vortexed for 2 min, after which the water and ethyl acetate were allowed to react naturally. The upper phase was collected and dried with nitrogen, and the internal granaticins were dissolved in 80% ethanol and directly measured using a TU-1900 ultraviolet -visible (UV) spectrophotometer (PERSEE, Beijing, China). A calibration curve ($R^2 = 0.9997$) was established using a series of granaticins in 80% ethanol solution ranging from 0.1 to 0.3 mg/L (Eq. (1)). The encapsulation efficiency of GA was calculated according to Eq. (2) (Ding et al., 2022):

$$Y = 3.55X - 0.1038 \quad (1)$$

$$\%EE = (W_{inner}/W_{total}) \times 100 \quad (2)$$

where EE is the encapsulation efficiency (%), W_{inner} is the inner granaticins amount (mg/mg), and W_{total} is the theoretical granaticins amount in the microcapsules (mg/mg).

2.6. Granaticins content

The modified method of Zhu et al. (Zhu et al., 2020) was used for determining the granaticins content. A Shimadzu UPLC instrument with an AQ-C18 (4.6 mm × 250 mm; 5 μm) column was used to separate the granaticins and the absorbance was determined at 580 nm. The chromatographic conditions were as follows: solvent A, 1% (*v*/v) acetic acid; solvent B, 100% acetonitrile; flow rate, 0.5 mL/min; temperature, 30 °C; and injection volume, 10 μL. The percentage of acetonitrile linearly increased from 25% to 75% after 40 min and returned to 25% after 30 min. The quantification of the main pigments, namely, granaticinic acid and granaticin B was carried out using calibration curves generated with standards within a relevant concentration range (quantity and peak area).

2.7. Physicochemical properties

2.7.1. Particle size and zeta potential

The average size and zeta potential of the granaticins and microcapsules in the milled and redissolved suspensions were determined with a Zetasizer Nano ZS90 instrument (Malvern Instruments, Malvern, UK). Six measurements were averaged at 25 °C. The GA refractive index was 1.42 (Singh et al., 2024).

2.7.2. Scanning electron microscopy (SEM)

The particle morphology of the microcapsules was determined using a CX-200 PLUS scanning electron microscope at 15 kV. The sample was sputter coated with a thin gold layer for 90 s. A representative image of the sample was obtained at a magnification of 200×. The morphology of the wet microparticles was evaluated via an n light microscope BK5000 (Cnoptec Instrument, Chongqing, China) at 100× magnification (Zhao, Liu, Duan, Xiao, & Liu, 2018).

2.7.3. Fourier transform infrared (FT-IR) spectroscopy

Granaticins microcapsule powder was prepared according to the modified method described by Zhao (Zhao, 2015). A stock solution of GA (10% *v*/v) was prepared by dissolving the powder in deionized water through magnetic stirring (700 rpm) at 30 °C for 1 h. Subsequently, granaticins was added to the solution, and the process was repeated. Following this, the microcapsules were freeze dried (BICOOL Instrument, Beijing, China) at −50 °C for 1–2 days in order to harvest the microcapsules. Thereafter, the samples were stored in sealed conical tubes at −18 °C for further analysis (Li et al., 2022).

2.7.4. Thermogravimetric analysis (TGA)

The thermal behaviors of the granaticins, GA, and the microcapsules were determined by thermogravimetric analysis (TGA) using a Q5000 thermogravimetric analyzer (TA Instrument, Newcastle, USA). A total of 5.0 mg each of granaticins powder, GA, and the microcapsules were packed in a blank aluminum pan and heated from 30 to 600 °C at a heating rate of 10 °C/min in a nitrogen atmosphere at a flow rate of 20 mL/min (Chen et al., 2021).

2.8. Storage stability

Granaticins powder and microcapsules were stored in centrifuge tubes at room temperature with direct light and at different temperatures (4 °C, 25 °C, and 37 °C) protected from light in a constant temperature incubator (DHP-9012, Bluepard, Shanghai, China) for 42 days for storage stability testing. The retention rate of these samples was analyzed every 7 days (Zhao, Liu, Duan, Xiao, & Liu, 2018), and the amount of each granaticins was determined by HPLC. The retention rate was calculated by Eq. (3):

$$Retention\ rate(\%) = Residual\ extract\ content/Initial\ extract\ content \times 100\# \quad (3)$$

2.9. Statistical analysis

In this study, the statistical analysis of the results was performed by the ANOVA multiple comparison method using Origin 2023 (Origin Lab, Northampton, USA). Tukey's multiple comparison test was used to determine the difference between the means. $p < 0.05$ indicated a statistically significant difference.

