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Microsphere formulation of quercetin-pectin from red dragon skin: Comparison of encapsulation techniques

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Abstract: **Background**: Quercetin can be enhanced by creating microspheres using polymers, with the quality of these microspheres influenced by manufacturing processes and polymer type. **Objectives**: This study compares high-speed stirring with ultra-turrax (method 1) and peristaltic dosing pumps (method 2) for producing quercetin-pectin microspheres. **Methods**: The method employed involves the production of quercetin-pectin microspheres by the ionic gelation process. Six formulas were created for each method, utilizing pectin extracted from red dragon skin with oxalic acid, as well as commercial apple pectin and orange peel pectin at concentrations of 1% and 1.5%. Parameters were evaluated, including FTIR, yield, moisture content (MC), polydispersity index, particle size, drug loading (DL), encapsulation efficiency (EE), Scanning Electron Microscope, Carr's Index, Hausner ratio, swelling index and *in vitro* drug release. **Results**: Results showed that the yield obtained ranged for method 1: 83.27-96.37%; yield for method 2: 84.13-93.87%; swelling index of method 1: 92.8±3.25-96.2±3.26%; swelling index of method 2: 94.5±3.41-97.7±2.43%; MC of method 1: 2.58±0.31-3.64±0.57%; MC of method 2: 2.93±0.15-3.64±0.27%; PDI of both method is 0.003%; DL of method 1 was 2.03±0.11-2.37±0.77%; DL of method 2 was 2.75±0.03-2.94±0.51%; EE of method 1 was 70.34±0.72-83.78±1.47%; EE of method 2 was 73.49±0.89-90.61±1.49% in vitro release up to 600 minutes for method 1 was 67.83±5.88-91.94±5.84%; for method 2 was 69.64±3.14-92.29±4.82. The FTIR profile exhibits resemblances between the two approaches, indicating the existence of O-H, C-O, and C-H groups, suggesting commonalities in the composition of quercetin and pectin molecules. The results of the study showed that microspheres produced using a custom-made peristaltic dosing pump yielded better DL, EE, release test, Carr's index, and Hausner ratio values compared to those produced using method 1 ($p > 0.05$). **Conclusion**: Peristaltic dosing pumps produce higher yields, exhibiting enhanced spherical morphology and demonstrate improved microsphere performance relative to ultra-turrax.

Keywords: Microspheres; Pectin; Peristaltic dosing pump; Quercetin; Ultra turrax

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INTRODUCTION

Quercetin is a flavonoid compound with a variety of potential health benefits, including antioxidant, anti-inflammatory, and potential anticancer properties (Shabir *et al.*, 2022). However, quercetin has several properties that make it difficult for the body to absorb and utilize it properly when consumed directly (Setyawan *et al.*, 2018). Therefore, quercetin encapsulation may be beneficial in increasing its bioavailability and effectiveness. One weakness of quercetin is its susceptibility to oxidation, light, and pH changes. Encapsulating quercetin in an appropriate encapsulant material, such as a polymer or lipid, protects it from elements that could destroy it during storage and digestion (Kurniawan, Setyawan and Hariyadi, 2024). Another limitation of quercetin is its low solubility in water, which hinders the body's absorption when ingested directly. Encapsulating quercetin inside an appropriate system enhances its solubility, facilitating improved absorption in the gastrointestinal tract.

Encapsulation safeguards quercetin from digestive enzymes and pH fluctuations, prolonging its interaction with the intestinal mucosa and enhancing absorption (Dey, Ghosh and Giri, 2020). Encapsulation enhances the regulation of quercetin release. This data can establish a preferred release profile, such as slow or targeted release to some areas of the digestive system, thereby enhancing the body's utilization of quercetin. Utilizing appropriate encapsulation technology can augment the effectiveness of quercetin in delivering certain health benefits by targeting its absorption to particular areas of the body, such as the small intestine or other designated tissues (Li *et al.*, 2023). Consequently, quercetin encapsulation can enhance its bioavailability, stability and efficacy as a supplement or

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pharmaceutical agent, thereby improving its capacity to deliver the intended health advantages (Grgic *et al.*, 2020). Several methods can be used to form encapsulation, including drop extrusion, spray drying, emulsification, coacervation, and ionic gelation (Kurniawan, Setyawan, and Hariyadi, 2024). One of the most frequently used methods is the ionic gelation method, which offers several advantages, including low cost, minimal equipment requirements, and the avoidance of high temperatures and organic solvents. This method is suitable for encapsulating hydrophobic active substances such as quercetin (Kurozawa and Hubinger, 2017). Ionic gelation is a method that involves cross-linking polyelectrolytes with oppositely charged ions to form a hydrogel. The type of polymer and crosslinker used is critical in determining the quality of the resulting microencapsulation. Factors influencing microencapsulation properties include the type of polymer, the ratio of drug to polymer, the type of crosslinker, crosslinker concentration, and manufacturing method (Hariyadi, Hendradi, and Sharon, 2019). Mixing the polymer-drug with the crosslinker is crucial when preparing microspheres using the ionic gelation technique, as it significantly influences their quality. Mixing using an ultra-turrax is one of the devices or technologies used in making microspheres or micron particles. Ultra thurrax allows excellent control over the size of the particles produced. By varying parameters such as rotor speed, air pressure, or type of material used, operators can achieve the desired particle size with high precision (Xu *et al.*, 2016).

In this research, the quality of microencapsulation was optimized by comparing two different technical ionic gelation methods, equalizing the type of polymer, drug-polymer ratio, type of crosslinker, and concentration of crosslinker used. The two methods used are high mixing using an ultra-turrax and mixing using a peristaltic dosing pump. Peristaltic dosing pumps are crucial in pharmaceutical, water treatment, laboratory, and chemical industries for precise liquid dosages, ensuring ingredient addition and product consistency in manufacturing microspheres, and are regulated by precise controls (Tamadon *et al.*, 2019). Comparing ionic gelation methods using ultra-thin syringes and peristaltic dosing pumps aims to optimize the microsphere manufacturing process by evaluating encapsulation efficiencies, particle size homogeneity, and physicochemical characteristics, including size, morphology, stability, porosity, and release profiles of the active ingredients. This comparison is also important for determining the more efficient method in terms of time, energy, and cost, and assessing its scalability for industrial applications. Ultra-thurrax tends to produce homogeneous dispersions with high mechanical energy, while peristaltic dosing pumps offer more precise control, making them more suitable for large-scale or sensitive materials. In this research, three types of pectin were also used, namely two types of commercial pectin (apple and orange peel) and pectin from dragon fruit peel, which was extracted using oxalic acid because dragon fruit peel pectin which was extracted using oxalic acid solvent provided the profile and characteristics that were closest to with commercial pectin (Kurniawan, Hariyadi and Setyawan, 2024).

