Viability of *Lactobacillus plantarum* TISTR 2075 in Carrot Tablet after Fluidized bed drying

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ABSTRACT

In the present study, carrot tablet containing *Lactobacillus plantarum* TISTR 2075 was produced. The strain was cultivated in 10% (w/v) artichoke juice at 37 °C for 24 h. The 50% (v/w) overnight culture of this strain (8.32 log CFU/ml) with 3% (w/w) gelatin and 5% (w/w) different protective agents [monosodium glutamate (MSG), maltodextrin (MD), inulin and fructo-oligosaccharide (FOS)] were mixed with dried carrot powder prior to the tablet process. To obtain dried carrot tablets with moisture content below 11% (Uppal et al., 2002), the wet carrot tablets were dried in fluidized bed dryer at various temperatures of 50, 60 and 70 °C. The optimum drying times were 30 min at 50, 60 °C and 20 min at 70 °C. The results revealed that viability of the strain depend on drying temperatures and times with survival rate of 53.67-71.31%. Furthermore, the addition of some protective agents significantly improved the strain viability during drying. At all drying conditions, MSG was considered as the most efficient protective agent providing high survival rate of 81.53%, followed by MD with survival rate of 79.55 % after drying at 50 °C for 30 min. Only MSG provided the high survival rate of the strain of 74.01 and 78.22 % after dryings at 60 °C for 30 min and 70 °C for 20 min, respectively. Additionally, the strain in dried carrot tablets had significantly ability to survive during exposure to simulated gastrointestinal tract. Survival rate of the strain were in range of 56.63-95.87% depending on types of protective agents and drying conditions. Moreover, MSG could improve survival rate of the strain in dried carrot tablet during storage at 4 °C for 90 days. A temperature dependent prediction model based on Arrhenius theory was developed to determine the strain viability for long term-storage.

Keywords: *L. plantarum* TISTR 2075, Fluidized bed drying, Protective agents

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Introduction

Non-dairy products containing probiotic organisms is still ongoing interest since consumers can receive high nutritive values from non-dairy products especially vegetables and health benefits from probiotics. Many studies have used vegetables as alternative raw materials for non-dairy products because they can reduce some limitations of dairy products including vegetarian, individuals with lactose intolerance and cholesterol-restricted diets (Granato et al, 2010; Ranadheera et al, 2010). Moreover, vegetables can serve as a substrate for growth promoting, activating metabolism and delivery of selected probiotics into the gastrointestinal tract (GIT), due to containing nutrients, minerals, native prebiotics, dietary fiber and antioxidants. For example, some vegetables (e.g. cabbage, red beet, celery, tomato and carrot) could be used as carrier for delivery, enhancing viability and controlled release of viable probiotic cells ($\sim 10^6$-10^8 CFU/mL) (e.g. *Lactobacillus*, *Bifidobacterium* and *Bacillus*) after passing through GIT by reducing their sensitivity to unfavorable environment condition (Koh et al, 2010; King et al, 2007; Karovicova and Kohajdova, 2003). The accepted amount of live probiotics in products should be at least 10^6 CFU/g at time of consumption (Singh, 2014).

Producing a dried vegetables incorporating probiotics in form of tablets through fluidized bed drying is a good way to preserve the nutrients in vegetables and to convey adequate amount of viable probiotic into consumers. Fluidized bed drying is suitable to produce dried non-dairy products because it is economic technique, easy to control and high heat and mass transfer. Moreover, dried products are convenience have an increased shelf life (Sokhansanj and Jayas, 2006). In addition, this process was successfully used for the preparation of dried granules or powder containing lactic acid bacteria. According to Bensch et al. (2014), viability of *L. plantarum* in maltodextrin granules was decreased from 7.0×10^9 to 2.7×10^9 CFU/g after fluidized bed drying at 40 ºC for 30 min. Although fluidized bed drying provides a mild condition for drying microorganism, it may inactivate the viable cells due to the removal of water which induced conformational changes in proteins and cell membranes. Morgan et al, 2006 and Carvalho et al, 2002 have reported that the addition of protective agents (e.g. MSG, MD and prebiotics) could improve the viability of lactic acid bacteria prior to drying and storage by retaining amounts of residual moisture in dried powder.

