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Survivability of *Lactobacillus acidophilus* and quality attributes of puffed pounded-unripe rice supplemented with probiotics using a fluidized-bed coating method

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Abstract

Value-added breakfast cereals are receiving increased interest due to changes in lifestyles and modern trends of health awareness among consumers. Among various cereal grains, puffed pounded-unripe rice (PPUR) was chosen because of its excellent health beneficial properties. Feasibility of supplementing the probiotic bacteria *Lactobacillus acidophilus* (LA5) into PPUR was investigated using a fluidized-bed coating method. Suitable fermentation conditions were tested based on a pH criterion of 4.5. Three types of cell host solutions as sucrose, skim milk and maltodextrin were examined as suitable carriers, and optimal coating condition was determined. Product qualities were evaluated for stability. Results showed that milk fermented for 24 hrs gave the highest viable cell count of approximately 10^9 to 10^{10} CFU/mL when optimally mixed with distilled water at milk: water ratio of 45:105. Skim milk was determined as the most suitable host giving the highest retention of LA5 cell count. Coating condition at a feed rate of 10 mL/min, coating time of 5 mins and constant temperature at 50°C gave the highest survival rates of LA5. Moisture contents and water activity (a_w) values of all coated products ranged from 3.38-3.72% wb and 0.201-0.232, respectively. Regarding textural properties, coated PPUR showed increased hardness and crispiness compared with uncoated samples, while the variation of coating conditions had no significant effects. Dynamic changes in viable LA5 cell count, textural properties, a_w, peroxide value and thiobarbituric acid values fitted well with various kinetic orders. Findings indicated the feasibility of adding probiotics to dry products associated with suitable coating solution and offered guidance for product development and process design.

1. Introduction

Nowadays, customers are increasingly attracted by the additional health-associated benefits offered by functional foods beyond meeting basic nutritional needs (Hasler, 2002). Probiotic-enriched foods have become a well-known group that has recently emerged as a significant product category in food markets, especially those claiming to promote gastrointestinal health (de Vrese and Schrezenmeir, 2008; Bosnea *et al*., 2017).

Probiotics comprise live microorganisms that actively contribute to gut health. They can be delivered using a food matrix as a vehicle to the desired destination with a controlled-released time. To meet most health benefits, viable probiotic cell counts should be sufficient at the time of consumption or the expiration date (Bosnea *et al*., 2017). Therefore, product development has challenged researchers to design appropriate food vehicles for probiotics.

Probiotic products have been developed to meet consumers' lifestyles in various food matrices, from those with high water activities and an expected shelf-life of weeks such as yogurt, to dry products with low water activities and an expected shelf-life of months, e.g. infant formula (Weinbreck *et al*., 2010). Dairy products have successfully emerged in functional food markets but due to their short shelf-life and strict storage conditions, dry products with prolonged shelf-life are often an alternative choice for consumers. However, reduced probiotic survival is affected by food matrix and

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storage conditions such as temperature, humidity and atmospheric oxygen (Teixeira *et al*., 1995; Anal and Singh, 2007; Weinbreck *et al*., 2010) and still needs to be addressed.

To protect probiotic cells against severe environmental factors, encapsulation techniques have been successfully applied to liquid-based products such as dairy products (Kailasapathy, 2006; Krasaekoopt *et al*., 2003; Weinbreck *et al*., 2010). However, scant information is available concerning applications of dry food products with low water activity. Therefore, a greater understanding regarding the feasibility of adding probiotics to a dry food matrix such as breakfast cereals would be interesting for product development.

Among several encapsulation techniques, fluidized-bed coating is an effective method to successfully add natural extracts to cereals as reported in (Palamanit *et al*., 2013; Duangkhamchan and Siriamornpun, 2015; Solíls-Morales *et al*., 2009). However, no research exists on trials focusing on improving functional properties of breakfast cereals by addition of probiotics. Therefore, here, a top-spray fluidized-bed coating technique was applied for coating puffed pounded-unripe rice with a solution containing viable probiotics (*Lactobacillus acidophilus*, LA5). Optimal fermentation of milk, probiotic concentration and carrier as well as suitable coating conditions were examined. Stability of viable microorganisms and quality attributes of coated puffed pounded-unripe rice were assessed during storage.

