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Encapsulation of *Pediococcus pentosaceus* RSU-Nh1 into pectin-sodium alginate and chitosan coating

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Abstract Lactic acid bacteria (LAB) are beneficial to the human intestines, they inhibit the growth of pathogenic microorganisms and are used for lactic acid fermentation. LAB are partially digested when they pass through the digestive system, resulting in reduced survival rate. Encapsulation can be used to enhance the survival of probiotic bacteria as protection against harsh conditions in the gastrointestinal tract. Results indicated that the initial cell numbers of *Pediococcus pentosaceus* RSU-Nh1, free cells survived with 7.68 log CFU/ml, whereas encapsulation using sodium alginate combined with various pectins extracted from pomelo and passion fruit peel exhibited free cell survival with 7.35 and 8.12 log CFU/ml, respectively. Encapsulation by combining chitosan with shrimp shells and fish scales showed cell survival rates of 8.59 and 8.08 log CFU/ml, respectively, while encapsulation with pectin from passion fruit coated with chitosan from shrimp shells showed the highest number of *Ped. pentosaceus* RSU-Nh1 with 9.24 log CFU/ml in gastrointestinal simulation. It revealed that encapsulation improved the survival of *Ped. pentosaceus* RSU-Nh1 in simulated gastrointestinal conditions.

Keywords: encapsulation, lactic acid bacteria, pectin, chitosan, simulation

Introduction

Most probiotic bacteria belong to the lactic acid bacteria (LAB) group. They include *Lactobacilli* species which are commonly selected as probiotics

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because they display several important properties such as high tolerance to acid and bile, capability to adhere to the intestinal tract, inhibition of pathogenic bacteria, resistance to antibiotics, production of exopolysaccharides and reduction of cholesterol (Fijan, 2014). Some beneficial effects of probiotics comprise of anti-pathogenic activity, anti-cancer activity, anti-allergic activity, anti-diabetic activity, anti-obesity activity and angiogenic activity (George Kerry *et al.*, 2018). Probiotics also play an important role in health and wellbeing (Food and Agriculture Organization (FAO), 2001). Some LAB are used as probiotics; they are characterized by the production of lactic acid with bacteriocins as the main growth inhibition substances. Bacteriocins are a group of potent antimicrobial peptides produced by microorganisms (Mokoena, 2017). Da Costa *et al.* (2019) reported the wide variety of bacteriocins produced by LAB. They show great potential applications in food biopreservation since the majority are regarded as Generally Recognized as Safe (GRAS). Bacteriocins have been found in most LAB genera including *Lactococcus*, *Lactobacillus*, *Leuconostoc*, *Pediococcus*, and some *Streptococcus* (Silva *et al.*, 2018).

Microencapsulation is a process whereby probiotic cells are entrapped in an encapsulating matrix or membrane which protects them from unfavorable environmental conditions and incompatibilities (Shori, 2017). Encapsulation is widely used in various applications of food and bioindustry for functional foods (Gómez-Mascaraque *et al.*, 2015), drug delivery (Martínez Rivas *et al.*, 2017) and probiotics bacteria (Peredo *et al.*, 2016).

Khandare and Patil (2016) reported that encapsulation of lactic acid bacteria for higher bacteriocin production was an interesting technology to improve food safety. Common encapsulation materials are carbohydrate polymers such as gum arabic, gelatin, dextran, alginate, chitosan and pectin (Wandrey *et al.*, 2010).

The fisheries and seafood processing industry has now gained global popularity. In Thailand, many people like to eat seafoods and large amounts of waste are generated from seafood production each year as shrimp shells, fish scales and crab shells. Recently, public interest in recycling and reducing this waste through new innovations has increased (FAO, 2009). The seafood processing industry produces large amounts of by-products and discards them: heads, tails, skins, scales, viscera, backbones and shells. These residual parts are a considerable source of proteins, lipids, pigments and small molecules, while the shells are a good source of chitin (Hamed *et al.*, 2016). The chitin content in shrimp shells is very high and can be converted into chitosan after deacetylation. Chitosan is a linear polysaccharide of natural origin, characterized by biodegradability and non-toxicity (de Queiroz Antonino *et al.*,

2017). It is a very expensive ingredient of foods, cosmetics and pharmaceutical products (Islam *et al.*, 2017; Gao *et al.*, 2012) and it can be formed into thin durable sheets to encapsulate and protect materials in beads for drug delivery systems (Harish Prashanth and Tharanathan, 2007). Chitosan can also be used in agriculture for fertilizers, animal feed and biopesticides (Ravi Kumar, 2000). Several studies have reported that chitosan can be extracted from crab shells (Kaya *et al.*, 2016), mussel shells (Song *et al.*, 2019), fish scales and shrimp shells (Tungse *et al*., 2016).

Different varieties of fruits produced in Thailand and other countries were popular and widely consumed. Fruit peels generated annually after processing, packing, distribution and consumption amount to about 1.5 million tons (Wadhwa *et al.*, 2013). In Thailand, fruit waste is increasing every year such as pomelo peels (Suklampoo *et al.*, 2012), pineapple peels (Choonut *et al.*, 2014), mangosteen pericarp (Ibrahim *et al.*, 2017), mango peels (Kantrong and Eshtiaghi, 2018) and durian rind (Suwannarat *et al.*, 2019). These fruit peels contain large amounts of pectin as a natural complex heteropolysaccharide and carbohydrate (Sundarraj and Ranganathan, 2017). This pectin is in the form of soluble fibers that can regulate the digestive system, decrease the risk of colon cancer and reduce cholesterol levels (Zhang *et al.*, 2015). However, pectin is also a potential prebiotic for probiotic bacteria (Floch, 2014). Here, encapsulation of *Pediococcus pentosaceus* RSU-Nh1 by extrusion technique using sodium alginate, pectin and chitosan as the matrix was investigated to determine cell survival under simulated gastrointestinal conditions.

