Sains Malaysiana 51(7)(2022): 2073-2085
http://doi.org/10.17576/jsm-2022-5107-11

Survivability and Release Process of *Lactobacillus plantarum* SU-LS36 Encapsulated with Native and Modified Taro Starch Under Simulated Digestive Conditions

(Kemandirian dan Proses Pelepasan Pengkapsulan *Lactobacillus plantarum* SU-LS36 dengan Kanji Taro Asli dan Terubah Suai dalam Keadaan Simulasi Pencernaan)

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Received: 10 July 2021/Accepted: 9 December 2021

ABSTRACT

This research aimed to evaluate the viability, survivability, and release process of the encapsulated *Lactobacillus plantarum* SU-LS36 in the simulated gastric juice (SGJ), simulated intestinal juice (SIJ), and simulated colon juice (SCJ). We tested four types of encapsulations: native taro starch (NTS), modified taro starch (MTS) by heat moisture treatment (HMT), autoclaving-cooling-2 cycles (AC-2C), and maltodextrin (commercial encapsulant). We found that *L. plantarum* SU-LS36 with AC-2C-modified taro starch (MTS) showed the highest viability in SGJ (6.95 log CFU/g), SIJ (7.09 log CFU/g), and SCJ (7.85 log CFU/g) after incubation up to 4 h. AC-2C MTS dissolved or released more rapidly from its encapsulant material in the colon in SCJ than in NTS, HMT MTS, and maltodextrin. The longest time release of *L. plantarum* SU-LS36 encapsulated in AC-2C MTS was 3 h in SIJ conditions, 2 h in SGJ, and the fastest (1 h) in SCJ. The encapsulated *L. plantarum* SU-LS36 was released through a dissolution process (SGJ and SCJ) and by pancreatin activity (SIJ). Conclusively, AC-2C MTS could maintain the viability of *L. plantarum* SU-LS36 cells to the colon at 6.04 log CFU/g and fulfilled the minimum requirement of biovalue (MBV) probiotics set forth by the US FDA (6-7 log CFU/g).

Keywords: Encapsulation; *Lactobacillus plantarum* SU-LS36; simulated digestion *in-vitro*; survivability; taro starch

ABSTRAK

Penyelidikan ini bertujuan untuk menilai kebolehhidupan, kemandirian dan proses pembebasan pengkapsulan *Lactobacillus plantarum* SU-LS36 dalam jus gaster simulasi (SGJ), jus usus simulasi (SIJ) dan jus kolon simulasi (SCJ). Kami menguji empat jenis pengkapsulan: kanji ubi keladi asli (NTS), kanji ubi keladi terubah suai (MTS) dengan rawatan kelembapan haba (HMT), kitaran penyejukan-2 autoklaf (AC-2C) dan maltodekstrin (pengkapsulan komersial). Kami mendapati bahawa *L. plantarum* SU-LS36 dengan kanji ubi keladi (MTS) diubah suai AC-2C telah menunjukkan kebolehhidupan yang tertinggi dalam SGJ (6.95 log CFU/g), SIJ (7.09 log CFU/g) dan SCJ (7.85 log CFU/g) selepas pengeraman sehingga 4 jam. AC-2C MTS larut atau dibebaskan dengan lebih cepat daripada bahan pengkapsulan dalam kolon di SCJ berbanding NTS, HMT MTS dan maltodekstrin. Pelepasan masa terpanjang *L. plantarum* SU-LS36 yang terkandung dalam AC-2C MTS ialah 3 jam dalam keadaan SIJ, 2 jam dalam SGJ, dan terpantas (1 jam) dalam SCJ. *L. plantarum* SU-LS36 terkapsul telah dilepaskan melalui proses pelarutan (SGJ dan SCJ) dan oleh aktiviti pancreatin (SIJ). Secara kesimpulannya, AC-2C MTS boleh mengekalkan kebolehhidupan sel *L. plantarum* SU-LS36 ke kolon pada 6.04 log CFU/g dan memenuhi keperluan minimum nilai bio probiotik (MBV) yang ditetapkan oleh FDA AS (6-7 log CFU/g).

Kata kunci: Kanji ubi keladi; kemandirian; *Lactobacillus plantarum* SU-LS36; pengkapsulan; simulasi pencernaan *in-vitro*

Introduction

Microencapsulation of probiotics increases their survivability and viability in the digestive tract (Shori 2017). Improving survivability is feasible by focusing on controlling the release of probiotics from microcapsules (Rokka & Rantamaki 2010). The design of an encapsulated probiotic delivery system aiming to provide a controlled release is vital and must consider the physiological aspects of the complexity of the digestive tract (Rokka & Rantamaki 2010). Appropriate pH, time, peristaltic pressure, and probiotic bacteria fermentation are taken into account when developing various methods of delivering encapsulated probiotics into the digestive tract (Shori 2017). The formulation of polymer mixture as the wall material of capsules is equally important (Dias et al. 2017). These microencapsulate materials modify the release rate and change the characteristics of the microencapsulated probiotic (Basu et al. 2018).

Cook et al. (2011) and Homayouni et al. (2012) reported that probiotics have criteria to qualify, including 1) being non-pathogenic and present in normal microbiota in the host intestine, 2) able to survive acidic conditions in the stomach and high levels of bile salts in the small intestine, 3) able to attach and colonize some parts of the host's intestinal tract, 4) able to produce organic acids and antimicrobial compounds against pathogens, and 5) able to grow in large-scale production systems and survive the storage conditions. Probiotic bacteria such as plantaricin, nisin, and acidophilin are capable of producing antibacterial bioactive peptides (bacteriocins) that kill pathogenic bacteria in the intestine (Sulistiani 2018). Moreover, probiotic bacteria can act as immunomodulators by stimulating macrophages, monocytes, T lymphocytes, immunoglobulin G, and immunoglobulin A (Homayouni et al. 2012). *Lactobacillus plantarum* SU-LS36 is a recommended candidate for indigenous probiotic for stronger antibacterial activities than acidophilin and nisin, high viability (9.08 log CFU/mL), ability to inhibit the growth of six pathogenic bacteria (*Escherichia coli, Pseudomonas aeruginosa, Listeria monocytogenes, Bacillus subtilis, Staphylococcus aureus*, and *Salmonella enteritidis*), ability to survive in low acidity (pH 3), and bile salt tolerance (Sulistiani 2018).