2. Materials and methods

2.1 Strains

Lactobacillus acidophilus (LA5) was used as the probiotic strain. The culture was prepared as previously described by Dimitrellou *et al.* (2016) and then used to inoculate the feed media.

To activate the cells, LA5 was cultured in MRS broth at 37°C for 16-18 hrs. About 5% of activated LA5 was transferred into milk sterilized at 110°C for 5 mins. Sterile reconstituted milk of 50 mL was inoculated under anaerobic condition (5% CO_2) at 37°C for 18-24 hrs and subsequently used as a starter.

2.2 Fermentation of milk

Milk (100 ml) was pasteurized at 85°C for 15 mins. After cooling to ambient temperature, LA5 was added to the pasteurized milk and then inoculated under anaerobic condition (5% CO_2) at 37°C for various fermentation periods of 0, 3, 6, 9, 12, 18 and 24 hrs. Strain growth was analyzed by the pour plate method in MRS agar. The pH was recorded at each fermentation period, and time taken to reach 4.2-4.6 was considered as the optimal fermentation period (Dimitrellou *et al*., 2016).

2.3 Determination of optimal concentration of coating solution

Fermented milk obtained from section 2.2 was mixed with distilled water at various mixing ratios, including 45:105, 60:90 and 75:75 (fermented-milk:distilled-water). The mixtures were tested in the top -spray fluidized-bed coating equipment as shown in Figure 1. All coating parameters employed in this section were kept constant: a feed rate of 8 ml/min, coating time of 15 mins and air temperature of 50°C. The mixture with the highest cell count was further used as a suitable coating solution.

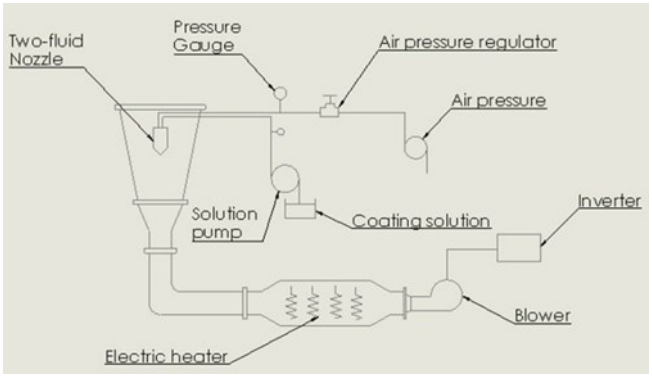

Figure 1. Top-spray fluidized-bed equipment (Duangkhamchan and Siriamornpun, 2015).

2.4 Determination of optimal carrier

To enhance the survival of microorganisms entrapped on the surface of the puffed pounded-unripe rice (PPUR) sample, a carrier was selected from three solutions of skim milk, maltodextrin and sucrose with a concentration of 10% (w/v). For each solution, LA5 was added with an initial number of 10^9-10^{10} CFU/mL, and subsequently subjected to the fluidized-bed coating process under constant operating parameters including air temperature 50°C, coating solution feed rate 5 mL/ min and coating time 10 mins. The survival of LA5 after coating was analyzed using MRS agar. Moisture content (MC, wet basis), water activity (a_w) and textural properties (hardness and crispiness) were also determined and used as criteria for selecting a suitable carrier.

2.5 Optimal fluidized-bed coating condition

Operating parameters in the fluidized-bed coating process were selected as coating solution feed rate (6.7, 7.5 and 10 ml min^{-1}) and coating time (5, 7.5 and 10 mins) to compare results of coating yield or the total amount of coating solution (50 mL) used in each experiment. Corresponding coating conditions are

presented in Table 1. Because the survival of probiotics is sensitive to heat, the air temperature was kept constant at 50°C during the coating process. Optimal fluidized-bed coating condition was determined based on survival percentage, physical and textural properties.

Table 1. Coating conditions for determining optimal fluidized-bed coating parameters.

