Shehata *et al. J Pure Appl Microbiol,* **13**(**1**), 609-617 | March 2019
Article 5425 | https://dx.doi.org/10.22207/JPAM.13.1.70

Print ISSN: 0973-7510; E-ISSN: 2581-690X

RESEARCH ARTICLE

Plant Extracts in Probiotic Encapsulation: Evaluation of their Effects on Strains Survivability in Juice and Drinkable Yogurt During Storage and an *in-vitro* Gastrointestinal Model

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Abstract

The present study concerned with the evaluation of the adding value from the addition of plant extracts, including those from moringa, fennel, sage and green tea, during alginate encapsulation on the viability of probiotic bacteria (*L. plantarum* DSM 20205 and *P. acidilactici* DSM 20238) in fruit juice (i.e., kiwi, prickly pear and carrot juice) and drinkable yoghurt throughout storage at 4°C. The results revealed that the survival rates of *L. plantarum* DSM 20205 and *P. acidilactici* DSM 20238 cells encapsulated with 0.05% (w/v) moringa extract were significantly higher than those of cells encapsulated with fennel and saga after storage for 30 days. The *In vitro* digestibility behaviour and survival of the novel capsules were studied in terms of the survival of *L. plantarum* DSM 20205 and *P. acidilactici* DSM 20238 based on sequential exposure to simulated salivary, gastric and intestinal fluids. This novel encapsulation additive significantly increased the survival of *L. plantarum* DSM 20205 and *P. acidilactici* DSM 20238 compared with the control capsules cells in simulated digestive fluids. Therefore, the appropriate amount of moringa extract for use in culture encapsulation was determined after the addition to fruit juices and drinkable yoghurt, and the effect of this extract was compared with the effect of adding green tea extract (a standard plant extract). Green tea and moringa extracts enhanced the stability of probiotic beads in all products compared to the controls after storage. Encapsulated *L. plantarum* DSM 20205 and *P. acidilactici* DSM 20238 showed better survivabilities than the control capsules. The studied strains showed better survival in prickly pear juice and drinkable yoghurt throughout storage at 4°C for 30 days.

Keywords: Probiotic, Encapsulation, Plant extracts, Gastrointestinal model, Moringa.

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(Received: 03 January 2019; accepted: 11 February 2019)

Citation: Mohamed G. Shehata, Hagar S. Abd-Rabou and Sobhy A. El-Sohaimy, APlant Extracts in Probiotic Encapsulation: Evaluation of their Effects on Strain Survivability in Juice and Drinkable Yoghurtduring Storage and an *In vitro* Gastrointestinal Model, *J Pure Appl Microbiol*., 2019; **13**(**1**):609-617 doi: 10.22207/JPAM.13.1.70

INTRODUCTION

The increasing number of innovative foods that promote the consumers health has been taken a priority in the field of food industry over the last decades. The very wide acceptance of functional food products due to their health benefits[1]. According to FAO/WHO, 2001[2], "probiotics arel ive microorganisms that when administered in sufficient amounts gives the host a health benefit". In the early 1900s Elie Metchnikoff connected the longevity of Bulgarian peasants with their high consumption of fermented milk and the probiotics term has been used from this time. This is due to the bacteria present in yogurt which protect the gastrointestinal tract against the damaging effects of harmful bacteria[3]. In this study, survey have been done on several microorganisms and have revealed the benefits,such as decreasing cholesterol levels, reducing lactose intolerance, stimulating of the immune system, increasing mineral absorption, and relieving constipation as well as anti-hypertensive, anti-mutagenic and anti-carcinogenic effects, available to humans through the use of probiotics[4,5,6]. The impact of probiotics on human health has been a great advantage for the food industry because these microorganisms describe a significant division within the functional food industry[7].

The demand for functional foods has grown considerably in recent years, now accounting for 5% of the international food market[3]. This increase is correlated with consumer attention, as these products are a source of nutrients and act as a promoters of wellness and health[8,9]. Fermented milks and yogurts are the most famous food vehicles for the delivery of probiotics due to their high acceptance by consumers and superior nutritional value[10,11].