The encapsulated probiotic bacteria rapidly pass through the esophagus approximately 10-14 seconds after entering the mouth before reaching the stomach (Cook et al. 2012). The stomach is where the viability of probiotic bacteria decreases the most due to high acidity (pH 1-2.5) with a transit time of 2.5 ± 0.5 h (Liu et al. 2016). The time transit through the stomach, considering variants in pH between individuals, is affected by mealtime, sex, and age (Shori 2017). The encapsulated probiotic bacteria enter the small intestine 3.2±1.6 h after passing through the stomach (Homayouni et al. 2012). The estimated pH range of the small intestine is 6.15-7.35 in the proximal region, and 6.80-7.88 in the distal region (Basu et al. 2018). The encapsulated probiotic bacteria reach the colon where the pH is 5.26-6.72 in the ascending colon, and 5.20-7.02 in the descending colon with a transit time of 4.1 ± 1.5 h (Basu et al. 2018; Liu et al. 2016). The colon in the digestive tract is home to most of the native probiotic bacterial species, such as *Lactobacillus* sp. and *Bifidobacterium* spp. (Tao et al. 2019).

The encapsulant material should provide good protection against acids, enzymes, and bile salts. It should be free from antimicrobial and non-cytotoxic properties to prevent damaging the host probiotic bacteria (Chavarri et al. 2010). Common materials for encapsulants reported in previous studies included polysaccharides, oligosaccharides, and proteins of various origins (Cook et al. 2011; Dudkiewicz et al. 2020; Yao et al. 2020), alginate mixed with chitosan, and maltodextrin (Hernández-Carranza et al. 2014). There remains room for undertaking further research to obtain new natural resources of biopolymers to produce efficient wall material for probiotic microcapsules. In our previous research, we prove that the modified taro starch AC-2C (autoclaving-cooling 2 cycles) was a high potential encapsulant for *L. plantarum* SU-LS36 for its higher production yield (40.19%), high encapsulation efficiency (89.83%), protection from high temperature (70 °C), and the lowest decrease in viability up to 6-week shelf life at room temperature (Setiarto et al. 2020). However, to the best of our knowledge, there has been a scarcity of data on the use of taro starch for microencapsulation of probiotics for gastrointestinal delivery. Alfaro-Galarza et al. (2020) on the viability of taro and rice starch for encapsulation of *Lactobacillus paracasei* subsp. *paracasei* in simulated gastrointestinal conditions found that the former showed a better protective effect on bacteria.

The release process of encapsulated probiotics is determined based on diffusion, rupture, and dissolution parameters (Würth et al. 2015). In the diffusion process, the encapsulated material binds water, causing the material to expand and increase the porosity of the microencapsulation membrane to allow the release of probiotic cells (Chen et al. 2017). Rupture is a process of releasing probiotic cells characterized by degradation and

damage of microcapsule wall material due to enzymatic hydrolysis, strong acids (low pH), and pressure (Shori 2017). Dissolution refers to the release of probiotics because certain solvents are compatible with the viscosity and solubility of the encapsulant material (Cheow et al. 2014). Accordingly, this research aimed to evaluate the survivability and release of the *L. plantarum* SU-LS36 contained in microcapsules prepared with native and modified taro starch as the wall material in the SGJ, SIJ, and SCJ. Maltodextrin-encapsulated *L. plantarum* SU-LS36 was used as the positive control.

Materials and Methods

Materials

The raw material used in this research was Bogor taro tubers of Pandan variety (*Colocasia esculenta*) harvested at eight months from taro farms in Cijeruk Bogor West Java, Indonesia. The sampling process with three replications was conducted by randomly selecting taro from the farms, cutting off the stems and leaves. *L. plantarum* SU-LS36 as probiotic bacteria was provided from the Food Microbiology Laboratory, Research Center for Biology, Indonesian Institute of Science (LIPI).

Taro starch modification

Taro starch was extracted according to the method by Setiarto et al. (2020). Taro tubers were peeled, washed, and soaked in 1% NaCl mixture (3:4) for 1 h. After draining, the tubers were shredded and mixed with distilled water (3:1, w/v) for 1 min using a blender (Phillips, Eindhoven, The Netherlands), then the pulp was filtered using a double-folded cotton cloth. The taro liquid was centrifuged with a High-Speed Centrifuge (Kubota, Japan) at 7,000 × g for 10 min to obtain pellets as wet taro starch. The wet taro starch was oven-dried at 50 °C for 16 h to obtain dry taro starch which was crushed using a disk mill (Taian City Up International Trade Co. Ltd, China) and sieved to a size of 200 mesh.

We performed two modification processes of native taro starch (NTS). The first is the heat moisture treatment (HMT) adapted from Deka and Sit's method (2016). Taro starch (dry basis) was placed in a glass container, added with distilled water to obtain 25 g/100 g (w/w) of moisture content, and then the glass container was tightly closed and balanced for 48 h at room temperature. The taro starch was heated (110 °C, 3 h) in a hot air fast drying oven (DSR115, Isuzu, Japan), dried (50 °C, 16 h), pulverized in a disk mill (Taian City Up International Trade Co. Ltd, China) and sieved through a 200-mesh sieve.

The second modification is the autoclaving-cooling-2 cycles (AC-2C) treatment adapted from Setiarto et al. (2018). Native taro starch was mixed with distilled water with a ratio of 1:2 (w/v) and heated at 121 °C for 15 min using an autoclave (Hirayama HVE-50, Tokyo, Japan), then cooled in a refrigerator (4 °C, 24 h). The autoclaving-cooling treatment was repeated for two cycles, the yield was oven-dried (60 °C, 14 h) and milled with a pin disk mill (Taian City Up International Trade Co. Ltd, Shandong, China).

Microencapsulation *L. plantarum* SU-LS36 by spray drying

Microencapsulation of *L. plantarum* SU-LS36 by spray drying was carried out concerning the research of Setiarto et al. (2020). The cell biomass of *L. plantarum* SU-LS36 (10^{10} CFU/g) was mixed with encapsulants (1:1, w/w), namely NTS, HMT modified taro starch (MTS), AC-2C MTS, and maltodextrin, and dissolved in sterile water to obtain the final concentration of 10% (w/v). The microcapsule mixture was homogenized in a homogenizer (IKA-Ultra-Turrax T18basic, Staufen in Breisgau, Germany) (60 s, 7000 rpm), then spray dried with a spray dryer (Eyela, Tokyo, Japan) with 125 °C inlet temperature, 50 °C output temperature, 20 m^3/h drying air flow rate, 4 mL/min feed flow rate, and 0.196 MPa air pressure. The encapsulated *L. plantarum* SU-LS36 was packed in polyethylene packaging and stored at room temperature for three days until we analyzed it for viability, survivability, and the microcapsule release process in the digestive tract simulation (Rajam & Anandharamakrishnan 2015). The microencapsulation process by using modified taro starch as microencapsulant materials was carried out simultaneously.