Spraying time (min)	Feed rate (mL/min)
5	10
7.5	6.7
10	5

2.6 Stability of PPUR product supplemented with probiotics during storage

PPUR was kept in aluminium foil for stability analysis during the storage time of 28 days. Following the method of Yan *et al*. (2014), mean survival ratios (N/N_0) were fitted to a temperature-time model describing viable LA5 as a function of storage time and expressed as follows:

$$\frac{d\left(\frac{N}{N_0}\right)}{dt} = -k\left(\frac{N}{N_0}\right)^{1-n} \quad (1)$$

In this equation, N and N_0 denote LA5 cell counts at a specific time (t, day) and initial storage time, respectively where k is the rate constant (day^{-1}). The rate constant k was determined at different kinetic orders (n = 0, 0.5, 1, 1.5, 2) by converting the kinetic equation to a logarithm form (Yan *et al*., 2014). The linear relation was then employed to estimate all constants in equations 2 and 3 as expressed by:

$$\ln\left(\frac{N}{N_0}\right) = -kt + c \ \ (n = 1) \quad (2)$$

$$\left(\frac{N}{N_0}\right)^{1-n} = -kt + c \ \ (n \neq 1) \quad (3)$$

Among the different kinetic orders tested, the best fit was chosen based on the highest coefficient of determination (R^2).

Kinetic equations 1-3 were also employed for other attributes including a_w, hardness, stickiness, peroxide value (PV) and thiobarbituric acid (TBA) with the replacement of their ratios between values at specific and initial times.

2.7 Quality attributes of PPUR supplemented with LA5

2.7.1 Moisture content

About 3 g of PPUR was dried in a hot-air oven at 105°C for 72 hrs to reach equilibrium moisture content. Based on the standard method of AOAC (AOAC, 1995), moisture contents of samples were calculated using the initial weight and weight at equilibrium.

2.7.2 Water activity (a_w)

Water activity of PPUR was measured using a water activity meter (Aqualab, Decagon, USA).

2.7.3 Cell survival rate

About 10 g of PPUR were ground and then added to 90 mL of 0.1% peptone. The mixture was held at room temperature for 15 mins and then shaken for 2 mins. Subsequently, serial dilution was conducted and LA5 was enumerated using the pour plate method with MRS agar, inoculated at 37°C for 24-48 hrs. Three replications were conducted and the average cell survival rate was presented in CFU/mL.

2.7.4 Rancidity analysis

2.7.4.1 Analysis of peroxide value

The coated PUR samples were expected to be protected against the atmosphere; therefore, degree of hydroxide formation of the samples, considered as the rancidity index, was analyzed during storage. The peroxide value (PV) was measured according to the method proposed by Krik and Sawyer (1991) with slight modifications. Briefly, approximately four grams of blended PPUR were mixed with 10 mL chloroform and 15 mL glacial acetic acid and then shaken vigorously for 30 s. Meanwhile, 1 mL of potassium iodide was dropped wisely and subsequently kept in the dark for 5 min. In the final step, the mixture was titrated with 0.01 M sodium thiosulphate solution and a blank. The PV was calculated by:

$$PV = \frac{(V_1 - V_0)T \times 10^3}{M} \quad (4)$$

where V_1 and V_0 (mL) denote the volume of sodium thiosulphate solution and a blank without sample, respectively, M (g) the sample mass, and T the molarity of sodium thiosulphate. The PV was measured as three replicates and the average value was expressed in mill-equivalent of active oxygen per one kilogram (mEq/ active O_2/kg).

2.7.4.2 Analysis of 2-thiobarbituric acid (TBA)

In addition to PV, 2-thiobarbituric acid (TBA) of PPUR was measured based on the spectrophotometry method (Kirk and Sawyer, 1991). TBA solution was first prepared by dissolving 200 mg of TBA in butanol (100 mL). It was then filtered and stored in a fridge (4±1°C) for 2-3 days before use. Approximately 50 g of blended PPUR sample was added to the TBA solution. The mixture was heated in boiling water for 10 mins and then cooled to room temperature. A Beckman DU-640 Spectrophotometer (Beckman Coulter, Fullerton, USA) was employed to record the absorbance of the sample

mixture at 530 nm (A_s) as well as a blank (A_0). The TBA value was calculated by:

$$TBA = \frac{50 \times (A_s - A_b)}{C} \quad (5)$$

where C represents sample mass (mg). Based on the reaction of malonaldehyde (MDA) and thiobarbituric acid, TBA values averaged from three replications were expressed as mg of MDA per 100 g sample.