Objectives of the study

To investigate the effect of protective agents on the viability of *L. plantarum* TISTR 2075 during fluidized bed drying

To evaluate the survival rate of *L. plantarum* TISTR 2075 in dried carrot tablets after sequential exposure to simulated gastric juice and bile salt solution

To determine the survival of *L. plantarum* TISTR 2075 in dried carrot tablets during storage

To develop the prediction model for determining the viability of *L. plantarum* TISTR 2075 in dried carrot tablets during long term-storage

Materials and Methods

Preparation of *L. plantarum* TISTR 2075

L. plantarum TISTR 2075 was obtained from Microbiological Resource Centre, Thailand Institute of Scientific and Technological Research (TISTR). The strain was preserved in MRS broth (Difco™, France) containing 20% (v/v) of glycerol at -20 ºC. The strain was activated twice in MRS broth at 37 ºC for 24 h. The activated culture was inoculated into MRS broth and incubated at 37 ºC for 24 h as the inoculum. Inoculum (1% v/v) was then conducted into 250 ml Erlenmeyer flask containing 10% (w/v) of artichoke juice. The sample was incubated at 37 ºC for 24 h.

Preparation of dried carrot powder

Edible parts of carrot were sliced into small strips with metaltex and cleaned with saline water for prevention of enzymatic browning reaction. These carrot strips were dried by sun drying method until a constant weight about 10% moisture content. Sample was grounded with Hammer mill (Polymix, Switzerland) and was then separated with 60 mesh sieves. Dried carrot powder was stored in airtight container at 4 ºC until required.

Drying of carrot tablets using fluidized bed drying

Dried carrot powder was homogeneously mixed with 3% (w/w) of gelatin as binder and 50% (v/w) of sterile distilled water in the stand mixer. The ingredient mixture was pressed into tablets using handmade pill press with 1.0 cm diameter. The wet carrot tablets was dried in fluidized bed dryer (Sherrwood, England) at various temperatures of 50, 60 and 70 ºC. The sample was collected every 10 min to determine moisture content (AOAC, 2000).

Effect of protective agents on viability of *L. plantarum* TISTR 2075 in carrot tablets during fluidized bed drying

Each protective agent (5% w/w) including monosodium glutamate (MSG), maltodextrin (MD), inulin and fructo-oligasaccharide (FOS) was homogeneously mixed with dried carrot powder, gelatin (3% w/w) and *L. plantarum* TISTR 2075 culture (50% v/w) in the stand mixer. The ingredient mixture was pressed into tablets using handmade pill press with 1.0 cm diameter. The wet carrot tablets were dried in fluidized bed dryer at various temperatures of 50, 60 and 70 ºC with moisture content below 11%. The viable cells of the strain in each dried carrot tablet were determined viability.

Survival of *L. plantarum* TISTR 2075 in dried carrot tablets after sequential exposure to simulated gastrointestinal tract

The tolerance of *L. plantarum* TISTR 2075 in each dried carrot tablet to simulated gastrointestinal tract condition was determined by the modification method of Ranadheera et al. (2012). Simulated gastric juice was prepared by 0.3% (w/v) pepsin in 0.5% (w/v) NaCl sterilized by filter membrane and adjusted to pH 2 with HCL. Simulated small intestinal juice was prepared by the mixture of 0.1% (w/v) pancretin USP in 0.5% (w/v) NaCl sterilized by filter membrane and with 0.45% (w/v) bile salt which was adjusted to pH 8.0 with NaOH. Each dried carrot tablet (1 g) was transferred to 9 ml of simulated gastric juice solution. After incubation for 60 min at 37 ºC, the

gastric juice solution was removed by centrifugation and subsequently resuspended in 9 ml of simulated small intestinal juice solution. Samples were further incubated for 120 min at 37 ºC. The intestinal juice of each sample was then removed by centrifugation and viable cells of the strain in each carrot tablet were determined.

Stability of *L. plantarum* TISTR 2075 in dried carrot tablets during storage

The dried carrot tablets containing *L. plantarum* TISTR 2075 were kept in plastic zip bag at 4 ºC for 90 days. The viable cell counts were determined every 15 days.

