Trop J Nat Prod Res, February 2025; 9(2): 554 - 560 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Potential of Cell-Free Fermentation Supernatant from Yellow Passion Fruit Microspheres as a Novel Antibacterial Agent Against Multi-Drug Resistant Organisms

Esti Hendradi[1,2] , Tutiek Purwanti[1], Andang Miatmoko[1], Safarini Marwah[3], Isnaeni Isnaeni[1,4]

[1]*Department of Pharmaceutical Sciences, Faculty of Pharmacy, Universitas Airlangga, Mulyorejo, Surabaya, 60115, Indonesia;*
[2]*Nanotechnology and Drug Delivery System Research Group, Faculty of Pharmacy, Universitas Airlangga, Mulyorejo, Surabaya, 60115, Indonesia;*
[3]*Master Program of Pharmaceutical Sciences, Faculty of Pharmacy, Universitas Airlangga, Mulyorejo, Surabaya, 60115, Indonesia;*
[4] *Bachelor of Pharmacy Study Program, Faculty of Health Sciences, Universitas Muhammadiyah Surabaya, Mulyorejo, Surabaya, 60113, Indonesia*

ARTICLE INFO

Article history:
Received 13 December 2024
Revised 30 December 2024
Accepted 04 February 2025
Published online 01 March 2025

ABSTRACT

In the face of rising multidrug resistance organism (MDRO), the search for effective antibacterial agents is paramount. This study investigates the potential of encapsulated cell-free fermentation supernatant (CFFS) from yellow passion fruit as a novel antibacterial agent. Two formulas with a variation of 2.5% alginate-gelatin polymer were encapsulated using aerosolization technique. All formulations showed uniform spherical microparticles and were distributed without agglomeration. All formulas showed that there was no significant difference between the formulas and all quality attributes. From the evaluation results, brown microspheres were obtained with particle sizes between 42 - 58 µm, and %MC 2-4. Microspheres also showed a good swelling index. Formula 2 showed a denser and more rigid microsphere surface with observation using SEM at 5000x magnification. This is supported by the FTIR results where in formulas 1 & 2 a biopolymer network has occurred between alginate and gelatin. Microspheres have a larger inhibition zone against Methicillin Resistant *Staphylococcus aureus* (MRSA) than Extended Spectrum Beta Lactamase (ESBL) *Escherichia coli* of 14 mm and 13.5 mm, respectively. This condition is associated with a more complex inhibition mechanism in ESBL *Escherichia coli*. However, compared with CFSS and fresh juice of passion fruit, the antibacterial activity against MDROs like MRSA and ESBL *Escherichia coli* are decreased. This phenomenon is caused by the decrease in cell viability during the encapsulation process to 10^6 CFU/ml. Therefore, cell free fermentation supernatant microspheres from yellow fruit can be a good and potential antibacterial agent in inhibiting the growth of multidrug resistance organisms.

Keywords: Antibacterial activity, Passion fruit, Microsphere, Multidrug resistant organism, Fermentation supernatant

Introduction

Antibiotic resistance is a significant health issue in the world[1]. These phenomena cause increasing morbidity and mortality rates and also lead to more extended treatment periods and higher therapy expenses[2]. These bacteria, which mutate and adapt to antibiotics[3] and make inadequate therapy[4], are classified as Multi-drug Resistant Organisms (MDRO), by which the organisms are no longer vulnerable at least to one type of antibiotic in three or more categories[5]. ESBL *Escherichia coli* and MRSA are the most prevalent MDROs in Asia[6]. Vancomycin is currently the most often used antibiotic in MDRO therapy, but long-term use of this antibiotic lead to a higher risk of insensitivity to all bacteria[7]. Research regarding potential antibacterial alternatives has shifted from synthetic to herbal products. Herbal products have a complex activities and low side effects. The antibacterial activity of herbal products have been linked to the presence of bioactive compound[29].

***Corresponding author. E mail: esti-h@ff.unair.ac.id
Tel: +6281330175672

Citation: Hendradi E, Purwanti T, Miatmoko A, Marwah S, Isnaeni I. Potential of Cell-Free Fermentation Supernatant from Yellow Passion Fruit Microspheres as a Novel Antibacterial Agent Against Multi-Drug Resistant Organisms. Trop J Nat Prod Res. 2025; 9(2): 554 – 560 https://doi.org/10.26538/tjnpr/v9i2.20

Yellow passion fruit *(Passiflora edulis* forma *flavicarpa* Sims.) is one of the herbal products that has numerous biological activities, including antioxidant, analgesic, and anti-inflammatory properties, as well as antihypertensive, hepatoprotective, lung protective, anti-hyperlipidemic, antidiabetic, and antidepressant effects[8].

The yellow passion fruit is a tropical plant from Brazil that is grown extensively in China, South America, India, and Southeast Asia[8]. The yellow passion fruit has a higher acidity level (pH < 3.2) than other varieties due to the presence of citric and malic acids. Moreover, this fruit also has a high nutritional value, including vitamins A, B_2, and C, as well as non-nutritive phytochemicals, carotenoids, and polyphenols. It is also high in minerals such as K, P, Ca, Fe, Na, Mg, S, Cl, and protein. Previous research has shown that yellow passion fruit pulp can suppress the growth of *Vibrio cholera, Pseudomonas aeroginosa, Escherichia coli, Bacillus subtilis, Salmonella typhi, Staphylococcus aureus, and Streptococcus pyrogens*[9]. Meanwhile, cell-free fermentation supernatant (CFFS) of yellow passion fruit shows good inhibitory strength against *Escherichia coli and Staphylococcus aureus*, as well as the ability to inhibit MDRO such as ESBL *Escherichia coli* and MRSA[10].