2.7.5 Textural analysis

Textural properties including hardness and crispiness of coated PPUR were measured by using a TA-XT2i Texture Analyzer (Stable Microsystems Ltd., UK) equipped with a 5-blade Krammer shear cell. The test was performed by compressing the samples (200 g) at a speed rate of 1 mm s^{-1} until the blades completely cut all pieces of PPUR. Peak force (g) and the number of peaks obtained from the force-deformation curve were recorded and expressed as the hardness and crispiness, respectively. An average of five measurements for each sample was presented.

3. Results and discussion

In this work, the feasibility of adding LA5 cell onto PPUR as the breakfast cereal product was analyzed. Experimental scenarios were orderly conducted to examine the optimal process. The fermented milk was first prepared with the suitable condition under which its pH reached 4.5 and subsequently mixed with distilled water as the base coating solution. Due to heat sensibility, the type of carrier was suitably selected based on LA5 cells retained after exposure to heat in a fluidized bed coating process. Next, the appropriate coating parameters were determined in order to obtain good physical and textural properties with remaining a maximum number of living bacteria. Eventually, dynamic changes in all attributes during storage were analyzed.

3.1 Optimal condition for preparing fermented milk

Figure 2 shows variations of LA5 growth (log CFU/ mL) and pH of fermented milk at different fermentation times (hr). The pH value played an important role in LA5 growth, decreasing from 6.82 to 4.5 at fermentation time of 24 hrs, while the growth of LA5 increased from 7.55 to 12.68 log CFU/mL corresponding to a suitable pH of 4.5 at fermentation time of 24 hrs. This was considered as the optimal fermentation condition that produced the highest number of microorganisms with pH in a suitable range of 4.2-4.6 (Dimitrellou *et al*., 2016).

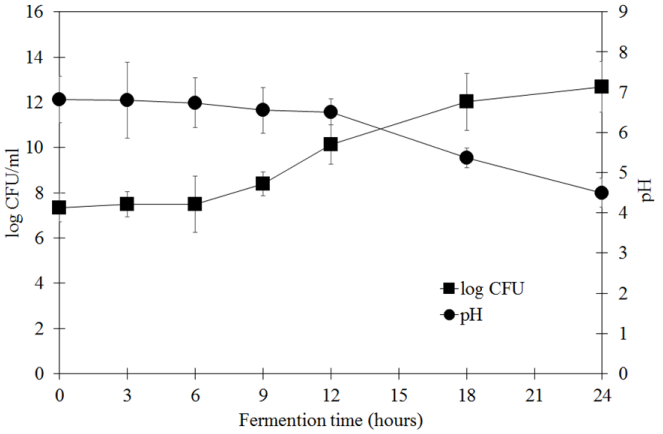

Figure 2. Optimal condition for preparing fermented milk containing LA5.

3.2 Optimal concentration of coating solution

Table 2 shows the survival percentage of LA5 and moisture content of PPUR coated with different ratios of fermented milk and distilled water. The ratio with the highest percentage of LA5 survival after the coating process was chosen as the most suitable. According to results in Table 2, the initial count of LA5 and moisture content of PPUR were not significantly different, ranging from 0.97×10^{10} to 1.48×10^{10} CFU/mL and 3.86 to 4.53% wb, respectively.

After coating, viable LA5 count changed with different amounts of distilled water in the solution, ranging from 3.82×10^8 CFU/mL to 5.21×10^8 CFU/mL. Survival increased at higher fermented milk ratios, resulting in higher cell count. Maximum LA5 cell viability was found when using 75 mL of fermented milk followed by 45 mL and 60 mL. Table 2 also shows the survival percentage of LA5. This decreased as water reduced from 105 mL to 90 mL. This observation could be attributed to higher water evaporated, resulting in lower temperature for the cells. Increase in water also increased the MC values of the samples but they remained at a safe level.