Materials and methods

Preparation of chitosan

Two samples: shrimp (*Macrobrachium rosenbergii*) shells and Nile tilapia (*Oreochromis niloticus*) scales were collected from a seafood restaurant at Si Mum Mueang Market (Pathum Thani, Thailand). Both samples were washed thoroughly to remove all impurities and dried overnight at 80 ℃. Chitosan extraction was modified from Benhabiles *et al*. (2012). For demineralization, all samples were soaked in 2 M hydrochloric acid (HCl; QRëC, New Zealand) and stirred continuously for 30 min followed by washing with water. Next, deproteinization was conducted with 2 M sodium hydroxide (NaOH) and stirred constantly at 50 ℃ for 2 h. After that, all samples were washed with clean water, then decolored with 95% (v/v) ethanol for 10 min and washed before drying at 80 ℃ overnight. Finally, the chitin was deacetylated (1 g: 20 ml) in 2 M NaOH and stirred at 100 ℃ for 2 h. All samples were neutralized with clean water several times and then dried at 80 ℃ overnight.

Bacterial strain and sensitivity test

Pediococcus pentosaceus RSU-Nh1 was reported to produce bacteriocin in a previous study. Two percent of *Ped. pentosaceus* RSU-Nh1 culture was transferred to 50 ml MRS (MRS; Merck, Germany) broth and incubated at 37 ℃ for 18 h. *Ped. pentosaceus* RSU-Nh1 was then swabbed onto MRS agar and dropped with 10 µl of 0.4% (w/v) chitosan prepared in 0.1 M acetic acid (Labscan (Asia), Thailand). The sample was then incubated at 37 ℃ for 48 h under anaerobic conditions. Sensitivity testing was performed by drop plate assay according to the method of Herigstad *et al.* (2001). An aliquot of 10 ml *Ped. pentosaceus* RSU-Nh1 was centrifuged at 12,000 rpm for 10 min and the cell free supernatant was discarded. The pellet was resuspended in 0.1% (w/v) peptone (Bacto^{TM} Peptone; Becton, Dickinson and Company, France) and centrifuged at 12,000 rpm for 10 min. The pellet was collected and mixed with 10 ml sterilized water for encapsulation.

Encapsulation by pectin-sodium alginate extrusion

Pectin-sodium alginate was prepared from 3% (w/v) pomelo pectin (PoP) or passion fruit pectin (PaP) combined with 4% (w/v) sodium alginate (Chemipan; China) and added with 40 ml sterilized water. Subsequently, 10 ml of *Ped. pentosaceus* RSU-Nh1 suspension was mixed with pectin-sodium alginate solution. The mixture was transferred to a 50 ml syringe and dropped into an aqueous solution containing 0.15 M calcium chloride ($CaCl_2$; UNILAB, New Zealand). The beads were soaked with $CaCl_2$ solution for 30 min, then removed from solution and washed with 0.1% peptone solution. This method was modified from Bepeyeva *et al*. (2017).

Encapsulation of sodium alginate coated by chitosan

Beads prepared for sodium alginate encapsulation as described were immersed in 0.4% (w/v) shrimp shell chitosan (ShC) or fish scale chitosan (FiC) containing 0.1 M acetic acid for 40 min according to the method of Huang *et al.* (2015).

Encapsulation of pectin-sodium alginate coated by chitosan

Ped. pentosaceus RSU-Nh1 was encapsulated using pectin-sodium alginate which was coated with chitosan as described previously. The beads were prepared using sodium alginate combined with four treatments including

i) pomelo pectin and shrimp shell chitosan (PoP + ShC), ii) pomelo pectin and fish scale chitosan (PoP + FiC), iii) passion fruit and shrimp shell chitosan (PaP + ShC), and iv) passion fruit and fish scale chitosan (PaP + FiC).

Encapsulation efficiency (EE) calculation

Beads from the different treatments were 10-fold serially diluted using 0.1% peptone. Drop plate assay was performed as described by Herigstad *et al.* (2001) to determine the number of bacteria.

To evaluate the survival rate of the microencapsulated bacteria, encapsulation efficiency (EE) was determined following the modified method of Lotfipour *et al*. (2012). The EE was calculated using the equation:

$$EE = \frac{logN}{logN_0} \times 100\%$$

where N is the number of viable entrapped cells released from the beads and N_0 is the number of free cells added to the biopolymer mixture immediately before the production procedure.

Simulated gastro-intestinal condition

The simulation was modified from the method of Farias *et al*. (2019). Simulated gastric juice (SGJ) was performed by mixing 9 g sodium chloride (NaCl; Scharlau, Spain) and 3 g of pepsin (SIGMA Life Science; China) in 1 L sterilized water and the pH was adjusted to 2. Meanwhile, 1 L of sterilized water containing 0.835 g potassium chloride (KCl; UNIVAR, New Zealand), 0.22 g calcium chloride ($CaCl_2$; UNILAB, New Zealand) and 1.386 g sodium bicarbonate ($NaHCO_3$; UNIVAR, New Zealand) was prepared as the simulated intestinal juice (SIJ) and adjusted to pH 7.

For the free cell analysis, a pellet of *Ped. pentosaceus* RSU-Nh1 was suspended in 10 ml sterilized water and 1 ml bacterial suspension was transferred to the SGJ and incubated at 37 ℃ for 60 and 120 min. At each time period, 1 ml of the mixture was serially diluted and spotted on MRS agar using the drop plate method. After 120 min, the mixture was centrifuged at 12,000 rpm for 10 min and the pellet was collected. Subsequently, 9 ml of SIJ was added and incubated under agitation conditions for 240 min. Then, the sample was diluted and spotted on MRS agar using the drop plate assay.