Fermentation is known as a natural process that can increase the nutritional profile of passion fruit and antimicrobial activity by promoting the growth of good bacteria[11]. The CFFS of passion fruit is a perfect vehicle for probiotic cultures because it provides the necessary nutrients and environment for bacteria to grow[12]. The CFFS of passion fruit has modest inhibitory activity against ESBL *Escherichia coli* and MRSA. Apart from the organic compounds contained in the fruit, it also contains bacteriocin, which synergizes to increase antibacterial

activity[11]. Bacteriocin is a bactericidal probiotic toxin that attacks bacterial target membranes and causes intracellular chemical leakage[13]. Probiotics from the Lactobacillus group[11], such as *Lactobacillus reuteri*[14], are typically found in fermented passion fruit.

To make probiotics effective for humans, the number of living microorganisms should be more significant than 6 log CFU/g or provide a daily dose of 10^6-10^9 CFU/ml living bacteria[15]. The most serious concern is probiotic cell death caused by the environment factors. Hence, a safe production method for bacterial viability is required. In food biotechnology, microencapsulation can also be used to enclose microorganisms by isolating them from the external environment with a hydrocolloid covering, allowing the cells to be released at the appropriate time in the gut compartment. Their advantages include preventing interfacial inactivation, stimulating secondary metabolite generation and excretion, and ensuring ongoing usage. The physical-chemical features of the capsules influence the viability of encapsulated cells, including the type and concentration of the coating material, particle size, initial cell counts, and bacterial strains[16].

Microencapsulated probiotics have been used to maintain the life of various bacteria. The viability of *Lactobacillus casei* can be maintained in extreme conditions and through the digestive system in silk sericin-alginate microspheres[30]. Microencapsulation of *Lactobacillus acidophilus* La-05 helps preserve its viability during storage, exposure to stress conditions, and stimulated gastrointestinal digestion[31]. *Lactobacillus plantarum* in alginate-gelatin encapsulation is also known to have good antibacterial activity during storage, and simulated GIT conditions[32].

Alginate polymer is one of the suitable polymer hydrocolloids for probiotic microencapsulation[32]. These polymers are utilized to preserve active molecules and probiotics because they are able to protect the substance in acidic conditions and release it in alkaline conditions[19]. Alginate will cross-link with Ca^{2+} ions and form a strong egg box structure[32]. However, these polymers may risk of particle leakage because of the porous structure[19]. Coating the microcapsules with gelatin can reduce their exposure to oxygen during storage and improve their stability at low pH[16]. By adding gelatin to the alginate polymer, it forms a more rigid microcapsule with a smoother surface[32].

Another issue is that the large quantity of low weight molecule sugar and organic acids in passion fruit makes drying process of microsphere being difficult due to its sticky nature, which leads to a low yield during processing. The inclusion of polysaccharides, such as maltodextrin, helps alleviate this issue[12]. Gelatin combined with maltodextrin has also been shown to improve cellular viability and phenolic component retention over the maltodextrin alone[14].

As scientific research continually uncovers the hidden potential of natural sources. Research related to the ability of passion fruit CFFS to inhibit MDRO such as ESBL *Escherichia coli* and MRSA has been known[19], and the presence of storage time and enzymatic processes is known to be able to reduce its antibacterial effectiveness. The combination of alginate and gelatin polymer microspheres is known to be able to maintain moderate antibacterial activity of probiotics against both bacteria until day 45[32]. The novelty of this research is the application of microencapsulation in cell free fermentation supernatant of passion fruit using a combination of alginate-gelatin polymers to maintain viability and increase its antibacterial effectiveness. Evaluation of CFFS microspheres was carried out including physical properties and antibacterial activities against ESBL *Escherichia coli* and MRSA .

Materials and Methods

Plant source and determination

Yellow passion fruit was collected from a local garden in Sidoarjo, West Java, Indonesia (-7.389437703847348, 112.58939396165188), which was harvested in April 2023. Passion fruit plants were identified and determined based on taxonomic characters of leaves, flowers, fruits, and identified by Herbarium Malangensis, Department of Biology, Faculty of Mathematics and Natural Sciences, State University of Malang as *Passiflora edulis* forma *flavicarpa* Sims.

Sample preparation, fermentation, and characterization

Yellow passion fruits pulp was washed and divided aseptically into two parts, then the pulp was weighed as much as 20 g and put into 100 mL of de Man-Rogosa and Sharpe (MRS) broth media (Merck Millipore) to be fermented with a orbital shaker (SHO-2D, Daihan Scientific, Korea) at 150 rpm and 37°C for 24 hours[10]. The fermentation broth was taken and then centrifuged. The supernatant put into 10 mL portion in vials. The solution was stored at 8°C and each vial was used for one microsphere formula, also for identification of isolate biochemical reaction (Gram straining, catalase, and motility test) and verification using VITEK 2 (bioMérieux, France).