Accelerated storage test

The dried carrot tablets containing *L. plantarum* TISTR 2075 were incubated in hot air oven at 50, 60, 70 and 80 ºC. At 50 ºC, samples were collected every 2 h until 24 h of exposure; at 60 ºC, every 2 h until 18 h; at 70 ºC, every 1 h until 5 h; and at 80 ºC, every 15 min until 2.25 h to determine the residual viable counts.

Viable cell counts

Each sample was weighed into a sterile stomacher bag and homogeneously mixed with 0.85% (w/v) NaCl solution by stomacher (Seward, UK) at high speed for 120 s. The viability and survival rate (%) of *L. plantarum* TISTR 2075 in dried carrot tablets were determined by the standard plate count method on MRS agar plate. The plates were incubated at 37 °C for 24 h. The viable cell counts were expressed as the $\log_{10}$ CFU/g. The survival rate (%) was defined as following: $(N/ N_0) \times 100$, where N represented the number of viable cells (CFU/g) after exposure and N_0 denotes the initial viable cell count (CFU/g) prior exposure.

Statistical analysis

Results were expressed as the mean ± S.D. of two determinations. The data were analyzed by analysis of variance (ANOVA) with significant at P □ 0.05. Significant different among mean values were detected by using Duncan's multiple range tests. All statistical analyses were performed using SPSS software Version 20.

Results and Discussion

Drying of carrot tablets in fluidized bed drying

To decrease moisture content in carrot tablets, carrot tablets were dried in fluidized bed drying at various temperatures of 50, 60 and 70 °C. As shown in Figure 1, it was found that drying at higher temperature dehydrated the wet carrot tablets faster than the lower temperature to reach the equilibrium moisture content or below 11%. The moisture content dramatically decreased from 44 to 11% after drying at 50 and 60 °C for 30 min while drying at 70 °C provided only 20 min. These drying time values of carrot tablets were used for the further step.

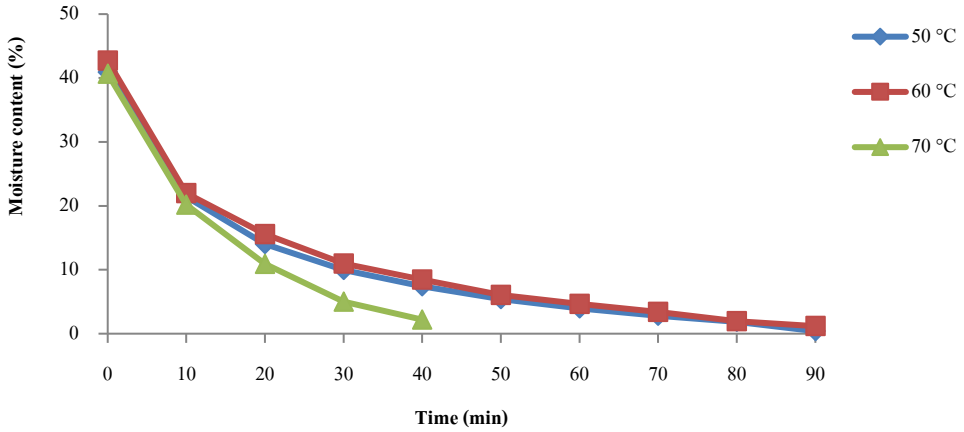

Figure 1. Drying curves of carrot tablets containing *L. plantarum* TISTR 2075 during fluidized bed drying at various temperatures of 50, 60 and 70 °C

Effect of protective agents on survival of *L. plantarum* TISTR 2075 in carrot tablets after fluidized bed drying

Preliminarily, the viability of *L. plantarum* TISTR 2075 in carrot tablets were determined after fluidized bed drying. The strain showed high survival rate of 71.31, 53.67 and 64.76 % after fluidized bed dryings at 50, 60 °C for 30 min and 70 °C for 20 min, respectively (Table 1). It was observed that the reduction of the strain viability increased as time increased. Obviously, drying temperature is important factor which affects to the viability of microorganisms during drying process. The viability loss was probably due to removal of water which may cause irreversible changes in the structural and functional integrity of cell membranes and proteins (Shokri et al., 2015). In addition, the reductions of viable cells do not depend on only drying temperature but also the time of heat exposure (Chavez and Ledboer, 2007).