3. Results and discussion

3.1. Model adequacy for the optimization of granaticins microcapsules

The data fitting about the RSM was evaluated by plotting the predicted data and experimental values (Leylak et al., 2021). Fig. S2 shows the relation between the actual and predicted values of encapsulation efficiency. The results showed that the relationship was approximately linear, and the predicted value was in good agreement with the experimental value. The residual points in the normality test results (Fig. S3A) were close to a straight line, which indicated that the experimental RSM data followed a normal distribution. The relationship between the residuals and the predicted response is shown in Fig. S3B. The random scattering of the residuals indicated that the variance of the original observations was constant and independent of the response value. The outlier t-plot for encapsulation efficiency is shown in Fig. S3C, with all data points within the ±4.82 interval, indicating that the RSM was a good approximation of the fitted model.

3.2. Optimization of granaticins microencapsulation

Single-factor experiments were carried out on the wall material, GA concentration, wall/−core ratio, embedding temperature and embedding time. Based on the results of the single-factor experiment, GA was the best wall material for covering granaticins, and the GA concentration, wall/core ratio and embedding temperature had significant effects on the encapsulation efficiency (Fig. S4). Therefore, the above three single factors were selected for the response surface optimization experiment (Aslan Türker & Doğan, 2022).

The complete matrix of the experimental plan and experimental encapsulation efficiency during the execution of the BBD are presented in Table 1. The highest encapsulation efficiency (91.46%) of the microcapsules was obtained at the third trial, which was carried out with a GA concentration of 10%, a wall-to-core ratio of 4 and an embedding temperature of 30 °C.

The ANOVA results for the extract microencapsulation model are shown in Table 2. A p-value <0.001 indicated that the model was extremely significant, and a p-value >0.05 was not significant, suggesting that the quadratic model was highly significant and reliable for fitting the effects of the GA concentration, wall/core ratio and embedding temperature on the encapsulation efficiency of the microcapsules. Moreover, the R^2 values of encapsulation efficiency (0.9931) and the Adj. R^2 (0.943) was close to 1, and adequate precision was 25.8679, indicating a high degree of correlation between the actual and predicted values, which could be used for the estimation of the encapsulation efficiency (Dharma et al., 2016). The adequate precision value indicates that the signal/noise ratio is expected to be >4 (Dutta, Shaik, Ganesh, & Kamal, 2017). As adequate precision value was 25.8679, and it was found that the adequate. The precision provided a sufficient signal for optimization.

The relation between the response values and selected variables is obtained as follows:

$$Y = 87.40 - 5.14A + 13.09B - 3.18C - 4.96AB + 0.065AC - 1.39BC - 30.09A^2 - 8.66B^2 - 12.68C^2$$

where Y is the encapsulation efficiency (%) of the granaticins microcapsules, A is the GA concentration, B is the wall/core ratio, and C is the embedding temperature.

The ANOVA results showed that the model was very significant, and the linear effects A, B, and C and quadratic effects AB, $A,^2$ B^2 and C^2 had extremely significant effects on encapsulation efficiency over the selected range (p value<0.05). In addition, the influence of three factors on encapsulation efficiency decreased in the following order: wall/core ratio (B) > GA concentration (A) > embedding temperature (C). Based on these results, it could be inferred that the quadratic regression model fully describes the microencapsulation process of the extract.

3.3. Optimization and model validation of microencapsulation

The anticipated model obtained the maximum encapsulation efficiency encapsulation efficiency. The optimum circumstances were a GA concentration of 9.24%, wall/−core ratio of 4.81 *w*/w and embedding temperature of 29.15 °C, resulting in a theoretical encapsulation efficiency of 93.19% for the resin microcapsules.

The verification of the experimental conditions was conducted at a GA concentration of 9.20%, a wall/core ratio of 4.8 w/w and an embedding temperature of 29 °C, under which the experimental results (93.64%) were not significantly different from the predicted results (93.38%), indicating that the BBD-based model was considered sufficient to represent the experimental results. The results revealed that the concentration of GA in the microcapsules ranged from 9% to 10% with a greater encapsulation efficiency, implying that high GA concentrations might retain a higher extract content in the microcapsules. This phenomenon can be related to GA's high emulsifying ability in oil-in-water (O/W) emulsions (Zhang et al., 2022), as well as the possible that a force formed between GA and granaticins.