Microencapsulation of cell free fermentation supernatant of yellow passion fruit by aerosolization technique

Two microspheres formula were made with different matrix compositions (Table 1). Sodium alginate (low viscosity, Sigma-Aldrich) and maltodextrin (food grade, Baolingbao Biology Co, Ltd.) were weighed according to the formula, then dissolved in 100 mL of purified water, stirred at a speed of 1,000 rpm for 10 minutes. Calcium chloride (Anhydrous powder, Reag, Ph.Eur, Merck) weighed as many as 11.1 grams, then dissolved in 100 mL of purified water, and stirred until dissolved. All solution were sterilized by autoclaving (Huxley HL-340, Somatco, Taiwan) at 121°C for 15 minutes. The polymer solutions and 10 mL CFFS were sprayed using nozzle aerosolization into calcium chloride solution while stirred at a speed of 1,000 rpm, then left for 90 minutes. The microspheres formed were separated using a Buchner funnel, while being washed until the water were free of from calcium chloride. The bovine gelatin (Gel strength 225 g Bloom, Type B, Sigma Aldrich) was weighed according to the formula, dispersed in 100 mL of 40°C sterile water until swelling. Wet microspheres are dispersed in the swollen gelatin solution and stirred in a water bath at a speed of 1,000 rpm for 10 minutes. The solution was separated using a Buchner funnel. The microspheres were dried using a freeze dryer (DK-FD12T, Biobase, China) for 96 hours. The characterizations of the microspheres and microbial assay were carried out[20].

Physical characterization of formulation

Organoleptic: Organoleptic evaluation is performed visually by observing powder form, color, and odor.

Determination of swelling index and % moisture content: Moisture content analysis was measured using a moisture analyser (HB43-5, Mettler Toledo, Germany) of 500 mg microspheres and replicated three times. And for swelling index, 50 mg of microspheres were weighed and added 20 mL of sterile water in test tube. Observations were made at 37°C for 5, 30, 60, and 120 minutes. The microspheres filtered and aerated for 20 seconds and weighed as the final weight[21]. Swelling index is calculated by the equation1[17].

$$swelling\ index = \left|\frac{initial\ weight - final\ weight}{initial\ weight}\right| x\ 100\% \quad \ldots\ldots(1)$$

where initial weight and final weight are the dry and swollen weights of the sample, respectively. The swelling index of each microsphere was calculated three times, and the average was taken to record the final results.

Analysis of molecular structure using FTIR spectroscopy

Evaluation of the occurrence of cross-linking reactions was carried out by infrared spectra examination using FTIR Spectrophotometer (Alpha II, Bruker, Germany). The result of the examination was compared to the infrared spectrum of sodium alginate, gelatin, and microspheres.

Morphology of the microspheres

The microsphere shape was observed using an optical binocular microscopy (CX23, Olympus, Japan) coupled to a digital camera at 1000x magnification and a scanning electronic microscope (SEM) (Flexsem 100, Hitachi High Technologies, Japan) in 500-5000x magnification. The particle size distribution was determined by Particle Size Analyzer (LA-960, Horiba, Ltd., Japan).

Microbial assay and microsphere of cell free fermentation supernatant of yellow passion fruit

Cell viability and antibacterial activity were evaluated by comparing the free cells and microspheres. The cell viability was determined using the total plate count method in MRS broth medium up to 10^{10} dilution. Incubation was performed for 30 hours at 35°C. Colonies were counted using a colony counter (8500, Funke Gerber, Germany). On the other hand, the antibacterial activity was measured by diffusion well method using a combination of two medium, nutrient agar (NA) (Merck Millipore) and MRS broth, according to the test bacteria (ESBL and MRSA, which were obtained from Dr. Soetomo Regional Public Hospital, Surabaya, West Java, Indonesia). The MRSA used a 25%: 75% (w/v) MRS-NA medium combination and ESBL *Escherichia coli* used a 50%: 50% (w/v) MRS-NA medium combination[22]. The volume of test bacteria tested was 5 uL. In this study, vancomycin 32 ppm was used as a positive control for MRSA and *Staphylococcus aureus*, while cefixime 8 ppm was used as a positive control for ESBL *Escherichia coli & Escherichia coli*. Incubation was performed at 37°C for 24 hours. The diameter of the growth inhibition zone was measured with a digital vernier calliper (JIGO-150, Taffware, Indonesia).

Statistical Analysis

All results were subjected to one way analysis of variance (ANOVA) (Version 25, IBM SPSS Statistic, 2017) using a completely randomized design with three replications for all treatments. Tukey post hoc was performed. The differences between means were tested at a significance value of $p < 0.05$.

Results and Discussion

Yellow passion fruit (*Passiflora edulis* forma *flavicarpa* Sims*)* is a passion fruit species that has higher acidity and pulp yield than other varieties. The presence of phenol content in yellow passion fruit makes this fruit have many activities. The pulp of yellow passion fruit has the largest total phenolic content compared to other parts, which is 1297.32 ± 13.43 mg/100 g dry weight[33]. Fermentation supernatant of passion fruit in MRS media is used in this formula as the raw material for microspheres. Fermentation was carried out to enhance the antibacterial activity[34].

Table-1. Formula of CFFS passion fruit microsphere in alginate gelatin matrix

Ingredients	Function	Formula 1	Formula 2
Cell free fermentation supernatant (CFFS) of yellow passion fruit	Probiotic	1.2 x 10^{14} CFU/mL	1.2 x 10^{14} CFU/mL
Sodium alginate	Polymer	2% (w/v)	1.25% (w/v)
Maltodextrin	Substrate	5% (w/v)	5% (w/v)
$CaCl_2$	Cross linker	11.1 % (w/v)	11.1 % (w/v)
Gelatin	Polymer	0.5% (w/v)	1.25% (w/v)

CFFS of yellow fruit experienced a significant increase in the diameter of the inhibition zone against Gram-positive and Gram-negative bacteria[10]. Before the production process is carried out, the supernatant from fermentation is characterized first and the values are given in Table 2. The supernatant was brown in color, pH value was 7.0+0.1 before and decreasing to 2.5+0.1 after 24 hours fermentation. The decrease in pH that occurs during passion fruit fermentation indicates the presence of lactic acid bacteria that convert lactose into lactic acid[34]. The presence of lactic acid bacteria contained in CFFS yellow passion fruit is proven by the total plate count in MRS media that was $1.2x10^{14}$ CFU/mL. From the bioassay identification, it is known that the lactic acid bacteria contained in CFFS yellow fruit are Gram-positive with *coccus* and *bacillus* morphology. Based on the results of the VITEK-2 analysis, several colonies suspected to be *Lactobacilli* and *Streptococci* were identified. With the presence of lactic acid bacteria in the supernatant, the selected formulation method is microencapsulation.