To enhance the viable cells of this strain during fluidized bed drying, four protective agents including MSG, MD, inulin and FOS were evaluated. Results showed that each protective agent enhanced the strain viability with different degrees of protective effect resulting in different survival rate. Among these protective agents, MSG and MD significantly ($p<0.05$) improved viability of the strain with high survival rate after fluidized bed drying followed by inulin, while FOS did not demonstrate protective effect comparing to control. With the addition of MSG, the

highest survival rate of 81.53, 74.01 and 78.22 % were obtained after fluidized bed dryings at 50, 60 °C for 30 min and 70 °C for 20 min, respectively.

In this experiment, MSG provided the highest protective effect on the strain viability from high temperature. This was probably because it can stabilize the protein structure by the reactions between amino groups of the protectant compound and the carboxyl groups of the microorganism proteins, retain greater amounts of residual moisture in dried powder, form matrix with cell wall of microorganisms which can prevent cell injury caused by temperature and counter-ion for K^+ to balance the intracellular charge accumulated by bacteria under osmotic stress (Carvalho et al., 2002; King and Su, 1993).

Table 1. Effect of protective agents on survival rate of *L. plantarum* TISTR 2075 in carrot tablets after fluidized bed drying

Protective agent	50 °C, 30 min		60 °C, 30 min		70 °C, 20 min	
	Viability (log CFU/g)	Survival rate (%)	Viability (log CFU/g)	Survival rate (%)	Viability (log CFU/g)	Survival rate (%)
Control	8.0934±0.0073 ^{c}	71.3068±1.7783 ^{c}	7.9685±0.0129 ^{d}	53.6725±1.1692 ^{d}	8.0453±0.0109 ^{b}	64.7564±0.3089 ^{b}
MSG	8.1614±0.0064 ^{a}	81.5340±1.8605 ^{a}	8.1206±0.0047 ^{a}	74.0112±1.3904 ^{a}	8.1430±0.0113 ^{a}	78.2235±3.6117 ^{a}
MD	8.1492±0.0044 ^{ab}	79.5455±1.4427 ^{ab}	8.1004±0.0024 ^{b}	70.9040±0.9660 ^{b}	8.0607±0.0105 ^{b}	67.0487±0.2625 ^{b}
Inulin	8.1399±0.0137 ^{b}	76.7045±3.0270 ^{b}	8.0682±0.0026 ^{c}	66.3842±0.1309 ^{c}	7.7634±0.0053 ^{c}	32.9513±1.0731 ^{c}
FOS	8.0792±0.0025 ^{c}	68.4659±0.1484 ^{c}	7.9777±0.0064 ^{d}	54.2373±0.3657 ^{d}	7.5798±0.0253 ^{d}	20.9169±1.6398 ^{d}

* Values in the same conditions with difference superscript lowercase letters (a-d) are significantly different using Duncan's multiple range tests ($p < 0.05$).

Viability of *L. plantarum* TISTR 2075 in dried carrot tablets after sequential exposure to simulated gastrointestinal tract

In the present study, the ability of fluidized bed dried *L. plantarum* TISTR 2075 were tested for their tolerance on simulated gastrointestinal tract. After sequential exposure to simulated gastric for 60 min and intestinal juice for 120 min, the strain in dried carrot tablets exhibited high tolerance with high survival rate of 77.68-87.30% which was higher than free cells (39.52%) (Table 2), It was due to bacterial cells located in intracellular space, pores and capillaries of carrot cell wall and due to the composition of carrot including cellulose, hemicelluloses and lignin which are not digested by appropriate enzymes in gastrointestinal system of human (Kochar and Sharma, 1992). Moreover, gelatin in dried carrot tablets can enhance the survival rate by gel forming that can entrap the cells (Rokka and Rantamaki, 2010).

