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ENHANCED SURVIVAL OF PROBIOTICS BY ENCAPSULATION WITH PLANT EXTRACTS DURING FOAM-MAT DRYING AND UNDER SIMULATED GASTROINTESTINAL CONDITIONS

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ABSTRACT

Survival of probiotic bacteria, *Pediococcus pentosaceus* ARG-MG12 encapsulated in sodium alginate beads with different plant extracts (onion, soybean, and lotus root) were tested under simulated gastrointestinal conditions compared with free cells. Encapsulated *P. pentosaceus* ARG-MG12 in sodium alginate solution with onion and soybean extracts revealed the highest survival under bile salt. The survival increased proportionately with increasing extracts concentrations. Microencapsulation enhanced acidic survival of all probiotic strains compared to free cells. *P. pentosaceus* ARG-MG12 co-encapsulated with 3% soybean extracts showed the highest survival of 9.98 log CFU/mL ($p \leq 0.05$) after bile salt exposure for 3 hr, while the control including microencapsulated cells without extracts exhibited 8.66 log CFU/mL of survival. Alginate coating of the soybean extracts co-encapsulated probiotic increased survival during foam-mat drying at 70°C to 98.39% compared to that of uncoated which showed 87.55% survival. Co-encapsulated *P. pentosaceus* ARG-MG12 stored in hard gelatin capsule and aluminium foil bag at 8°C for eight weeks showed higher survival than room temperature.

Key words: Foam-mat drying, probiotic, co-encapsulated, plant extracts

INTRODUCTION

Probiotic bacteria (*Pediococcus pentosaceus*) are described as "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host" (FAO/WHO, 2001). The health prosperity outcomes of probiotics such as promoting good digestion, lactose intolerance depletion, serum cholesterol reduction, anti-carcinogenic and anti-tumor activity, lowering of allergic symptoms, immune function invigoration, and diarrhea avoidance, are newly updated by Sanders, *et al*. (2013). Even so, in order to provide the beneficial health significances, the viable probiotic counts in food products that provide as delivery systems desires to be tremendous, suggesting lowest level of living cells should be at least 6-7 log CFU/mL before devouring (Nualkaekul *et al*., 2012). Hence, maintaining of probiotic cells in the functional food products during process and storage is a crucial component.

Microencapsulation has denoted to be a preference protective technique of probiotics from unfavorable surroundings (Ribeiro, *et al*., 2014). Alginate is largely protective of cells material through its economical, integrity, and bio-compatibility (Krasaekoopt *et al*., 2003). Many scientists found that alginate included with other components, occurrence, Thai herbal extracts (Chaikham, 2015), cereal extracts (Michida *et al*., 2006), gelatin (Chaikam *et al*., 2013), chitosan (Nualkaekul *et al*., 2013), pectin, hemicellulose (Chávarri *et al*., 2010), resistant starch (Sultana *et al*., 2000), inulin, galacto-oligosaccharides and fructo-oligosaccharides (Krasaekoopt & Watchara-poka, 2014; Sathyabama *et al*., 2014), can be used to gain the survival of numerous probiotic cells in food and drinks products throughout storage and under an acidic condition along the intestinal tract.

Pediococcus pentosaceus ARG-MG12 was used to be encapsulated in this study. It was able to survive and capable of promptly fermentation process. Accordingly, physico-chemical parameters

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like pH and acidity were found seemly for development of starter culture in both pickled samples that the culture demonstrated to be a suitable starter for pickled production and facilitate the expansion of sensory acceptable pickled mustard (Tanganurat & Chareonchai, 2017). Consequently, it must be emphasized that a particular characterisation of starter strains is key to ensure a meaningful practical performance of a culture, indicating the potential usefulness of this strain for development of starter cultures and controlled fermentation processes.

Foam-mat drying is a procedure in which liquid or gelatinous food is whipped to a durable foam by incorporating a bulky size of air in the existence of a foaming agent and suddenly subsequently dried. Thus, it is vital to supplement foaming agents to promote foaming and improve the strength of the foams, respectively (Sankat & Castaigne, 2014). The foam is then spread onto a tray and dried by various methods to shorten the time. The benefits of this technique are being relatively plain and inexpensive process, accelerated drying time at lower temperatures, the yield powder is able of instant rehydration in cold water, elevated product quality and also less effect on the survival of bacteria (Ratti & Kudra, 2006; Kadam *et al*., 2011; Krasaekoopt & Bhatia, 2012; Tanganurat *et al*., 2015).