3.4. Influence of different variables on encapsulation efficiency

Based on the second-order polynomial equation of the RSM, a 3-D response surface diagram (Fig. S5) was established to intuitively analyze the impact of various factors on the encapsulation efficiency (Amiri, Shakeri, Sohrabi, Khalajzadeh, & Ghasemi, 2019). As Fig. S5A and S4b show, the encapsulation efficiency gradually increased as the GA concentration increased from 5.00% to 9.20% and then decreased. The highest encapsulation efficiency value of 93.64% was obtained at a GA concentration of 9.20%, indicating that the wall material with a relatively high GA concentration can maintain a higher level of granaticins in the microcapsules, which can be attributed to the good emulsifying ability of GA at the oil–water interface (Zhang et al., 2022).

Table 2 shows that the wall/core ratio significantly affected encapsulation efficiency (p-value <0.001). As shown in Fig. S5A and S5B, the encapsulation efficiency increased with increasing wall/−core ratio in the range of 3–5 *w*/w, reaching a peak of 93.64% at 4.8. Fig. S5B and S5C illustrate that the encapsulation efficiency of the microcapsules first increased and then decreased with increasing embedding temperature, which may be related to the different interactions between the core and wall materials at different temperatures.

3.5. Physicochemical analysis of microcapsules

3.5.1. Particle size and zeta potential of the microcapsules

Particle size is an important index for evaluating the potential application of microcapsules. As shown in Table S1, the average particle sizes of the granaticins, GA, and microcapsules were 145.80 ± 1.97 nm, 250.87 ± 8.14 nm, and 206.37 ± 2.51 nm, respectively, which indicated that the granaticins bound well to GA. The zeta potential of the microcapsules was significantly greater than that of the granaticins, as shown in Table S1, which indicated that the structure of the wall material was altered by the action of the granaticins, and the interaction between the two made the system more stable (Labhasetwar & Dorle, 1991).

3.5.2. Microcapsule morphology

As shown in Fig. 1A, B and C, the morphology of the granaticins presented an irregular smooth surface, and the shapes of the GA and microcapsules were irregular single-crystal structures. The structural characteristics of the freeze-dried microparticles were determined by the sublimation of the frozen surface, where the removal of water resulted in a porous structure without shrinkage (Aguilera & Stanley, 1999). The same observations of Hibiscus calyces' extracts were made by other researchers (Piovesana & Noreña, 2018). Meanwhile, as

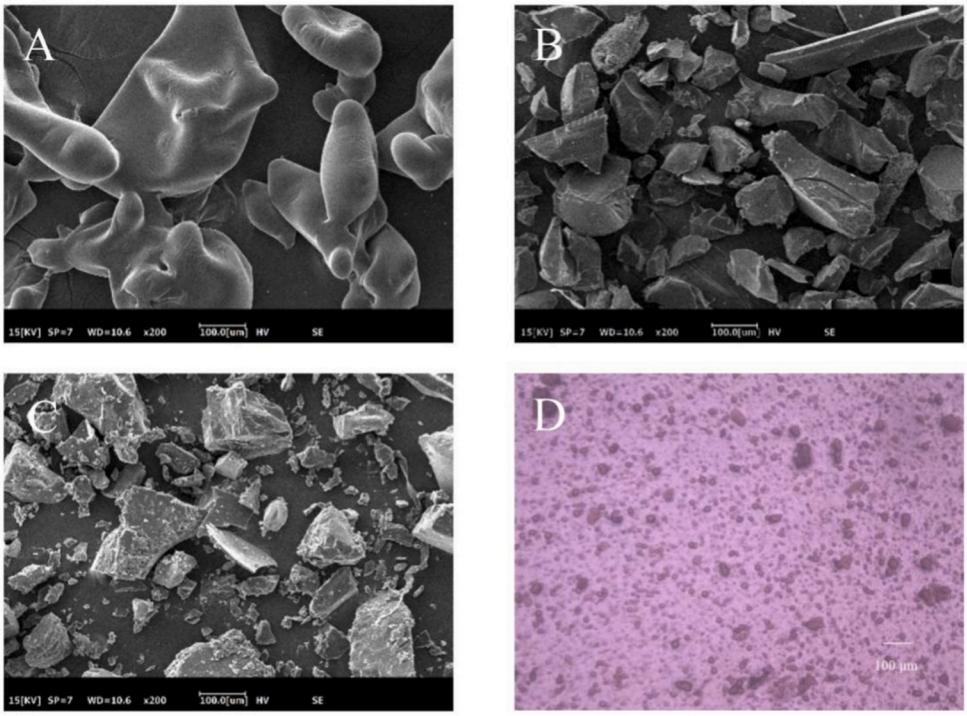


Fig. 1. Morphology structure. (A) SEM of granaticins. (B) SEM of GA. (C) SEM of microcapsules. (D) OM of microcapsules.

illustrated in Fig. 1B and C, the structure of the microencapsulated samples remains unchanged, indicating their structural stability. This implies that granaticins binds well to GA, and the optical microscope (OM) of microcapsules (Fig. 1D) presents spherical microcapsules with a chamber filled with granaticins, further indicating a proper encapsulation.

