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Modified taro starch as alternative encapsulant for microencapsulation of *Lactobacillus plantarum* SU-LS 36

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Abstract: Taro starch was modified and used as an alternative encapsulant for the microencapsulation of *Lactobacillus plantarum* SU-LS 36 by spray drying. Modification of taro starch was conducted by heat moisture treatment (HMT) and 2 autoclaving-cooling cycles (AC-2C). Microencapsulation of *L. plantarum* SU-LS 36 by spray dryer was done at constant air inlet (125 °C) and outlet temperature (50 °C), feed flow rate (4 mL min^{-1}), drying air flow rate (20 m^3 h^{-1}) and air pressure (0.196 MPa). The modified taro starch AC-2C as an encapsulant material was able to produce round-shaped microcapsules and provided optimal protection during spray drying. The modified taro starch AC-2C is very promising to be used as an encapsulant for *L. plantarum* SU-LS36 since it showed better production yield (40.19%), high encapsulation efficiency (89.83%), protected the encapsulated bacteria from high temperature (70 °C), and showed the lowest viability decreasing during storage up to 6 weeks at room temperature.

Keywords: 2 autoclaving-cooling cycles; microencapsulation; probiotic; resistant starch; spray drying

The demand for probiotic products is increasing because they have been proved as immunomodulators, preventing diarrhoea and treating intestinal inflammation (Gadhiya et al. 2015). *Lactobacillus plantarum* SU-LS36 has the potential to be applied to probiotics because it has antibacterial activity, survives in conditions of low acidity (pH 3), bile tolerance and grows at 45 °C (Sulistiani 2018). Probiotics are generally presented in the form of capsules, powders, pills and tablets which have many advantages (Gadhiya et al. 2015). The minimum number of probiotics recommended for human health is 10^8 CFU (Arslan-Tontul & Erbas 2017). The low pH of gastric acid and bile salts are the main causes of a significant decrease in the viability of probiotic cells after absorption in the digestive tract (Lisová et al. 2013).

Microencapsulation provides protection for probiotic cells against unfavourable conditions during food

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processing, distribution, storage, and along the digestive tract (Dos Santos et al. 2019). Encapsulant material should not have cytotoxicity and antimicrobial activity (Brinques & Ayub 2011). Encapsulant agents are required to be able to promote suitable conditions for the survival of probiotics and increase their stability during storage and also they can be released in a controlled manner in the colon (Brinques & Ayub 2011). Spray drying techniques are most widely applied in the food industry considering economic aspects, ease of application, they can produce smaller capsules and formulation conditions for cell viability retention (Dianawati et al. 2013). The survival rate of bacteria after spray drying depends on a number of factors, including culture strains, drying conditions and characteristics of encapsulant materials (Dos Santos et al. 2019).

Resistant starch (RS) is a part of starch that cannot be hydrolysed by gastric acid and digestive enzymes in the small intestine so that it enters the colon to be fermented by a probiotic (Ashwar et al. 2018). The potential of RS is to be used as an encapsulant material for the delivery of targeted probiotic bacteria into the colon (Ashwar et al. 2018). Setiarto et al. (2018) reported that modified taro flour using fermentation and two autoclaving-cooling cycles can improve its RS content and prebiotic properties. This study aims to produce encapsulated *L. plantarum* SU-LS36 by spray drying using modified taro starch as an encapsulant and evaluate its encapsulation efficiency, microstructure properties, heat resistance, and viability during storage at room temperature.

MATERIAL AND METHODS

Material. The main raw material used in this study was Bogor taro of Pandan *(Colocasia esculenta)* with eight months harvest age, from Cijeruk, Bogor, West Java, Indonesia. *Lactobacillus plantarum* SU-LS 36 was obtained from Laboratory of Food Microbiology, Research Centre for Biology, Indonesian Institute of Science (LIPI).