Nowadays, there are fewer facts on the influence of alginate merged with plant extracts for improving probiotics stability during foam-mat drying. Besides, this exploration scope was to observe the effect of encapsulation with plant extracts on the survival of probiotic powder, including *P. pentosaceus* in hard gelatin capsule during storage for eight weeks at different conditions.

MATERIALS AND METHODS

Plant extracts preparation

Plants, such as lotus (*Nelumbo nucifera* Gaertn.) root, onion (*Allium cepa* L.), and soybean (*Glycine max* L.), were purchased from the local market in Pathum Thani provinces, Thailand. All plants were dried using a tray dryer (Mammert, Germany) at 55°C for 12 hr before powdering. Following, powder (100 g) were extracted with distilled water (800 mL) by shaking (60 rpm) at 60°C for 2 hr and continuously drained through filter paper (No. 1 Whatman, Spain) then all filtrates were dehydrated using a hot air oven at 65°C for 20-24 hr.

Probiotic culture

Cell pellets of *P. pentosaceus* ARG-MG12 was prepared (Tanganurat *et al*., 2015) and washed twice with sterile phosphate buffered saline (PBS) and gathered by centrifugation at 5,000 rpm for 10 min and then resuspended to serve the concentration of 10 log CFU/mL in the same volume by sterile 0.85% NaCl solution since extrusion and drying process.

Preparation of encapsulated probiotic culture

The encapsulation was derived from Krasaekoopt and Kitsawad (2010). For plant extracts selection, the cell mass were inoculated in 10 mL sterile 0.85% NaCl solution of bacterial culture and were aseptically included 2% w/v sodium alginate, 5% v/v glycerol, 0.1% v/v tween80 and 1% w/v of plant extracts solutions. The suspension with culture was dropped through a needle (0.11 mm) into sterile hardening solution (0.05 M $CaCl_2$) for 30 min, then the encapsulated beads were rinsed by distilled water. The plant extracts that shows the highest viable cell count on the simulated gastric tolerance of encapsulated cells was chosen for the study on contrary concentrations of plant extracts (0, 1, 2 and 3% w/v). Eventually, the most viable cell count from various conditions was chosen for the foam-mat drying experiment.

Cell survival under acidic and intestinal conditions

To examine the tolerance level of co-encapsulated probiotic with plant extracts against stimulated gastric juice and bile salt condition. Artificial gastric acid and bile salt solution were prepared (Charteris *et al*., 1998). Beads were inoculated into *in vitro* acidic at 37°C for 3 hr and dissolved in new MRS media fill out with 0.6% w/v of bile salt (Sigma) at 37°C for 3 hr. The resistance cells were determined after acceptable diluting by pour plate count on MRS agar (Hyronimus *et al*., 2000) and survival efficiency (%) was calculated using (1):

$$\% \text{ Survival efficiency} = (N/N_0) \times 100$$

Where N denoted the number of resistance cells (log CFU/mL) under acidic and intestinal juices, and N_0 denoted the number of free cells (log CFU/mL) in suspension.

Foam-mat drying

Foams were produced by 5 g beads with 100 mL of soymilk mixing sample at 5% egg albumin with high-speed mixer for 15 min (Free cell was acted as control). The foam was poured into a Teflon tray with the foam height of 5 mm, then dehydrated in a hot air oven at 60 and 70°C for 2-3 hr. After drying, the flakes were shredded from the tray and merged into powder (Krasaekoopt & Bhatia, 2012; Tanganurat *et al*., 2015). Subsequently, the powder was kept in the no. 1 gelatin capsule and put into laminate bag for additional analysis.

Viability of probiotic powder in gelatin capsule during storage

During storage (-4, 8°C and room temperature), these powders were evaluated at different time periods (0, 30 and 60 days). The viable count was obtained using the pour plate method after 48 hr at 37°C with MRS agar. The cell survival after drying process was expressed as log CFU/g (Hyronimus *et al*., 2000).