MATERIALS AND METHODS

In this research, a quercetin-pectin microsphere formulation was prepared using the ionic gelation method, and optimization was performed using two different techniques when mixing the pectin-quercetin polymer with the crosslinker. In the first method, a mixing technique using an ultra-turrax (Method 1) is employed, while in the second method, a mixing technique utilizing a peristaltic dosing pump (Method 2) is used. This study was performed at the Multi-Purpose Laboratory of the Faculty of Pharmacy, Airlangga University. Three types of pectin were also used in this study: pectin extracted from red dragon fruit skin using oxalic acid, and two types of commercial pectin (apple and orange peel). The utilized red dragon fruit skin was of the *Hylocereus polyrhizus* variety. The fruit segments were treated to a controlled drying procedure in a food dehydrator at 50°C for four hours. Following the drying phase, the resultant material was carefully ground into a fine powder.

An extensive analysis was performed to evaluate the moisture content of the acquired powder. Following this evaluation, 25 g of the dried raw material was measured. The extraction solvent consisted of 1000 mL of oxalic acid at a pH of 4.00. The extraction procedure was conducted utilizing a hot plate magnetic stirrer, with the temperature maintained at 75°C for 120 minutes. After the extraction operation, the resultant filtrate underwent a filtration step. The precipitation of pectin was subsequently performed by adding 96% alcohol to the filtrate, according to an exact 1:1 ratio between the filtrate and the alcohol. The coagulation persisted for 24 hours. The mixture was filtered to obtain wet pectin, and subsequently dried using a freeze dryer at -40°C under a pressure of 200 mTorr for 96 hours (Kurniawan, Setyawan, and Hariyadi, 2025). Tables 1 and 2 show the formulations of microspheres.

Method 1

The pectin solutions were prepared separately at different concentrations, each 100 ml, using distilled water. They were then stirred using an ultra-turrax at 3600 rpm for 10 minutes, as shown in Table 1. Weighed 0.2 g of quercetin and dissolved it in 20 ml of ethanol, stirred until homogeneous, then slowly added to the previous pectin solution and stirred using an ultra-turrax at 3600 rpm for 30 minutes until homogeneous to form a pectin-quercetin solution. A 5.5% $CaCl_2$ solution was prepared in 100 ml of distilled water, which will be used as a crosslinker. The pectin-quercetin solution was added gradually to the $CaCl_2$

solution while stirring at 3600 rpm for 35 minutes using an ultra-turrax. The microparticles formed were separated from the $CaCl_2$ solution by centrifugation at 2500 rpm for 6 minutes and then washed using distilled water. Microparticles were resuspended in 5% maltodextrin as a lyoprotectant while stirring using an ultra-turrax at 3600 rpm for 10 minutes. The quercetin microparticle suspension was dried using a freeze dryer at -50°C for 96 hours. Method 1 produces six formulas, namely FA1, FB1, FC1, FD1, FE1 and FF1.

Method 2

The pectin solution was prepared separately at different concentrations. A total of 100 ml of distilled water was used, and then it was stirred using an ultra-turrax at 5000 rpm for 10 minutes, as shown in Table 2. Weighed 0.2 g of quercetin and dissolved it in 20 ml of ethanol, stirred until homogeneous, then slowly added to the previous pectin solution and stirred using an ultra-turrax at 5000 rpm for 30 minutes until homogeneous to form a pectin-quercetin solution. A 5.5% $CaCl_2$ solution was prepared in 100 ml of distilled water, which will be used as a crosslinker. The pectin-quercetin solution was added gradually to the $CaCl_2$ solution using a peristaltic pump at a rate of 2 ml/min, while stirring with a magnetic stirrer at 1000 rpm for 60 minutes. The microparticles formed were separated from the $CaCl_2$ solution by centrifugation at 3500 rpm for 6 minutes. The supernatant was discarded, and the particles were washed using distilled water. This process involved adding sufficient distilled water to the quercetin coagulant and then centrifuging at 3500 rpm for 6 minutes (three times, for replication). The microparticles were resuspended in 5% maltodextrin as a lyoprotectant and stirred gently with moderate agitation using a stir bar until a homogeneous solution was achieved. The quercetin microparticle suspension was dried using a freeze dryer at -50°C for 96 hours. Method 2 produces six formulas, namely FA2, FB2, FC2, FD2, FE2 and FF2.

Evaluation of microsphere parameters

Yield

The yield value of nearly 100% demonstrates the effectiveness of the manufacturing method in producing optimal microparticles. Quercetin microparticles are weighed, and the percentage is calculated based on the formula below (Kalalo *et al.*, 2022):

$$yield = \frac{weight\ of\ microparticles}{weight\ of\ polymer + quercetin + lyoprotectant} x\ 100\%$$

Moisture content

Measure the moisture content of quercetin microparticles using a moisture analyzer at a temperature of 110°C for 10 minutes (Kurniawan, Hariyadi and Setyawan, 2024).

Carr's index and the Hausner-ratio

Carr's index and Hausner-Ratio are determined using the formula (Hariyadi *et al.*, 2022):

$$\text{Carr's index} = \frac{tapped\ density - bulk\ density}{tapped\ density}\ 100\%$$

$$\text{Hausner ratio} = \frac{tapped\ density}{bulk\ density}\ 100\%$$

FTIR

Microsphere powder was mixed with KBr powder until a smooth mixture was achieved at a ratio of 1:100. After mixing, the sample was placed in a sample holder and then pressed until it formed a thin, even layer that did not break. Measurements were carried out in the wavelength range of 4000-400 cm^{-1} (Hariyadi *et al.*, 2020).

Pectin and microsphere morphology

Morphological evaluation of dried quercetin microspheres using Scanning Electron Microscope (SEM) instruments. The sample to be tested is first coated with gold on its surface. Samples ready to be tested are inserted into the Hitachi Flexsem 100 SEM testing instrument equipped with a microscope using a high voltage of 20 kV with a magnification of 7500x (Hariyadi *et al.*, 2022).

Polydispersity index (PDI)

Observations were made using an optical microscope, and measurements were taken using Optilab software. Start by placing the microspheres to be observed on a glass slide or other suitable surface. Then, 300 particles were measured and grouped based on the smallest and largest particle sizes of all samples, and they were then divided into several intervals and classes. The average diameter value was determined, and a particle size distribution curve was created using the formula (Rosita *et al.*, 2022).

$$\frac{\sum nd}{\sum n}$$

n = number of microparticles observed
d = particle size

$$\alpha = \frac{weight\ before\ swelling - \ weight\ after\ swelling}{weight\ before\ swelling}\ 100$$

Swelling index

The swelling index is a measurement used to determine the extent to which a material can swell or expand when exposed to specific substances, such as water. The swelling index test was performed by weighing 100 mg of microparticles and adding 5 mL of PBS (pH 7.4) to the vial. This test is carried out for 24 hours and 30 hours. After the specified time, the wet microparticles are filtered using filter paper. After no PBS droplets remain, the wet microparticles are transferred to dry filter paper until they are no longer wet and no PBS is left. Wet microparticles were dried using an oven at 37ºC for 2 hours or until the weight of the microparticles became constant. After drying, the microparticles were weighed and determined as the degree of swelling (α) based on the formula.

$$\alpha = \frac{weight\ before\ swelling - \ weight\ after\ swelling}{weight\ before\ swelling}\ 100$$

Encapsulation efficiency (EE) and drug loading (DL)