The demand for functional foods has grown considerably in recent years, now accounting for 5% of the international food market[3]. This increase is correlated with consumer attention, as these products are not only as a source of nutrients but also as promoters of wellness and health[8,9]. Fermented milks and yogurts are the most famous food vehicles for the delivery of probiotics due to their high acceptance by consumers and superior nutritional value[10,11].

Encapsulation has been shown to be an alternative method for the safeguarding of probiotics from detrimental environmental factors[12]. Sodium alginate iscommonly used for this purpose because of its simplicity, low cost and biocompatibility[13]. Many researchers have reported that alginate combined with other materials, for example, Hi-maize starch[14], inulin, galactooligosaccharides and fluctooligosaccharides[15,16], gelatine[12], chitosan, pectin, and glucomannan[17,18], can be used to improve the survival of different probiotic strains under gastrointestinal environments and in food products during storage.

Currently, the influence of alginate blended with plant extracts on the survival of probiotics in different beverages and foods is not well understood. There are many evidence in the literature regarding, the potential of some antioxidant extract from natural plants containing high contents of phenolic and flavonoids compounds (Maisuthisakul, *et al*.[19], Siriwatanametanon, *et al.*[20], Abdel-Razek, *et al.* [21] (2017), Badr *et al.*[22] and Shehata *et al.* [23]). Consequently, the objective of this investigation was to determine the impact of alginate encapsulation with certain plant extracts on the stability of probiotic bacteria, including *L. plantarum* DSM 20205 and *P. acidilactici* DSM 20238, in fruit juices and drinkable yogurt during storage at 4°C for 28 days.

MATERIALS AND METHODS

Preparation of plant extracts

Herbal plants, such as fennel (*Foeniculum vulgare*), moringa (*Moringa oleifera*), sage (*Salvia officinalis*) and green tea (*Camellia sinensis L.*), were purchased from a market in Alexandria, Egypt, in 2017. All plants were dehydrated in an oven at 55°C for 2 days before powdering. Water extracts were made (1:5) (w/v) then extracts were filtered through Whatman No. 1 filter paper (Whatman,Spain). All filtrates were then lyophilized using a freezedrier (Dura-Dry MP freezedrier FTS System, USA) at -50 °C for 15–20 h.

Probiotic cultures

Probiotic bacteria, including *Lactobacillus plantarum* DSM 20205and *Pediococcus acidilactici* DSM 20238, were purchased from Egypt Microbial Culture Collection (EMCC), Ain shams, Egypt. Cell pellets of *L. plantarum* DSM 20205 and *P. acidilactici* DSM 20238, lyophilized cells were

subculture in autoclaved MRS broth at 37 °C for 20h. Activated cells were centrifuged at 3000 xg for 20 min, washed twice with 0.1% (w/v) autoclaved water peptone. Cells log were adjusted to 1010 cfu/ml prior encapsulation.

Encapsulation of probiotics

Probiotic capsules were produced with some modifications in accordance with the procedure described by Chaikham, *et al*.[12]. Cells were mixed with Sodium alginate sterile solution (Sigma-Aldrich, UK) and plant extracts were added in different concentrations (0.05–0.2%, w/v). Capsules were formed using 0.5 mm sterile needle by injecting the previous mixture in to 0.5 M sterilized calcium chloride solution and then kept for gelation for 30 min. After gelation the beads were washed with sterile saline at 0.85 percent (w / v) and kept at 4 ° C.

Enumeration of immobilized probiotics

Briefly, one gram of probiotic capsules was diluted with 99 ml 0.1M autoclaved phosphate buffer (pH7) (Merck, Germany) and grinded for 10 min, decimal dilutions were done then plating on MRS agar. Plates were anaerobically incubated at 37 °C for 24–72 h.

Viability of probiotic encapsulated with herbal extracts during storage

Ten grams of *L. plantarum* DSM 20205 and P. acidilactici DSM 20238 immobilized with plant extracts were kept in a glass bottle and stored at 4 °C for 30 days. Survival rates were monitored weekly.

Preparation of simulated digestive fluids and *in-vitro* digestion of encapsulated probiotics

Simulated salivary fluid (SSF), simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) were prepared according to the method proposed by Minekus *et al*.[25], and the details of the solutions including the stock solutions are presented in Table 1.