3.5.3. Structural characteristics of microcapsules

The FTIR spectra of the granaticins, GA and microcapsules are shown in Fig. S6. The absorption peaks of granaticins at 3371 cm^{-1}, 2937 cm^{-1}, 1714 cm^{-1}, 1607 cm^{-1}, 1418 cm^{-1} and 1270 cm^{-1} represented O—H stretching vibrations, =C-H stretching vibrations (saturated carbon), C═O stretching vibrations, C═C telescopic vibrations, C—H telescopic vibrations and C—C skeletal vibrations, respectively. The characteristic absorption peaks of GA at 3407 cm^{-1}, 2930 cm^{-1}, 1620 cm^{-1} and 1074 cm^{-1} were associated with O—H stretching, CH- stretching, C═O stretching/N-H bending and C—O stretching, respectively, which was consistent with previous studies (Han et al., 2020; Kang, Lee, Kim, & Chang, 2019). As shown in Fig. S6, compared with those in the granaticins spectrum, the absorption peaks at 2934 and 1607 cm^{-1} were redshifted, which indicates that the stretching of the O—H vibration (-COOH)/C-H and the telescopic vibration of the benzene ring telescopic vibrations were restrained. Compared with those in the granaticins spectrum, the absorption peaks at 2937 and 1611 cm^{-1} were redshifted, which indicated that the vibrations of C—H and the aromatic system of the granaticins backbone were suppressed. In addition, the broader absorption peak (3371 cm^{-1}) indicated that intramolecular hydrogen bonds can form between granaticins and GA, which could explain and confirm why appropriate GA concentrations have a positive effect on the microencapsulation of granaticins (Zhang et al., 2022).

3.5.4. Thermal stability of microcapsules

The TGA and derivative thermogravimetric analysis (DTGA) patterns of the granaticins, GA and microcapsules are shown in Fig. 2. The mass loss peak of the granaticins occurred at 179 °C, while that of the microcapsules peaked at 310 °C, which was related to thermal degradation of the sample. The maximum degradation temperature (Tm) and mass

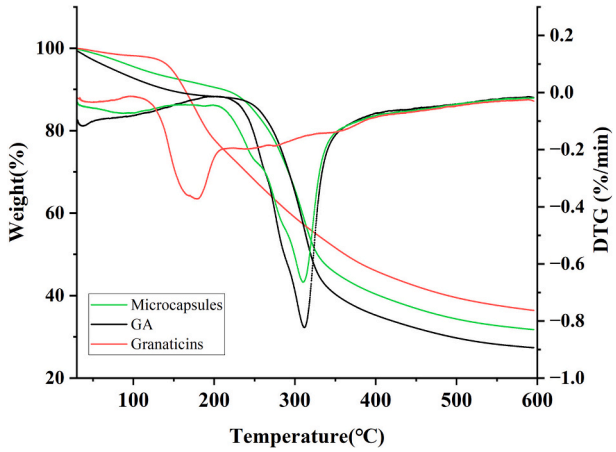


Fig. 2. TGA and DTG of granaticins, GA and microcapsules.

loss (%) of each sample in Table S2 showed that the microcapsules had a greater Tm than did the granaticins, which implies that more intermolecular interactions formed between the granaticins and GA, indicating that microencapsulation enhanced the thermal stability of the granaticins, as the wall material has a protective effect on heating (Cao et al., 2024).

3.6. Storage stability

The chemical stability of unstable components during long-term storage is an important indicator for microcapsule evaluation (Ding et al., 2022). A stability study was conducted to investigate the effect of sunlight exposure at room temperature and in the dark on the conversion of granaticins. HPLC showed that granaticinic acid (Compound 1) and MM44785 (Compound 2) were converted to Compound 3, granaticin (Compound 4) and granaticin (Compound 5); however, granaticin B was converted to granaticins again over time due to the instability of