The amount of quercetin trapped in the microparticle system was determined directly by calculating the total concentration in the microparticles compared to the concentration of quercetin added to the formula. The quercetin content was determined by dissolving 100 mg of quercetin-pectin microparticles in 100 mL of ethanol using sonication for 60 minutes, until the microparticles were completely dissolved. Then, it was filtered and analyzed using UV spectrophotometry with a wavelength of 370 nm. Measurements were replicated three times. (Kalalo *et al.*, 2022)

$$EE = \frac{quercetin\ weight\ in\ sample}{,theoretical\ quercetin\ weight}\ 100\%$$

$$DL = \frac{quercetin\ weight\ in\ sample}{microparticle\ sample\ weight}\ 100\%$$

In-vitro release

This test was conducted using a phosphate buffer solution of pH 7.4, which was shaken in a water bath at 100 rpm and 37°C, and was replicated three times. After that, samples were taken at 15, 30, 60, 120, 180, 240, 300, 360, 420, 480, 540 and 600 minutes, totaling 5.0 mL. When taking the sample, the same volume was replaced, and then the absorbance of each sample was observed using UV-Vis spectrophotometry at the maximum wavelength of quercetin, namely 370 nm. This test procedure has been reviewed and received an ethical approval letter from the UAD research ethics committee, No: 022410144/2024.

RESULTS

Pectin morphology

Fig. 1 shows the three types of pectin used in the formulation by SEM. It can be observed that the morphology of red dragon fruit skin pectin is similar to that of apple pectin and orange peel pectin. This result supports previous research indicating that the pectin in red dragon fruit skin is similar to that found in apple and orange peel, as determined by FTIR analysis. Pectin is derived from various natural sources and is readily available and inexpensive to obtain. Due to this, it is a cost-effective option for pharmaceutical formulations (Kurniawan, Hariyadi, and Setyawan, 2024).

Yield, moisture content (MC), polydispersity index (PDI), drug loading (DL), encapsulation efficiency (EE), Carr's index and Hausner-Ratio, swelling Index

Tables 3 and 4 show the characteristics resulting from methods 1 and 2. During manufacturing, data were obtained indicating that the yield, DL, and EE values of the microspheres produced using method 2 were higher than those produced using method 1, as were the Carr index, Hausner ratio, and Swelling index values. However, this difference was not statistically significant ($p>0.05$). The yield values produced by both methods equally meet the requirements, where the MC value requirement for microspheres is 2-5%, and both methods can produce MC values within that range. The yield values for both methods also produced several microspheres with diameters greater than 80%. This indicates that the effectiveness of the results obtained using these two methods is excellent. This is because freeze-drying is a very suitable method for this process. The swelling index values resulting from methods 1 and 2 are not statistically different. This indicates that the pectin can expand, which will affect the amount and time of quercetin release from the microspheres. The DL and EE values indicate that the microspheres produced using method 2 have higher values than those produced using method 1; however, the difference is not statistically significant ($p > 0.05$). This indicates that manufacturing using a peristaltic dosing pump will produce microspheres with the desired characteristics, making it easier to incorporate quercetin into the pectin polymer matrix. The same results are shown by the Carr index and Hausner ratio values, where method two also produces better values than method one, but the difference is not statistically significant. This indicates that the microspheres produced by method 2 have better flow properties than those produced by method 1. The Carr Index and Hausner Ratio can also indicate how well the microspheres can be compressed into certain dosage forms, such as tablets.

FTIR

For the FTIR profile, fig. 2 and 3 show the FTIR results from methods 1 and 2. From the FTIR test results, methods 1 and 2 produced the same intensity pattern at wave numbers 3411, 1600, 1380, and 1010, indicating the presence of the same C-H, C=O, C-O, and C-O-C stretching functional groups as those in quercetin (Aun *et al.*, 2023). A change in the absorption intensity pattern at wave numbers 100-500 in the resulting microsphere indicates a structural change caused by the influence of the $CaCl_2$ crosslinker and pectin polymer.

Microsphere morphology

For the morphology by SEM, Figs. 4 and 5 show the SEM results of microspheres produced from methods 1 and 2. The results of the SEM analysis indicate that method 2 yields a more spherical microsphere morphology visually, compared to method 1. In method 2, many spherical microspheres are formed, while in method 1, there are more irregular shapes. This will result in microspheres made using method 2 having good flow properties and a better release of quercetin than those made using method 1. This is reflected in the DL, EE, Carr index, and Hausner ratio values of the microspheres made using method 2, which are higher than those made using method 1. This indicates that the morphology of the resulting microspheres influences the release of quercetin and its physical properties.

In-vitro release

Fig. 6 and 7 show *the in vitro* release profile of quercetin-pectin microspheres produced using methods 1 and 2. In

the in vitro release test, values were also produced that were not significantly different between the two types of methods used (p>1). However, the amount of quercetin released when made using method two is higher than when using method 1. These results indicate that the encapsulation process in method 2 is more efficient than that in method 1. The peristaltic dosing pump produces a more spherical microsphere shape, enabling better absorption of quercetin.

DISCUSSION

Pectin possesses the distinctive characteristic of undergoing physical and ionic cross-linking in response to external stimuli. This property enables pectin to construct a three-dimensional hydrogel network readily. The level of esterification has a significant impact on the pectin gelation process. With a high degree of methyl-esterification, Pectin is exceptionally responsive to an acidic environment, causing it to form gels at low pH levels and in the presence of high sugar concentrations.

Alternatively, pectin, which has a low level of methyl esterification, can form gels when divalent cations are present. The egg-box model explains this cross-linking process. Combining microencapsulation techniques with pectin polymers offers various advantages, including protection of active substances from external factors, pH-responsive release control, increased solubility and bioavailability, and safe biocompatibility (Xu *et al.*, 2024). Additionally, pectin derived from plant sources supports the development of halal and environmentally friendly products, utilizing organic waste, such as the skin of red dragon fruit, to promote sustainability. The easily modified nature of pectin also allows for broader and more specific microencapsulation applications for high-quality pharmaceutical products. Cross-linking is the process of creating a network inside polymer solutions, which improves their mechanical properties and viscoelastic behavior. The bonds, which are not stable, can be broken down under normal physiological conditions through physical or chemical cross-linking. Chemical cross-linking, which involves the use of covalent agents, improves mechanical stability but can potentially compromise polymer integrity and lead to increased toxicity.

The ionic gelation technique involves the interaction of polymers with opposite charges and cross-linking agents with complementary charges. Unlike other methods, polyelectrolyte complexation is solely based on electrostatic interactions between polyions that are either positively or negatively charged, without the need for cross-linking agents. The innovative technique of dual cross-linking integrates both physical and chemical elements, resulting in decreased toxicity and enhanced stability (Bejenaru *et al.*, 2024).

Furthermore, it has been verified that elevated temperatures enhance the gelation capacity in both high-methylated and low-methylated pectins. Therefore, hydrogen bonds and hydrophobic interactions play a significant role in pectin gelation. Pectin's capacity to undergo gelation under various external stimuli employing sol-gel technology enables the creation of three-dimensional networks in the form of hydrogels (Nesic *et al.*, 2022).