Survival of encapsulated probiotics in fruit juices and yogurt during storage

Assessing the viability of encapsulated probiotics in fruit juices and drinkable yogurt, 10 g ofencapsulated probiotics were inoculated aseptically into 90 ml of pasteurized kiwi, prickly pear and carrot juices or 90g of milk before storing at 4 °C. post inoculation, samples were taken at 0, 7, 14, 21 and 28days in order to quantify viable numbers as CFU/mlon MRS agar[12]. Product pH changes were monitored.

Data analysis

Results presented as standard deviation ± average. Variance analysis (ANOVA) was performed using (SPSS 16. Inc., USA). Duncan's multiple range tests ($P<0.05$) determined the significant differences between the means of treatment groups.

RESULTS AND DISCUSSION

Viability of *L. plantarum* DSM 20205 and *P. acidilactici* DSM 20238 encapsulated with plant extracts during storage

This study is investigating the potential for using plant extracts to increase the stability of probiotic strains during storage at 4°C. Plant extracts, including fennel, moringa, saga and green tea, typically include high quantity of antioxidant components[1,26,27]. In this study, encapsulated probiotic cells and free cells were assessed (Table 2). The results showed that 0.05% moringa extract could noticeably increase the survival rates of *L. plantarum* DSM 20205 and *P. acidilactici* DSM

Table 1. Details of the composition of stock solutions used to prepare simulated digestive fluids. The final volume of each simulated fluid was adjusted to 500 ml with distilled water

Components	Stock Concen. (M)	SSF (pH 7.0) Concen. (mM)	SGF (pH 3.0) Concen. (mM)	SIF (pH 7.0) Concen. (mM)
KCl	0.5	20.1	6.9	6.8
KH_2PO_4	0.5	8.7	0.9	0.8
$NaHCO_3$	1	23.6	25	91
NaCl	2	-	47.2	38.4
$MgCl_2(H_2O)_6$	0.15	0.3	0.12	0.33

SSF = Simulated Salivary Fluid, SGF = Simulated Gastric Fluid, SIF = Simulated Intestinal Fluid.

20238 cells when compared to control and other plant extract treatments (P<0.05). Fennel and saga extracts had the smallest impacts on the survival of probiotic cells compared to the other extracts. Similar findings were obtained by Chavarri et al.[17] for *B. bifidum* and *L. Gasseri* encapsulated with quercetin during storage at 4°C for 28 days. The effects of the extracts of different species of green tea on the survival of *Bifidobacterium animalis* spp. *lactis* LAFTI-B94, *Lactobacillus paracasei* LAFTI-L26 and *Lactobacillus acidophilus* LAFTI-L10were studied by Lopez de Lacey et al.[27]; they concluded thatthe addition of these extracts during encapsulation had a positive effect on the viability of probiotic cells. This positive effectcanbe attributed to the antioxidant activity of these plant extracts, and this activity is important to the viability and stability of probiotic bacteria.

In the present investigation, during refrigerated storage, the addition of 0.05% moringa extract to calcium alginate as the encapsulating material had a positive effect on probiotic cells. To date, it has been reported to improve probiotic stability by encapsulation with moringa extract. Referring to previous studies in this area, Coz-Bolaɒos *et al.*[28] and Wang et al.[29] revealed that *Moringa oleifera* has a high content of antioxidant compounds. Hence, this extract can therefore create an anaerobic environment that promotes probiotic survival due to its oxygen - scouring properties[30]. This finding was confirmed by Shah et al.[31], who found that fruit juices containing antioxidant compounds showed superior probiotic bacteria stability during 6 weeks of storage compared with the control sample, which was consistent with the positive effect of moringa extract on the survival and stability of probiotic strains. In summary, *L. plantarum* DSM 20205 and *P. acidilactici* DSM 20238 encapsulated with 0.05% moringa extract shown the highest survivability throughout storage at 4°C. Consequently, this amount of extract was elected for evaluating the effects of refrigerated storage on novel encapsulated probiotic cells in fruit juices and drinkable yogurt compared to the negative control treatment and treatments with

Table 2. Stability of two probiotic strains encapsulated with and without plant extracts during refrigerated storage