Microencapsulation is one of the appropriate technologies to maintain the stability and viability of probiotics under harsh conditions. Encapsulated probiotics are known to have a better survival ability than unencapsulated cells during storage and gastric transit. Their concentration strongly influences the process of encapsulation of probiotics using polymers. Microencapsulation was carried out in two different formulas using the alginate and gelatin polymer ratio (Table 1), and each formula was triple replicated. From previous studies, the optimum concentration of sodium alginate ranged from 0.75% - to 2%. Using a polymer mixture of alginate and gelatin in a concentration of 2.5% produces homogeneous beads and is not affected by the process[21].

Table-2. Characteristic of CFFS passion fruit fermented in MRS media

Parameters	Characteristics
Odor	Specific smell of passion fruit
pH	7.0±0.1 (0 hours), 2.5±0.1 (24 hours)
Inhibitory activity at 0-hour incubation	- (*S. aureus* and *E. coli*)
Inhibitory activity at 24 hours incubation	+ (*S. aureus* and *E. coli*)
Lactic acid bacteria screening	+ (based on Gram staining, morphology, catalase and motility test, conformed to VITEK-2)

The core system of encapsulation contains probiotics, maltodextrin, and alginate, was made using the ionic gelation method with aerosolization techniques and $CaCl_2$ as a cross-linker solution. Maltodextrin was used as a substrate for probiotics, so it was hoped that the nutrients for probiotics were appropriate during the manufacturing and storage processes. The aerosolization technique uses a controlled pressure of 40 Psi with nozzle size and spray distance, which have been optimized in previous research[23]. Figure 1 shows that dry microsphere in the entire formulas were brown and odorless powder. The microparticles formed are expected to be uniform, spherical, non-agglomerated, and range in size form micro to nano in size. The results of the study found that the microspheres' size and shape were around 42 – 58 µm, spherical (Figure 2), and uniform. Another advantage of this aerosolization technique is that there is no invasive probiotic treatment. After the core system was formed, the bead was coated with gelatin to cover the porous surface of the bead.

Microsphere size is also strongly influenced by gelatin concentration. The higher the concentration of gelatin, the denser the microparticles. Based on the size of the microsphere particles, the size of formula 1 is smaller than formula 2, 42.43 µm and 57.68 µm, respectively. This shows that the encapsulation method used effectively coats the beads with gelatin. When the concentration between the two polymers is balanced, the surface formed will be rigid[32]. In this study, the surface of formula 1 microspheres still showed pores, whereas in formula 2, the surface of the microspheres looked denser and no pores were found (Figure 3).

In the evaluation of physical properties, it is necessary to analyze the molecule structure using FTIR to confirm that the biopolymer network forms in the microparticles. The egg box structure is the interaction between divalent ions (Ca^{2+}) from the $CaCl_2$ cross-linking solution with free carboxylate (COO^-) in the G monomer of alginate to form a strong microparticle structure[24]. The mechanism of egg box shape formation is schematically depicted in Figure 6 through three stages, (a) the interaction of Ca^{2+} ions with a single G monomer unit to form a monocomplex; (b) forming egg box dimers through pairing between monocomplexes; (c) lateral association of several egg box dimers to produce multicomplexes. Lateral interactions between dimers are known to be mediated not only by Ca^{2+} ions, but also by Na^+ ions, H_2O molecules, and H^+ ions bonds between the hydroxyl and carboxyl groups of the G monomer unit residues[35]. The bonds between gelatin, alginate, and $CaCl_2$ influence the quality of encapsulation process. The FTIR spectrum of each formulation was depicted in Figure 3. All formula showed a broad peak at 3300-3200 cm^{-1} (O-H vibration) due to high content of –OH groups. The increase in peak intensity at ~1600 cm^{-1} is characteristic of the presence of $-CONH_2$ groups, indicating that there was binding between the anions of alginate and the cations of gelatin (Figure 4)[24].

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table-3. Physical characteristic evaluation of CFFS passion fruit microsphere formula

Formula	Organoleptic	Particle size (μm)	MC (%)	*Swelling index*			
				5 mins	30 mins	1 h	2 h
Formula 1	Brown powder. odorless	42.43 ± 0.20	2.17 ± 0.04	30.00	35.09	36.05	40.52
Formula 2	Brown powder. odorless	57.68 ± 0.18	4.37 ± 0.02	26.51	30.60	33.15	35.95

Note: MC represent %moisture content; the units of time used in the swelling index test are mins, which is minutes, and h is hours.