Polymers play a crucial role in the formation of microspheres in drug formulations. Polymers can provide physical stability to microspheres, prevent clumping or agglomeration of drug particles and maintain the size and shape of microspheres during storage and administration (Han *et al.*, 2022). Pectin is a natural polymer easily broken down by the body and is generally considered safe for use in pharmaceutical applications. Pectin is soluble in water, allowing the formation of a stable solution or dispersion for the encapsulation process. This ability facilitates the use of pectin to form encapsulated microspheres or nanoparticles (Gawkowska, Cybulska, and Zdunek, 2018).

Microspheres can be used as controlled drug delivery systems that can be loaded with drugs and are designed to release drugs gradually in the body, allowing for more controlled drug dosing and prolonged release. Some drugs have low solubility in body fluids. In this case, microspheres can be used to increase the solubility of the drug by incorporating it into a water-soluble matrix or layer (Nayak *et al.*, 2018). Microspheres can be designed to release drugs gradually over time, which is beneficial for drugs that require gradual dosing or sustained release in the body. Microspheres enable precise dosing, which is essential for drugs requiring careful dose measurement, such as critical drugs or drugs with a narrow dose range (Das, Ahmed and Saha, 2019). Microspheres in pharmaceutical formulations often offer advantages, including enhanced therapeutic effectiveness, improved dose control, and a potential reduction in side effects. Microspheres can also increase the stability and availability of drugs in the body (Jat *et al.*, 2018). Microsphere formulations can reduce the required doses, increasing patient compliance with the treatment regimen (Bhadraiah *et al.*, 2023).

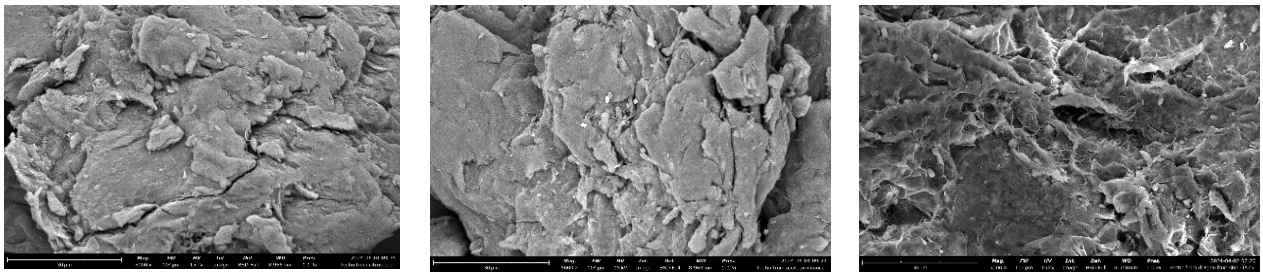
Freeze-drying, also known as lyophilization, is used in pharmaceuticals, food, and biotechnology to remove water from heat-sensitive biological or chemical materials by freezing and then sublimating the water under low pressure. Freeze-drying allows for the preservation of the structure and biological activity of the dried material. This process does not involve high heating, which can damage or alter the structure or activity of the material, allowing for the yield of microspheres with the desired properties to be obtained (Bhatta, Janezic, and Ratti, 2020).

Table 1: Quercetin-pectin microspheres formulations using method 1

Material	FA1	FB1	FC1	FD1	FE1	FF1
Quercetin hydrate	0.2%	0.2%	0.2%	0.2%	0.2%	0.2%
Commercial orange peel pectin	1%	1.5%	-	-	-	-
Commercial apple pectin	-	-	1%	1.5%	-	-
Pectin from red dragon fruit skin	-	-	-	-	1%	1.5%
$CaCl_2$	5.5%	5.5%	5.5%	5.5%	5.5%	5.5%
Maltodextrin	5%	5%	5%	5%	5%	5%

Table 2: Quercetin-pectin microspheres formulations using method 2

Material	FA2	FB2	FC2	FD2	FE2	FF2
Quercetin hydrate	0.2%	0.2%	0.2%	0.2%	0.2%	0.2%
Commercial orange peel pectin	1%	1.5%	-	-	-	-
Commercial apple pectin	-	-	1%	1.5%	-	-
Pectin from red dragon fruit skin	-	-	-	-	1%	1.5%
$CaCl_2$	5.5%	5.5%	5.5%	5.5%	5.5%	5.5%
Maltodextrin	5%	5%	5%	5%	5%	5%



(Commercial orange peel pectin) (Commercial apple pectin) (Red dragon fruit peel pectin)

Fig. 1: Morphology of commercial orange peel pectin, commercial apple pectin and red dragon fruit peel pectin extracted with oxalic acid (C) with 5000x magnification.

Table 3: Microsphere characteristics of method 1

Formula	Parameters							
	Yield (%)	MC (%)	PDI	DL (%)	EE (%)	Carr index (%)	Hausner ratio (%)	Swelling index (%)
FA1	84.13±2.58	2.93±0.41	0.0033±2.78x10^{-7}	2.03±0.11	75.56±1.25	13.21±2.43	1.16±0.05	92.83±3.25
FB1	96.37±3.24	3.64±0.57	0.0033±5.83 x10^{-7}	2.19±0.11	83.78±1.47	13.70±2.32	1.14±0.06	95.87±2.83
FC1	83.27±1.92	2.58±0.31	0.0033±4.52 x10^{-7}	2.28±0.05	72.45±2.52	13.5±2.32	1.16±0.05	96.24±3.26
FD1	88.60±2.27	2.98±0.83	0.0033±2.73 x10^{-7}	2.37±0.05	80.86±1.31	13.00±2.95	1.17±0.06	95.29±3.23
FE1	85.35±2.83	2.83±0.94	0.0033±3.89 x10^{-7}	2.15±0.34	70.34±0.72	15.28±2.31	1.12±0.008	94.82±5.62
FF1	88.47±3.52	3.49±0.33	0.0033±4.91 x10^{-7}	2.37±0.77	78.18±1.47	11.65±2.28	1.14±0.012	93.71±6.15

Table 4: Microsphere characteristics of method 2

Formula	Parameters							
	Yield (%)	MC (%)	PDI	DL (%)	EE (%)	Carr index (%)	Hausner ratio (%)	Swelling index (%)
FA2	89.33±3.48	3.19±0.31	0.0033±4.17 x10^{-7}	2.87±0.78	81.83±1.03	12.87±2.25	1.15±0.03	94.65±3.18
FB2	93.87±2.67	4.47±0.38	0.0033±3.49 x10^{-7}	2.94±0.51	90.61±1.49	11.70±2.09	1.13±0.03	95.41±3.95
FC2	85.40±4.13	4.96±0.41	0.0033±3.82 x10^{-7}	2.91±0.45	81.28±1.33	12.50±2.89	1.14±0.04	96.79±2.63
FD2	88.39±3.51	3.17±0.53	0.0033±4.73 x10^{-7}	2.99±0.89	86.24±1.38	13.00±2.22	1.15±0.03	97.74±2.43
FE2	84.13±2.51	2.93±0.15	0.0033±7.23 x10^{-7}	2.75±0.03	73.49±0.89	15.33±1.89	1.18±0.03	95.83±5.14
FF2	86.40±2.47	3.64±0.27	0.0033±6.47 x10^{-7}	2.80±0.04	82.18±1.10	8.40±2.76	1.09±0.03	94.53±3.41