Statistical analysis

All the investigations were performed in triplicate (n=3) and then analyzed using ANOVA. Significantly different averages were split applying Duncan's multiple-range test (DMRT) and t-test. The statistical significance tests were expressed at 95% significance interval ($p \leq 0.05$).

RESULTS AND DISCUSSION

Viability of co-encapsulated cell under simulated gastric and intestinal environments

Plant extracts including lotus root, onion and soybean mostly contain prebiotic as potential antioxidant activities of bioactive components (Al-Sheraji *et al*., 2013). Onions and soybean are prosperous in inulin and FOS as called prebiotic. From previously research, lotus root is also sources of carbohydrates constituting potential prebiotics that find out the internal flora and osmotic shifts modulation in the gastric acid conditions are mechanisms of the prebiotic effect that determined to protect and improve the probiotic survival and activity at the storage interval (Gandomi *et al*., 2016). In order to provide health-promoting, probiotics need survive to harsh acid gastric and carry on at high levels in the intestinal conditions (cell concentration between 6 and 8 log CFU/mL or g of intestinal transit) (Marteau & Rambaud, 1993). Gastrointestinal survival efficiency (%) of viable free and encapsulated *P. pentosaceus* (without extracts) was shown in Figure 1. The encapsulated cells had greater gastrointestinal transits survival efficiency compared with free cells. Similar termination was reported by Gandomi *et al*. (2016) which exhibited a protective response of alginate on encapsulated probiotic bacteria survival under exposure to gastric acid and intestinal fluids. This work suggested that the viability of encapsulated probiotic strain with and without plant extracts during stimulated gastrointestinal tracts conditions was observed (Figure 2). The results noticeably displayed that soybean extract could extend the numbers of survival cells of *P. pentosaceus* ARG-MG12 through others comparison ($p \leq 0.05$). The result shows that lotus root extracts had no significance in probiotic survival improvement.

Figure 2 demonstrates the action of plant extracts on the encapsulated tolerance cells within extracts during 180 min culture while under *in vitro* gastric acid and intestinal conditions. Co-encapsulated cells indicated higher tolerance than the encapsulated cells without extracts cultured under artificial gastrointestinal environments. The inclusion of soybean extract was more efficient than lotus root and onion extracts in enhancing the gastric tolerance of *P. pentosaceus*. Hence, different

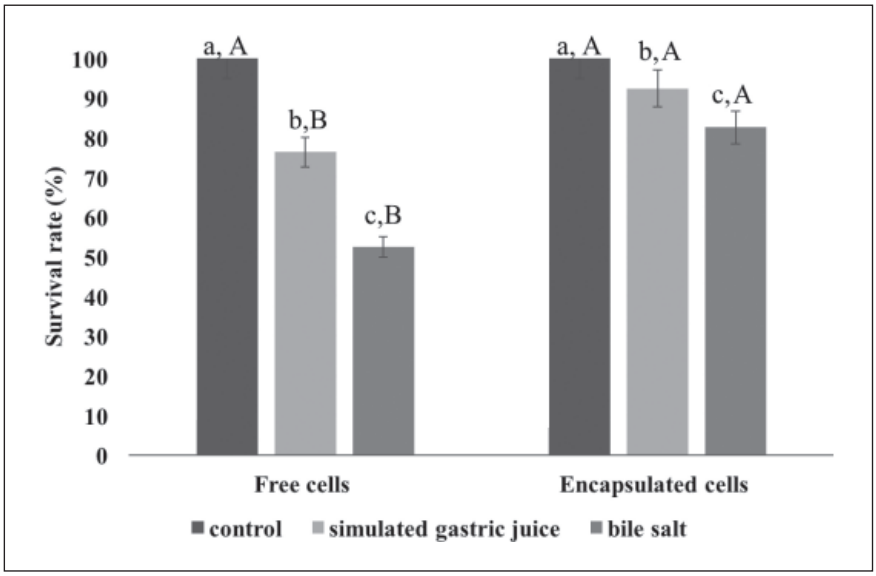

Fig. 1. Gastrointestinal survival rate (%) of free and encapsulated *P. pentosaceus* (without extracts). Different lowercase letters represent statistical difference observed on the same column group ($p \leq 0.05$), whereas different uppercase letters represent statistical differences observed during the gastrointestinal transition ($p \leq 0.05$).