Table-4. Cell viability and antibacterial activity of sample

Formula	Viable cell count (CFU/mL)	Inhibition Zone (mm)			
		Staphylococcus aureus	*Escherichia coli*	MRSA	ESBL *Escherichia coli*
Fresh juice	NA	17.82 ± 1.08	17.98 ± 0.66	16.29 ± 1.34	15.95 ± 2.05
CFFS	1.2 x 10^{14}	17.49 ±1.31	17.10 ± 0.71	15.50 ± 1.10	14.95 ± 1.94
Formula 1	1.24x10^{8}	14.11 ± 2.07	13.02 ± 1.75	14.19 ± 1.88	13.36 ± 1.72
Formula 2	1.27x10^{8}	14.66 ± 2.31	14.28 ± 2.35	13.73 ± 1.77	14.48 ± 1.92

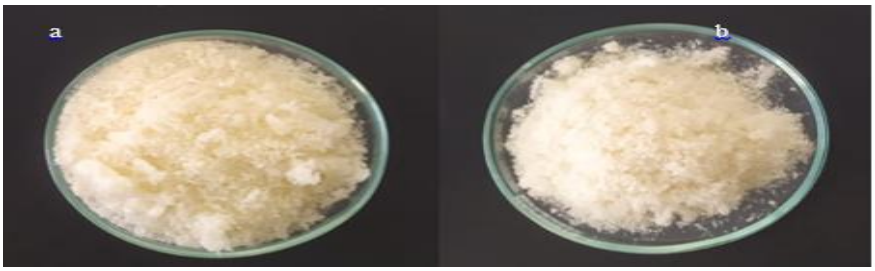

Figure 1. Organoleptic of microsphere; a) Formula 1; b) Formula 2

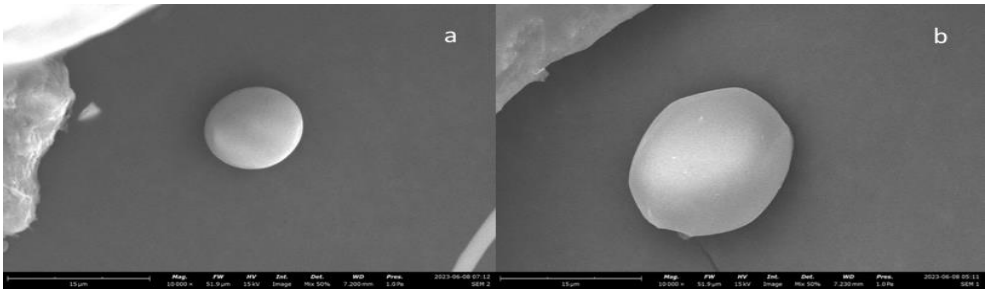

Figure 2. The shape of microsphere using optical microscopy with a magnification of 1000x; a) Formula 1; b) Formula 2

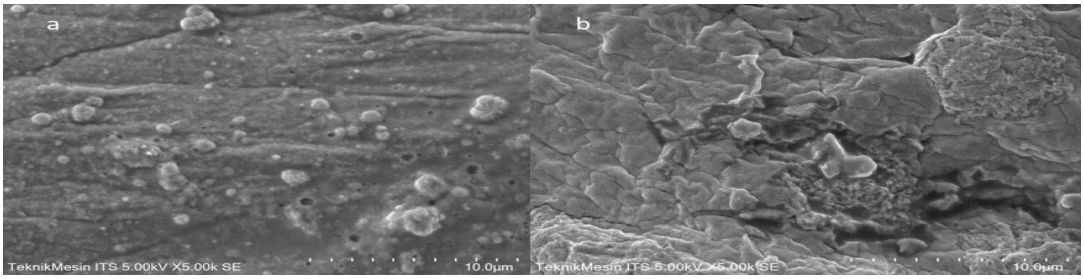

Figure 3. Surface appearance of microspheres using scanning electron microscopy (SEM) with magnification of 5.000x; a) Formula 1; b) Formula 2

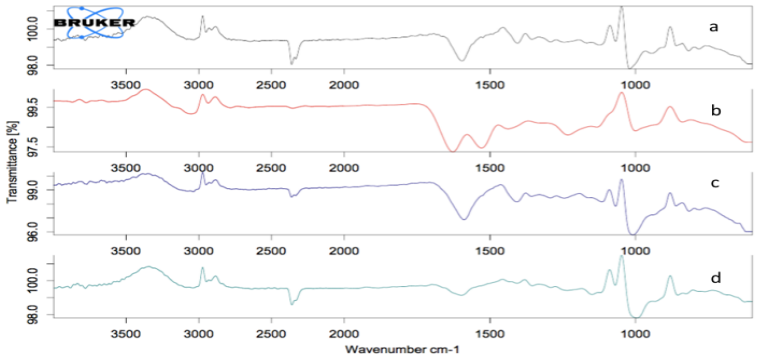

Figure 4. Fourier transform infrared spectroscopy (FTIR) spectra of each formula at a wavelength of 400 - 4000 cm^{-1}; a) Alginate; b) Gelatin; c) Formula 1; d) Formula 2.

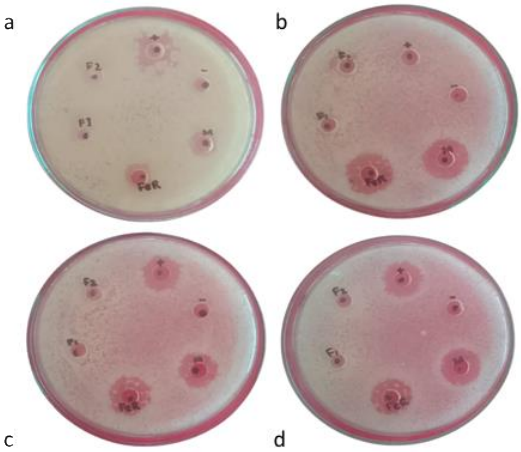

Figure 5. Inhibition zone of fresh juice of passion fruit (M); CFFS of passion fruit (Fer); Formula 1 Microsphere CFFS (F1); Formula 2 Microsphere CFFS (F2) against each pathogenic bacteria a) ESBL *Escherichia coli*; b) *Escherichia coli*; c) MRSA; d) *Staphylococcus aureus*