For micro-encapsulation, 1 g of the beads was treated in SGJ followed by SIJ using the free cell conditions as mentioned above. Finally, all beads were broken in each time period and filtered through a wire mesh sieve for analysis of cell survival. Surviving cells were dropped onto an MRS agar plate and incubated at 37 ℃ for 48 h under anaerobic conditions.

Statistical analysis

All data were analyzed using the statistical software package IBM SPSS version 22. Statistical significance was determined by one-way analysis of variance (ANOVA) and Duncan's Multiple Range Test. All differences were considered significant at $P < 0.05$. Results were presented as mean ± standard deviation.

Results

Extraction of chitosan and activity test

Products from the two extraction steps of demineralization and deproteinization were derived as chitin. Here, weights of chitin extraction from shrimp shells and fish scales were 41.11 g and 53.04 g, respectively. After deacetylation, chitin was converted into chitosan. Post extraction, chitosan weights of shrimp shells and fish scales were 31.09 g and 39.88 g, respectively. However, yield percentage of shrimp shell chitosan extraction was slightly lower than fish scale chitosan extraction because the raw shrimp shells contained some flesh. The color of chitosan powder from shrimp shells was white to light orange, whereas chitosan powder from fish scales was white (Table 1). Results from activity testing showed that chitosan can be used with *Ped. pentosaceus* RSU-Nh1.

Table 1. Chitosan extraction from shrimp shells and fish scales

Sample	Fresh weight (g)	Weight (g)		Yield of chitosan (%)
		Chitin	Chitosan	
Shrimp shells	100	41.11	31.09	75.62
Fish scales	100	53.04	39.88	75.19

Encapsulation efficiency (EE)

Cell count of *Ped. pentosaceus* RSU-Nh1 before and after encapsulation using the four different treatments were determined. Results showed that cell numbers ranged from 9.89 to10.48 log CFU/ml with no significant differences between the two groups. Encapsulation efficiencies (EE) of all treatments varied at 94.3 to 97.2%. The treatment using pomelo pectin and shrimp shell chitosan (PoP + ShC) gave the highest survival rate (97%), whereas the treatment using passion fruit pectin and fish scale chitosan (PaP + FiC)

recorded the lowest survival rate (94%) with the other two treatments at approximately 96% survival rate (Table 2).

Table 2. Viable cell count (mean ± SD) before and after encapsulation

Treatment	Cell number (log CFU/ml)		Encapsulationefficiency (EE %)
	Before encapsulation	After encapsulation	
PoP	10.78 ± 0.20 [a]	10.35 ± 0.11 [b]	96.0
PaP	10.78 ± 0.20 [a]	10.24 ± 0.24 [b]	94.9
ShC	10.30 ± 0.01 [a]	9.89 ± 0.23 [b]	96.0
FiC	10.56 ± 0.04 [a]	10.15 ± 0.13 [b]	96.1
PoP + ShC	10.78 ± 0.20 [a]	10.48 ± 0.12 [b]	97.2
PoP + FiC	10.78 ± 0.20 [a]	10.42 ± 0.16 [b]	96.6
PaP + ShC	10.78 ± 0.20 [a]	10.38 ± 0.20 [b]	96.2
PaP +FiC	10.78 ± 0.20 [a]	10.17 ± 0.15 [b]	94.3

Pomelo pectin (PoP); passion fruit pectin (PaP); shrimp shell chitosan (ShC); fish scale chitosan (FiC); pomelo pectin and shrimp shell chitosan (PoP + ShC); pomelo pectin and fish scale chitosan (PoP + FiC); passion fruit pectin and shrimp shell chitosan (PaP + ShC); passion fruit pectin and fish scale chitosan (PaP + FiC);
Values (mean ± SD) followed by the same lower case letter on the same line. The compares the same treatment to different status. One-way ANOVA and Duncan's test were performed.

Table 3. Viability of free cells and cells encapsulated with pomelo and passion fruit pectin of *Ped. pentosaceus* RSU-Nh1 for simulated gastric and intestinal juices

LAB species	Simulation time (min)	Free cell number (log CFU/ml)	Encapsulated cell number (log CFU/ml)	
			PoP	PaP
Ped. pentosaceus RSU-Nh1	0	10.52 ± 0.16 [Aa]	10.35 ± 0.11 [Aa]	10.24 ± 0.24 [Aa]
	60	8.22 ± 0.08 [Ab]	8.41 ± 0.25 [Ab]	8.47 ± 0.13 [Ab]
	120	8.09 ± 0.08 [Ab]	7.46 ± 0.15 [Bc]	8.29 ± 0.06 [Ab]
	240	7.68 ± 0.23 [Bc]	7.35 ± 0.11 [Cc]	8.12 ± 0.11 [Ac]

PoP = pomelo pectin; PaP = passion fruit pectin.
Values (mean ± SD) followed by the same capital letters on the same line and the same lower case in the same column. The first compares the same time to different treatment. The second compare the same treatment to different time. One-way ANOVA and Duncan's test were performed.

Gastrointestinal simulation

Survival of encapsulated cells with pectin

Ped. pentosaceus RSU-Nh1 encapsulated with various pectin-sodium alginate treatments was investigated and compared with free cells. Results indicated that initial cells of three treatments consisting of free cells, PoP and PaP were about 10 log CFU/ml. After 60 min, the viability of all treatments decreased to 8 log CFU/ml with the PoP treatment showing a constant decline to 7 log CFU/ml at 120 min, while the others exhibited stable numbers. The treatment using PaP showed the highest viable cell number (8 log CFU/ml) at 240 min, whereas the cell count of free cells and PoP which survived was 7 log CFU/ml as shown in Table 3.