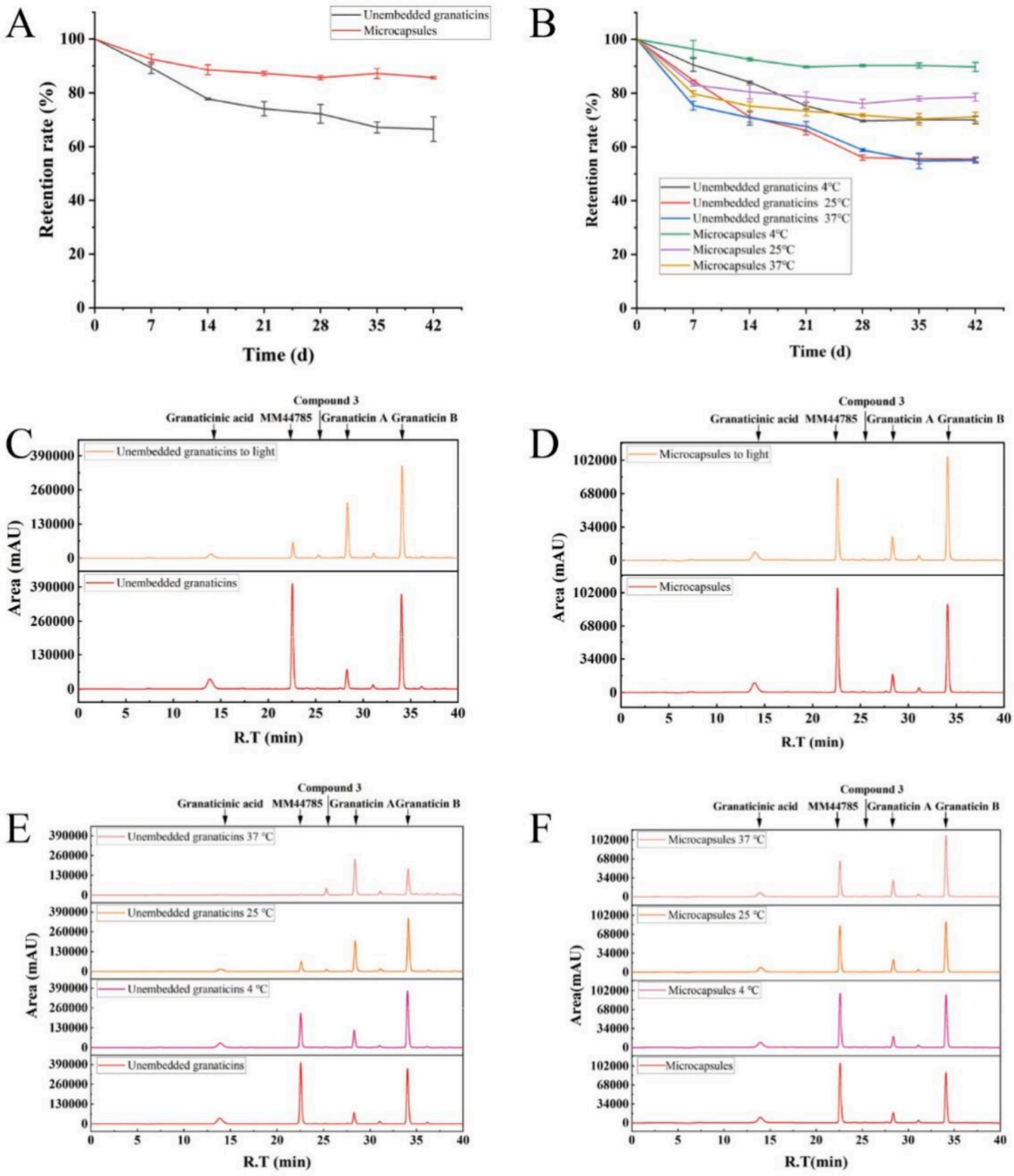


Fig. 3. Granaticins without embeding and microcapsules after sunlight and different temperatures storage for 42 days.
(A) Retention rates after sunlight storage.
(B) Retention rates after different temperatures storage.
(C) HPLC of unembedded granaticins before and after sunlight storage.
(D) HPLC of granaticins in microcapsules before and after sunlight storage.
(E) HPLC of granaticins before and after different temperatures storage.
(F) HPLC of granaticins in microcapsules before and after different temperatures storage.

granaticin B under acidic conditions.