Taro starch extraction. Taro starch was first extracted by applying the technique from Airul et al. (2014) with a few modifications. Taro tuber was peeled, washed, and soaked in a mixture of 1% NaCl (3 : 4) for 1 h. It was then shredded and mixed with distilled water (3 : 1) for one minute using a blender (Phillips, Amsterdam, Netherlands). The obtained taro pulp filtrate was allowed to settle overnight to let the starch sink to the bottom of the beaker glass. Double fold cotton cloth was used to filter the taro pulp. Taro pulp was centrifuged using a high-speed centrifuge (Kubota, Tokyo, Japan) at 7 000 *g* for 10 min to obtain taro starch. After that, it was oven dried at 50 °C until constant weight was reached. Finally, the dry taro starch was ground using a disk mill (Taian City Up International Trade Co. Ltd, Shandong, China) (5 min) and it was sieved with a size of 200 mesh (74 μm).

Heat-moisture treatment (HMT). The taro starch modification using HMT was obtained following Deka & Sit (2016). Forty-five grams of taro starch (dry-based) was placed into a glass container, and distilled water was added to it while stirring until the water content reached 25% (w/w). Then, the glass container was sealed, balanced for 48 h at room temperature, then heated at 120 °C in an electric oven (Shimizu, Tokyo, Japan) for three hours.

Autoclaving-cooling treatment. The autoclaving-cooling method of taro starch followed the procedure by Setiarto et al. (2018). The taro starch was added distilled water (1 : 2), heated in an autoclave (Hitachi, Tokyo, Japan) (121 °C, 15 min), then chilled in a refrigerator (4 °C, 24 h). The autoclaving-cooling treatment was also completed with two cycles.

Microencapsulation by spray drying. *L. plantarum* SU-LS36 was cultivated in MRS broth (Oxoid Ltd., Hampshire, England) (1 : 100) and incubated (24 h, 37 °C). *L. plantarum* SU-LS36 cell biomass was harvested using a high-speed centrifuge 6500 (Kubota, Tokyo, Japan) (5 000 *g*, 20 min, 4 °C). *L. plantarum* SU-LS36 cell biomass (10^{10} CFU g^{-1}) was mixed with encapsulant material at a 1 : 1 ratio which had a final concentration of 10% (w/v) as follows: a) *L. plantarum* SU-LS36/native taro starch; b) *L. plantarum* SU-LS36/HMT taro starch; c) *L. plantarum* SU-LS36/AC-2C taro starch, d) *L. plantarum* SU-LS36/maltodextrin. Sterile distilled water was used as a solvent for encapsulant materials. *L. plantarum* SU-LS36 cell biomass and encapsulant material were homogenized using a high-speed homogenizer (IKA-Ultra-Turrax T18 basic; Munich, Germany) (60 sec, 13 700 g). Microencapsulation of *L. plantarum* SU-LS 36 by spray dryer (Eyela, Tokyo, Japan) (0.5 mm nozzle diameter) was done at constant air inlet (125 °C) and outlet temperature (50 °C), feed flow rate (4 mL min^{-1}), drying air flow rate (20 m^3 $hour^{-1}$) and air pressure (0.196 MPa) (Rajam & Anandharamakrishnan 2015). The encapsulated *L. plantarum* SU-LS36 was packaged in polyethylene packaging and stored at room temperature until analysis.

Microencapsulation yield determination. The microencapsulation yield was determined according to Ra-

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jam and Anandharamakrishnan (2015), using the following equation:

$$\text{Microencapsulation yield (\%)} = \frac{\text{Mass of spray drying products recovered from collector (g)}}{\text{Mass of solids in the processed suspension (g)}} \times 100$$

Viability of encapsulated *L. plantarum* SU-LS 36 and encapsulation efficiency (EE) measurement. Viability of *L. plantarum* SU-LS 36 was determined as the number of colony-forming units per gram dry matter (log CFU g^{-1}). The values are the averages of three replicates. Encapsulation efficiency (%) of probiotic microorganisms was calculated using the equation: $(N_t/N_o) \times 100$, according to Rajam & Anandharamakrishnan (2015). N_o was the probiotic calculated based on the feed solution before spray drying (log CFU g^{-1}) and N_t was the probiotic calculated based on microcapsules produced after spray drying (log CFU g^{-1})