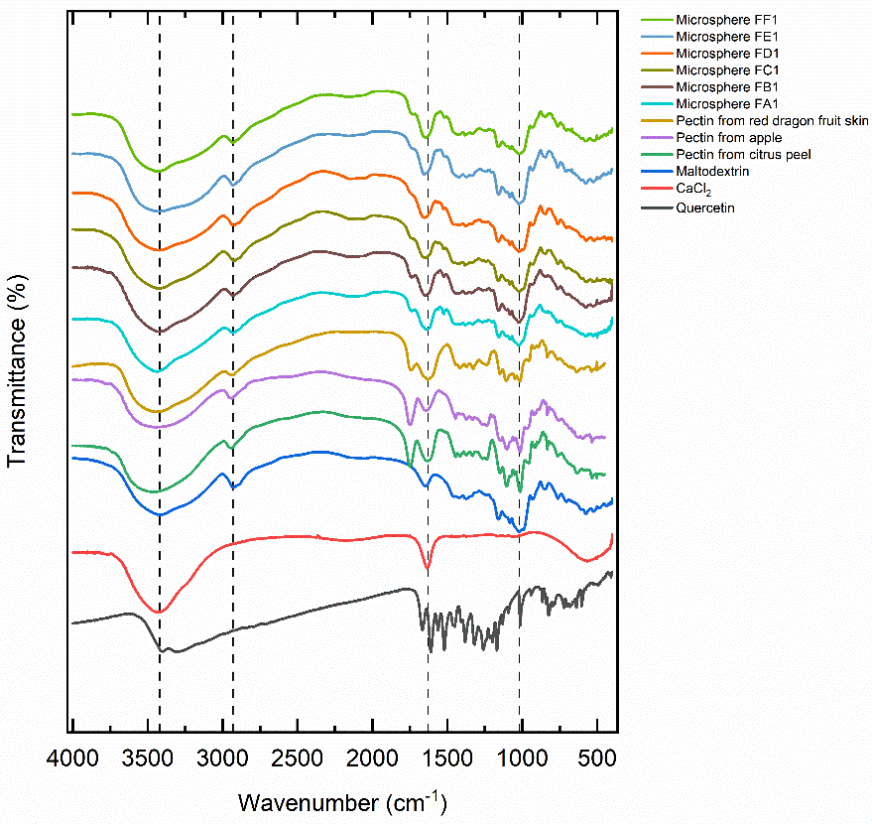


Fig. 2: FTIR of microspheres of method 1

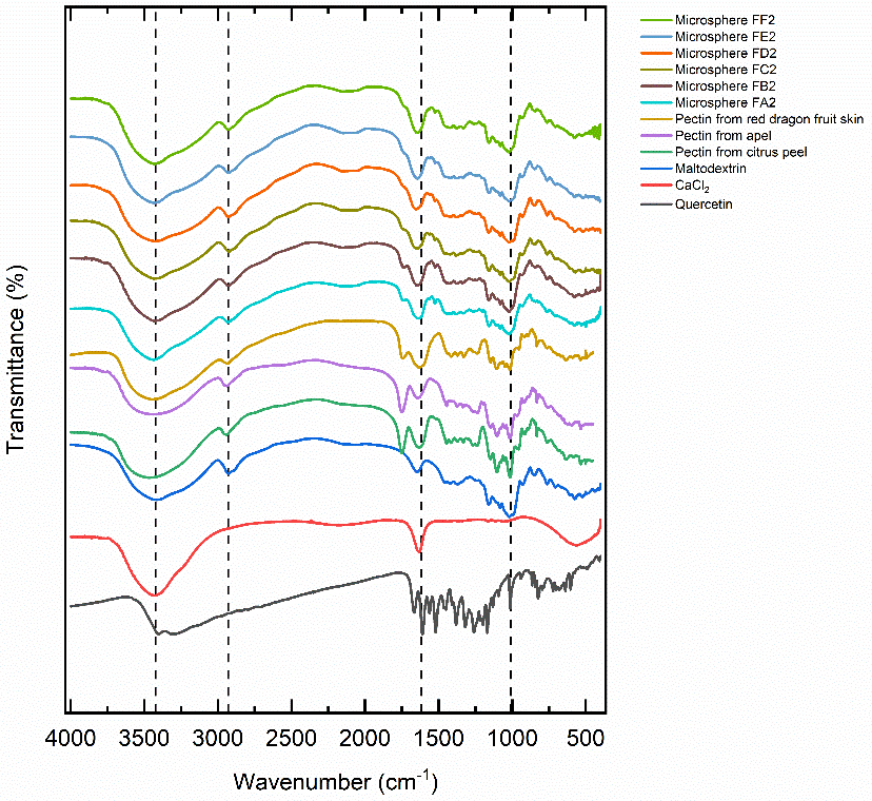
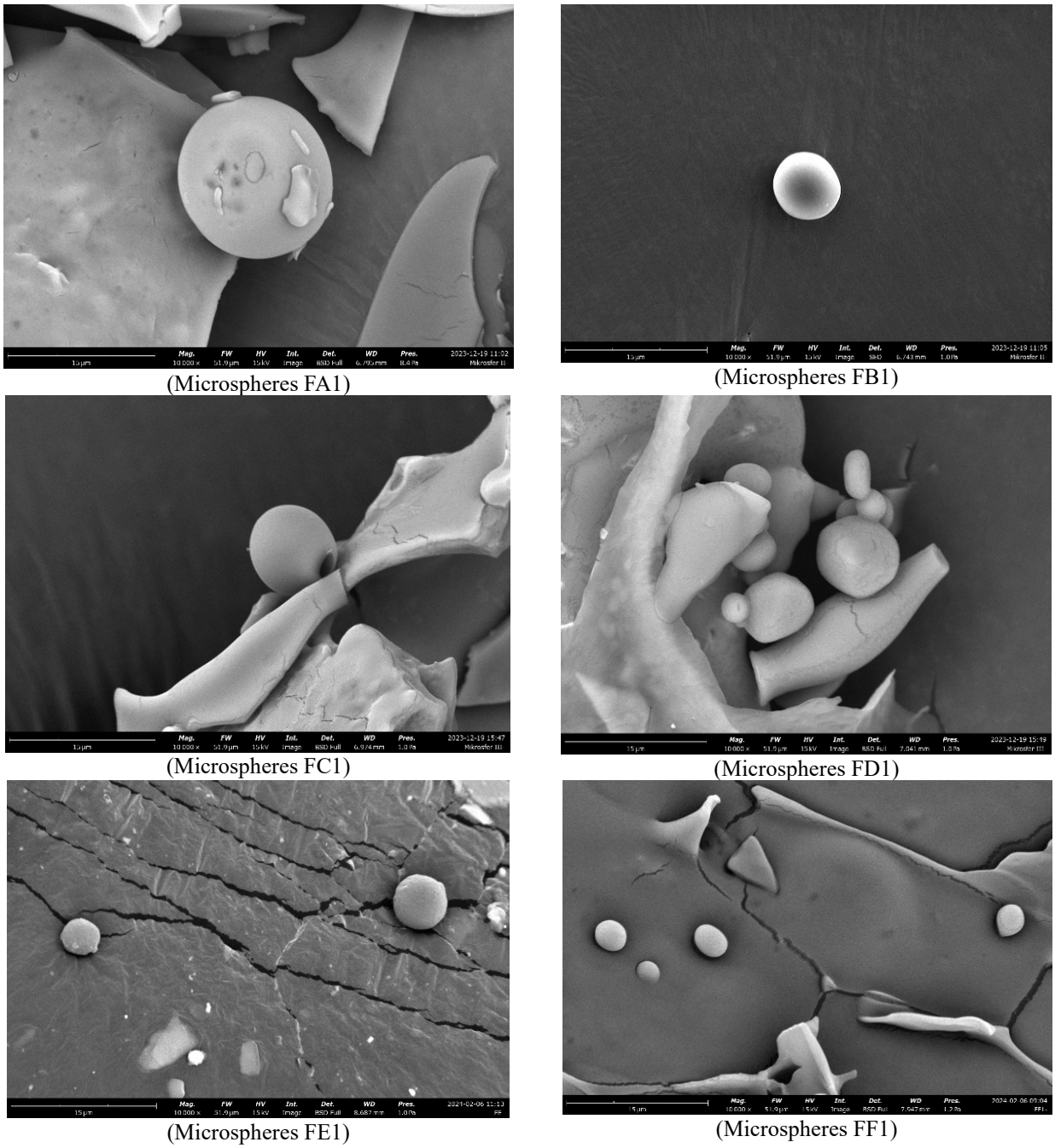