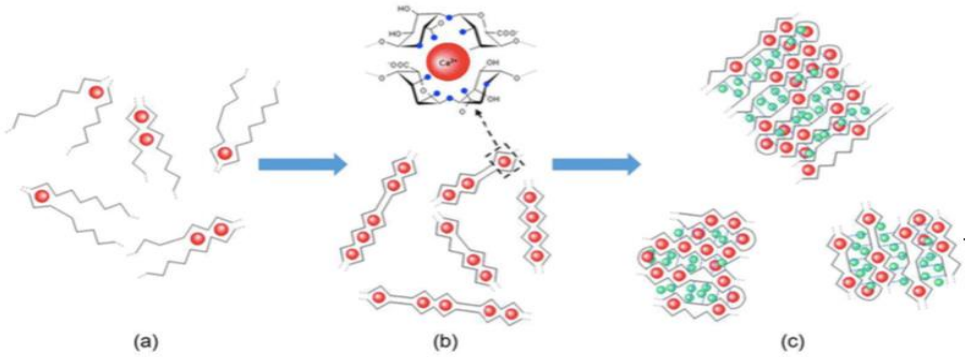

Figure 6. Gelation mechanism of Ca-alginate, a. single monomer units cross-linked with ions; b. egg box shape dimer; c. lateral association between egg box dimers; The red dots represent Ca^{2+} ions, while the green ones represent Na^{+} ions. The blue dots represent oxygen atoms, and the dashed blue lines represent hydrogen bonds[35].

Probiotic microspheres must be fully swollen with target receptors to be therapeutically effective, in which the properties of each polymer material influence the effectiveness. Probiotics are expected to work in the intestines, but the acidic conditions in the stomach can increase the risk of probiotic death. Alginate will expand according to the pH of its environment, where the beads are stable in acidic conditions and will expand in the intestines[36]. Alginate expands to 10 times its original size when it absorbs water. On the other hand, gelatin can prevent liquid from entering the beads for 2 hours and acts as a "shield" during the bead at the beginning of the digestive process[25]. Swelling index test results showed that all formulations expanded well. The bead continuously increased for up to 2 hours. Theoretically, gastric emptying time is 2 hours after oral phase. Therefore, microspheres fully expanded once they entered the intestine. During 2 hours of testing, the weight of the microspheres increased significantly by 36-41 times from its dry weight. However, there was no significant difference between the development capabilities of formula 1 and formula 2. This is because the alginate concentration is at its optimum condition.

During storage, hydrogel beads should also be able to maintain the water content within the matrix. A high moisture content risks the growth of unwanted microorganisms, and a low moisture content can make the beads brittle[21]. Moisture content also affects probiotic viability. In the optimum conditions, probiotic microspheres' moisture content should be maintained at around 5-10%. In this study, the

average %MC for all formulas was 3.45% and the values are given in Table 3. The low %MC from the optimum range caused the viability of lactic acid bacteria in CFFS of yellow passion fruit microspheres to decrease significantly. The viability value of lactic acid bacteria is very important because there is a decrease in effectiveness in the number of inadequate bacteria. In this study, the viable cell counts in the probiotic suspension (free cells) used for microencapsulation was 1.2 x 10^{14} CFU/mL and the number of microsphere plate counts was 1.24x10^{8} CFU/ml. Probiotics require an adequate number of cells to provide therapeutic effects, although there was a significant decrease but the viability of lactic acid bacteria in microspheres is still adequate as an antibacterial.

In general, the *Lactobacillus* group inhibits the growth of MRSA by actively penetrating and releasing toxic metabolites to the bacteria. In ESBL *Escherichia coli*, on the other hand, probiotics form anti-biofilms preventing bacteria from transmitting pathogenic agents to host cells[26]. The cell viability and antibacterial activity of free cells was compared to the microsphere formula. The inhibitory potencies of microsphere against MRSA and ESBL *Escherichia coli* were 12.33 mm and 12.13 mm, respectively (Table 3 & Figure 5). The order of inhibitory ability against the ESBL *Escherichia coli* test bacteria is Fresh juice > CFFS > Formula 2 > Formula 1. Meanwhile, for MRSA, the order of inhibitory ability was Fresh juice > CFFS > Formula 1 > Formula 2 (Table 4).

The all test groups showed good antibacterial activity in this study. There is a significant difference in the inhibitory ability between microspheres, CFFS, and fresh juice. In this study, the fresh juice has the most significant inhibitory effect on both ESBL *Escherichia coli* and MRSA due to the complex polyphenol compounds contained in the fruit. Meanwhile, in CFFS, the contents of the fruit are used not only to inhibit pathogenic bacteria but also for bacterial growth. The inhibitory zone of CFFS against MRSA and ESBL *Escherichia coli* was 15.50 mm and 14.95 mm, respectively. The previous inhibitory zone of CFFS was more potent against MRSA. The previous research showed that the fermentation potential of passion fruit is more substantial in MRSA than ESBL *Escherichia coli* due to its inhibitory activities[27,28]. While in microspheres, the inhibitory zone formed was 14 mm and 13.5 mm for MRSA and ESBL *Escherichia coli*, respectively. The antibacterial activity of microspheres was lower than CFFS and fresh juice. The decrease in cell viability of microsphere resulted in a decrease in the inhibitory potential of all tested bacteria compared to CFFS and fresh juice. The decreasing cell viability to 10^{6} CFU/ml causes a decrease in the diameter of inhibition zone by 3-4 cm. Interventions during the microencapsulation and freeze-drying processes are the leading causes of decreased viability. The difference in polymer concentration did not significantly affect the viability and antibacterial activity of the microspheres. To demonstrate the viability and antibacterial activity of microspheres, polymer destruction using acid solution needs to be carried out during microsphere sample preparation so that the effect of encapsulation can be ignored.