Microstructure analysis (SEM). The sputter coater (Hitachi E102 Ion Sputter; Tokyo, Japan) was used to cover the *L. plantarum* SU-LS36 encapsulated samples. An acceleration voltage of 20.0 kV was used to analyse all samples of encapsulated *L. plantarum* SU-LS36. The Hitachi S 2400 scanning electron microscope (SEM) (Hitachi, Tokyo, Japan) was used to record and analyse the *L. plantarum* SU-LS36 microcapsules. *L. plantarum* SU-LS36 encapsulated images were captured at 10 000 × magnification (Ying et al. 2013). The particle size of encapsulated *L. plantarum* SU-LS36 was analysed using Image-J free software for processing digital images according to Java program created by researchers at the Research Services Branch, National Institute of Mental Health, Bethesda (Maryland, USA) (Collins 2007).

Heat resistance evaluation. One gram sample of *L. plantarum* SU-LS36 encapsulated with native taro starch, maltodextrin, HMT and AC-2C modified taro starch was weighed in glass tubes. The sample was added 10 mL sterile Aqua Dest distilled water and it was homogenised until a suspension was obtained. Samples in the form of suspension were incubated in the water bath (Memmert, Schwabach, Germany) at various temperatures, i.e. 50 °C, 60 °C, 70 °C, 80 °C, for 30 min (Dianawati et al. 2013). Survivability of *L. plantarum* SU-LS36 (non-encapsulated) free cells in heat resistance analysis was determined in this study. At the end of the treatment, viability analysis of *L. plantarum* SU-LS36 was done.

Determination of viability of encapsulated *L. plantarum* SU-LS36 during storage. *L. plantarum* SU-LS36 encapsulated was vacuum packed using low density polyethylene (LDPE) aluminium foil-coated plastic packaging (Xuzhou First Chemical Co. Ltd, Jiangsu, China). The packaged product was stored in closed glass jars at a room temperature of 27 ± 1 °C for 6 weeks. Storage study was carried out by analysing the viability of encapsulated *L. plantarum* SU-LS36 for 6 weeks of storage by sampling every week. Viability of *L. plantarum* SU-LS36 was calculated by the cultivation method on MRS agar. Evaluation of encapsulated *L. plantarum* SU-LS36 cell viability during storage is described by the equation according to Dianawati et al. (2013):

$$\log \frac{N_t}{N_i} = kT \times t \Rightarrow \log N_t = \log N_0 + kT$$

where: N_t – the number of *L. plantarum* SU-LS36 cells that live in a certain storage period (6 weeks) (CFU g^{-1}), N_i – the number of *L. plantarum* SU-LS36 cells at initial storage (CFU g^{-1}), kT – the level of specific viability loss of *L. plantarum* SU-LS36 cells at 27 °C every week, t – storage time (weeks).

Statistical analysis. There were three replications in this experiment, where the statistical analyses were implemented to process the research data. Duncan's statistical test was applied to examine the considerable differences at the level of $P < 0.05$ using the SPSS 18.0 statistical software.

RESULTS AND DISCUSSION

Encapsulation efficiency (EE) and yield. As shown in Figure 1A, the microencapsulation of *L. plantarum* SU-LS36 using AC-2C modified taro starch produced a microcapsule yield of 40.19% which was not significantly different ($P < 0.05$) from maltodextrin as a common encapsulant (42.09%). Meanwhile *L. plantarum* SU-LS36 encapsulated with HMT modified taro starch gave the lowest yield of 31.70%. This study showed that AC-2C modified taro starch yielded equal productivity with maltodextrin. The encapsulation yield of microcapsules with resistant starch (Hi-maize) for probiotics *L. casei, L. brevis* and *L. plantarum* was 48.46%, 43.01% and 43.85%, respectively (Etchepare et al. 2016). Etchepare et al. (2016) concluded that probiotic encapsulation by emulsification techniques using Hi-Maize starch-alginate can increase the storage stability of microencapsulated bacteria.