Plant extracts	Concen. (%w/v)	Number of survival cells (CFU/g beads) Day 0	Day 7	Day 14	Day 21	Day 28	Cell loss (log CFUs)
Lactobacillus plantarum DSM 20205							
Control	0	9.69 ±0.04^{a}	8.34 ±0.08^{b}	7.41 ±0.15^{c}	6.25± 0.18^{d}	4.58 ± 0.18^{e}	5.11 ± 0.14^{A}
Green Tea	0.05	9.42 ±0.09^{a}	8.64 ±0.09^{b}	7.93 ±0.13^{c}	6.96 ±0.22^{d}	6.38 ± 0.07^{e}	3.04 ± 0.14^{E}
	0.1	9.33 ±0.11^{a}	8.25 ± 0.2^{b}	7.68 ±0.24^{c}	6.51± 0.08^{d}	5.85 ± 0.14^{e}	3.48 ± 0.12^{D}
Moringa	0.05	9.32 ±0.13^{a}	8.87 ±0.23^{a}	8.06 ±0.28^{b}	7.50 ±0.07^{c}	5.65 ± 0.10^{d}	3.67 ± 0.04^{D}
	0.1	9.58 ± 0.09^{a}	8.56 ± 0.15^{b}	8.04 ± 0.28^{c}	7.36 ± 0.19^{d}	5.75 ± 0.25^{e}	3.83 ± 0.025^{CD}
Fennel	0.05	9.18 ± 0.17^{a}	8.34 ± 0.27^{b}	7.47 ± 0.24^{c}	6.67 ± 0.10^{d}	4.60 ± 0.34^{e}	4.58 ± 0.22^{BC}
	0.1	9.28 ± 0.30^{a}	8.63 ± 0.10^{b}	7.30 ± 0.04^{c}	6.41 ± 0.09^{d}	4.83 ±0.06^{e}	4.45 ± 0.32^{C}
Marmaria	0.05	9.38 ±0.19^{a}	8.34 ± 0.20^{b}	7.94 ± 0.21^{c}	6.64 ± 0.12^{d}	4.76 ±0.1^{e}	4.61 ± 0.18^{BC}
	0.1	9.36 ± 0.12^{a}	8.04 ±0.15^{b}	7.35 ± 0.13^{c}	6.38 ± 0.31^{d}	4.49 ±0.18^{e}	4.87 ± 0.30^{ABC}
Pediococcus acidilactici DSM 20238							
Control	0	9.63 ± 0.12^{a}	8.30 ± 0.12^{b}	7.36 ± 0.03^{c}	6.41 ± 0.18^{d}	4.38 ±0.19^{e}	5.24 ± 0.30^{A}
Green Tea	0.05	9.71 ± 0.06^{a}	8.48 ± 0.06^{b}	8.09 ± 0.11^{c}	7.64 ±0.12^{d}	6.13 ± 0.08^{e}	3.58 ± 0.13^{D}
	0.1	9.47 ± 0.25^{a}	8.52 ± 0.10^{b}	7.43 ± 0.17^{c}	7.48 ± 0.14^{c}	5.73 ± 0.10^{d}	3.73 ± 0.35^{CD}
Moringa	0.05	9.82 ± 0.13^{a}	8.73± 0.21^{b}	8.01± 0.26^{c}	7.36 ± 0.24^{d}	6.24 ± 0.21^{e}	3.22 ± 0.33^{E}
	0.1	9.81 ± 0.08^{a}	8.84± 0.11^{b}	7.78 ± 0.16^{c}	7.28 ± 0.23^{d}	6.02 ± 0.21^{e}	3.79 ± 0.26^{CD}
Fennel	0.05	9.51 ± 0.04^{a}	8.58 ±0.15^{b}	7.65 ± 0.07^{c}	6.57 ± 0.34^{d}	4.86 ± 0.08^{e}	4.65 ± 0.13^{BC}
	0.1	9.28 ± 0.25^{a}	8.34±0.32^{b}	7.36 ± 0.13^{c}	6.43 ± 0.11^{d}	4.81 ± 0.09^{e}	4.46 ± 0.28^{C}
Marmaria	0.05	9.33 ± 0.30^{a}	8.0 ± 0.14^{b}	7.81±0.18^{b}	6.03 ± 0.07^{c}	4.41 ± 0.20^{d}	4.92 ± 0.30^{AB}
	0.1	9.57 ± 0.12^{a}	8.10±0.11^{b}	7.70 ±0.20^{c}	5.77 ± 0.14^{d}	4.74 ± 0.08^{e}	4.83 ± 0.07^{ABC}

Means in the same row or column followed by the same lower case or capital letters respectively are not significantly different (P<0.05). Each data point is the average of three replications.

green tea extract as the positive control.