Conclusion

In summary, our findings highlight the potential mechanisms of cell free fermentation supernatant of yellow passion fruit's microsphere impede the growth and proliferation of MDROs like MRSA and ESBL *Escherichia coli*, emphasizing their potential as potent antibacterial agents. CFFS microspheres from yellow fruit using alginate and gelatin polymers gave good evaluation results on physical characterization and antibacterial activity against MRSA and ESBL *Escherichia coli*. Although there was no significant difference between the two microsphere formulas, formula 2 gave a more compact microsphere surface appearance so that the risk of leakage could be minimized. A further research, further research is needed related to the ability of these microspheres to maintain their viability and antibacterial activity both in storage time, extreme storage conditions, and the influence of the digestive system.

Authors Declaration

The authors hereby declare that the work presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgement

The authors gratefully acknowledge the assistance of the Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia for providing their laboratory, instrument facility, and financial support with Halal Excellent Research 2020, Chancellor's Decree No. 448/UN3/2020.

Conflict of interest statement

The authors declare no conflict of interest.

References

1. Selvin J, Maity D, Sajayan A, Kiran GS. Revealing antibiotic resistance in therapeutic and dietary probiotic supplements. J. Glob. Antimicrob. Resist., 2020; 22: 202-205.
2. Sharma A, Luvsansharav UO, Paul P, Lutgring JD, Call DR, Omulo S. Multi-country cross-sectional study of colonization with multidrug-resistant organisms: protocol and methods for the Antibiotic Resistance in Communities and Hospitals (ARCH) studies. BMC Public Health, 2021; 21 (1): 1-9.
3. Munita JM, Arias CA. Antibiotic Resistance Mechanisms. Microbiol. Spectr., 2017; May: 95-99.
4. Ljungquist O, Kampmann C, Resman F, Riesbeck K, Tham J. Probiotics for intestinal decolonization of ESBL-producing Enterobacteriaceae: a randomized, placebo-controlled clinical trial. Clin. Microbiol. Infect., 2020; 26 (4): 456-462.
5. Qureshi S, Maria N, Zeeshan M, Irfan S, Qamar FN. Prevalence and risk factors associated with multi-drug resistant organisms (MDRO) carriage among pediatric patients at the time of admission in a tertiary care hospital of a developing country. A cross-sectional study. BMC Infect. Dis, 2021; 21 (1): 1-9.
6. Rodríguez-Villodres Á, Martín-Gandul C, Peñalva G, Guisado-Gil AB, Crespo-Rivas JC, Pachón-Ibáñez ME. Prevalence and risk factors for multidrug-resistant organisms' colonization in long-term care facilities around the world: A review. Antibiotics, 2021; 10 (6): 1-22.
7. Peivasteh-Roudsari L, Pirhadi M, Karami H, Tajdar-Oranj B, Molaee-Aghaee E, Sadighara P. Probiotics and food safety: an evidence-based. J. food saf. hyg., 2019; 5 (1): 1-9.
8. He X, Luan F, Yang Y, Wang Z, Zhao Z, Fang J, Wang M, Zuo M, Li Y. *Passiflora edulis*: An Insight into Current Researches on Phytochemistry and Pharmacology. Front. pharmacol., 2020; 11: 1-16.
9. Thokchom R, Mandal G. Production Preference and Importance of Passion Fruit (*Passiflora edulis*): A Review. J. Agric. Eng., 2017; 4 (1): 27-30.
10. Marwah S, Rosyidah I, Mertaniasih N, Hamzah M, Novianti K, Primaharinastiti R, Rahmawaty D, Isnaeni I. In vitro antibacterial activity of cell free fermentation supernatant of *Passiflora edulis* forma *flavicarpa* Sims fruit fermented by de Man, Rogosa and Sharp media. Jordan J. Biol. Sci., 2020; 13 (4): 469 – 474.
11. Nurrosyidah I, Isnaeni I, Mertaniasih N. Potential probiotic from indigenous Indonesian red passion fruit (*Passiflora edulis* Sims). Sys Rev Pharm., 2020; 11 (8): 123 - 130.
12. Dias C, dos Santos Opuski de Almeida J, Pinto S, de Oliveira Santana F, Verruck S, Müller C, Prudêncio E, de Mello Castanho Amboni R. Development and physico-chemical characterization of microencapsulated *Bifidobacteria* in passion fruit juice: A functional non-dairy product for probiotic delivery. Food Biosci., 2018; 24 : 26 – 36.
13. Amer SA, Abushady HM, Refay RM, Mailam MA. Enhancement of the antibacterial potential of plantaricin by incorporation into silver nanoparticles. J. Genet. Eng. Biotechnol, 2021; 19 (1): 1-16.
14. Monteiro S, Beserra Y, Oliveira H, de Bittencourt Pasquali M. Production of probiotic passion fruit (*Passiflora edulis* sims f. *flavicarpa* deg.) drink using *Lactobacillus reuteri* and microencapsulation via spray drying. Foods, 2020; 9 (335): 1-14.