Furthermore, results showed that the addition of each protective agent also improved the survival rate of the strain after exposure to simulated gastrointestinal tract condition. In comparison with control, fluidized bed dried *L. plantarum* TISTR 2075 showed significantly higher survival rate (95.87 and 80.00%) in dried carrot tablets with MSG and MD, respectively while inulin and FOS did not enhance the strain viability. Furthermore, it was observed

that fluidized bed drying at high temperature of 70 °C affected to the ability of the strain to tolerance to GI conditions. Interestingly, MSG and MD provided protective effect against harsh conditions. This was because they can form shell or matrix on the surface of carrot tablets (Liliana and Vladimir, 2013; Cook et al., 2012).

Table 2. Survival rate of 50, 60 and 70 °C fluidized bed dried *L. plantarum* TISTR 2075 in carrot tablets after sequential exposure to simulated gastrointestinal tract

Protective agents	Survival rate (%)		
	50°C, 30 min	60°C, 30 min	70°C, 20 min
Control	87.2973 ± 0.1880 ^{bc}	82.4120 ± 2.1632 ^{b}	77.6820 ± 0.1355 ^{b}
MSG	95.8700 ± 1.1527 ^{a}	87.1707 ± 1.7885 ^{a}	83.4429 ± 0.1133 ^{a}
MD	89.0319 ± 0.5786 ^{b}	83.4981 ± 0.0770 ^{b}	80.0018 ± 1.0025 ^{ab}
Inulin	85.7143 ± 0.1957 ^{c}	81.6139 ± 0.8603 ^{b}	65.3294 ± 0.0163 ^{c}
FOS	80.6506 ± 0.7328 ^{d}	77.4541 ± 0.8409 ^{c}	56.6269 ± 3.1064 ^{d}
Free cell		39.5238 ± 2.5529	

* Values in the same columns with difference superscript lowercase letters (a-d) are significantly different using Duncan's multiple range tests ($p < 0.05$).

Survival of *L. plantarum* TISTR 2075 in dried carrot tablets during storage at 4 °C

As shown in Figure 2, it was observed that the survival of *L. plantarum* TISTR 2075 in dried carrot tablets was reduced during storage at 4 °C only 0.01-2.24 log CFU/g depending on protective agents comparing to control (0.16-2.24 log CFU/g). The highest survival of the strain was belonged to the MSG treatment (0.01-2.08 log CFU/g) because MSG can express the antioxidant property to protect the cell membrane from lipid oxidation (Singh and Ahluwalia, 2012). Moreover, low temperature can induce the survival of microorganism by decreasing the metabolism of bacteria (Bucio et al., 2005).

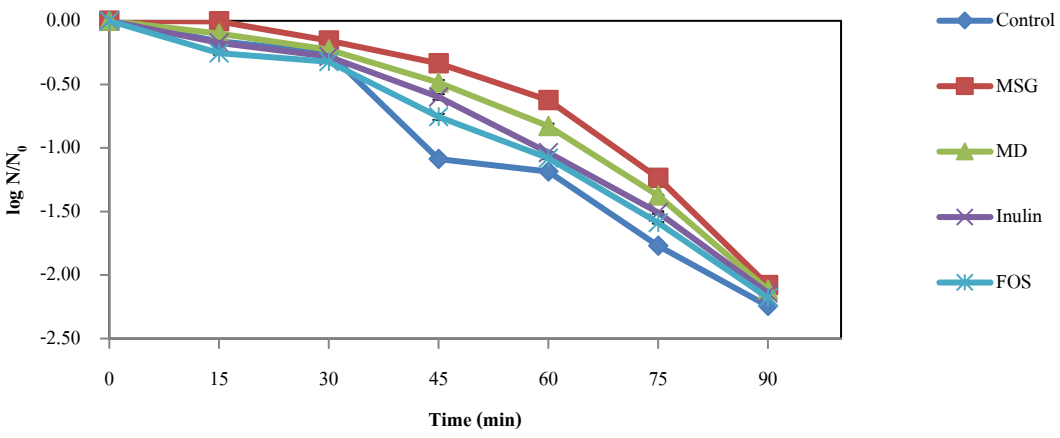

Figure 2. Survival of *L. plantarum* TISTR 2075 in dried carrot tablets during storage at 4 °C

Prediction of storage stability of *L. plantarum* TISTR 2075 in dried carrot tablets