Fig. 3A shows that the retention of granaticins without embedding was 66.50%, while after 42 days of storage under sunlight, the retention of the microcapsules was 1.29 times greater. Compared with those in the unembedded microcapsule, the proportion of granaticins lost in the microcapsules was significantly lower, and the highly active compound granaticin B eventually tended to remain at a higher level (Fig. 3C and D). Table S3 also shows that the amount of granaticinic acid decreased sharply from 188.24 μg/mg to 65.75 μg/mg in the unembedded granaticins, while the amount of granaticinic acid in the microcapsules decreased by only 22.91%. The results showed that the retention rates of microcapsules were greater than those of unembedded granaticins at different storage temperatures (4, 25 and 37 °C); among them, the retention rate of microcapsules reached 89.76% at 4 °C, while that of unembedded granaticins reached only 70.07% under the same treatment (Fig. 3B). HPLC chromatograms (Fig. 3E and F) revealed that the loss of granaticins intensified as the temperature increased, and granaticinic acid and granaticinic derivative MM44785 were almost undetectable in unembedded granaticins stored at 37 °C. Moreover, Table S3 also shows that granaticin B exhibited a high and stable plateau (increased by 6.60%, 15.50% and 21.65% at 4, 25 and 37 °C, respectively), while the amount of granaticin B decreased greatly in the unembedded samples, which was attributed to the good embedding ability of GA, which reduced its contact with external factors (Chranioti & Tzia, 2014).

Overall, microencapsulation improved the sunlight and thermal stability of granaticins, and the conversion between granaticins in microcapsules was inhibited as a result of the encapsulation process during high-temperature storage. Therefore, encapsulating GA as a wall material could significantly improve the stability of the core material.

4. Conclusion

The microencapsulation of granaticins using GA by the freeze-drying method has been shown to significantly improve the stability of these compounds. Optimal encapsulation conditions (GA 9.2% (*v*/v), a wall/−core ratio 4.8 (*w*/w), encapsulating temperature 29 °C) resulted in a high encapsulation efficiency of 93.64% and produced microcapsules with an average particle size of 206.37 ± 2.51 nm. Stability studies showed that microencapsulated granaticins exhibited enhanced stability, with significant retention improvements for granaticin B in both sunshine and different temperature storage. This work is also the first to clarify the conversion trends of granaticins and microencapsulation can also inhibit the conversion of granaticin B to granaticin A, preserving the more effective bioactive compound. Consequently, utilizing GA as a wall material is a promising technique for improving the stability of granaticins derived from *Streptomyces vilmorinianum* YP1.

CRediT authorship contribution statement

Xuechen Si: Writing – original draft, Methodology, Data curation, Conceptualization. **Zuoyun Yuan:** Methodology. **Huilin Li:** Methodology. **Yunping Zhu:** Writing – review & editing, Resources, Funding acquisition. **Yawen Zhou:** Methodology. **Jia Liu:** Methodology. **Zhichao Wu:** Methodology.

Declaration of competing 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

Data will be made available on request.

Acknowledgments

This work was supported by the National Natural Scientific Foundation of China (no.32272253, 31771960) and the Science & Technology Plan Program of Beijing (Z221100007122006).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2024.101548.

References

Agarry, E. I., Wang, Z. R., Cai, T., Kan, J. Q., & Chen, K. W. (2022). Chlorophyll encapsulation by complex coacervation and vibration nozzle technology: Characterization and stability study. *Innovative Food Science & Emerging Technologies, 78*, Article 103017. https://doi.org/10.1016/j.ifset.2022.103017

Aguilera, J. M., & Stanley, D. W. (1999). *Microstructural principles of food processing and engineering*.

Amiri, S., Shakeri, A., Sohrabi, M. R., Khalajzadeh, S., & Ghasemi, E. (2019). Optimization of ultrasonic assisted extraction of fatty acids from Aesculus hippocastanum fruit by response surface methodology. *Food Chemistry, 271*, 762–766. https://doi.org/10.1016/j.foodchem.2018.07.144

Aslan Türker, D., & Doğan, M. (2022). Ultrasound-assisted natural deep eutectic solvent extraction of anthocyanin from black carrots: Optimization, cytotoxicity, in-vitro bioavailability and stability. *Food and Bioproducts Processing, 132*, 99–113. https://doi.org/10.1016/j.fbp.2022.01.002

Barcza, S., Brufani, M., Keller-Schierlein, W., & Zähner, H. (1966). Metabolic products of microorganisms. 52. *Granaticin B. Helvetica chimica acta. 49*(6), 1736–1740. https://doi.org/10.1002/hlca.19660490603

Cao, Y., Gou, Q., Song, Z., Zhang, L., Yu, Q., Zhu, X., & Li, S. (2024). Smart carrageenan/carboxymethyl cellulose films combined with zein/gellan gum microcapsules encapsulated by composite anthocyanins for chilled beef freshness monitoring. *Food Hydrocolloids, 153*, Article 110059.