The results from this study showed that AC-2C modified taro starch was able to provide protection as the best encapsulant for the microencapsulation of *L. plantarum* SU-LS36 with encapsulation efficiency (EE) of 89.83% (Figure 1B). Maltodextrin had encap-

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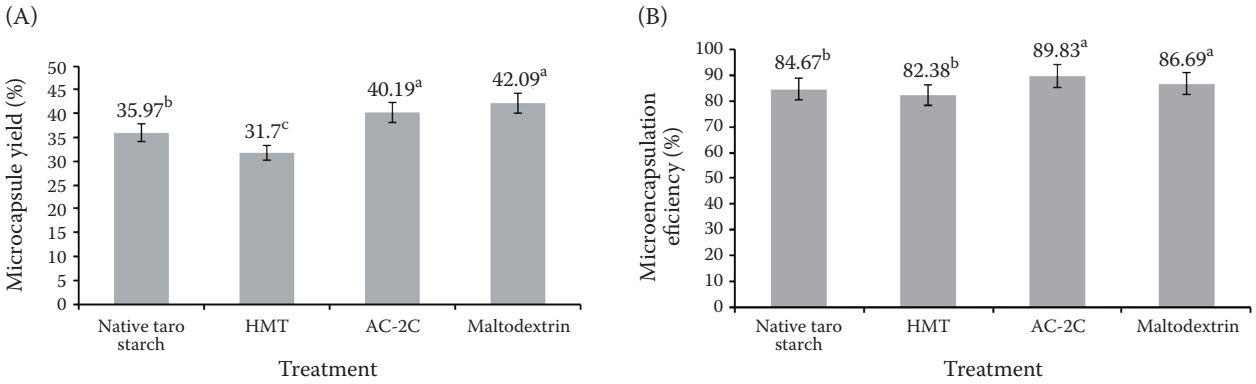

Figure 1. Encapsulated *L.plantarum* SU-LS36 with various encapsulants: yield production (A), encapsulation efficiency (B)

Yield production and encapsulation efficiency are expressed by different typescript letters of the bar chart, where the noticeable difference occurs at the $P < 0.05$ level

sulation efficiency (EE) of 86.69% when it was not significantly different ($P < 0.05$) from AC-2C taro starch. AC-2C taro starch showed the best heat resistance to protect *L. plantarum* SU-LS36 during spray drying. Meanwhile, native taro starch and HMT taro starch were also able to provide protection for *L. plantarum* SU-LS36 with encapsulation efficiency (EE) of 84.67% and 82.38%, respectively (Figure 1B).

The microencapsulation of *L. plantarum* (MTCC 5422) with fructooligosaccharides (FOS) showed encapsulation efficiency (EE) between 70% and 73% (Rajam and Anandharamakrishnan 2015). Dos Santos et al. (2019) reported that the microencapsulation of *L. acidophilus* La-5 by using inulin (10%) provided a high level of probiotic encapsulation efficiency (EE) at 86.5% and a high survival rate for acid conditions 78.7%. The study from Dutra-Rosolen et al. (2019) reported encapsulation efficiency of 94.61%, which shows that the combination of whey and inulin is efficient in protecting *L. lactis* R7 during spray drying.

Microstructure of encapsulated *L. plantarum* SU-LS36. *L. plantarum* SU-LS 36 free cells (non-encapsulated) were stems with straight, rounded, 0.9–1.2 μm wide and 3–8 μm long size, occurring individually, in pairs or in short chains (Figure 2E). Modified AC-2C taro starch was able to produce the surface structure of spherical