The accelerated storage test was used to predict the stability of *L. plantarum* TISTR 2075 in 50 °C dried carrot tablets with MSG under storage temperature of 50, 60, 70 and 80 °C. The change of the strain stability after followed drying was shown in Figure 3 and the specific degradation rate (k, h^{-1}) of dried carrot tables with *L. plantarum* TISTR 2075 was obtained from the regression lines between cell viability and storage time as shown in Table 3. The correlation between temperature and k value can be described by the Arrhenius equation as shown in equation 1.

$$k = Ae^{(-Ea/RT)} \quad [1]$$

Where A is pre-exponential constant, E_a is the energy of activation ($kJ.mole^{-1}$), R is the universal gas constant (8.32 $J.mole^{-1}.K$) and T is the absolute temperature (K). When taking the logarithm of both sides of equation 1, the equation 2 was obtained.

$$\log k = -(Ea/2.303R) \times (1/T) + \log A \quad [2]$$

When the log k values were plotted against [1/T], the regression equation for the strain with MSG [log k = -3.535 x (1000/T) + 10.11] was obtained as shown in Figure 4. After solving the obtained equations for 4 °C, the predicted k value was calculated. Therefore, the predicted models for estimating the longevity of this strain at 4 °C was obtained by placing the values of N_0 and k_4 in equation 3 and model was shown in Table 4.

$$\log N = \log N_0 - kt \quad [3]$$

Table 3. Specific rate of degradation of *L. plantarum* TISTR 2075 in 50 °C dried carrot tablets with MSG

Protective agent	Specific rate of degradation,k (h^{-1}) (R^2)			
	50 °C	60 °C	70 °C	80 °C
MSG	0.170(0.972)	0.213(0.920)	0.871(0.828)	1.516(0.931)

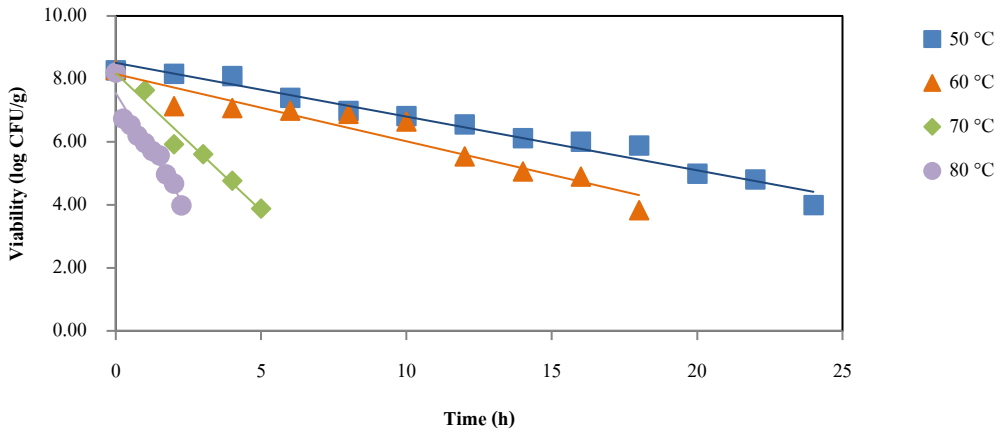

Figure 3. Viability of *L. plantarum* TISTR 2075 in 50 °C dried carrot tablets with MSG

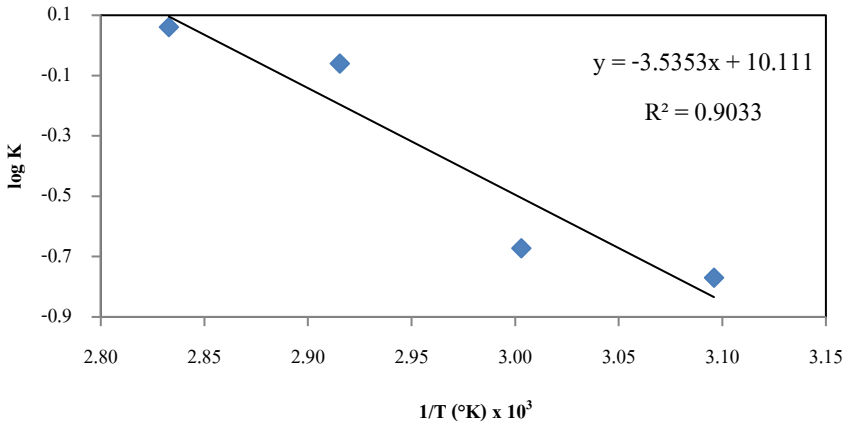

Figure 4. Arrhenius plot of specific rate of degradation (k) of *L. plantarum* TISTR 2075 in 50 °C dried carrot tablets with MSG