Chen, T. T., Zhang, Z. H., Wang, Z. W., Chen, Z. L., Ma, H. L., & Yan, J. K. (2021). Effects of ultrasound modification at different frequency modes on physicochemical, structural, functional, and biological properties of citrus pectin. *Food Hydrocolloids, 113*, Article 106484. https://doi.org/10.1016/j.foodhyd.2020.106484

Chranioti, C., & Tzia, C. (2014). Arabic gum mixtures as encapsulating agents of freeze-dried fennel oleoresin products. *Food and Bioprocess Technology, 7*, 1057–1065. https://doi.org/10.1007/s11947-013-1074-z

Deng, M. R., Li, H., & Li, Y. (2019). *Method for increasing yield of granaticin and granaticin B*. CN110484576A.

Dhakal, S. P., & He, J. (2020). Microencapsulation of vitamins in food applications to prevent losses in processing and storage: A review. *Food Research International (Ottawa, Ont.), 137*, Article 109326. https://doi.org/10.1016/j.foodres.2020.109326

Dharma, S., Masjuki, H. H., Ong, H. C., Sebayang, A. H., Silitonga, A. S., … Mahlia, T. M. I. (2016). Optimization of biodiesel production process for mixed Jatropha curcas–Ceiba pentandra biodiesel using response surface methodology. *Energy Conversion and Management, 115*, 178–190. https://doi.org/10.1016/j.enconman.2016.02.034

Ding, Z., Wang, X., Wang, L., Zhao, Y., Liu, M., … Wang, Z. (2022). Characterisation of spray dried microencapsules with amorphous lutein nanoparticles: Enhancement of processability, dissolution rate, and storage stability. *Food Chemistry, 383*, Article 132200. https://doi.org/10.1016/j.foodchem.2022.132200

Dutta, S. G., Shaik, A. B., Ganesh, K. C., & Kamal, A. (2017). Statistical optimization of production conditions of β-glucosidase from *bacillus stratosphericus* strain SG9. *3 Biotech, 7*(3), 221. https://doi.org/10.1007/s13205-017-0866-7

Frattini, M. G., Djaballah, H., & Kelly, T. J. (2011). *Cdc7 kinase inhibitors and uses thereof*. AU2011224457A1.

Han, C., Xiao, Y., Liu, E., Su, Z., Meng, X., & Liu, B. (2020). Preparation of ca-alginate-whey protein isolate microcapsules for protection and delivery of *L. bulgaricus* and *L. paracasei*. *International Journal of Biological Macromolecules, 163*, 1361–1368. https://doi.org/10.1016/j.ijbiomac.2020.07.247

Hu, Y., Li, Y., Zhang, W. L., Kou, G. N., & Zhou, Z. Q. (2018). Physical stability and antioxidant activity of citrus flavonoids in Arabic gum-stabilized microcapsules: Modulation of whey protein concentrate. *Food Hydrocolloids, 77*, 588–597. https://doi.org/10.1016/j.foodhyd.2017.10.037

Iwasaki, S., & Omura, S. (2007). Search for protein farnesyltransferase inhibitors of microbial origin: Our strategy and results as well as the results obtained by other groups. *The Journal of Antibiotics, 60*(1), 1–12. https://doi.org/10.1038/ja.2007.1

Kang, Y. R., Lee, Y. K., Kim, Y. J., & Chang, Y. H. (2019). Characterization and storage stability of chlorophylls microencapsulated in different combination of gum Arabic and maltodextrin. *Food Chemistry, 272*, 337–346. https://doi.org/10.1016/j.foodchem.2018.08.063

Kordjazi, T., Mariniello, L., Giosafatto, C. V. L., Porta, R., & Restaino, O. F. (2024). Streptomycetes as microbial cell factories for the biotechnological production of melanin. *International Journal of Molecular Sciences, 25*(5). https://doi.org/10.3390/ijms25053013. Article 5.

Labhasetwar, V. D., & Dorle, A. K. (1991). A study on the zeta potential of microcapsules during ageing. *Journal of Microencapsulation, 8*(1), 83–85. https://doi.org/10.3109/02652049109021861

Leylak, C., Özdemir, K. S., Gurakan, G. C., & Ogel, Z. B. (2021). Optimisation of spray drying parameters for *lactobacillus acidophilus* encapsulation in whey and gum Arabic: Its application in yoghurt. *International Dairy Journal, 112*, Article 104865. https://doi.org/10.1016/j.idairyj.2020.104865

Li, J., Hou, X., Jiang, L., Xia, D., Chen, A., Li, S., … Zhang, Z. (2022). Optimization and characterization of Sichuan pepper (*Zanthoxylum bungeanum* Maxim) resin microcapsule encapsulated with β-cyclodextrin. *LWT, 171*, Article 114120.