Survival of chitosan-microencapsulated cell viability

Enumeration of *Ped. pentosaceus* RSU-Nh1 microencapsulated chitosan (roughly 10 log CFU/ml) was carried out before immersion in simulated gastric and intestinal juices. After 60 and 120 min, decreasing viability of cell numbers was established in all treatments. The survival rate of microencapsulated cells coated with shrimp shell and fish scale chitosan were the highest at 8.59 ± 0.11 and 8.08 ± 0.07 log CFU/ml, respectively and is significantly different when compared with the treatment using free cells (Table 4).

Table 4. Viability of free cells and cells encapsulated with chitosan coated *Ped. pentosaceus* RSU-Nh1 for simulated gastric and intestinal juices

LAB species	Simulation time (min)	Free cell number (log CFU/ml)	Chitosan-encapsulated cell number (log CFU/ml)	
			ShC	FiC
Ped. pentosaceus RSU-Nh1	0	10.52 ± 0.16 ^{Aa}	9.87 ± 0.23 ^{Ba}	10.15 ± 0.13 ^{Ba}
	60	8.22 ± 0.08 ^{Bb}	9.35 ± 0.11 ^{Ab}	9.24 ± 0.06 ^{Ab}
	120	8.09 ± 0.08 ^{Bb}	8.84 ± 0.21 ^{Ac}	8.34 ± 0.12 ^{Ac}
	240	7.68 ± 0.23 ^{Bc}	8.59 ± 0.11 ^{Ac}	8.08 ± 0.07 ^{Ad}

ShC = shrimp shell chitosan; FiC = fish scale chitosan.
Values (mean ± SD) followed by the same capital letters on the same line and the same lower case in the same column. The first compares the same time to different treatment. The second compare the same treatment to different time. One-way ANOVA and Duncan's test were performed.

Survival of encapsulated cells with pectin coated by chitosan

Evaluation of cell viability of *Ped. pentosaceus* RSU-Nh1 encapsulated with pectin-sodium alginate coated with chitosan was determined (Table 5). Survival cell numbers of the three treatments (PoP + ShC, PoP + FiC and PaP + FiC) decreased from 10 to 8 log CFU/ml, while the free cell treatment displayed cell decrease from 10 to 7 log CFU/ml at 0 and 240 min, respectively. The treatment of PaP + ShC showed the highest cell numbers that had survived in simulated gastric and intestinal juices at 9.24 ± 0.06 log CFU/ml. Results of analysis of variance showed different significance levels between the various groups at 240 min simulation time.

Table 5. Viability of free cells and cells encapsulated with pectin and chitosan and coated with *Ped. pentosaceus* RSU-Nh1 for simulated gastric and intestinal juices

Simulation time (min)	Free cell number (log CFU/ml)	Encapsulated cell number (log CFU/ml)			
		PoP		PaP	
		ShC	FiC	ShC	FiC
0	10.52 ± 0.16^{Aa}	10.48 ± 0.12^{Aa}	10.43 ± 0.16^{Aa}	10.38 ± 0.20^{Aa}	10.17 ± 0.15^{Ba}
60	8.22 ± 0.08^{Bb}	8.49 ± 0.10^{Bb}	8.75 ± 0.26^{Bb}	9.57 ± 0.14^{Ab}	9.29 ± 0.26^{Ab}
120	8.09 ± 0.08^{Cb}	8.26 ± 0.05^{Cc}	8.31 ± 0.27^{Cc}	9.34 ± 0.12^{Ab}	8.81 ± 0.21^{Bc}
240	7.68 ± 0.23^{Dc}	8.04 ± 0.04^{Cd}	8.23 ± 0.20^{Cc}	9.24 ± 0.06^{Ac}	8.52 ± 0.07^{Bc}

PoP = pomelo pectin; PaP = passion fruit pectin; ShC = shrimp shell chitosan; FiC = fish scale chitosan.

Values (mean ± SD) followed by the same capital letters on the same line and the same lower case in the same column. The first compares the same time to different treatment. The second compare the same treatment to different time. One-way ANOVA and Duncan's test were performed.

Results indicated that microencapsulation of passion fruit pectin coated by shrimp shell chitosan might be suitable for probiotics to increase cell viability through the gastrointestinal tract.

Discussion

Encapsulation is a process for preserving probiotic viability by entrapment within a coating using hydrocolloidal materials. This method can protect cells from harsh conditions i.e. bile salts, high acidity and low pH (Călinoiu *et al.*, 2019; Iravani *et al.*, 2015). The encapsulated material is usually called the active, internal, core, fill, or payload phase, while the material used for encapsulation is called the coating membrane, capsule, shell, matrix, carrier

material or external phase (Kavitake *et al.*, 2018). Encapsulation of internal substances by a coating membrane can be achieved by various methods such as freeze drying (Carvalho *et al.*, 2002), spray drying (O'Riordan *et al.*, 2001), emulsion technique (Annan *et al.*, 2008), extrusion technique (Reid *et al.*, 2007) and by coating as microencapsulation (Krasaekoopt *et al.*, 2004).

Encapsulation is used for cell protection, especially lactic acid bacteria (LAB) or probiotics, during passage through the gastrointestinal tract (Totosaus *et al.*, 2013). Lactic acid bacteria such as *L. plantarum* (Carvalho *et al.*, 2002), *Bifidobacterium adolescentis* (Annan *et al.*, 2008), *Lactobacillus acidophilus* (Gebara *et al.*, 2013) and *Ped. pentosaceus* (Yao *et al.*, 2018) were reported using different coating materials including starch (Sultana *et al.*, 2000), gelatin (Annan *et al.*, 2008), gum arabic (Dikit *et al.*, 2015), pectin (Sandoval-Castilla *et al.*, 2010) and chitosan (Huang *et al.*, 2015).