Table 4. Prediction model for long-term stability of *L. plantarum* TISTR 2075 in 50 °C dried carrot tablets with MSG during storage at 4 °C

Sample	Prediction model
	4 °C
MSG	$\log N = \log N_0 - 2.23 \times 10\text{-}3t$

To validate the prediction model in Table 3, the theoretical viability was calculated from these equations and the experiment viability obtained from stability tests was compared. As shown in Figure 5, it was found that the stability of this strain did not show significant difference between predicted and experimental viability of the strain in dried carrot tablets with MSG during storage at 4 °C for 90 days. This could confirm that the prediction equation could be used to determine viability of the strain in dried carrot tablets during storage at 4 °C. From the results, it can be summarized that accelerated storage test is a rapid and simple technique for estimation of the product stability.

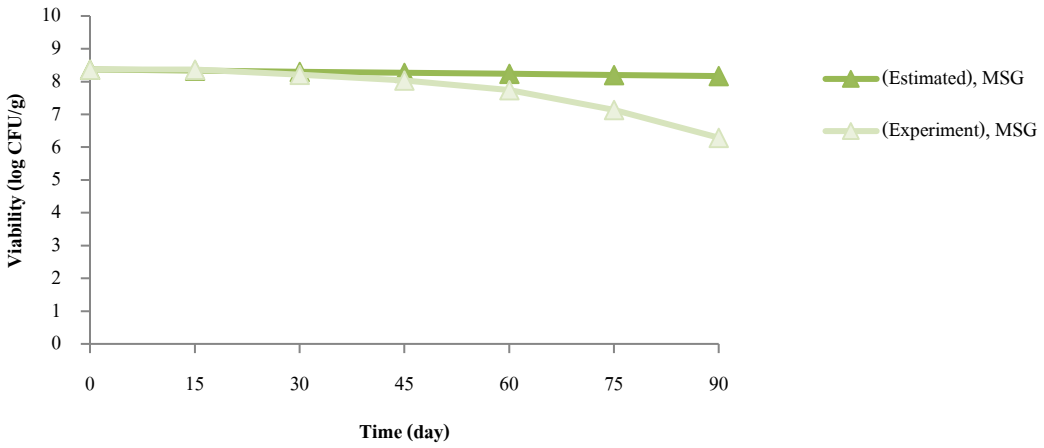

Figure 5. Comparison of the estimated and the experimentally measured viability of *L. plantarum* TISTR in 50 °C dried carrot tablets with MSG during storage at 4 °C

Conclusion

According to these results, it is possible to prepare the carrot tablets as a good carrier with high viable cell numbers of probiotic *L. plantarum* TISTR 2075 using fluidized bed drying as food supplements. The viability of the strain in carrot tablets depended on drying temperatures, drying times and types of protective agents. After fluidized bed drying, high viable cells of 10^6-10^8 CFU/g were achieved in carrot tablets which met the level requirements for probiotic products (at least 10^6 CFU/g at time of consumption). Moreover, MSG provided protective effect of the strain during fluidized bed drying, exposure to GIT and storage for days. Furthermore, the accelerated storage testing was a rapid and simple method for estimating the long-term shelf-life of this probiotic product.

References

AOAC. Official methods of the association of official analytical chemists. Washington D.C.: Association Official Analytical Chemist 2000; 14.

Bensch G, Ruger M, Wassermann M. Flow cytometric viability assessment of lactic acid bacteria starter cultures produced by fluidized bed drying. Appl Microbiol Biot 2014; 98: 4897–4909.

Bucio A, Hartemink R, Scharama JW, Verreth J, Rombouts FM. Survival of *Lactobacillus plantarum* 44a after spraying and drying in feed and during exposure to gastrointestinal tract fluids in vitro. J Gen Appl Microbiol 2005; 51: 221-227.