Survivability evaluation of encapsulated *L. plantarum* SU-LS36 in SGJ

Survivability of encapsulated and non-encapsulated *L. plantarum* SU-LS36 was evaluated in SGJ (Shah et al. 2016). MRS broth (Oxoid Ltd., Hampshire, England) was adjusted to pH 2.0 with 1 M HCl and sterilized by autoclaving at 121 °C for 15 min. Then, pepsin (3200 U/mL - Sigma Aldrich, Saint Louis, MO, USA), 7.2 mM $CaCl_2$, 3 mM $MgCl_2$, 98 mM NaCl, 24 mM KCl, and 12.8 mM KH_2PO_4 were filtered through 0.2 µm sterile

membrane filters and suspended in a sterile MRS broth 0.3% (v/v). Exactly 0.5 g microcapsules of *L. plantarum* SU-LS36 and an equivalent amount of *L. plantarum* SU-LS36 free cells were dissolved in 9.5 mL of sterile SGJ, placed in a screw-covered vial containing N_2 flow (20 m^3/h), and then incubated at 37 °C with a constant stirring at 130 rpm. Samples were taken before digestion (0 h) and after 1, 2, 3, and 4 h to evaluate the viability of *L. plantarum* SU-LS36 cells (log CFU/g) under SGJ conditions. The survivability was calculated as the log-transformation ratio between the viability of *L. plantarum* SU-LS36 (log CFU/g) that survived before (N_o) and after digestion in SGJ (N) as follows in equation (1):

% Survivability *L. plantarum* SU-LS36 in SGJ, SIJ, SCJ =

$$\frac{\log \text{CFU/g N}}{\log \text{CFU /g No}} \times 100\%$$

SURVIVABILITY EVALUATION OF ENCAPSULATED *L. plantarum* SU-LS36 IN SIJ

We compared the survivability of *L. plantarum* SU-LS36 encapsulated by spray drying and non-encapsulated *L. plantarum* SU-LS36 cells under SIJ conditions in a procedure by Shah et al. (2016) with modifications to better mimic the concentration of porcine pancreatin (Sigma Aldrich) and duodenal bile salts (Sigma Aldrich) to those found in the human digestive tract. Exactly 300 mg of *L. plantarum* SU-LS36 encapsulated and an equivalent amount of *L. plantarum* SU-LS36 free cells without encapsulation was suspended in 2.5 mL of saline buffer in a 30 mL glass bottle, then pancreatin and bile salts were dissolved in 0.1 M $NaHCO_3$. It was added to the sample to obtain the final concentration of 4.4 g/L bile salt and then incubated for 4 h. Samples were taken at the beginning of the process (0 h) and after 1, 2, 3, 4 h of incubation. Aliquots from the results of SIJ were diluted in phosphate buffer saline (PBS) and grown on MRS agar to be enumerated living *L. plantarum* SU-LS36. The survivability was calculated as the log-transformation ratio of the viability (log CFU/g) of *L. plantarum* SU-LS36 that survive before (N_o) to after placement in SIJ condition (N).

SURVIVABILITY EVALUATION OF ENCAPSULATED *L. plantarum* SU-LS36 IN SCJ

The survivability of encapsulated and non-encapsulated *L. plantarum* SU-LS36 was evaluated in SCJ conditions. SCJ was prepared by dissolving bile salts (Sigma Aldrich) in MRS broth to a final concentration of 2% (v/v) until pH 7.0 and then sterilized. *L. plantarum* SU-LS36 spray dry microcapsules (0.5 g) and *L. plantarum* SU-LS36 free cells (0.5 g) were dissolved in 9.5 mL of sterile SCJ and incubated at 37 °C with constant stirring at 100 rpm. Samples were taken at the beginning of the process (0 h) and after 1, 2, 3, 4 h of incubation to evaluate the viability of *L. plantarum* SU-LS36 cells (log CFU/g) under SCJ conditions (Rajam & Anandharamakrishnan 2015). We found higher viability of encapsulated *L. plantarum* SU-LS36 cells in the SCJ (log CFU/g) than in the non-encapsulated *L. plantarum* SU-LS36-free cells (log CFU/g). The survivability was calculated as the log-transformation ratio of the viability (log CFU/g) of *L. plantarum* SU-LS36 that survive before (N_o) and after placement in SCJ condition (N).

RELEASE PROCESS ANALYSIS OF ENCAPSULATED *L. plantarum* SU-LS36 IN SGJ, SIJ, AND SCJ

The release process of *L. plantarum* SU-LS36 encapsulated in SGJ, SIJ, and SCJ were observed by scanning electron microscopy (SEM Hitachi S 2400, Tokyo, Japan) at each sampling time of 0, 60, 120, 180, and 240 min. Sputter coater (Hitachi E102 Ion Sputter, Tokyo, Japan) with a 20-nm thick gold layer and conductive double-sided carbon tape was used to coat the encapsulated *L. plantarum* SU-LS36 sample. Visualization was done by placing the film on a sheet of aluminum covered with double-sided tape and then coated with gold as thick as 20-30 nm. A voltage of 20.0 kV (SEM Hitachi S 2400, Tokyo, Japan) was used to analyze the release of *L. plantarum* SU-LS36 encapsulated with AC-2C MTS at 5,000 - 10,000 × magnification (Arslan-Tontul & Erbas 2017).

STATISTICAL ANALYSIS

This experiment used three replications and statistical analyses to evaluate the survivability evaluation of encapsulated *L. plantarum* SU-LS36 in SGJ, SIJ, and SCJ. The analysis of variance with Duncan's statistical post-hoc test was applied to examine the considerable differences at the level of $p < 0.05$ using the Statistical Package for the Social Sciences (SPSS) 18.0 statistical software (IBM, Armonk, NY, USA).

RESULTS AND DISCUSSION

SURVIVABILITY AND RELEASE OF ENCAPSULATED *L. plantarum* SU-LS36 IN SGJ

The survivability and viability of encapsulated *L. plantarum* SU-LS36 and non-encapsulated *L. plantarum* SU-LS36 were tested in SGJ during the 4 h incubation period by sampling before digestion (0 h) and after 1, 2, 3, and 4 h which can be refer from Table 1.

The results indicated that microencapsulation with taro starch and maltodextrin has maintained the viability and survivability of *L. plantarum* SU-LS36 when passing through SGJ. The *L. plantarum* SU-LS36 AC-2C MTS had higher viability and survivability than HMT MTS, and NTS ($p<0.05$) in SGJ up to 4 h incubation because the decrease of viability rate was only 0.47 log CFU/(g×h) and the survivability rate declined from 97.63 to 77.19%. Meanwhile, the resistance of maltodextrin-encapsulated *L. plantarum* SU-LS36 in SGJ during 4 h incubation was non-significantly different from those of *L. plantarum* SU-LS36 AC-2C MTS ($p^3 0.05$) as shown from the viability (decreased at 0.53 log CFU/(g×h) rate) and survivability (declined from 97.67 to 75.00%) which can be referred from Table 1.