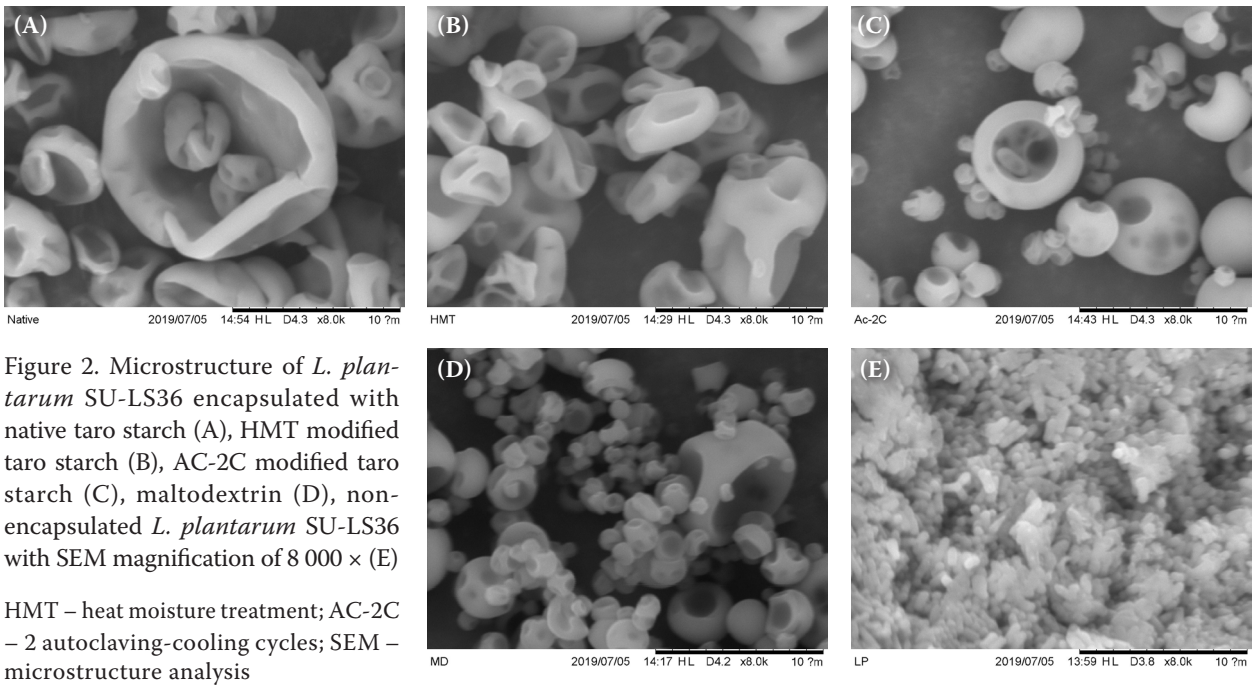

Figure 2. Microstructure of *L. plantarum* SU-LS36 encapsulated with native taro starch (A), HMT modified taro starch (B), AC-2C modified taro starch (C), maltodextrin (D), non-encapsulated *L. plantarum* SU-LS36 with SEM magnification of 8 000 × (E)

HMT – heat moisture treatment; AC-2C – 2 autoclaving-cooling cycles; SEM – microstructure analysis

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L. plantarum SU-LS36 microcapsules shaped like a ball of 50–60 μm in size (Figure 2C). The spherical microstructure provides optimal protection for *L. plantarum* SU-LS36 microcapsules during spray drying with the highest encapsulation efficiency (EE) of 89.83%. The structure of *L. plantarum* SU-LS36 microcapsules formed by maltodextrin had a spherical morphology similar to the AC-2C modified taro starch structure but with a smaller size of 20–40 μm (Figure 2D).

Meanwhile, *L. plantarum* SU-LS36 encapsulated with native taro starch and HMT taro starch showed an irregular, hollow surface structure with a size of 70–80 μm and it had many cavities (Figure 2A and 2B). The structure was irregular, providing relatively less optimal protection for encapsulated *L. plantarum* SU-LS36. The spherical structure of encapsulated *L. plantarum* SU-LS36 was more resistant during spray drying with storage at room temperature compared to the other treatments (Figure 2C and 2D).

Maltodextrin as a result of dextrin hydrolysis has a simple chemical structure and a low degree of polymerization (DP 10–20) (Anekella & Orsat 2013). This affects the structure and size of the microcapsules formed by maltodextrin, which are round with a small size (20–40 μm). The AC-2C modified taro starch showed a low degree (40–60) of polymerisation (DP) (Setiarto et al. 2018) to form a spherical microcapsule-like surface structure that resembles maltodextrin but with a larger microcapsule size (50–60 μm). Meanwhile native taro starch and HMT taro starch still have complex chemical structures with a high degree of polymerization (DP > 100) (Simsek & El 2015). This is likely the formation of microcapsule structures that tend to be irregular with a fairly large size of microcapsule (70–80 μm).