Survival of herbal extract-encapsulated probiotics and control capsules cells in simulated digestive fluids

Probiotics can survive under acidic stomach conditions and throughout the intestines in suitable numbers between 106 and 108 cfu/g. However, their viability must be conformed early in any study[18,32,33]. The survival or viability of the control capsules cells (CC) contains calcium alginate only and novel capsules (NC) contains herbal extracts of *L. plantarum* DSM 20205 and *P. acidilactici* DSM 20238 in simulated digestive fluids (SSF, SGF, and SIF) are displayed in Figs. 1 & 2.

These s trains showed a steadily loss of viability in simulated digestive fluids, but their sensitivity to SSF, SGF and SIF differed considerably. Dramaticaly reductions in the number of probiotic cells after exposure to simulated mouth fluid (SSF) were observed for both cells control capsules (CC) and novel capsules (NC) *L. plantarum* DSM 20205 and *P. acidilactici* DSM 20238 cells (Fig. 1 and 2), and a significant loss in (CC) viability corresponds to ~ 0.5 log cfu/ml. During the second step, exposure to SGF, which is a two-hour process and includes normal ingested foods also present in the stomach[34], there was a remarkable decrease in both (CC) and (NC) numbers compared to the initial state after the first hour of incubation, but the cell numbers increased in the second hour. Novel capsules (NC) showed significantly better survivability ($p < 0.05$) than was seen with (CC), and the NC counts were 1.3 and 1 log cfu/ml for moringa extract and green tea extract-encapsulated cells), respectively, compared to 1.76 log cfu/ml for (CC). The last step, which is exposure to SIF, was the most important part and the target of this work as this step determines if these beneficial bacteria can reach the intestine in reasonable live numbers to be able to exert their effects. Our novel capsules (NC) successfully improved the survival relative to ordinary (CC). The same results were obtained by Mandal *et al.* (2006) in their study on free *Lactobacillus casei*

Table 3. Survivability of probiotics encapsulated with and without plant extracts in fruit juices during refrigerated storage