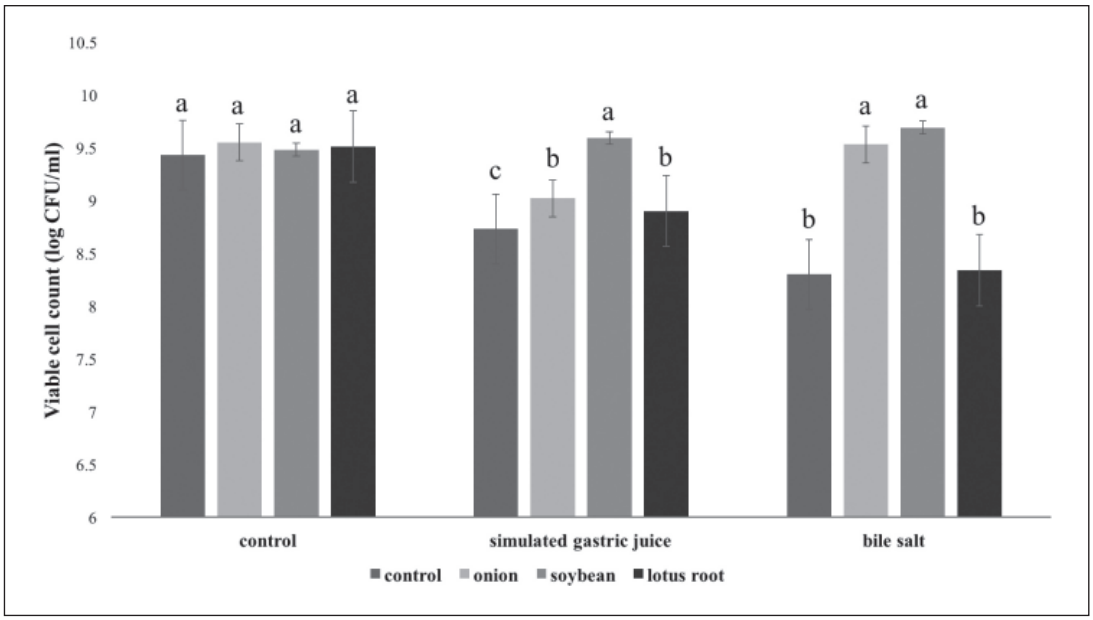

Fig. 2. Viability of *P. pentosaceus* immobilized within plant extracts during 180 min incubation in the presence of simulated gastric juice with and without extracts. Values with the same letters in the same column group are not significantly different ($p > 0.05$).

concentrations (0, 1, 2, and 3% w/v) of soybean extract were investigated. The stability of cells immobilized under gastrointestinal fluids was raised when the presence of 3% soybean extracts ($p \leq 0.05$) as shown in Figure 3.

The viability of cells was 8.66, 8.72, 9.20 and 9.98 log CFU/g with 0, 1, 2 and 3% soybean extract, respectively. Moreover, the cells' tolerance with 3% soybean extract was greater than that co-encapsulated cells with 1 and 2% extract. It was certainly found that the amounts of the viable cell raised with promoting the extract levels, notably soybean extract revealed a beneficial effect on this strain. Agreeing findings were acquired with micro-encapsulated cells during 180 min incubation in gastrointestinal tract conditions (Michida *et al*., 2006). The addition of 3% extract composed a better counteraction effect in the co-encapsulated cells than encapsulated cells without extract. Generally, this can describe that extract concentration is a relevant factor that affects the stability of probiotic cells. The corresponding result was reported, which indicated the protective effect of inulin on encapsulated probiotic survival; meanwhile, exposure to gastrointestinal tracts (Nazzaro *et al*., 2009). This concentration of soybean extract was preferred to study the stability of *P. pentosaceus* ARG-MG12 after foam-mat drying experiment.

Presently, these observations revealed that the stability of probiotics encapsulated with soybean extract can be improved. Prebiotics may support as an alternative to probiotics for longevity stability while the functional food and beverages shelf-life, and also processing resistance that indicates an effective impact on the consistency of outputs shall encourage prebiotics as a trial to probiotics. Moreover, tolerance cells to acidic and intestine fluid may be considered as other favorable properties of prebiotics cause an alleviation of intestinal environments and prolong the osmotic retention of water in the intestines (Crittenden & Playne, 2009; Sivieri *et al*., 2014).