Heidebach et al. (2012) claimed that probiotic microcapsules must be smaller than 100 μm. Probiotic microcapsule particle sizes smaller than 350 μm are not preferred because of poor solubility. Gong et al. (2019) analysed changes in the viability of probiotic cells during spray drying. Death of probiotic cells during spray drying occurs due to damage to the phospholipid bilayer membrane. Gong et al. (2019) reported that the survival rate of spray-dried probiotics can be maintained by regulating external temperatures to < 64 °C.

Survivability of encapsulated *L. plantarum* SU-LS36 after heat treatment. The encapsulated *L. plantarum* SU-LS36 with AC-2C taro starch has the highest heat resistance survivability among the others. AC-2C taro starch was able to provide the best heat resistance and protection for *L. plantarum* SU-LS36 at temperatures of 50, 60 and 70 °C for 30 min (Table 1). The high

Table 1. Survivability and heat resistance of encapsulated *L. plantarum* SU-LS36 with various encapsulants at 50, 60, 70, 80 °C for 30 min

Encapsulant materials	Viability before treatment (log CFU g^{-1})	50 °C		60 °C		70 °C		80 °C	
		Viability (log CFU g^{-1})	Survivability (%)	Viability (log CFU g^{-1})	Survivability (%)	Viability (log CFU g^{-1})	Survivability (%)	Viability (log CFU g^{-1})	Survivability (%)
Native taro starch	8.80 ± 0.09	6.83 ± 0.05	77.61 ± 0.79^{c}	5.19 ± 0.07	58.98 ± 0.83^{e}	3.42 ± 0.11	37.73 ± 1.02^{g}	2.15 ± 0.02	23.63 ± 028^{i}
HMT	8.07 ± 0.05	6.86 ± 0.06	85.01 ± 0.85^{b}	5.55 ± 0.04	68.77 ± 0.36^{d}	4.13 ± 0.09	45.57 ± 0.95^{f}	2.89 ± 0.01	31.76 ± 0.17^{h}
AC-2C	9.06 ± 0.04	8.17 ± 0.04	90.14 ± 0.68^{a}	6.85 ± 0.05	75.58 ± 0.59^{c}	5.55 ± 0.08	61.24 ± 0.87^{e}	4.15 ± 0.03	45.79 ± 0.35^{f}
Maltodextrin	9.10 ± 0.06	7.90 ± 0.08	86.81 ± 0.91^{b}	6.44 ± 0.02	70.77 ± 0.28^{d}	5.25 ± 0.04	57.69 ± 0.48^{e}	3.78 ± 0.04	41.54 ± 0.46^{g}
L. plantarum SU-LS36 free cell (non- encapsulated)	9.13 ± 0.08	5.08 ± 0.02	55.64 ± 0.26^{e}	2.12 ± 0.04	23.22 ± 0.45^{i}	0.00 ± 0.00	0 ± 0.00^{j}	0.00 ± 0.0	0.00 ± 0.00^{j}

Survivability of encapsulated *L. plantarum* SU-LS36 with the different superscript letters within a row is significantly different at $P < 0.05$; HMT – heat moisture treatment; AC-2C – 2 autoclaving-cooling cycle

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content of resistant starch type 3 (RS3) in AC-2C taro starch is able to protect, coat and contribute to the resistance of encapsulated *L. plantarum* SU-LS36 from an adverse heating effect. Maltodextrin was also able to provide adequate heat protection in maintaining the viability of *L. plantarum* SU-LS36 because of its survivability still above 50% under heating conditions of 50, 60 and 70 °C (Table 1). The AC-2C taro starch and maltodextrin were able to encapsulate *L. plantarum* SU-LS36 more perfectly by forming a spherical microstructure that can minimize the penetration process of heat transfer into the structure of *L. plantarum* SU-LS36 cell. Maltodextrin and AC-2C taro starch play an optimal role as an encapsulant material and eggshell for *L. plantarum* SU-LS36 cell that was able to withstand the effects of heating so as to maintain viability and survivability of *L. plantarum* SU-LS36.