On the other hand, NTS and HMT taro starch were protected by maintaining high viability and survivability of *L. plantarum* SU-LS36 in SGJ for 2 h which can be referred from Table 1. The viability of NTS-encapsulated *L. plantarum* SU-LS36 decreased at an average rate of 0.74 log CFU/ (g×h) in SGJ, and the survivability declined from 97.61 to 65.38%. Meanwhile, HMT MTS-encapsulated *L. plantarum* SU-LS36 experienced a decreased viability at an average rate of 0.79 log CFU/ (g×h) and declined survivability from 98.04 to 59.88% which can be refer from Table 1. The non-encapsulated *L. plantarum* SU-LS36 showed significant viability and survivability only up to 1 h in SGJ, after which the viability decreased at an average rate of 1.43 log CFU/ (g×h) which can be refer from Table 1.

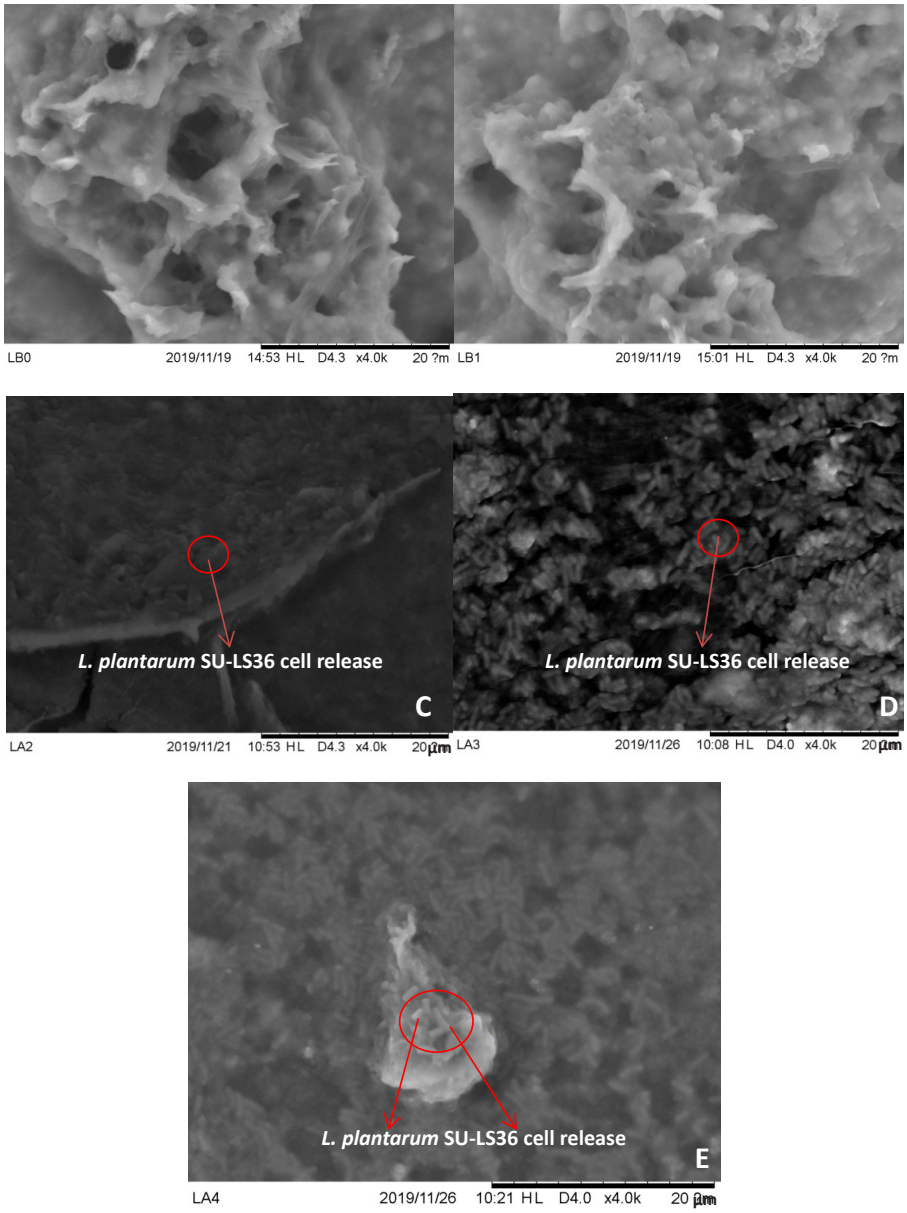
Basu et al. (2018), Chavarri et al. (2010), and Cook et al. (2012), also reported higher survivability of probiotic bacteria in the matrix of probiotic-fortified alginate microcapsules than non-probiotics alginate microparticles in SGJ. Additionally, Dos Santos et al. (2019) reported a high resistance of *Lactobacillus acidophilus* La-5 during digestion in SGJ when the probiotics were incorporated into the synbiotic growth media containing low sugar, guava pulp (12.5%), and inulin (2.0%). *L. acidophilus* La-5 had high survivability in the simulation of stomach fluid *in vitro* due to the slow degradation of inulin microcapsules under acidic conditions. Encapsulation of *Lactobacillus* sp. with resistant starch type 4 (RS4) provided a physical barrier to environmental stresses, thereby reducing the loss of viability of *Lactobacillus* sp. which is unavoidable during testing in SGJ (Ying et al. 2013). Hi-maize resistant starch with a concentration of 1% has been used for microencapsulation of *Lactobacillus acidophilus* in the form of alginate beads in both wet and freeze-dried microparticles (Etchepare et al. 2016). Hi-maize provides better protection for *L. acidophilus* after exposure to a simulated stomach fluid when freeze-dried microparticles were used. The viability of Hi-Maize-encapsulated probiotic cultures was stable in 30-day shelf life in freeze-dried form and 13.5 days in the wet form at room temperature (25 °C).

SEM images show that *L. plantarum* SU-LS36 cells encapsulated with AC-2C MTS in SGJ began to release from the modified taro starch after 2 h which can be refer from Figure 1(C) and continued at a significant rate on the 3rd and 4th hour which can be refer from Figure 1(D) and 1(E). The release time that started at the 2nd to the 4th hour was probably through the dissolution process due to low pH which can be refer from Figure 1(C), 1(D), and 1(E). The modified taro starch was dissolved due to the low pH of SGJ so that the *L. plantarum* SU-LS36 cells can be released from the structure of the microcapsules which can be refer from Figure 1(A). The modified taro starch was very suitable as encapsulant material because it was resistant to acid and pepsin hydrolysis in the stomach, so *L. plantarum* SU-LS36 cells were only released in small amounts of encapsulant material within 2 h by dissolution process which can be refer from Figure 1(C).