Fig. 3: FTIR of microspheres of method 2



(Microspheres FA1) (Microspheres FB1)

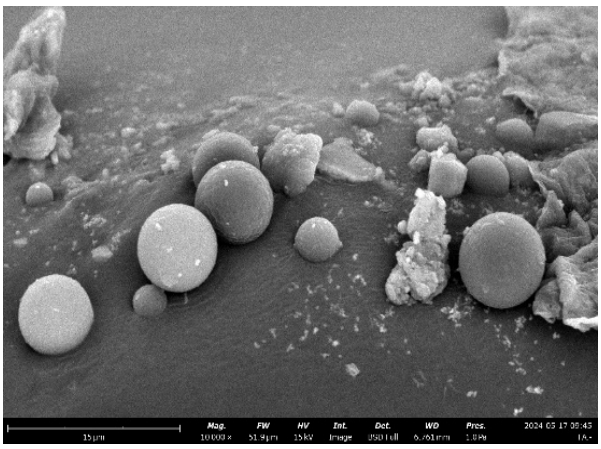
(Microspheres FC1) (Microspheres FD1)

(Microspheres FE1) (Microspheres FF1)

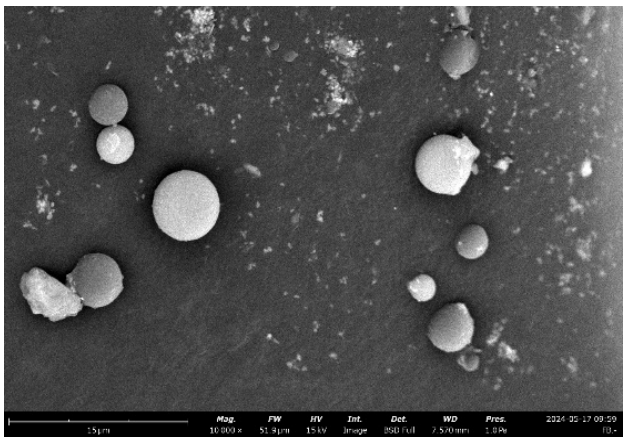
Fig. 4: SEM of microspheres of method 1 (10000x magnification)

The freeze-drying process reduces the risk of damage to the material through a controlled freezing and water removal process. This allows the integrity of the microsphere particles to be maintained, increasing the final yield. Freeze drying produces the final product in a lighter and denser form than conventional drying methods. This reduces the volume and weight of the product, which in turn can increase the yield relative to the initial weight or volume of the material. Products produced through freeze-drying tend to have better shelf stability than those produced through other drying methods. This can increase the product's shelf life and minimize losses during storage. The freeze-drying process allows for the addition of materials, such as protectants or binding agents, which can enhance the characteristics of the resulting microspheres and ultimately increase the yield (Krakowska-Sieprawska *et al.*, 2022).

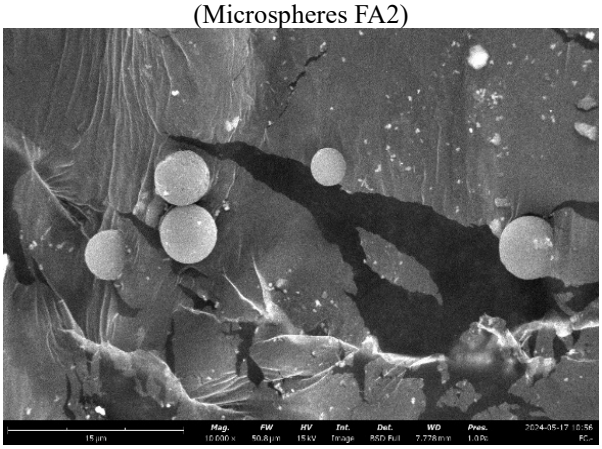
The PDI assessment parameters have nearly identical values and are not significantly different between the two methods used. The PDI value shows that the microspheres are evenly distributed and monodisperse. PDI is a parameter used to characterize the particle size distribution in a sample. When the PDI value approaches 0, the particle size distribution is homogeneous or monodisperse,



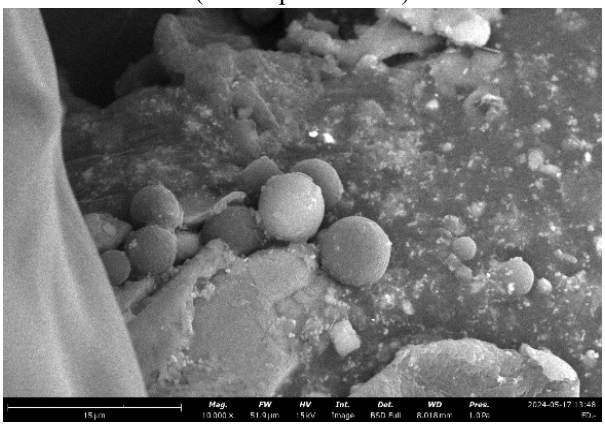
(Microspheres FA2)