Nguyen, C. T., Nguyen Di, K., Phan, H. C., Kha, T. C., & Nguyen, H. C. (2024). Microencapsulation of noni fruit extract using gum Arabic and maltodextrin – Optimization, stability and efficiency. *International Journal of Biological Macromolecules, 269*, Article 132217. https://doi.org/10.1016/j.ijbiomac.2024.132217

Ogilvie, A., Wiebauer, K., & Kersten, W. (1975a). Inhibition of leucyl-transfer ribonucleic acid synthetasymol. *The Biochemical Journal, 152*(3), 511–515. https://doi.org/10.1042/bj1520511

Ogilvie, A., Wiebauer, K., & Kersten, W. (1975b). Stringent control of ribonucleic acid synthesis in Bacillus subtilis treated with granaticin. *The Biochemical Journal, 152*(3), 517–522. https://doi.org/10.1042/bj1520517

Ozturk, B., Argin, S., Ozilgen, M., & McClements, D. J. (2015). Formation and stabilization of nanoemulsion-based vitamin E delivery systems using natural biopolymers: Whey protein isolate and gum Arabic. *Food Chemistry, 188*, 256–263. https://doi.org/10.1016/j.foodchem.2015.05.005

Piovesana, A., & Noreña, C. P. Z. (2018). Microencapsulation of bioactive compounds from Hibiscus calyces using different encapsulating materials. *International Journal of Food Engineering, 14*(1), 20170170. https://doi.org/10.1515/ijfe-2017-0170

Ricicová, A., & Podojil, M. (1965). Quantitative assay of granaticin by the microbiological diffusion method. *Folia Microbiologica, 10*(5), 299–301. https://doi.org/10.1007/BF02871029

Singh, S., Arya, H., Sahu, W., Reddy, K. S., Nimesh, S., Alotaibi, B. S., … Kumar Bhatt, T. (2024). Green synthesized silver nanoparticles of *Terminalia bellirica* leaves extract: Synthesis, characterization, in-silico studies, and antimalarial activity. *Artificial Cells, Nanomedicine, and Biotechnology, 52*(1), 238–249. https://doi.org/10.1080/21691401.2024.2339429

Van, C. K., Nguyen, P. T. N., Nguyen, T.-T. T., & Ach, L. G. (2024). Microencapsulation of *Citrus latifolia* peel essential oil by spray-drying using maltodextrin: Characterization, antimicrobial activities, and release profile. *LWT, 197*, Article 115825.

Walter, K. (1974). *Granaticin B and method of preparing same*. US3836642A.

Zhang, Z. H., Yu, B., Xu, Q., Bai, Z., Ji, K., … Xiao, R. (2022). The physicochemical properties and antioxidant activity of Spirulina (Artrhospira platensis) chlorophylls microencapsulated in different ratios of gum Arabic and whey protein isolate. *Foods (Basel, Switzerland), 11*(12), 1809. https://doi.org/10.3390/foods11121809

Zhao, C. C. (2015). *Study on effective method to improve the stability of natural red kermel color. M. Eng.* Thesis. Tianjin: Tianjin University of Science and Technology.

Zhao, T., Liu, F., Duan, X., Xiao, C., & Liu, X. (2018). Physicochemical properties of lutein-loaded microcapsules and their uptake via Caco-2 monolayers. *Molecules (Basel, Switzerland), 23*(7), 1805. https://doi.org/10.3390/molecules23071805

Zhao, T., Liu, F., Duan, X., Xiao, C., & Liu, X. (2018). Physicochemical properties of Lutein-Loaded microcapsules and their uptake via Caco-2 Monolayers. *Molecules, 23*(7). https://doi.org/10.3390/molecules23071805

Zheng, S., Zhao, H., Yuan, Z., Si, X., Li, Z., Song, J., … Wu, H. (2023). The analysis of the glycosyltransferase gene function from a novel Granaticin producer, Streptomyces Vilmorinianum. YP1. *Current Microbiology, 80*(4), 103. https://doi.org/10.1007/s00284-023-03192-5

Zhu, Y., Shang, X., Yang, L., Zheng, S., Liu, K., & Li, X. (2020). Purification, identification and properties of a new blue pigment produced from Streptomyces sp. A1013Y. *Food Chemistry, 308*, Article 125600. https://doi.org/10.1016/j.foodchem.2019.125600