TABLE 1. Survivability and viability of *L. plantarum* SU-LS36 encapsulated with native taro starch (NTS), heat moisture treatment modified taro starch (HMT MTS), autoclaving-cooling-2 cycles modified taro starch (AC-2C MTS) and maltodextrin in SGJ

Encapsulant material	Viability (log CFU/g)	Survivability (%)	Viability (log CFU/g)	Survivability (%)	Viability (log CFU/g)	Survivability (%)	Viability (log CFU/g)	Survivability (%)	Viability (log CFU/g)	Survivability (%)
	0 h		1 h		2 h		3 h		4 h	
NTS	8.60±0.07	97.61±0.75^{a}	7.78±0.02	88.31±0.25^{b}	6.89±0.01	78.21±0.15^{c}	6.02±0.08	68.33±0.82^{d}	5.76±0.02	65.38±0.21^{d}
HMT MTS	7.99±0.08	98.04±0.81^{a}	7.10±0.05	87.12±0.53^{b}	6.28±0.06	77.06±0.64^{c}	5.37±0.04	65.89±0.41^{d}	4.88±0.05	59.88±0.54^{e}
AC-2C MTS	8.79±0.05	97.63±0.54^{a}	8.26±0.06	91.74±0.62^{a}	7.94±0.02	88.19±0.23^{b}	7.23±0.07	80.30±0.72^{b}	6.95±0.03	77.19±0.31^{c}
Maltodextrin	8.83±0.06	97.67±0.63^{a}	8.19±0.04	90.60±0.45^{a}	7.26±0.08	80.31±0.81^{b}	7.02±0.06	77.65±0.61^{c}	6.78±0.07	75.00±0.71^{c}
Without encapsulant	8.98±0.10	91.48±098^{a}	6.48±0.09	66.01±0.90^{d}	5.11±0.04	52.05±0.42^{e}	4.08±0.05	41.56±0.53^{f}	3.05±0.01	31.07±0.11^{g}

Means with different superscript letters within a row and column are significantly different at $p<0.05$



Magnification 5000×

FIGURE 1. The scanning electron microscope (SEM) images of *L. plantarum* SU-LS 36 encapsulated with AC-2C MTS before digestion – (A) 0 h, and (B) after 1 h, (C) 2 h, (D) 3 h, and (E) 4 h digestion in SGJ

SURVIVABILITY AND RELEASE OF *L. plantarum* SU-LS36 ENCAPSULATED IN SIJ

Table 2 shows that the viability and survivability of non-encapsulated *L. plantarum* SU-LS36 and capsulated with native (NTS) and modified (HMT and AC-2C) taro starch, and maltodextrin during 4 h incubation in SIJ. We found that *L. plantarum* SU-LS36 encapsulated with AC-2C MTS had higher viability and survivability than the NTS ($p<0.05$). The viability of *L. plantarum* SU-LS36 encapsulated with AC-2C MTS decreased at an average rate of 0.46 log CFU/(g×h), and the survivability declined from 98.34 to 78.52%. Similarly, *L. plantarum* SU-LS36 maltodextrin and HMT MTS showed resistance

to SIJ during the 4 h incubation as indicated from the non-significantly different viability and survivability from those in *L. plantarum* SU-LS36 AC-2C MTS ($p \geq 0.05$). The viability of maltodextrin-encapsulated *L. plantarum* SU-LS36 decreased between 0 and 4 h of incubation with an average rate of 0.47 log CFU/ (g×h), and the survivability declined from 98.45 to 77.65%.

Meanwhile, HMT MTS protected by maintaining viability and survivability of *L. plantarum* SU-LS36 in SIJ within 4 h which was observed from the decreased viability at an average rate of 0.41 log CFU/ (g×h) and declined survivability from 96.38 to 75.72% which can be refer from Table 2. Also, the viability of *L. plantarum* SU-LS36 NTS decreased at an average rate of 0.60 log CFU/ (g×h) and the survivability declined from 99.20 to 71.25% in SIJ during 4 h which can be refer from Table 2. The non-encapsulated *L. plantarum* SU-LS36 showed high viability and survivability until the 1st in SIJ before its viability decreased at an average rate of 1.36 log CFU/(g×h) which can be refer from Table 2.

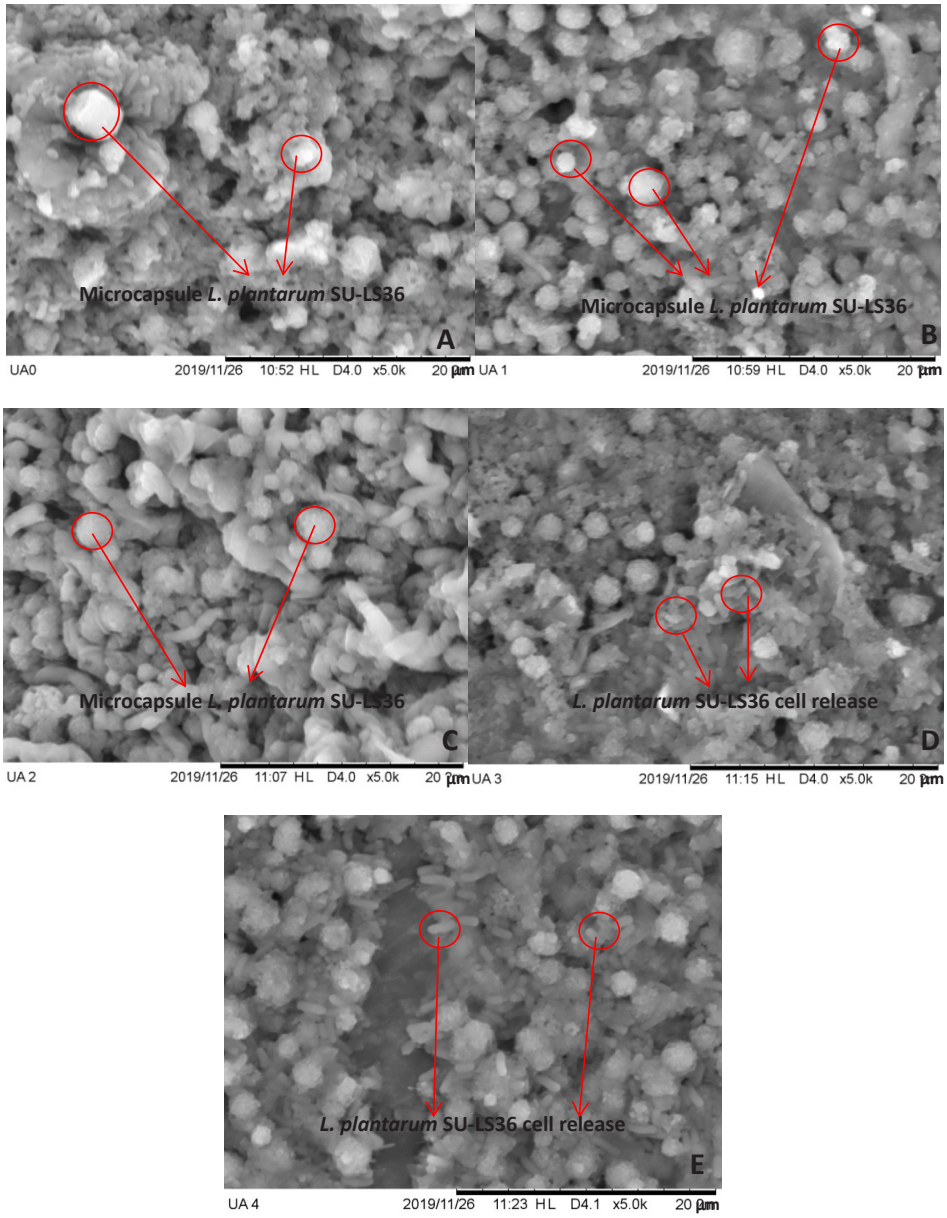
SEM analysis showed that *L. plantarum* SU-LS36 cells encapsulated with AC-2C MTS in SIJ treatment began to release from modified taro starch at the 3rd hour, but the number of released cells at the 3rd and 4th was relatively small which can be refer from Figure 2(D) and 2(E) through an enzymatic hydrolysis process. The modified taro starch was hydrolyzed due to the complex activity of pancreatic amylase contained in the SIJ, so *L. plantarum* SU-LS36 cells could be separated from the structure of the microcapsules which can be refer from Figure 2(A). Accordingly, the modified taro starch was highly compatible as an encapsulant material because it was resistant to the hydrolysis of the digestive enzyme complex (porcine pancreatin) in the intestine so *L. plantarum* SU-LS36 cells were gradually released in small amounts over 4 h from their microencapsulated materials.