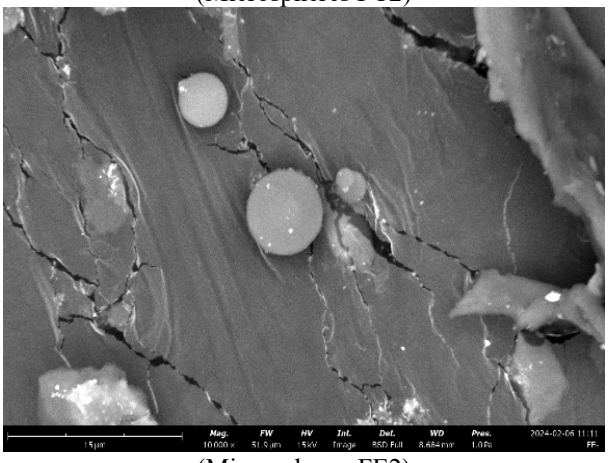
(Microspheres FB2)



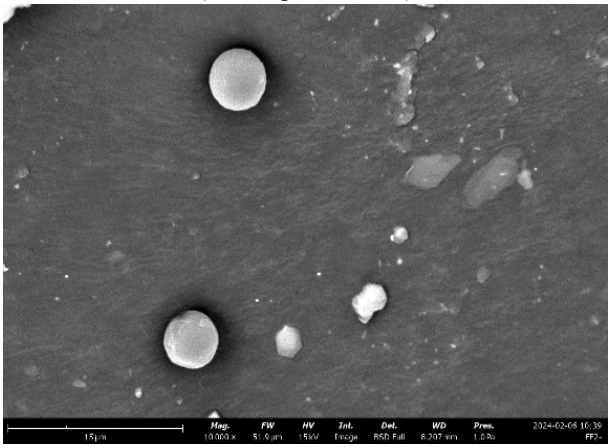
(Microspheres FC2)



(Microspheres FD2)



(Microspheres FE2)



(Microspheres FF2)

Fig. 5: SEM of microspheres of method 2 (10000x magnification)

meaning that the particle sizes in the microspheres are almost uniform. Monodisperse particles exhibit consistent physical and chemical properties. This is useful in many applications where microsphere performance must be consistently predicted. When the particle sizes in a sample are homogeneous, production processes or laboratory experiments can be replicated more efficiently, and similar results can be expected over time. When the particle sizes in a sample are homogeneous, production processes or laboratory experiments can be replicated more efficiently, and similar results can be expected over time. With a uniform particle size distribution, no particles will be wasted because the size matches the needs of a particular application. This can reduce material waste and production costs (Danaei *et al.*, 2018).

Good compressibility is essential to ensure consistent quercetin dosage and physical stability of the microspheres. Changes in the Carr Index and Hausner Ratio over time can provide clues about the physical