Furthermore, the optimal ratio of fermented milk and distilled water was selected based on the highest survival percentage of the cells after exposure to heat in the coating process. Solutions with fermented milk: water ratios of 45:105 and 60:90 equally gave the maximum survival percentage but for economic concerns, the ratio with a lower amount of fermented milk was selected as more suitable. Therefore, the ratio of fermented milk and distilled water at 45:105 was further used for the coating solution.

3.3 Optimal carrier

The major purpose of encapsulation is to protect bacteria against severe environmental factors.

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Table 2. Survival percentage of LA5 and moisture content before and after fluidized-bed coating with different concentrations of fermented milk.

FM: water	Before coating		After coating		
(mL:mL)	Survival^{ns} (CFU/mL)	MC^{ns} (%wb)	Survival (CFU/mL)	Survival (%)	MC (%wb)
45:105	$0.97\pm0.92\times10^{10}$	4.24±0.26	$3.82\pm0.37^{a}\times10^{8}$	4.02 ± 0.54^{b}	4.75 ± 0.11^{b}
60:90	$1.28\pm0.25\times10^{10}$	3.86±0.24	$3.90\pm0.71^{a}\times10^{8}$	3.05 ± 0.04^{a}	4.55 ± 0.17^{b}
75:75	$1.48\pm0.28\times10^{10}$	4.53±0.19	$5.21\pm0.51^{b}\times10^{8}$	3.56 ± 0.33^{b}	3.98 ± 0.08^{a}

Values with different superscript within the same column are significantly different.
FM, Fermented milk; ns, not significant.

Table 3. Survival percentage of LA5 and moisture content before and after fluidized-bed coating with different carriers.

Carrier	Before coating		After coating		
	Survival^{ns} (CFU/mL)	MC^{ns} (%wb)	Survival (CFU/mL)	Survival (%)	MC (%wb)
Sucrose	$3.74\pm0.49'10^{10}$	4.37±0.16	$2.49\pm0.28^{b}'10^{8}$	0.66 ± 0.01^{b}	4.35±0.21
Skim milk	$3.03\pm0.53'10^{10}$	4.34±0.17	$2.51\pm0.29^{b}'10^{8}$	0.82 ± 0.09^{c}	4.55±0.13
Maltodextrin	$3.26\pm0.10'10^{10}$	4.42±0.09	$1.10\pm0.38^{a}'10^{8}$	0.33 ± 0.15^{a}	3.98±0.18

Values with different superscript within the same column are significantly different. ns, not significant.

Appropriate cell carriers contribute to survive during processing and storage and time of release (Anal and Singh, 2007; Champagne and Fustier, 2007). Table 3 presents the survival percentage of LA5 as affected by cell-carrier type.

Fluidized-bed coating is a process in which heat and mass transfer take place simultaneously in one operation. At intermediate heat (50°C), bacteria require a suitable host during the process so that they can survive until the encapsulation process is complete. Here, three types of carriers as sucrose, milk powder and maltodextrin were mixed with fermented milk containing LA5 at 3.2-3.7x10^{10} CFU/mL, as shown in Table 3. After coating at 50°C, survival percentages of LA5 were significantly different, decreased after coating as normally found for lactic bacteria which is sensible to heat (Zhang *et al*., 2018). Coating solution mixed with skim milk gave the highest survival percent of 0.82, implying that skim milk was a suitable host during the process, followed by sucrose and maltodextrin with survival percentages of 0.66 and 0.33, respectively. A plausible explanation for this result was that protein-based materials have higher oxygen and moisture barriers as well as heat resistance (Krochta and Nisperos-Carriedo, 1994; Gennadios, 2002). This is consistent with Dimitrellou *et al*. (2016) stating that skim milk could protect lactic bacteria during spray drying due to its heat protective capacity (Corcoran *et al*., 2004). The amount of water used for each coating solution was kept constant; therefore, moisture content of coated PPUR was insignificant (3.98-4.55%wb).