Doherty et al. (2012) have extensively evaluated the possibility of whey protein microbeads to encapsulate *Lactobacillus rhamnosus* GG and determine stability and viability of such microcapsules in the simulated stomach fluid followed by simulated intestinal fluid. The results showed a low decrease in the viability of probiotics during incubation in simulated stomach fluid, but there was a release of probiotics in more than 30 min in the simulated intestinal fluid. The release of probiotic bacteria was not recorded in the saline phosphate buffer (pH 7), so the release was attributed to the synergistic effect of pH and the enzyme activity of the intestinal fluid. Chavarri et al. (2010) showed that the initial viability of probiotic cells was correlated with the rate of probiotic cells released from the alginate matrix into milk. In addition, alginate microcapsules coated with chitosan could retain the encapsulated probiotic bacteria during exposure to simulated stomach fluid, but probiotic bacteria were completely released in intestinal fluids within 2 h, allowing the control of the release of probiotics in the small intestine, but not to the farthest regions of the colon.

TABLE 2. Survivability and viability of *L. plantarum* SU-LS36 encapsulated with native taro starch (NTS), heat moisture treatment modified taro starch (HMT MTS), autoclaving-cooling-2 cycles modified taro starch (AC-2C MTS) and maltodextrin in SIJ

Encapsulant material	Viability (log CFU/g)	Survivability (%)	Viability (log CFU/g)	Survivability (%)	Viability (log CFU/g)	Survivability (%)	Viability (log CFU/g)	Survivability (%)	Viability (log CFU/g)	Survivability (%)
	0 h		1 h		2 h		3 h		4 h	
NTS	8.73±0.05	99.20±0.54^{a}	7.89±0.08	89.66±0.83^{b}	7.15±0.01	81.25±0.12^{b}	6.81±0.04	77.39±0.43^{c}	6.27±0.01	71.25±0.13^{c}
HMT MTS	7.98±0.04	96.38±0.43^{a}	7.51±0.02	90.71±0.24^{a}	7.26±0.03	87.68±0.32^{b}	6.82±0.03	82.37±0.32^{b}	6.27±0.02	75.72±0.21^{c}
AC-2C MTS	8.88±0.03	98.34±0.35^{a}	8.26±0.04	91.47±0.42^{a}	8.09±0.06	89.59±0.63^{b}	7.24±0.05	80.18±0.51^{b}	7.09±0.04	78.52±0.42^{c}
Maltodextrin	8.90±0.02	98.45±0.23^{a}	8.18±0.06	90.49±0.65^{a}	7.91±0.04	87.50±0.42^{b}	7.25±0.01	80.20±0.12^{b}	7.02±0.03	77.65±0.33^{c}
Without encapsulant	9.18±0.07	93.51±0.72^{a}	7.18±0.05	73.14±0.54^{c}	5.78±0.08	58.88±0.81^{d}	4.53±0.02	46.15±0.22^{e}	3.71±0.02	37.79±0.24^{f}

Means with different superscript letters within a row and column are significantly different at $p<0.05$



Magnification 5000×

FIGURE 2. The scanning electron microscope (SEM) images of *L. plantarum* SU-LS36 encapsulated with AC-2C MTS before digestion – (A) 0 h, and (B) after 1 h, (C) 2 h, (D) 3 h, and (E) 4 h digestion in SIJ

SURVIVABILITY AND RELEASE OF ENCAPSULATED *L. plantarum* SU-LS36 IN SCJ

L. plantarum SU-LS36 encapsulated with AC-2C MTS had the highest viability and survivability in SCJ incubation up to 4 h of other taro starch microcapsules ($p<0.05$) which can be refer from Table 3, during which the viability decreased at an average rate of 0.27 log CFU/(mL×h) and the survivability declined from 98.89 to 87.03%. Meanwhile, the maltodextrin-encapsulated *L. plantarum* SU-LS36 showed viability and survivability that was not significantly different from those of *L. plantarum* SU-LS36 AC-2C MTS ($p\geq0.05$). Meanwhile,

the decrease rates in viability and survivability of maltodextrin-encapsulated *L. plantarum* SU-LS36 were not significantly different from those of *L. plantarum* SU-LS36 AC-2C MTS ($p \geq 0.05$), namely 0.34 log CFU/(mL×h) and 97.86 to 83.04% which can be refer from Table 3.

NTS and HMT MTS taro starch provide significant protection by maintaining the viability and survivability of *L. plantarum* SU-LS36 in SCJ for 3 h which can be referred from Table 3. The NTS-encapsulated *L. plantarum* SU-LS36 experienced a decreased viability at an average rate of 0.55 log CFU/(g×h) and a declined survivability from 97.84 to 72.08%. Similarly, the decreased rates of viability and survivability of *L. plantarum* SU-LS36 encapsulated with HMT MTS was 0.50 log CFU/(g×h) and 96.90 to 73.55%, respectively, which can be refer from Table 3. Meanwhile, the viability and survivability of *L. plantarum* SU-LS36 free cells were on the acceptable level within 1 h incubation in SCJ before its viability significantly decreased at an average rate of 1.15 log CFU/(g×h).