15. Meybodi N, Mortazavian A. Probiotic Supplements and Food Products: A Comparative Approach. Biochem. Pharmacol., 2017; 6 (2): 1-7.
16. Nazzaro F, Orlando P, Fratianni F, Coppola R. Microencapsulation in food science and biotechnology. Curr. Opin. Biotechnol., 2012; 23 (2): 182 – 186.
17. Gull N, Khan SM, Butt MTZ, Khalid S, Shafiq M, Islam A, Asim S, Hafeez S, Khan RU. In vitro study of chitosan-based multi-responsive hydrogels as drug release vehicles : a preclinical study. RSC Adv., 2019; 9: 31078 – 31091.
18. Kobo G, Kaseke T, Fawole O. Micro-Encapsulation of Phytochemicals in Passion Fruit Peel Waste Generated on an Organic Farm: Effect of Carriers on the Quality of Encapsulated Powders and Potential for Value-Addition. Antioxidants, 2022; 11 (8): 1-30.
19. Frakolaki G, Giannou V, Kekos D, Tzia C. A review of the microencapsulation techniques for the incorporation of probiotic bacteria in functional foods. Crit Rev Food Sci Nutr, 2021; 61 (9): 1515-1536.
20. Hariyadi DM, Pathak Y, Hendradi E, Erawati T, Hidayah I, Santoso E. Formulation of metformin-loaded alginate microspheres by ionotropic gelation aerosolization technique, Sanins Mayalsiana, 2020; 49 (10): 2513-2525.
21. Purwanti T, Satriawan RA, Hariyadi DM. The effect of the comparison of sodium alginate-gelatin levels on microspheres characteristics (Produced by ionic gelation method aerosolized technique). Int. J. Drug Deliv. Technol., 2020; 10 (2): 301-306.
22. Marwah M, Poernomo AT, Hendradi E. Study of growth curve of Lactobacillus plantarum FNCC 0026 and its antibacterial activity. J. farm. Ilmu kefarmasian Indones., 2023; 10 (1): 38-43.
23. Hariyadi DM, Hendradi E, Kurniawan TD. Alginate Microspheres Encapsulating Ciprofloxacin HCl: Characteristics, Release and Antibacterial Activity. Int. j. pharm. res. health sci., 2019; 7 (4): 3020-3027.
24. Putra INSA, Tutiek H, Melani D. Characterization of microspheres in alginate-gelatin matrix with ionotropic gelation method and aerosolization technique. Res J Pharm Technol, 2020; 13 (9): 4239-4243.
25. Asshafa RN, Purwanti T, Hariyadi DM. Effect of Combination Sodium Alginate-Gelatin 1 %: 2 % Content in Characteristic and Antimicrobial Activity of Probiotic Microspheres *Lactobacillus acidophilus*. Proceeding of the 1st International Conference in Medicine and Health Sciences; 2016, 31 August – 01 September 2016; Jember, Indonesia. Jember: UNEJ e-Proceeding.
26. Selvin J, Maity D, Sajayan A, Kiran GS. Revealing antibiotic resistance in therapeutic and dietary probiotic supplements. J Glob Antimicrob Resist, 2020; 22: 202-205.
27. Shim YH, Lee SJ, Lee JW. Antimicrobial activity of lactobacillus strains against uropathogens. Pediatr. Int., 2016; 58 (10): 1009-1013.
28. Aprelita ND, Poernomo AT, Nurrosyidah IH, Isnaeni I, Rahmawaty D, Kusumawat I. Growth inhibitory effect of red and yellow fruits against MRSA and ESBL-producing bacteria. J. farm. Ilmu kefarmasian Indones., 2023; 10 (1): 87-91.
29. Nurrosyidah I, Mertaniasih N, Isnaeni I. Antibacterial activity of probiotics cell-free fermentation filtrate from Passiflora edulis Sims. Against pathogen bacteria. Res J Pharm Technol, 2022; 15 (12): 5767-5773.
30. Apiwattanasiri P, Charoen R, Rittisak S, Phattayakorn K, Jantrasee S, Savedboworn W. Co-encapsulation efficiency of silk sericin-alginate-prebiotics and the effectiveness of silk sericin coating layer on the survival of probiotic *Lactobacillus casei.* Food Biosci.. 2022; 46 (101576): 1 – 10.
31. Lopes LAA, Carvalho EdSF, Magalhaes NSS, Madruga MS, Athayde AJAA, Portela IA, Barao CE, Pimentel TC, Magnani M, Stamford TCM. Microencapsulation of *Lactobacillus acidophilus* La-05 and incorporation in vegan milks : physicochemical characteristics and survival during storage, exposure to stress conditions, and simulated gastrointestinal digestion. Food Res Int., 2020; 135 (109295): 1-8.
32. Marwah S, Poenomo AT, Diyah NW, Mahdiyyah AA, Hendradi E. Microencapsulation of *Lactobacillus plantarum* FNCC 0026 : physical properties of microencapsules and antibacterial activity against multi-drug resistance organism (MDRO). J. Res. Pharm., 2024; 28(2): 429 – 437.
33. Reis LCRd, Facco EMP, Salvador M, Flores SH, Rios AdO. Antioxidant potential and physicochemical characterization of yellow, purple, and orange passion fruit. J Food Sci Technol, 2018; 55(7):2679-2691.
34. Sinaga DP, Tampubolon DKA, Kambaren RF, Martgrita MM. Fermentation process effect to enhance antioxidant and antibacterial activity of phenolic compounds and its possible application to galactomannan polysaccharides : a review. Proceeding of the 1st Lekantara Annual Conference on Natural Science and Enviroment; 2021, 20–21 September 2021; Malang, Indonesia. Malang : IOP Publishing.
35. Hu C, Lu W, Mata A, Nishinari K, Fang Y. Ions-induced gelation of alginate: Mechanisms and applications. Int. J. Biol. Macromol., 2021; 177: 578-588.
36. Liu H, Xie M, Nie S. Recent trends and applications of polysaccharides for microencapsulation of probiotics. Food front., 2020; 1 (1): 45-59.