Fruit juices	Herbal extracts	Number of survival cells (CFU/ml fruit juices)					Cell loss (log CFUs)
		Day 0	Day 7	Day 14	Day 21	Day 28	
Lactobacillus plantarum DSM 20205							
Kiwi	Control	9.51 ±0.30^{a}	8.44± 0.14^{b}	7.47 ±0.18^{c}	6.23 ±0.06^{d}	4.78 ±0.17^{e}	4.73±0.42^{AB}
juice	Moringa	9.54 ± 0.08^{a}	8.58±0.17^{b}	7.53 ±0.06^{c}	6.43 ±0.15^{d}	5.21 ±0.15^{e}	4.32±0.21^{BCDE}
	Green Tea	9.24 ± 0.11^{a}	8.44±0.17^{b}	7.84±0.06^{c}	6.44±0.17^{d}	5.10± 0.09^{e}	4.13±0.17^{DEF}
Prickly	Control	9.55 ±0.19^{a}	8.44 ±0.06^{b}	7.47 ±0.05^{c}	6.51 ±0.04^{d}	5.26 ± 0.12^{e}	4.29±0.10^{CDE}
pear	Moringa	9.40 ±0.43^{a}	8.67 ±0.07^{b}	7.62 ±0.09^{c}	6.57 ±0.17^{d}	5.47±0.25^{e}	3.93±0.17 ^{DEFG}
juice	Green Tea	9.26 ± 0.17^{a}	8.57 ± 0.21^{b}	7.41 ± 0.08^{c}	6.45 ± 0.12^{d}	5.36 ± 0.09^{e}	3.89±0.10 ^{DEFG}
Carrots	Control	9.58 ± 0.17^{a}	8.13 ± 0.15^{b}	7.10 ± 0.10^{c}	6.13 ± 0.06^{d}	4.97 ± 0.15^{e}	4.61±0.17^{ABC}
juice	Moringa	9.50 ± 0.19^{a}	8.43 ± 0.10^{b}	7.41 ± 0.10^{c}	6.39 ± 0.09^{d}	5.19 ± 0.16^{e}	4.30±0.31^{BCDE}
	Green Tea	9.34 ±0.07^{a}	8.28 ± 0.14^{b}	7.51 ± 0.10^{c}	6.54 ± 0.07^{d}	5.32 ± 0.24^{e}	4.02±0.16^{DEFG}
Pediococcus acidilactici DSM 20238							
Kiwi	Control	9.69±0.13^{a}	8.16±0.15^{b}	7.23±0.15^{c}	6.43±0.12^{d}	4.81±0.19^{e}	4.87±0.29^{A}
juice	Moringa	9.51±0.08^{a}	7.92±0.10^{b}	7.32±0.03^{c}	6.59±0.09^{d}	5.27±0.19^{e}	4.24±0.20^{CDEF}
	Green Tea	9.22±0.07^{a}	8.07±0.11^{b}	7.58±0.06^{c}	6.72±0.11^{d}	5.12±0.08^{e}	4.10±0.16^{DEF}
Prickly	Control	9.46±0.20^{a}	8.12±0.08^{b}	7.08±0.17^{c}	6.50±0.19^{d}	5.33±0.12^{e}	4.13±0.14^{DEF}
pear	Moringa	9.50±0.24^{a}	8.44±0.19^{b}	7.31±0.11^{c}	6.75±0.11^{d}	5.66±0.39^{e}	3.83±0.40^{FG}
juice	Green Tea	9.51±0.27^{a}	8.47±0.22^{b}	7.53±0.08^{c}	6.83±0.11^{d}	5.86±0.15^{e}	3.64±0.26^{G}
Carrots	Control	9.55±0.25^{a}	7.80±0.17^{b}	6.94±0.07^{c}	6.11±0.07^{d}	5.19±0.14^{e}	4.36±0.13^{BCD}
juice	Moringa	9.40±0.17^{a}	8.10±0.10^{b}	7.21±0.11^{c}	6.35±0.10^{d}	5.40±0.16^{e}	4.00±0.26 ^{DEFG}
	Green Tea	9.28±0.25^{a}	8.23±0.11^{b}	7.35±0.12^{c}	6.51±0.07^{d}	5.3±0.10^{e}	3.95±0.16 ^{DEFG}

Means in the same row or column followed by the same lowercase or capital letters respectively are not significantly different ($P<0.05$). Each data point is the average of three replications.

NCDC-29 cells revealed to different concentrations of bile salts; they noticed a decrease in the cell counts from 9.34 to 5.60 log cfu ml^{-1}. These results show that the presence of plant extracts will protect bacterial cells throughout the simulated gastric-intestinal system. This effect may be due to their strong antioxidant activity, which has been confirmed by many previous studies[27]. As mention above, these results strongly support the improvement of the survival of probiotic bacteria in the human digestive system by encapsulating probiotic bacteria with plant extracts.

Survival of probiotics encapsulated with plant extracts in fruit juices and drinkable yogurt during storage at 4°C

The survivals of *L. plantarum* DSM 20205 and *P. acidilactici* DSM 20238 encapsulated with 0.05% moringa extract and green tea extract during storage at 4°C for 30 days were evaluated after aseptically transferring the encapsulated substance into various fruit juices, like kiwi, prickly pear and carrotjuices. The results in Table 3 show that the number of cells of all encapsulated cultures in each fruit juice decreased continuously ($P<0.05$) with increasing storage time. Moreover, all cultures survived better in prickly pear juice than in kiwi and carrotjuices.