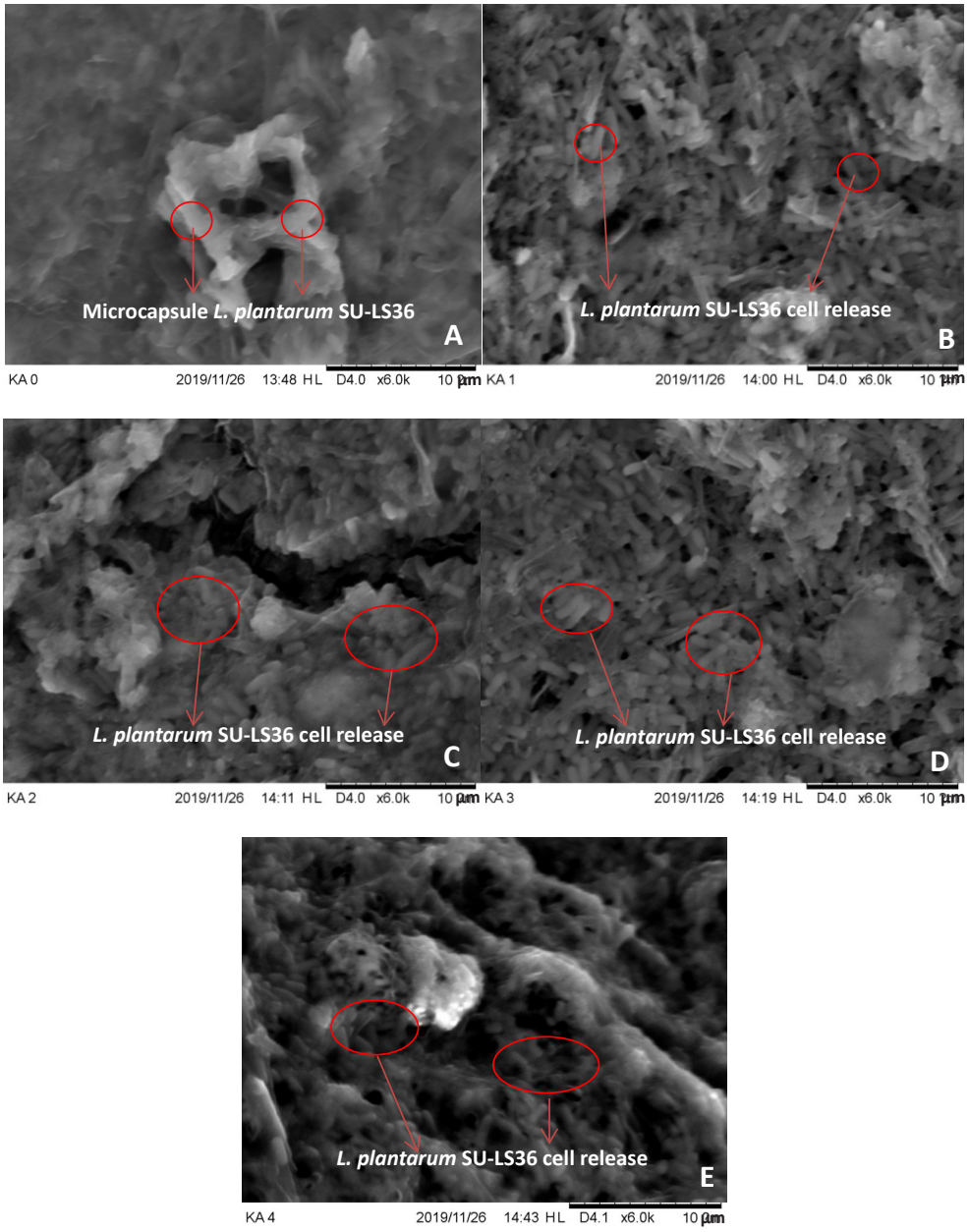
Similar results were reported by Li et al. (2016) who found a decrease in the number of microencapsulated *Lactobacillus casei* from 0.77 to 2 log CFU/g in 0.5% bile and from 1.27 to 3 log CFU/g in 1% bile after 6 h incubation. The survival of microencapsulated probiotics in bile greatly depends on the concentration of the encapsulated agent and on the species that are being microencapsulated (Sohail et al. 2012). Arslan-Tontul and Erbas (2017) reported that a total number of *L. plantarum* encapsulated by emulsion techniques remains above 6 log CFU/g after experiencing gastrointestinal digestion and 30-day storage. The number of free cells of *Lactobacillus lactis* R7 was reduced by 2.18 and 1.00 in the log cycle in the absence and presence of bile salts, respectively (Rosolen et al. 2019). However, *L. lactis* R7 microcapsules stored for 7 days maintained a decent number of cells. Likewise, Dianawati et al. (2013) showed that the use of prebiotics in the microencapsulation of *L. acidophilus* and *L. casei* not only provided an increase in the protection of probiotics but also the growth of these microorganisms in the simulated digestive tract.

The encapsulated *L. plantarum* SU-LS36 cells in SCJ began to release from MTS after 1 h which can be refer from Figure 3. The release of *L. plantarum* SU-LS36 from microcapsules probably occured through a dissolution process in which the taro starch was dissolved in SCJ so *L. plantarum* SU-LS36 cells could escape and release the microcapsule structure. The number of *L. plantarum* SU-LS36 cells released from modified taro starch continued to increase in the 2nd and 3rd hour until finally all *L. plantarum* SU-LS36 cells were released from the encapsulant material in the 4th hour which can be refer from Figure 3(C), 3(D) and 3(E). The AC-2C MTS was very suitable as an encapsulant material because it was resistant to hydrolysis of SGJ and digestive enzymes in the SIJ but easily dissolved in SCJ, so *L. plantarum* SU-LS36 cells could be released completely from the microencapsulated material in the colon.

TABLE 3. Survivability and viability of *L. plantarum* SU-LS36 encapsulated with native taro starch (NTS), heat moisture treatment modified taro starch (HMT MTS), autoclaving-cooling-2 cycles modified taro starch (AC-2C MTS) and maltodextrin in SCJ

Encapsulant material	Viability (log CFU/g)	Survivability (%)	Viability (log CFU/g)	Survivability (%)	Viability (log CFU/g)	Survivability (%)	Viability (log CFU/g)	Survivability (%)	Viability (log CFU/g)	Survivability (%)
	0 h		1 h		2 h		3 h		4 h	
NTS	8.62±0.02	97.84±0.21^{a}	8.16±0.05	92.66±0.52^{a}	7.88±0.07	89.44±0.72^{b}	7.22±0.01	81.95±0.12^{b}	6.35±0.04	72.08±0.41^{c}
HMT MTS	8.01±0.01	96.90±0.13^{a}	7.62±0.04	92.18±0.41^{a}	7.03±0.06	85.04±0.62^{b}	6.51±0.03	78.75±0.31^{c}	6.08±0.03	73.55±0.32^{c}
AC-2C MTS	8.92±0.03	98.89±0.32^{a}	8.65±0.01	95.90±0.12^{a}	8.30±0.02	92.02±0.23^{a}	8.05±0.02	89.25±0.22^{b}	7.85±0.02	87.03±0.21^{b}
Maltodextrin	8.85±0.03	97.86±0.33^{a}	8.40±0.02	92.89±0.22^{a}	8.03±0.04	88.79±0.42^{b}	7.73±0.01	85.48±0.11^{b}	7.51±0.03	83.04±0.30^{b}
Without encapsulant	9.32±0.02	94.94±0.20^{a}	7.22±0.02	73.55±0.23^{c}	6.12±0.03	62.34±0.34^{d}	5.26±0.03	53.58±0.31^{e}	4.57±0.05	46.55±0.51^{f}