Viability of encapsulated cell during foam-mat drying

Co-encapsulated probiotic cell foam was produced by using 5% egg albumin then dried in an oven at 60, 65 and 70°C. The survival of probiotic was enumerated compared among before and after foam-mat drying process, as shown in Figure 4. It was noticed that the number of probiotic of these products was decreased when high temperature was used. The co-encapsulated probiotic powder as high as 7.92 log CFU/g (dry basis) after drying process, which was higher than the free cell had reduced less than 1.0 log cycles indicating less effect of foam-mat drying on the survival of these bacteria (Krasaekoopt & Bhatia, 2012; Tanganurat *et al*., 2015). Although, the number of free cell and survival rate in foam-mat drying powder at 65 and 70°C was significantly lower than 60°C whereas the percentage of co-encapsulated cell powder survivability had no significantly among before and after drying ($p > 0.05$).

The initial cell suspension of individual lactic acid bacteria contained 8 log CFU/mL which 1 log

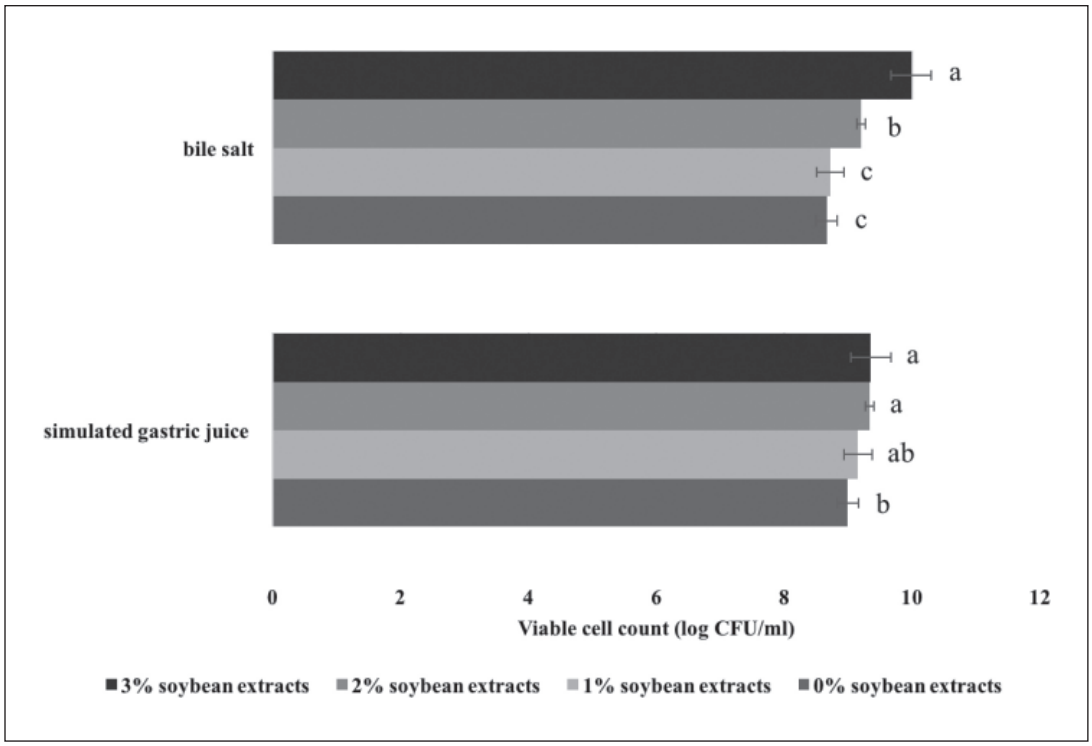

Fig. 3. Tolerance of *P. pentosaceus* immobilized within soybean extract in the presence of simulated bile juice and effect of extract concentrations on viability at 0, 1, 2 and 3%. Values with the same letters in the same row group are not significantly different ($p > 0.05$).