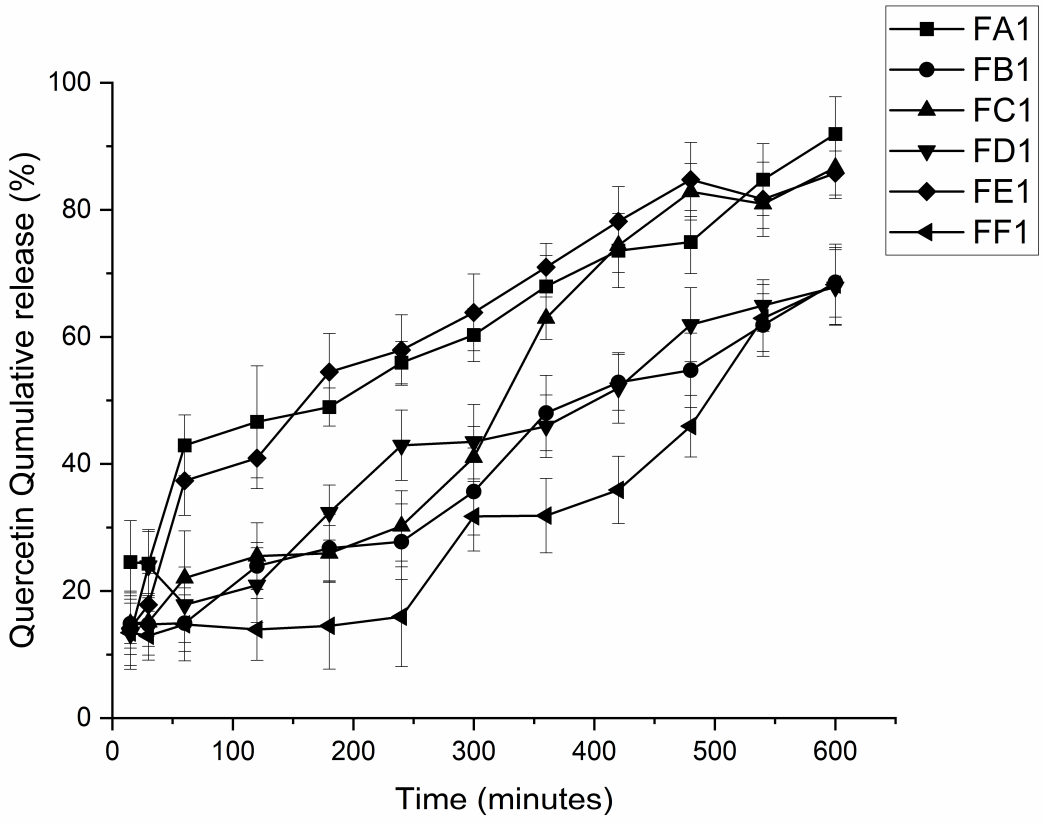


Fig. 6: *In-vitro* release study from method 1

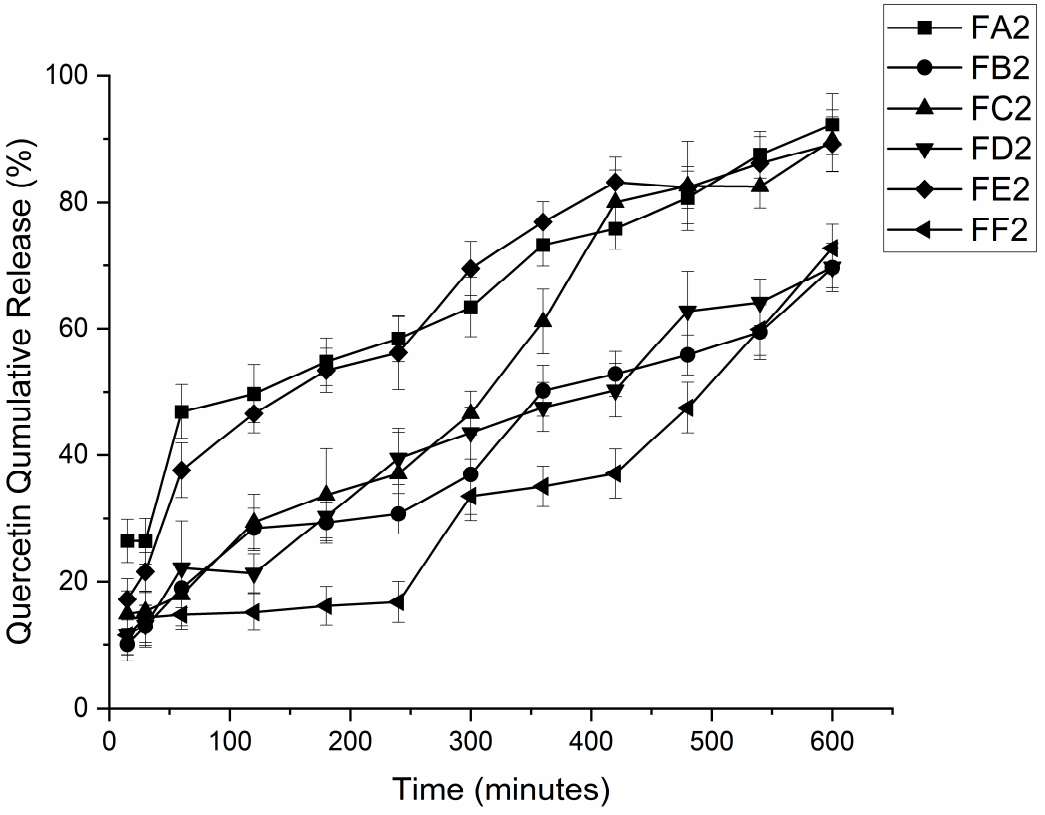


Fig. 7: *In-vitro* release study from method 2

stability of the microsphere formulation. A sharp decrease in the Carr index and Hausner ratio values indicates a problem that needs to be addressed, such as a change in particle size or moisture absorption (Buanz, 2021). A homogeneous particle size distribution is critical in the microsphere manufacturing process. Ultra-Turrax can provide a more uniform particle size distribution, which is essential for applications such as manufacturing drugs or other pharmaceutical ingredients. Ultra-Turrax is typically designed to work efficiently, optimizing the microsphere manufacturing process by reducing production time and waste (Cabrera-Trujillolo and Filomena-Ambrosio, 2018).

Instruments like the ultra-turrax are often equipped with easy-to-use interfaces, which allow operators to monitor and manage the process accurately. This allows for quick adjustments in the event of changes in production parameters or desired particle specifications. Ultra-turrax can often work with various materials, including pharmaceutical ingredients, nutraceuticals, or other chemicals used to manufacture microspheres. This capability allows flexibility in product and formulation development (Mangione *et al.*, 2023). Although Ultra-Turrax can work with various materials, some may require more convenient processing with this equipment. Some materials may require a more balanced or sticky formulation to be processed successfully with the Ultra-Turrax, limiting flexibility in product formulation selection. Although the ultra-turrax can produce tiny particles, there are limits to how fine the particles can be. Additional technologies or manufacturing methods may be required (Watkins *et al.*, 2010).

Peristaltic dosing pumps operate on the principle of peristalsis, where fluid is pumped through an elastic tube by repeatedly contracting and relaxing the tube. However, dosing pumps are designed to accurately control the fluid flow rate, delivering consistent and precise dosing (Dey, Mondal, and Sengupta, 2019). With advancements in medical sciences and the discovery of new drugs, it has become clear that more accurate and efficient techniques of injecting therapeutic fluids, particularly over extended periods, are necessary. The peristaltic pump is utilized in nearly all medical monitoring procedures involving fluid movement. The precise administration of medications is necessary in hospitals, elderly care facilities, clinics, and when providing care to patients in their own homes.

This pump is suitable for administering slow liquid pharmaceutical injections to reduce pain in various inpatient treatments. It can also feed tube patients who are unable to consume solid foods. Given the crucial significance of peristaltic pump applications in medical institutions and the previous design efforts made on this instrument, this research aims to design a versatile pump model and create the necessary plans for its industrial production using interaction design (Faraji, Razavi and Fatouraee, 2013).

The advantages of using a peristaltic dosing pump include delivering accurate dosing, ease of use and maintenance, and high safety, as the pumped fluid does not come into contact with instrument parts. They can also be used for many fluids sensitive to contamination or chemical changes (Ramírez-carvajal, Puerto-López and López-Barrera, 2024). Peristaltic dosing pumps use the peristaltic principle, where the material passes through a replaceable pump hose. This reduces the risk of cross-contamination between product batches because no pump part comes into direct contact with the material. Peristaltic dosing pumps can work with various materials, including chemicals, pharmaceuticals, or nutraceuticals used to make microspheres. This makes it flexible and can be integrated with various product formulations. Peristaltic dosing pumps are generally easy to operate and maintain. They often have an intuitive user interface, allowing operators to quickly set up and monitor the process (Uzawa *et al.*, 2022).

The flow of material through the peristaltic dosing pump tends to be smooth and stable, resulting in the consistent addition of microspheres. This helps ensure that the distribution of ingredients in the mixture is homogeneous and that the quality of the microspheres is maintained. Brittle or sensitive materials often cannot withstand the high pressures of pumps. Peristaltic dosing pumps work to minimize stress on the material, making them suitable for brittle materials without the need for additional pumps or complicated modifications (Zehetbauer *et al.*, 2021). Some materials used in the production of microspheres may have high viscosity. Peristaltic dosing pumps may be less effective in handling high-viscosity materials because they can cause wear to the tubing and reduce dosing precision. This will make it challenging to use polymers with high concentrations because they have high viscosity; therefore, alternative methods are needed, such as using a pressurized sprayer gun. The hose on a peristaltic dosing pump can change dimensions over time, especially if exposed to certain chemicals. These dimensional changes can compromise dosing precision and result in variations in microsphere production yield. Peristaltic dosing pumps are generally more suitable for dosing applications that require low to moderate pressure. If high pressures are required for manufacturing microspheres, peristaltic dosing pumps may not be able to provide the desired performance, or additional pumps may be necessary (Yamatsuta *et al.*, 2019).

The process of dosing peristalsis may introduce air or oxygen into the material, especially if the material being pumped is susceptible to oxidation. This can be a problem in the manufacture of microspheres that require a non-oxygen environment to prevent material degradation or contamination. Although peristaltic dosing pump maintenance is relatively easy, it requires routine maintenance and periodic hose replacement to ensure

optimal performance. Failure to perform this maintenance may result in reduced dosing precision and increased risk of contamination. Although peristaltic dosing pumps provide precise dosing, flow rates can be variable, especially when operational conditions change or the hose experiences wear and tear. This can affect the consistency of microsphere production from one batch to another (Costi *et al.*, 2022).

CONCLUSION

Quercetin-pectin microspheres were prepared using the ionic gelation method with a crosslinker, employing two different techniques. In method one, the mixing technique was employed using an ultra-turrax; whereas in the second method, mixing was performed using a peristaltic dosing pump. The results of the microspheres made using a peristaltic dosing pump (method 2) show that the DL, EE, in vitro release value, Carr index, and Hausner ratio are better than those produced using method 1. The morphology of microspheres also showed that using method 2 produces a more spherical shape, which affects the quercetin release profile and leads to the development of dosage forms and improved therapeutic effectiveness. Furthermore, this research demonstrated that red dragon fruit skin pectin used in forming microspheres produces parameters of the same quality as commercial pectin.

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Authors' contributions
D.M.H. contributed to the concept and design research, as well as research supervision. M.F.K. and F.P.D. contributed to data collection, analysis, and drafting the script. D.S. participated in the research design, research, planning, and supervision. All authors have approved the final manuscript.

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Data availability statement
The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflict of interest
The authors declare that there is no conflict of interest regarding the conduct and publication of this research.

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