Nualkaekul *et al.*[35] showed that *Lactobacillus plantarum* encapsulated with chitosan-coated alginate beads could survive in pomegranate juice throughout storage at 4°C. Chaikham[36] mentioned the effect of alginate encapsulation with Thai herbal extracts, including extracts of yanang, pennywort and cashew flower, on the survival of *Lactobacillus casei 01, Lactobacillus acidophilus LA5* and *Bifidobacterium lactis Bb-12* suspended in melon, longan, maoberry and mulberry juices during storage at 4°C. Similar results were reported by Ding and Shah[33] in apple and orange juices containing encapsulated *Bifidobacterium longum, B. lactis* and *Lactobacillus plantarum* which could survive six weeks of storage at 4°C, whilst the free cells lost their viability during five weeks.

Our results show that encapsulation with 0.05% moringa extract or green tea extract significantly increased the stability of *L. plantarum* DSM 20205 and *P. acidilactici* DSM 20238 compared to control in fruit juices during storage (Table 3). Similar to fruit juices, during storage, the surviving populations of probiotics with plant extracts suspended in drinkable yogurt and

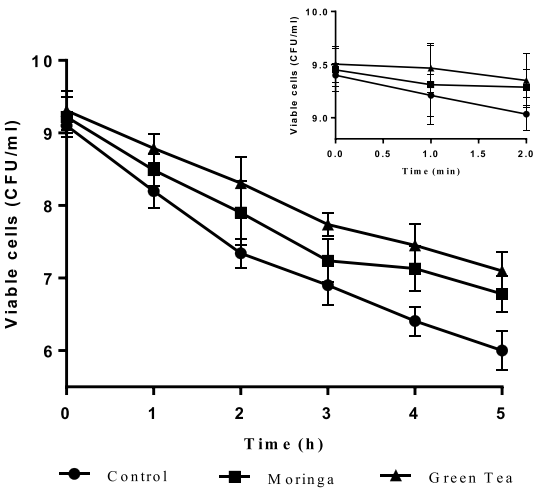

Fig. 1. Survival of control encapsulatedprobioticcells (CC) and novel encapsulatedprobioticcells (NC) with herbalextracts*L. plantarum*DSM 20205upon sequential exposure to simulated salivary fluid (SSF) for 2 min, simulatedgastric fluid (SGF) for 2 h and simulated intestinal fluid (SIF) for 3 h. The insetrepresents the survival of cells when exposed in SSF for 2 min.

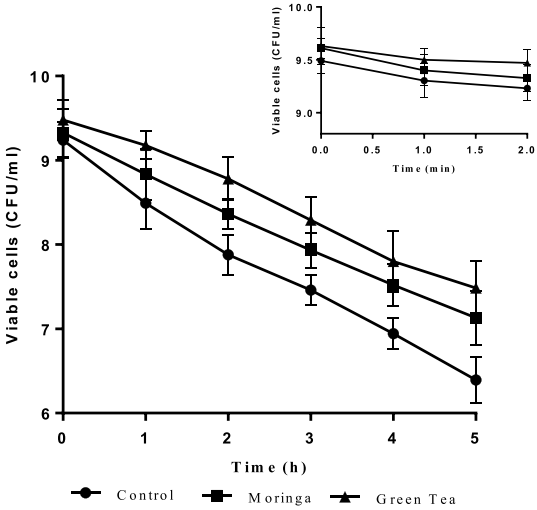

Fig. 2. Survival of control encapsulatedprobioticcells (CC) and novel encapsulatedprobioticcells (NC) with herbalextracts*P. acidilactici*DSM 20238upon sequential exposure to simulated salivary fluid (SSF) for 2 min, simulatedgastric fluid (SGF) for 2 h and simulated intestinal fluid (SIF) for 3 h. The insetrepresents the survival of cells when exposed in SSF for 2 min.