However, at temperatures of 70 and 80 °C, all microcapsule treatments for 30 min all microcapsule treatments showed a significant reduction in the number of viable probiotic cells. These results indicated that modified taro starch offers little protection for *L. plantarum* SU-LS36 after this temperature/time combination. Gbassi et al. (2009) reported that excessive heat treatment causes denaturation that damages the macromolecular structure of nucleic acids and bacterial cell proteins, which leads to bacterial cell death. In another study, Tárrega et al. (2010) reported that inulin combined with whey protein was able to provide thermal resistance in heat protection for *L. lactis* R7. Ashwar et al. (2018) reported that *Lactobacillus casei, Lactobacillus brevis* and *Lactobacillus plantarum* encapsulated with type 4 resistant starch (RS4 rice starch) had survivability up to 65 °C and began to show a decrease in viability at 75 °C. Ashwar et al. (2018) revealed that microencapsulation using RS4 rice starch can increase the thermal resistance of *Lactobacillus* sp. The AC-2C modified taro starch was able to provide better heat protection when compared to RS4 rice starch because it provides heat resistance for *L. plantarum* SU-LS 36 microcapsules at a temperature of 70 °C.

Viability of encapsulated *L. plantarum* SU-LS36 during storage. The viability of encapsulated *L. plantarum* SU-LS36 decreased at room temperature (27 °C) during a 6-week storage period (Figure 3A). A decrease in cell viability during storage every week is an indication of decreased quality of *L. plantarum* SU-LS36 growth. The best material for microencapsulation is chosen based on its ability to prevent the rate of decrease in viability of *L. plantarum* SU-LS36 during the storage period. The number of probiotic cells for consumption according to Arslan-Tontul et al. (2017) is a minimum of 10^8 CFU.

The AC-2C taro starch was able to provide the best protection for *L. plantarum* SU-LS36 over a storage period of 6 weeks at 27 °C. The encapsulated *L. plantarum* SU-LS36 with AC-2C taro starch had the lowest viability reduction rate of 0.41 log CFU g^{-1} every week (Figure 3E). Native taro starch had poor stability in maintaining the viability of *L. plantarum* SU-LS36 at 27 °C because it was stable only for 1-week storage time. This was indicated by the high rate of decrease in viability during the storage period at 27 °C that was 0.86 log CFU g^{-1} every week (Figure 3B). Meanwhile, HMT taro starch and maltodextrin showed fair stability in encapsulating of *L. plantarum* SU-LS36 because the rate of decrease in viability in the two treatments every week was 0.61 log CFU g^{-1} and 0.65 log CFU g^{-1}, respectively (Figures 3C and 3D). Maltodextrin was able to maintain the viability of *L. plantarum* SU-LS36 in accordance with IDF requirements (10^7 CFU g^{-1}) for 3-week shelf life. Rajam and Anandharamakrishnan (2015) encapsulated *L. plantarum* (MTCC5422) with FOS (fructooligosaccharide) and whey protein isolate using spray drying, so that the shelf life of probiotic microcapsules was obtained for 60 days at 4 °C. Ashwar et al. (2018) reported that RS4 rice starch was able to maintain the viability of *Lactobacillus* sp. microcapsules (> 7 log CFU g^{-1}) for 2 months at 4 °C. The AC-2C modified taro starch showed a better ability to maintain the viability of *L. plantarum* SU-LS36 microcapsules over a storage period of 6 weeks at 27 °C.

CONCLUSION

The present study showed that AC-2C modified taro starch can be applied as an alternative encapsulant material for probiotics. Microencapsulation by spray drying of *L. plantarum* SU-LS36 encapsulated with AC-2C modified taro starch yielded high encapsulation efficiency (89.83%) of microcapsules with round-shaped microstructures that protected the probiotic against a high temperature (70 °C) and maintained its viability for 6 weeks of storage at room temperature.