Carvalho AS, Silva J, Ho P, Teixeria P, Malcata FX, Gibbs P. Survival of freeze-dried *Lactobacillus Plantarum* and *Lactobacillus Rhamnosus* during storage in the presence of protectants. Biotechnol Lett 2002; 24: 1587-1591.

Cook MT, Tzortzis G, Charalampopoulos D, Khutoryanskiy VV. Microencapsulation of probiotics for gastrointestinal delivery. J Control Release 2012; 162: 56-67.

Granato D, Branco GF, Nazzaro F, Cruz AG, Faria JAF Functional foods and non dairy probiotic food development: trends, concepts and products. Compr Rev Food Sci Food Saf 2010; 9: 292–302.

Karovicova J, Kohajdova Z. Lactic acid fermented vegetable juice. Hort Sci 2003; (4)30: 152-158.

King VAE, Huang HY, Tsen JH. Fermentation of tomato juice by cell immobilized *Lactobacillus acidophilus*. Mid Taiwan J Med 2007; 12: 1-7.

King VAE, Su JT. Dehydration of *Lactobacillus acidophilus*. Process Biochem 1993; 28: 47-52.

Kochar GK, Sharma KK. Fiber content and its composition in commonly consumed Indian vegetables and fruits. J Food Sci Technol 1992; 29: 187–190.

Koh JH, Kim Y, Oh JH. Chemical characterization of Tomato Juice Fermented with Bifidobacteria. J Jpn Soc Food Sci 2010; 75: 428-432.

Kuar H. Processing Technologies for Pharmaceutical Tablets: A review. Int. Res. J. Pharm 2012; 3: 20-23.

Liliana SC, Vladmir VC. Probiotic encapsulation. Afr J Microbiol Res 2013; 7: 4743-4753.

Morgan CA, Herman N, White PA, Vesey G. Preservation of micro-organisms by drying; a review. J Microbiol Methods 2006; (2)66: 183–193.

Paz D, Dale RA, Barnett DA, Carpenter CC, Gaetner JF, Randolph TW. Effects of drying methods and additives on the structure, function and storage stability of subtilisin; role of protein conformation and molecular mobility. Enzyme Microb. Tech 2002; (6)31: 765-774.

Ranadheera CS, Evans CA, Adams MC, Baines SK. In vitro analysis of gastrointestinal tolerance and intestinal cell adhesion of probiotics in goat's milk ice cream and yogurt. Food Res Int 2012; 49: 619-625.

Ranadheera RDCS, Baines SK, Adams MC. Importance of food in probiotic efficacy. Food Res Int 2010; 43: 1-7

Rokka S, Rantamaki P. Protecting probiotic bacteria by microencapsulation: challenges for industrial applications. Eur Food Res Technol 2010; 231: 1-12.

Savedboworn W, Wanchaitanawong P. Viability and probiotic properties of *Lactobacillus plantarum* TISTR 2075 in spray-dried fermented cereal extracts. Maejo int J Sci Tech 2014; 9: 382-393.

Singh K, Ahluwalia P. Effect of monosodium glutamate on lipid peroxidation and certain antioxidant enzymes in cardiac tissue of alcoholic adult male mice. J Cardiovas Dis Res 2012; 3: 12-18.

Singh S, Kotl NG, Lal UR. Omnipresence of Probiotics in Diversified Clinical Practices. J Prob Health 2014; 3.

Sivakumar R, Saravanan R, Perumal AE, Iniyan S. Fluidized bed drying of some agro products. Renew Sust Energ Rev 2016; 61: 280-301.

Sokhansanj S, Jayas DS. Drying of foodstuff. In Handbook of Industrial Drying 2006; 521-546.

Srinivasakannan C. Modeling Drying Kinetics of Mustard in Fluidized Bed. Int J Food Eng 2008; (3)4.

Uppal DS, Ilyas SM, Silla SS. Quality and safety of animal feed in India. United Nations Asian and Pacific Center for Agricultural Engineering and Machinery 2002. Available from: http://www.unapcaem.org/activities%20files/a16/ animal%20feed%20&%20quality.pdf