3.4 Effect of fluidized-bed coating on survival percentage of LA5

The most suitable carrier as skim milk selected in the previous section was mixed with the coating solution, fermented milk and distilled water at the ratio of 45:105 to determine the optimal coating time. Table 4 shows the survival percentage of LA5 and moisture content before and after coating at different spraying times and feed rates. Each batch approximately contained the same initial count of LA5 (or the same amount of coating solution). The number of LA5 contained in the coating solution was 2.62-3.78x10^{10} CFU/mL. As bacteria LA5 are sensitive to heat, their survival decreased dramatically to 3.13-6.00x10^{8} CFU/mL after coating. This is commonly observed in many works when bacteria cells are subjected to heat; for instance, Paéz *et al*. (2012) presented the effects of heat treatment on the survivability of lactobacilli. With the variation of exposure times to heat, the coating treatment with shortest spraying time gave the highest survival percentage (1.60%), while LA5 bacteria were mostly destroyed after exposure to heat during 10 mins of spraying time at 99.17%.

3.5 Effect of fluidized-bed coating on physical and textural properties

Table 5 shows the physical and textural properties of PPUR coated at different coating conditions. Adding coating solution onto the sample surface slightly increased the moisture content; however, variations in spraying time and feed rate did not significantly affect the final MC.

By contrast, water activity decreased after coating but ranged in the safe level for dry products at 0.20-0.23. Typical properties of snack foods as hardness and crispiness of coated PPUR were determined. Hardness was not affected by process conditions, while crispiness of coated PPUR increased significantly, compared with

Table 4. Survival percentage of LA5 and moisture content before and after fluidized-bed coating with different operating conditions.

Operating Condition		Before coating		After coating		
Spraying time (min)	Feed rate (mL/min)	Survival^{ns} (CFU/mL)	MC^{ns} (%wb)	Survival (CFU/mL)	Survival (%)	MC (%wb)
5	10	3.78±0.99′10^{10}	3.05±0.08	6.00±0.39^{b}′10^{8}	1.60±0.11^{c}	3.13±0.12
7.5	6.7	2.62±0.31′10^{10}	2.92±0.10	2.85±0.11^{a}′10^{8}	1.08±0.05^{b}	3.39±0.31
10	5	3.74±0.49′10^{10}	3.12±0.11	3.13±0.60^{a}′10^{8}	0.83±0.11^{a}	3.25±0.14

Values with different superscript within the same column are significantly different. ns, not significant.

Table 5. Physical and textural properties affected by fluidized-bed coating conditions.

Operating Condition		MC (%wb)	Hardness^{ns} (N)	Crispiness (no. of peaks)	Water activity (a_w)
Spraying time (min)	Feed rate (mL/min)				
Uncoated	Uncoated	2.68±0.44^{a}	248.87±13.43	66.67±5.86^{a}	0.2672±0.02771^{b}
5	10	3.52±0.25^{b}	244.75±12.74	81.33±3.79^{b}	0.2010±0.00361^{a}
7.5	6.7	3.72±0.40^{b}	249.52±6.94	86.33±2.08^{b}	0.2160±0.00624^{a}
10	5	3.38±0.12^{b}	243.34±12.37	84.00±2.00^{b}	0.2323±0.01739^{a}

Values with different superscript within the same column are significantly different. ns, not significant.

uncoated ones (as shown in Table 5). This could be explained because the solidified coating solution layered onto the PPUR surface may result in a case-hardening effect. This finding is consistent with Duangkhamchan and Incheun (2016) presenting that significant changes in textural properties of PPUR coated by marigold extract were observed after coating. However, the varied process parameters tested did not significantly change the textural properties of coated PPUR, due to the same amount of coating solution used in this work.