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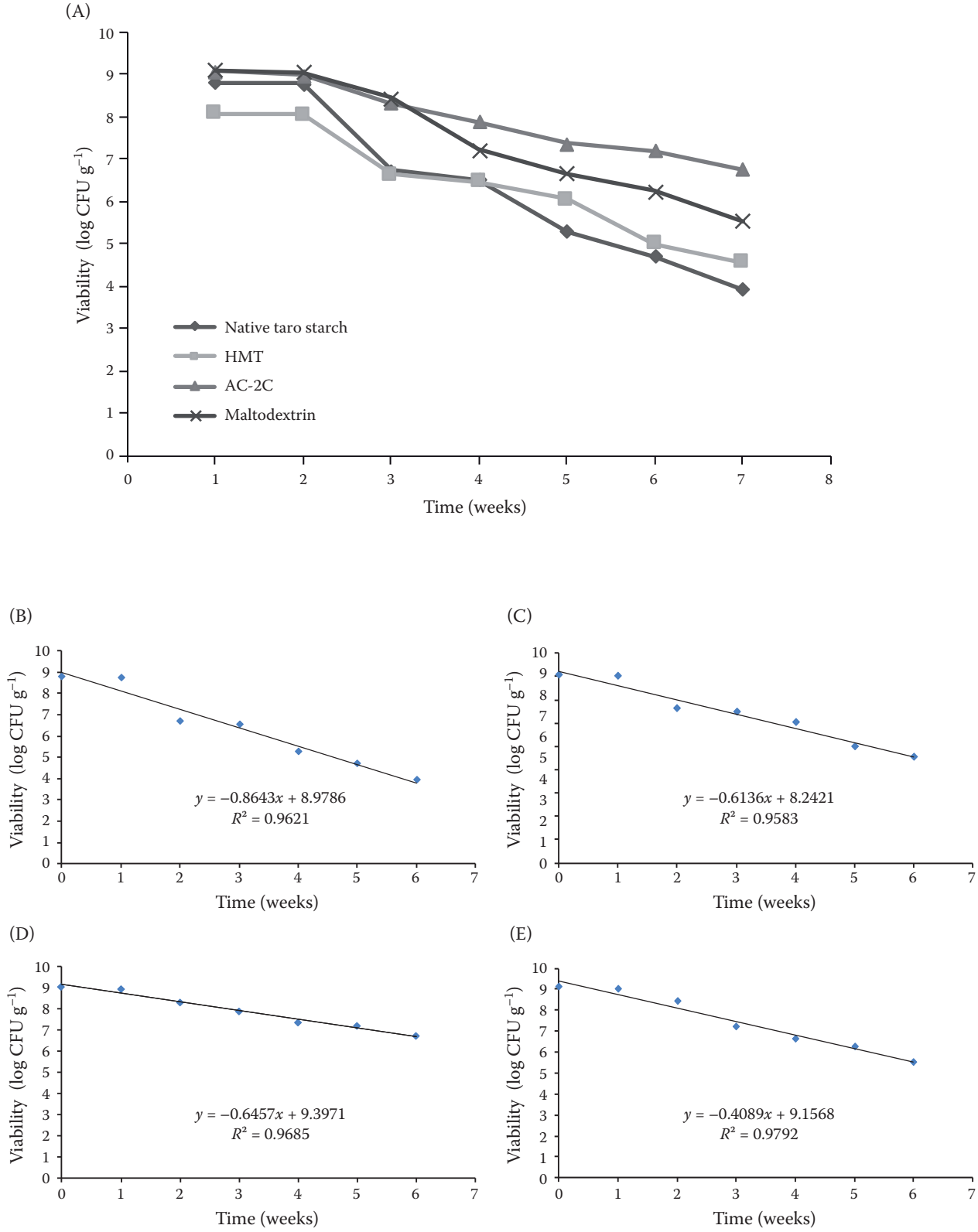

Figure 3. Viability of encapsulated *L. plantarum* SU-LS36 at room temperature (27°C) during the 6-week storage period (A), and reducing viability of *L. plantarum* SU-LS36 encapsulated with native taro starch (B), HMT taro starch (C), maltodextrin (D), AC-2C taro starch (E)

HMT – heat moisture treatment; AC-2C – 2 autoclaving-cooling cycles

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