The extrusion technique is the most popular to preserve probiotics because it is simple with low cost, and the gentle formulation conditions ensure high cell viability (Mart ń *et al.*, 2015). In 2017, Poornima and Sinthya used an extrusion method consisting of dropping droplets of an aqueous solution of polymer (0.6-3% sodium alginate) into a bath (0.05-1.5 M calcium chloride solution) using a pipette or syringe. Encapsulation with sodium alginate by the extrusion method was also reported by Sandoval-Castilla *et al.* (2010). Alginate is a linear heteropolysaccharide extracted from various algal species. This material is cheap, simple to use, biocompatible and can be formed using the extrusion technique.

Chitosan is a cationic polysaccharide composed of glucosamine and N-acetylglucosamine as a result of the deacetylation of chitin (Benhabiles *et al.*, 2012). It is widely used for coating bacteria cells to protect them from severe conditions. Chitosan coating was reported as better for protection in simulated gastric conditions than poly-l-lysine (Krasaekoopt *et al.*, 2004) and pea protein (Varankovich *et al.*, 2017). Moreover, chitosan can be extracted from waste such as fish scales that are plentiful in Thailand (Tungse *et al*., 2016). Research by de Queiroz Antonino *et al.* (2017) reported that chitosan was extracted from shrimp (*Litopenaeus vannamei* Boone) shells by demineralization with 1 M HCl, deproteinized with 1 M NaOH and deacetylated with 12.5 M NaOH to prepare white chitosan powder. Studies have also been carried out on chitosan extracted from *Metapenaeus monoceros* using 30% HCl and a concentration of 1.5 N NaOH for white chitosan powder preparation. If the concentration of acid and alkali or the time is inadequate for the demineralization, deproteinization, or deacetylization steps to prepare chitosan extraction, the powder may be a brownish color (Naznin, 2005). In this study, the shrimp shell chitosan was characterized as a white to light orange powder, while the color of fish scale

chitosan powder was white. Yields of chitosan extracted from shrimp shells and fish scales were 75%, similar to results of Oduor-Odote *et al*. (2005) who reported yield of chitosan from crustacean shell extraction consisting of crab (*Scylla serrata*), lobster (*Panulirus ornatus*) and prawn (*Penaeus indicus*) at 75.1%, 74.6% and 74.3%, respectively. Demir *et al.* (2016) and Kaya *et al.* (2016) reported yields of chitosan extracted from blue crab (*Callinectes sapidus*) shells at 77.78% and 76.03%, respectively. Many studies exhibited that LAB encapsulation in a chitosan-alginate complex is efficient in reducing the decline of viable cells in simulated gastro-intestinal conditions and also improves their survival during refrigerated storage (Chávarri *et al.*, 2010; Huang *et al.*, 2015). Encapsulation of *Lactobacillus reuteri* coated by commercial chitosan (coating agent) was reported by Huang *et al.* (2015). A concentration of 2% sodium alginate was dropped into 0.1 M $CaCl_2$ and coated by 0.4% chitosan. The gel beads were digested under high acidity in the stomach for 3 h. Results displayed that the survival rate of encapsulated *L. reuteri* was 3.37 log CFU/ml while survival of free cells was lower than 1 log CFU/ml. Therefore, encapsulation using chitosan-Ca-alginate gel beads improved the survival of *L. reuteri* in the digestive system, reduced injury to the cell membrane and also preserved adhesiveness and pathogen antagonism. Pectin is also widely used as a coating material. Pectin is a natural complex polysaccharide which comprises a functionally significant moiety of the primary cell walls of plants. Pectin is a carbohydrate found in all fruits and can be extracted from peels or pomace (Sundarraj and Ranganatha, 2017). Odun-Ayo *et al.* (2017) studied the encapsulation of *L. acidophilus* ATCC 4356 using citrus pectin (commercial) and found a significant increase in numbers of bacteria in the beads at day 7 and 14 (7.99 and 8.32 log CFU/g). Therefore, this pectin supplement maintained and improved the integrity and population of intestinal bacteria.

Combinations of probiotic bacteria and pectin were tested by Sen *et al.* (2014). They found that pectins were prebiotics for probiotic bacteria but not against the acidic pH in the stomach. Furthermore, research examined the encapsulation of pectin-alginate using 2% commercial pectin with 2% $CaCl_2$ for *L. acidophilus* la5 in SGJ for 60 and 120 min and then moved to intestinal juices up to 300 min. Results showed that numbers of free cells decreased from 8.48 to 7.77 log CFU/ml. Pectin microparticles also decreased from 6.96 to 6.28 log CFU/g in SGJ at pH 3 (Gebara *et al.*, 2013). However, Bepeyeva *et al.* (2017) reported that *L. casei*, encapsulated using 1%-3% pectin and 0.4% chitosan, survived in gastric acid and also displayed high numbers of viable bacteria released in the intestine at 9.6 log CFU/ml, whereas the residue of free cells was less than 4 log CFU/ml. Therefore, encapsulation of LAB cells using

pectin-chitosan was effective and protected the cells in a highly acidic environment. Probiotic bacteria provide beneficial effects to the host when administrated in amounts of more than 10^6 CFU/ml (Lee *et al.*, 2009; Possemiers *et al.*, 2010; Aureli *et al.*, 2011; Pimentel *et al.*, 2019). Food containing probiotic bacteria should be in the range of 10^8-10^9 CFU/ml before ingestion to ensure that an adequate minimum of 10^6-10^7 CFU/ml reach the colon (Nazzaro *et al.*, 2009).