3.6 Stability of PPUR supplemented with LA5 during storage

During storage, environmental parameters usually affect the physical, chemical and textural properties of snack products (Pathare and Byrne, 2011). An insight into kinetic behavior during storage is useful for process designers and management. Figure 3 shows the survival rate of LA5 cell supplemented in PPUR during storage of 28 days. The viability of LA5 cells decreased exponentially as a function of storage period from 6x10^{8} CFU/mL at the onset of the storage to 7.5x10^{6} CFU/mL. The viable cell count at the end of storage was not consistent with that reported in Santos Filho *et al.* (2019) in which LA5 remained approximately 10^{7} CFU/mL in cocoa juice after 42 days of refrigerated storage. Even though PPUR contained such low strain viability at 28 days, it was still in a range (10^{6}-10^{9} CFU/mL) which was sufficient to obtain a beneficial result (Vinderola *et al.,* 2000). Furthermore, microbial viability and product attributes were subjected to kinetics modeling with different orders. Based on the highest coefficient of determination (R^2), suitable choices of each attribute are presented in Table 6. All equations with R^2 values higher than 0.8 could serve as a basis for describing changes in attributes over a storage period of 28 days. All equations together with their constants are summarized in Table 7.

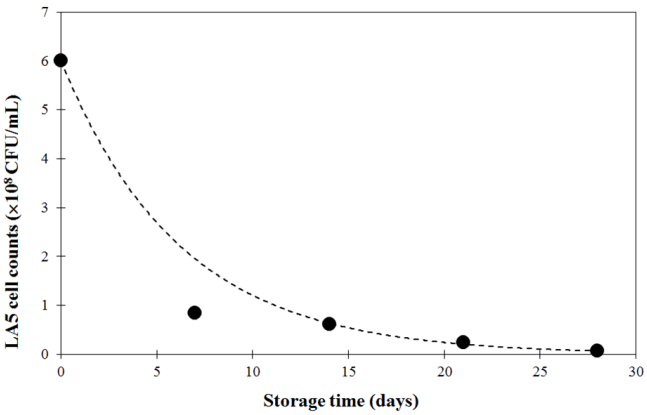

Figure 3. Kinetic behavior of LA5 viability during 28-day storage.

Table 6. Coefficients of determination (R^2) with different orders of kinetic equations.

Attribute	Coefficient of determination (R^2)				
	0th	0.5th	1st	1.5th	2nd
Moisture content	0.9709	0.9791	0.9856	0.9904	0.9935
Water activity (a_w)	0.8603	0.8575	0.8549	0.8523	0.8498
Hardness	0.9331	0.8985	0.857	0.8116	0.7656
Crispiness	0.9649	0.9641	0.9631	0.9621	0.9609
Population of *L. acidophilus*	0.6195	0.7782	0.9514	0.8998	0.7447
Peroxide value	0.8293	0.8465	0.8627	0.8779	0.8918
Thiobarbituric acid	0.9696	0.9601	0.9478	0.9327	0.9151

Table 7. Suitable kinetic equations for all attributes during 28 days of storage.

Attribute	Model
MC	$\frac{MC_0}{MC} = -0.0133t + 1.0139$
Water activity (a_w)	$\frac{a_w}{a_{w0}} = 0.0155t + 1.0111$
Hardness (H)	$\frac{H}{H_0} = -0.0204t + 1.035$
Crispiness (C)	$\frac{C}{C_0} = -0.0046t + 1.0012$
LA5 survival (%)	$\frac{LA5}{LA5_0} = e^{-0.1432t} + 0.6975$
Peroxide value (PV)	$\frac{PV_0}{PV} = -0.0134t + 1.0554$
TBA	$\frac{TBA}{TBA_0} = 0.0245t + 1.043$

4. Conclusion

Novel breakfast cereals were developed using the top -spray fluidized-bed coating process for probiotics supplementation. A suitable pH of 4.5 and a fermentation time of 24 hrs were considered to be optimal fermentation conditions, giving the highest number of microorganisms and pH at a suitable range of 4.2-4.6. Based on the highest survival percentage after coating, solution ratios of 45:105 and 75:75 were considered as optimal concentrations with survival percentages of 4.02 and 3.56, respectively. Among these solutions, the former was chosen with respect to economic concerns. In the coating test at 50°C, skim milk was selected as the best host for LA5 cells as it gave the highest survival percentage after the coating process. Both hardness and crispiness of PPUR after coating increased but were not significantly affected by operating conditions. Stability analysis during storage provided suitable kinetic equations for describing product quality attributes, with R^2 values mostly above 0.9. Our findings could be used as the basis for further product development and process design.

Conflict of interest

The authors declare no conflict of interest.

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