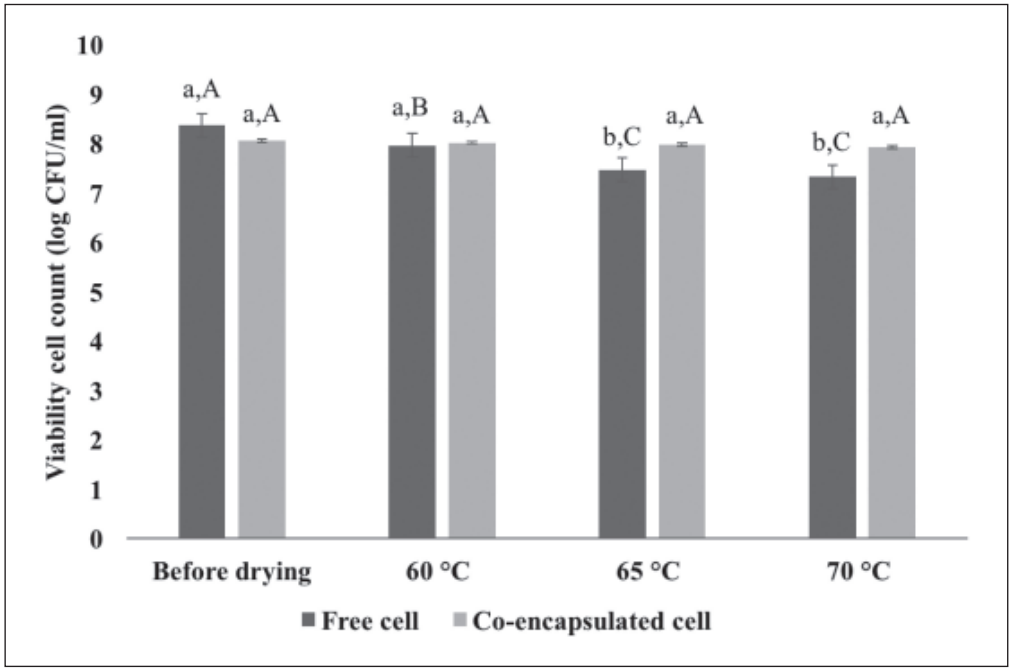

Fig. 4. Viability of *P. pentosaceus* immobilized within soybean extract during foam-mat drying and effect of drying temperature on viability at 60, 65 and 70°C. Different lowercase letters represent statistical difference observed on the same column group ($p \leq 0.05$), whereas different uppercase letters represent statistical differences observed during the drying period ($p \leq 0.05$).

CFU/mL cell loss (80-90% viability), immediately after the foam-mat drying process that exhibits less effect on probiotic population. On the other hand, higher cell loss after spray drying has been noted by Anekella and Orsat (2013) who proclaimed that the survival of probiotic cells in raspberry juice powder from spray drying method, decreased from 9.5 to 5 log CFU/mL when the inlet temperature elevated from 100 to 130°C (outlet temperature of 67-97°C). Furthermore, outlet temperatures greater than 85-90°C are injurious for probiotic that pass on cell membrane damage and affect functional properties (Lapsiri *et al*., 2012), next, the probiotic functionality and survival ability was lost (Ananta *et al*., 2005). The diminish viability obtained in the spray drying method can be characteristic of the outlet temperature remarked due to after drying the powder remains threatened to the high temperature until they are recovered (Alves *et al*., 2016). In this research, the foam-mat dried powder derived in higher cell viability after drying than the spray dried ones that represent the greater probiotic stability was attained in the foam-mat drying can be assigned to the below temperature used (60-70°C).

Cell survival during storage conditions

Survivors of *P. pentosaceus* powder in gelatin capsule were assessed after stored at -4, 8°C and ambient temperature (35°C) for eight weeks in sealed aluminium foil bags without vacuum. As shown in Figure 5 elucidated that the cell viability dropped ($p \leq 0.05$) with the rise of storage period. The different storage temperatures during 60 days storage are indicated the viable cell reduced through the 60 days of storage time. The most reduction (20%) was noticed within 60 days at room temperature and the decline rate was slower while stored at -4 and 8°C. Furthermore, the viable cell counts of probiotic powders decreased throughout the storage time that depletion was no significant compared to the free cell powders ($p > 0.05$).