In conclusion, this study investigated the protective effects of alginate-pectin with chitosan coating encapsulation of *Ped. pentosaceus* RSU-Nh1 for survival of cells in simulated digestion using the extrusion method. Results found that the cell survival of *Ped. pentosaceus* RSU-Nh1 encapsulated using passion fruit was about 10^8 CFU/ml, while free cells could survive at about 10^7 CFU/ml. The number of survived cells in encapsulated treatment using chitosan from shrimp shells and fish scales were similar. Encapsulation using passion fruit pectin coated with shrimp shell chitosan showed the highest survival with cell count of 9.24 log CFU/ml. Therefore, encapsulation of *Ped. pentosaceus* RSU-Nh1 with alginate-pectin coated with chitosan can protect bacteria under harsh gastro-intestinal conditions.

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References

Annan, N. T., Borza, A. D. and Hansen, L. T. (2008). Encapsulation in alginate-coated gelatin microspheres improves survival of the probiotic *Bifidobacterium adolescentis* 15703T during exposure to simulated gastro-intestinal conditions. Food Research International, 41:184-193.

Aureli, P., Capurso, L., Castellazzi, A. M., Clerici, M., Giovannini, M., Morelli, L. and Zuccotti, G. V. (2011). Probiotics and health: an evidence-based review. Pharmacological Research, 63:366-376.

Benhabiles, M. S., Salah, R., Lounici, H., Drouiche, N., Goosen, M. F. A. and Mameri, N. (2012). Antibacterial activity of chitin, chitosan and its oligomers prepared from shrimp shell waste. Food Hydrocolloids, 29:48-56.

Bepeyeva, A., de Barros, J. M. S., Albadran, H., Kakimov, A. K., Kakimova, Z. K., Charalampopoulos, D. and Khutoryanskiy, V. V. (2017). Encapsulation of *Lactobacillus casei* into calcium pectinate-chitosan beads for enteric delivery. Journal of Food Science, 82:2954-2959.

Carvalho A. S., Silva, J. Ho. P., Teixeira, P., Malcata1 F. X. and Gibbs, P. (2002). Survival of freeze-dried *Lactobacillus plantarum* and *Lactobacillus rhamnosus* during storage in the presence of protectants. Biotechnology Letters, 24:1587-1591.

Călinoiu, L. F., Ştefănescu, B., Pop, I., Muntean, L. and Vodnar, D. (2019). Chitosan coating applications in probiotic microencapsulation. Molecular Diversity Preservation International, 9:1-21.

Chávarri, M., Marañón, I., Ares, R., Ibáñez, F. C., Marzo, F. and Villarán, M. C. (2010). Microencapsulation of a probiotic and prebiotic in alginate-chitosan capsules improves survival in simulated gastro-intestinal conditions. Internation Journal of Food Microbiology, 142:185-189.

Choonut, A., Saejong, M. and Sangkharak, K. (2014). The production of ethanol and hydrogen from pineapple peel by *Saccharomyces Cerevisiae* and *Enterobacter Aerogenes*. Energy Procedia, 52:242-249.

da Costa, R. J., Voloski, F. L. S., Mondadori, R. G., Duval, E. H. and Fiorentini, Â. M. (2019). Preservation of meat products with bacteriocins produced by lactic acid bacteria isolated from meat. Journal of Food Quality, 2019:1-12.

de Queiroz Antonino, R., Lia Fook, B., de Oliveira Lima, V., de Farias Rached, R., Lima, E., da Silva Lima, R. and Lia Fook, M. (2017). Preparation and characterization of chitosan obtained from shells of shrimp (*Litopenaeus vannamei* Boone). Marine Drugs, 15:141.

Demir, D., Öfkeli, F., Ceylan, S. and Bölgen, N. (2016). Extraction and characterization of chitin and chitosan from blue crab and synthesis of chitosan cryogel scaffolds. Journal of the Turkish Chemical Society, 3:131-144.

Dikit, P., H-Kittikun, A. and Maneerat, S. (2015). Survival of encapsulated potentially probiotic *Lactobacillus plantarum* D6SM3 with bioemulsifier derived from spent yeast in simulated gastrointestinal conditions. Songklanakarin Journal of Science and Technology, 37:425-432.

Farias, T. G. S. d., Ladislau, H. F. L., Stamford, T. C. M., Medeiros, J. A. C., Soares, B. L. M., Stamford Arnaud, T. M. and Stamford, T. L. M. (2019). Viabilities of *Lactobacillus rhamnosus* ASCC 290 and *Lactobacillus casei* ATCC 334 (in free form or encapsulated with calcium alginate-chitosan) in yellow mombin ice cream. Lebensmittel-Wissenschaft and Technologie, 100:391-396.

Fijan, S. (2014). Microorganisms with claimed probiotic properties: an overview of recent literature. International Journal of Environmental Research and Public Health, 11:4745-4767.

Floch, M. N. (2014). Advances in nutrition: probiotics and prebiotics. Gastroenterology and Hepatology, 10:680-681.

Food and Agriculture Organization (FAO). (2001). Evaluation of health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria. FAO Food and Nutrition Paper, Cordoba, Argentina, pp. 1-5.

Food and Agriculture Organization (FAO^2). (2009). Fishery and aquaculture country profiles: the kingdom of Thailand. FAO Fisheries and Aquaculture Department. Retrieved from http://www.fao.org/fishery/facp/THA/en.

Gao, W., Lai, J. C. K. and Leung, S. W. (2012). Functional enhancement of chitosan and nanoparticles in cell culture, tissue engineering, and pharmaceutical applications. Frontiers in Physiology, 3:1-13.