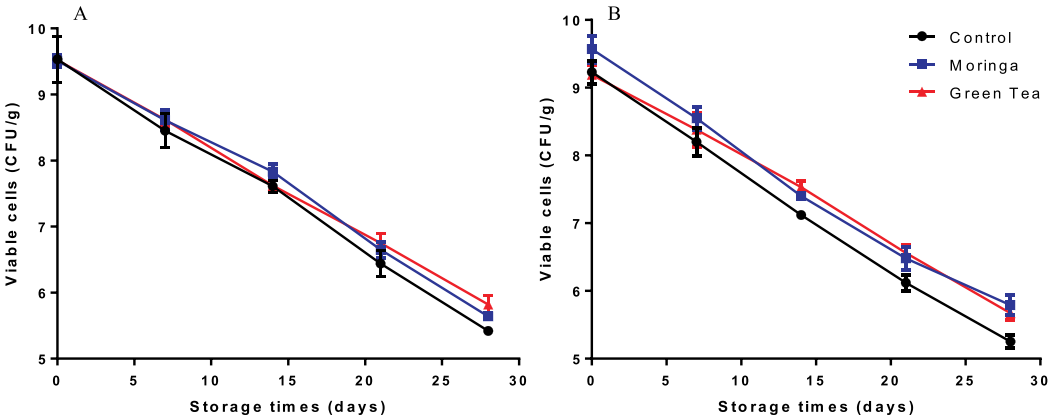

Fig. 3. Viable cells of control encapsulated probiotic cells (CC) and novel encapsulated probiotic cells (NC) with herbal extracts in drinkable yoghurt during refrigerated storage. (A) *Lactobacillus plantarum* DSM 20205, (B) *Pediococcus acidilactici* DSM 20238.

without them tended to decrease (Fig. 3). This investigation established that probiotics entrapped with 0.05% moringa extract or green tea extract survived better than probiotics encapsulated without plant extracts. The survival of *L. plantarum* DSM 20205 seemed to be higher than that of *P. acidilactici* DSM 20238 after 30 days of storage (Fig. 4). Our findings were consistent with the findings of Krasaekoopt and Watcharapoka[15] who reported the survivability of microencapsulated probiotics in a simulated digestive system, fruit juice and drinkable yogurt. Brinques and Ayub[37] studied the effects of immobilization techniques on the survival of lactobacilli in yogurt during refrigerated storage. The addition of green tea extracts has been positive impact on survival of *B. animalis* spp. *lactis* LAFTI-B94, *L. acidophilus* LAFTI-L10 and *L. paracasei* LAFTI-L26 during incubation for 72h at 37°C [27]. They found that the addition of green tea extract could lead to a favourable an anaerobic environment for probiotic bacteria due to the oxygen-scavenging and antioxidant characteristics.

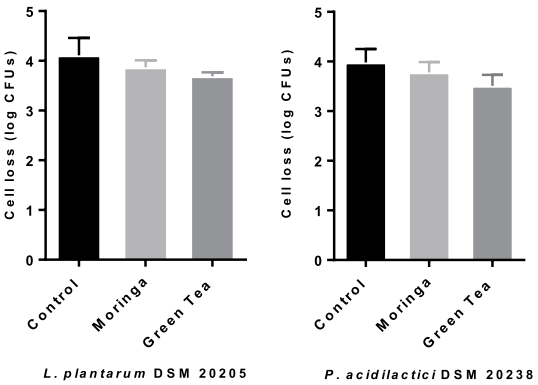

Fig. 4. Reduction of control encapsulated probioticcells (CC) and novel encapsulated probioticcells (NC) with herbal extracts in drinkable yoghurt at the end of storage at 4°C.

CONCLUSIONS

This study evaluated the effect of a novel encapsulation technique using calcium alginate and plant extracts on the stability of probiotic bacteria, including *L. plantarum* DSM 20205 and *P. acidilactici* DSM 20238,in fruit juices and drinkable yogurt during storage at 4°C for 28days. After 4 weeks of the storage period, the survivability of cells encapsulated with 0.05% moringa extract was significantly higher than those of probiotics encapsulated with fennel and sage extracts. Upon refrigerated storage, the extracts of both green tea and moringa improved the constancy of probiotic capsules in fruit juices and drinkable yogurt compared to the control capsules. Overall, the novel capsules improved the survival of *L. plantarum* DSM 20205 and *P. acidilactici* DSM 20238 in prickly pear juice and drinkable yogurt throughout storage. The novel capsules were sequentially subjected to simulated digestive fluids (SSF, SGF, and SIF) *In vitro*, and the results showed that the extracts enhanced the survival and intestinal adhering capacity and supported to keep a higher balance of probiotics in the human digestive system.

ACKNOWLEDGMENTS

None

CONFLICT OF INTEREST

The author declares that there are no conflict of interest.

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