Means with different superscript letters within a row and column are significantly different at $p<0.05$



Magnification 5000×

FIGURE 3. The scanning electron microscope (SEM) images of *L. plantarum* SU-LS36 encapsulated with AC-2C MTS before digestion – (A) 0 h, and (B) after 1 h, (C) 2 h, (D) 3 h, and (E) 4 h digestion in SCJ

The remaining cells of *L. plantarum* SU-LS36 encapsulated with MTS AC-2C which survived the simulated stomach fluid, simulated intestinal fluid, and simulated colonic fluid for 4 h were 6.95, 7.09, and 7.85 log CFU/g, respectively. The encapsulated *L. plantarum* SU-LS36 can be released from the MTS AC-2C encapsulant under SCJ conditions because MTS AC-2C, which acts as a lyoprotectant, on the peptidoglycan cell wall of *L. plantarum* SU-LS36 is easily dissolved in bile salts under SCJ conditions, resulting in a process of

releasing *L. plantarum* SU-LS36 from its encapsulation (Ashwar et al. 2016).

Therefore, AC-2C MTS was a compatible encapsulant for *L. plantarum* SU-LS36 because the bacteria were not entirely released from its encapsulant material after 2 h. It indicated that AC-2C MTS still protected *L. plantarum* SU-LS36 cell against hydrolysis in low pH of SGJ and porcine pancreatin in SIJ. Based on the SEM analysis, we observed that at the 2nd hour, not all but only a few *L. plantarum* SU-LS36 cells encapsulated with AC-2C MTS were released from the microcapsule. It was supported by the high survivability of *L. plantarum* SU-LS36 encapsulated with AC-2C MTS after 2 h in SGJ (88.19%), so that the AC-2C MTS can still protect *L. plantarum* SU-LS36 cells when it enters SIJ. Meanwhile, *L. plantarum* SU-LS36 encapsulated with AC-2C MTS were not released entirely from the microcapsules under 2 h in SIJ. It was evident by the high survivability of *L. plantarum* SU-LS36 encapsulated AC-2C MTS after 2 h in SIJ (89.59%). Therefore, AC-2C MTS was still able to provide protection against *L. plantarum* SU-LS36 cells when it entered SCJ. *L. plantarum* SU-LS36 cells were entirely released rapidly from AC-2C MTS encapsulant after 1 h dissolved in SCJ.

The protective capacity of AC-2C MTS microencapsulate against *L. plantarum* SU-LS36 can be calculated based on simulations of the normal human digestive tract. The viability, survivability, and release process of *L. plantarum* SU-LS36 from its encapsulant material in SGJ, SIJ, and SCJ conditions were carried out for 4 h which, according to Shori (2017) is the maximum transit time and emptied time for food into the stomach, intestines, and colon. More specifically, food stays for 2 h in the stomach, 2 h in the small intestine, and 4 h in the colon (Shori 2017).

The risk of untouchable transit of the probiotic microcapsule *L. plantarum* SU-LS36 remains and has been calculated in this study. The basis of this study was to calculate the decrease in cell viability of *L. plantarum* SU-LS36 (log CFU/g) under SGJ, SIJ, and SCJ for 4 h. The resistance of AC-2C MTS in protecting *L. plantarum* SU-LS36 cells against SGJ, SIJ, SCJ conditions was calculated in percentage with time intervals of 0, 1, 2, 3, and 4 h. The undissolved portion of probiotic microcapsules is expressed as non-viable cells because they are not counted in the cell enumeration using the pour-plate method on MRSA media. Accordingly, the number of microcapsules that did not reach the colon and were not released was counted as part of the dead (non-living) *L. plantarum* SU-LS36 cells.

In this study, the survivability rates of *L. plantarum* SU-LS36 encapsulated with AC-2C MTS and incubated for 2 h in SGJ (88.19% × 8.79 log CFU/g), 2 h in SIJ (89.59% × 7.75 log CFU/g), and 4 h in SCJ (87.03% × 6.94 log CFU/g) were 7.75, 6.94, and 6.04 log CFU/g, respectively. Meanwhile, the total dead and released cells in the same treatment were 11.81% (1.04 log CFU/g), 10.41% (0.81 log CFU/g), and 12.97% (0.90 log CFU/g), respectively. In other words, the total *L. plantarum* SU-LS36 encapsulated with AC-2C MTS that was released and died in SGJ, SIJ, and SCJ was 31.28% of the initial cell or 2.75 log CFU/g. Therefore, AC-2C MTS encapsulant had a good protective capacity because it was able to maintain the viability of *L. plantarum* SU-LS36 cells to the colon at 6.04 log CFU/g and meet the minimum requirement of biovalue (MBV) probiotic by the US FDA, i.e. at least 6-7 log CFU/g (Arslan-Tontul & Erbas 2017).

Conclusion

We proved that AC-2C MTS produced the best quality microcapsules compared to NTS, HMT MTS, and maltodextrin, particularly for its exceptional ability to maintain the survivability of *L. plantarum* SU-LS36 under SGJ, SIJ, and SCJ conditions. AC-2C MTS acted as the protector for *L. plantarum* SU-LS36 because it was resistant to hydrolysis under SGJ and SIJ conditions, so only a small number of *L. plantarum* SU-LS36 cells were released. Also, AC-2C MTS was easily dissolved in SCJ so *L. plantarum* SU-LS36 cells could be quickly released from its encapsulant material in the colon. The release time of the encapsulated *L. plantarum* SU-LS36 by AC-2C MTS in SIJ, SGJ, and SCJ conditions was 3 h, 2 h, and 1 h, respectively. The release process of the encapsulated *L. plantarum* SU-LS36 occurred through a dissolution (SGJ and SCJ) or a rupture by pancreatin activity (SIJ). MTS AC-2C had a good protective capacity because it was able to maintain the viability of *L. plantarum* SU-LS36 cells to the colon at 6.04 log CFU/g, and therefore, meet the MBV probiotic requirement set forth by the US FDA.

Acknowledgements

The authors would like to express their deepest gratitude to the Research Center for Biology National Research and Innovation Agency (BRIN) and the Science & Technology Postgraduate Scholarship from the Ministry of Research and Technology, the Republic of Indonesia for research funding. R. Haryo Bimo Setiarto, Harsi Dewantari Kusumaningrum, Betty Sri Laksmi

Jenie and Tatik Khusniati are the main contributors to this manuscript. Masrukhin and Sulistiani are member contributors to this manuscript.

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