Gebara, C., Chaves, K. S., Ribeiro, M. C. E., Souza, F. N., Grosso, C. R. F. and Gigante, M. L. (2013). Viability of *Lactobacillus acidophilus* La5 in pectin–whey protein microparticles during exposure to simulated gastrointestinal conditions. Food Research International, 51:872-878.

George Kerry, R., Patra, J. K., Gouda, S., Park, Y., Shin, H. S. and Das, G. (2018). Benefaction of probiotics for human health: a review. Journal of Food and Drug Analysis, 26:927-939.

Gómez-Mascaraque, L. G., Lagarón, J. M. and López-Rubio, A. (2015). Electrosprayed gelatin submicroparticles as edible carriers for the encapsulation of polyphenols of interest in functional foods. Food Hydrocolloids, 49:42-52.

Hamed, I., Özogul, F. and Regenstein, J. M. (2016). Industrial applications of crustacean by-products (chitin, chitosan, and chitooligosaccharides): a review. Trends in Food Science and Technology, 48:40-50.

Harish Prashanth, K. V. and Tharanathan, R. N. (2007). Chitin/chitosan: modifications and their unlimited application potential – an overview. Trends in Food Science and Technology, 18:117-131.

Herigstad, B., Hamilton, M. and Heersink, J. (2001). How to optimize the drop plate method for enumerating bacteria. Journal of microbiological methods, 44:121-129.

Huang, H. Y., Tang, Y. J., King, V. A., Chou, J. W. and Tsen, J. H. (2015). Properties of *Lactobacillus reuteri* chitosan-calcium-alginate encapsulation under simulated gastrointestinal conditions. International Microbiology, 18:61-69.

Ibrahim, U. K., Kamarrudin, N., Suzihaque, M. U. H. and Hashib, S. A. (2017). Local fruit wastes as a potential source of natural antioxidant: an overview. Materials Science and Engineering, 206:1-9.

Iravani, S., Korbekandi, H. and Zolfaghari, B. (2015). Phytosynthesis of nanoparticles. Nanotechnology and Plant Sciences, Springer International Publishing, Switzerland, pp. 203-258.

Islam, S., Khan, M. and Alam, A. N. (2017). Production of chitin and chitosan from shrimp shell wastes. Journal of the Bangladesh Agricultural University, 14:253-259.

Kantrong, A. and Eshtiaghi, M. N. (2018). Extraction of phenolic compounds from mango peel using subcritical water technique. Kasem Bundit Engineering Journal, 8:75-86.

Kavitake, D., Kandasamy, S., Devi, P. B. and Shetty, P. H. (2018). Recent developments on encapsulation of lactic acid bacteria as potential starter culture in fermented foods – a review. Food Bioscience, 21:34-44.

Kaya, M., Dudakli, F., Asan-Ozusaglam, M., Cakmak, Y. S., Baran, T., Mentes, A. and Erdogan, S. (2016). Porous and nanofiber α-chitosan obtained from blue crab (*Callinectes sapidus*) tested for antimicrobial and antioxidant activities, Lebensmittel-Wissenschaft and Technologie, 65:1109-1117.

Khandare, S. S. and Patil, S. D. (2016). Encapsulation of lactic acid bacteria in calcium alginate beads for higher bacteriocin production. International Journal of Life Sciences, 4:539-546.

Krasaekoopt, W., Bhandari, B. and Deeth, H. (2004). The influence of coating materials on some properties of alginate beads and survivability of microencapsulated probiotic bacteria. International Dairy Journal, 14:737-743.

Lee, Y. K., Margolles, A., Mayo, B., Ruas-Madiedo, P., Gueimonde, M., de los Reyes-Gavilán, C., Chapman, T. A., Chin, J. J., Crittenden, R., Donohue, D. and Tien Hung Chang, W., (2009). Handbook of probiotics and prebiotics. United States of America, pp. 4-5.

Lotfipour, F., Mirzaeei, S. and Maghsoodi, M. (2012). Evaluation of the effect of $CaCl_2$ and alginate concentrations and hardening time on the characteristics of *Lactobacillus acidophilus* loaded alginate beads using response surface analysis. Advanced Pharmaceutical Bulletin, 2:71-78.

Martín, M. J., Lara-Villoslada, F., Ruiz, M. A. and Morales, M. E. (2015). Microencapsulation of bacteria: A review of different technologies and their impact on the probiotic effects. Innovative Food Science and Emerging Technologies, 27:15-25.

Mart ínez Rivas, C. J., Tarhini, M., Badri, W., Miladi, K., Greige-Gerges, H., Nazari, Q. A. and Elaissari, A. (2017). Nanoprecipitation process: From encapsulation to drug delivery. International Journal of Pharmaceutics, 532:66-81.

Mokoena, M. P. (2017). Lactic acid bacteria and their bacteriocins: classification, biosynthesis and applications against uropathogens: a mini-review. Molecular Diversity Preservation International, 22:1-13.

Naznin, R. (2005). Extraction of chitin and chitosan from shrimp (*Metapenaeus monoceros*) shell by chemical method. Pakistan Journal of Biological Sciences, 8:1041-1054.

Nazzaro, F., Fratianni, F., Coppola, R., Sada, A. and Orlando, P. (2009). Fermentative ability of alginate-prebiotic encapsulated *Lactobacillus acidophilus* and survival under simulated gastrointestinal conditions. Journal of Functional Foods, 1:319-323.

Odun-Ayo, F., Mellem, J. and Reddy, L. (2017). The effect of modified citrus pectin-probiotic on faecal lactobacilli in Balb/c mice. Food Science and Technology, 37:478-482.

Oduor-Odote1, P. M., Struszczyk, M. H. and Peter, M. G. (2005). Characterisation of chitosan from blowfly larvae and some crustacean species from kenyan marine waters prepared under different conditions. Western Indian Ocean Journal of Marine Science, 4:99-107.