Even though the incorporation of soybean extracts improved the survival of encapsulated cells during simulated gastrointestinal tracts, no further preservative action distinguished on probiotic survival ability via the storage period. The reduction of *P. pentosaceus* counts expanded during the storage time, so that agreement with previous studies that mention the viable probiotic

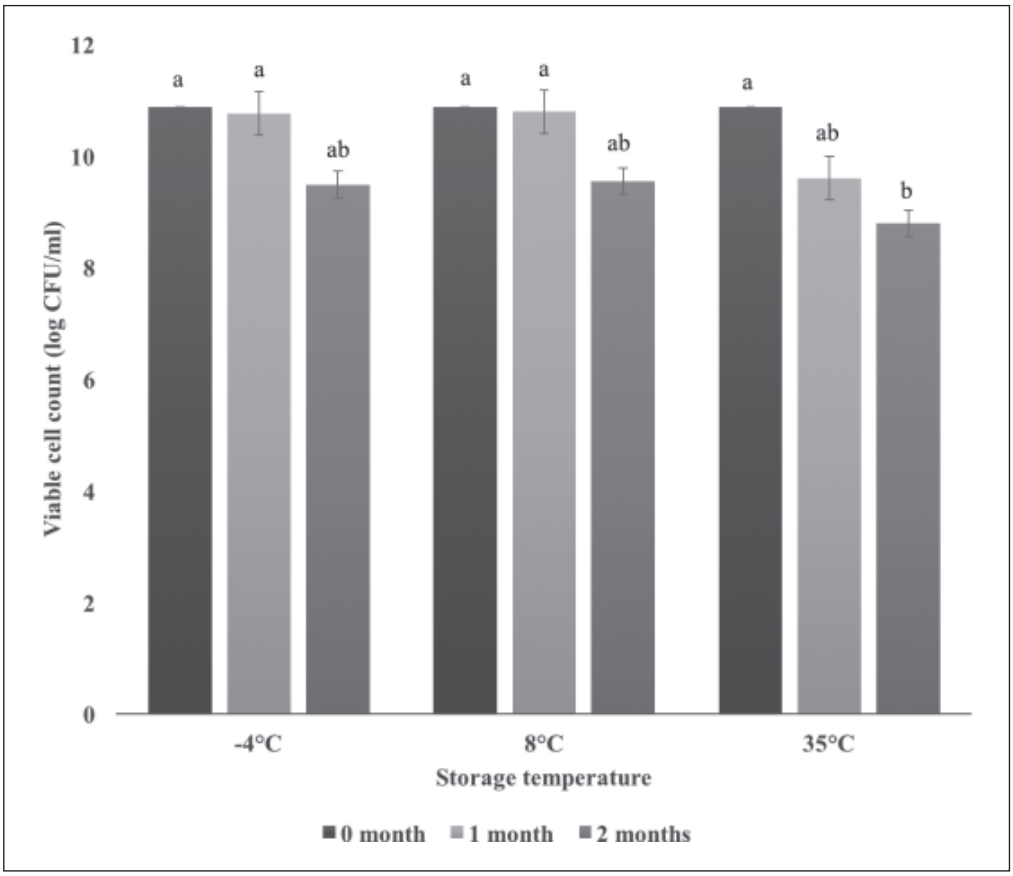

Fig. 5. Viability of *P. pentosaceus* encapsulated within soybean extract during storage at -4, 8 and room temperature (35°C). Values with the same letters in the same column group are not significantly different ($p > 0.05$).

cultures in dried powders reduced at the terminal of the storage (Nale *et al*., 2018; Tontul *et al*., 2018).

To sum up, the incorporation of alginate with plant extracts explained that encapsulated cells including soybean extracts improved ($p \leq 0.05$) the viability of *P. pentosaceus* under simulated gastrointestinal tract conditions during 180 min, as compared to control (without extracts). Furthermore, co-encapsulated bacteria revealed higher survival cell under gastrointestinal tracts compared to others. Soybean extract reinforcement enhanced the survival ability of co-encapsulated probiotic strain when foam-mat drying. Probiotic powder revealed a minimum level of living cells before consumption through 60 days of storage in the gelatin capsule. Mainly, the results of this study exhibited that the plant extracts may be used for transmitting probiotic bacteria to humans.

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