O'Riordan, K., Andrews, D., Buckle, K. and Conway, P. (2001). Evaluation of microencapsulation of a *Bifidobacterium* strain with starch as an approach to prolonging viability during storage. Journal of Applied Microbiology, 91:1059-1066.

Peredo, A. G., Beristain, C. I., Pascual, L. A., Azuara, E. and Jimenez, M. (2016). The effect of prebiotics on the viability of encapsulated probiotic bacteria. Lebensmittel-Wissenschaft and Technologie, 73:191-196.

Pimentel, T. C., Klososki, S. J., Rosset, M., Barão, C. E. and Marcolino, V. A. (2019). Fruit juices as probiotic foods. Federal Institute of Paraná, Paranavaí, Brazil, Elsevier, pp. 483-513.

Poornima, K. and Sinthya, R. (2017). Application of various encapsulation techniques in food industries. International Journal of Latest Engineering Research and Applications, 2:37-41.

Possemiers, S., Marzorati, M., Verstraete, W. and Van de Wiele, T. (2010). Bacteria and chocolate: a successful combination for probiotic delivery. International Journal of Food Microbiology, 141:97-103.

Ravi Kumar, M. N. V. (2000). A review of chitin and chitosan applications. Reactive and Functional Polymers, 46:1-27.

Reid, A. A., Champagne, C. P., Gardner, N., Fustier, P. and Vuillemard, J. C. (2007). Survival in food systems of *Lactobacillus rhamnosus* R011 microentrapped in whey protein gel particles. Journal of Food Science, 72:31-37.

Sandoval-Castilla, O., Lobato-Calleros, C., Garc á-Galindo, H. S., Alvarez-Ram írez, J. and Vernon-Carter, E. J. (2010). Textural properties of alginate–so beads and survivability of entrapped *Lactobacillus casei* in simulated gastrointestinal conditions and in yoghurt. Food Research International, 43:111-117.

Sen, A., Manuel, S. G. A. and Kale, R.D. (2014). Fruit waste pectin in enhancing the establishment of probiotic bacteria. Journal of Nutritional Health and Food Engineering, 1:124-126.

Shori, A. B. (2017). Microencapsulation improved probiotics survival during gastric transit. Hayati Journal of Biosciences, 24:1-5.

Silva, C. C. G., Silva, S. P. M. and Ribeiro, S. C. (2018). Application of bacteriocins and protective cultures in dairy food preservation. Frontiers in Microbiology, 594:1-15.

Song, X., Mei, J., Ye, G., Wang, L., Ananth, A., Yu, L. and Qiu, X. (2019). In situ pPy-modification of chitosan porous membrane from mussel shell as a cardiac patch to repair myocardial infarction. Applied Materials Today, 15:87-99.

Suklampoo, L., Thawai, C., Weethong, R., Champathong, W. and Wongwongsee, W. (2012). Antimicrobial activities of crude extracts from pomelo peel of khao-nahm-peung and khao-paen varieties. KMITL Science and Technology Journal, 12:55-61.

Sultana, K., Godward, G., Reynolds, N., Arumugaswamy, R., Peiris, P. and Kailasapathy, K. (2000). Encapsulation of probiotic bacteria with alginate–starch and evaluation of survival in simulated gastrointestinal conditions and in yoghurt. International Journal of Food Microbiology, 62:47-55.

Sundarraj, A. A. and Ranganathan, T. V. (2017). A review - pectin from agro and industrial waste. International Journal of Applied Environmental Sciences, 12:1777-1801.

Suwannarat, Y., Sawasdikarn, J. and Suwannarat, R. (2019). Extraction and application of pectin from durian rind. Rajabhat Rambhai Barni Research Journal, 13:25-37.

Tungse, W., Boonpattaro, D. and Suwanchatri, N. (2016). The study of chitosan from fish scale (*Lates calcarifer* and *Oreochromis niloticus*) and shrimp shell (*Litopenaeus vannamei*). Focus on Education and Culture for Community Development, Songkhla Rajabhat University, pp. 1069-1077.

Totosaus, A., de Jesús Ariza-Ortega, T. and de Lourdes Pérez-Chabela, M. (2013). Lactic acid bacteria microencapsulation in sodium alginate and other gelling hydrocolloids mixtures. Journal of Food and Nutrition Research, 52:107-120.

Varankovich, N., Martinez, M. F., Nickerson, M. T. and Korber, D. R. (2017). Survival of probiotics in pea protein-alginate microcapsules with or without chitosan coating during storage and in a simulated gastrointestinal environment. Food Scicience and Biotechnology, 26:189-194.

Wadhwa, M. M., Bakshi, P. S. and Makkar, H. P. S. (2013). Utilization of fruit and vegetable wastes as livestock feed and as substrates for generation of other value-added products. RAP Publication, Department of Animal Nutrition at GADVASU, Ludhiana, India, pp. 4-5.

Wandrey, C., Bartkowiak, A. and Harding, S. E. (2010). Materials for encapsulation: encapsulation technologies for active food ingredients and food processing. Springer Science and Business Media, LLC, pp. 31-100.

Yao, M., Li, B., Yeab, H., Huangab, W., Luoa, Q., Xiaoc, H., McClementsc, D. J. and Lia, L. (2018). Enhanced viability of probiotics (*Pediococcus pentosaceus* Li05) by encapsulation in microgels doped with inorganic nanoparticles. Lebensmittel-Wissenschaft and Technologie, 83:246-252.

Zhang, W., Xu, P. and Zhang, H. (2015). Pectin in cancer therapy: a review. Trends in Food Science and Technology, 44:258-271.

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