Effect of Drying Process and Storage Temperature on Probiotic *Lactobacillus casei* in Edible Films Containing Prebiotics

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Abstract

Incorporation of probiotics in edible film containing prebiotics is a novel approach for product development with potential usage in various functional food applications. The challenge of probiotic film is an ability to maintain adequate probiotic cultures throughout processing and storage. This study aimed to investigate the effect of various prebiotic edible films on the surviving rate of *Lactobacillus casei* TISTR 1463 during storage (4°C and room temperature). Each film consisted of *L. casei* TISTR 1463 (10–12 log CFU/mL) and 4% (w/v) prebiotic source (sodium alginate, gum arabic, konjac flour, pectin, or inulin). Film properties and survival rate of *L. casei* TISTR 1463 were monitored during storage every 5 d and shelf life prediction was calculated. Type of prebiotics significantly influenced the survival of *L. casei* and film strength after drying process ($p<0.05$). Film containing inulin had the highest survival retention of viable culture (87.4%) followed by sodium alginate (83.6%), konjac flour (80.3%), gum arabic (80.0%), and pectin (47.6%), respectively. Storage temperature also affected stability of the probiotic in prebiotic films ($p<0.05$). The viable cultures in sodium alginate, gum arabic, and inulin films had shelf life prediction of over 100 d at 4°C, whereas those stored at room temperature lasted for 5 day.

Keywords: Probiotic, *Lactobacillus casei*, Edible film, Prebiotic

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1. Introduction

The use of probiotics in functional foods has been growing due to their health benefits and potentials for product development. Probiotics are healthy microorganisms which play an important role in health maintenance and supporting gastrointestinal tract and digestive system (Heyman, 2000; Ogata *et al*., 1997). They have potential to reduce lactose intolerance, prevent carcinogens and decrease cholesterol-blood levels (Guerin-Danan *et al*., 1998; Yuki *et al*., 1999, Schrezenmeir and de Vrese, 2001). Prebiotics enhance the growth of probiotics inhabiting in colon and subsequently limit the growth of pathogenic bacteria (Saad *et al.*, 2013). Prebiotic-containing foods also have been reported to restrain or delay non-communicable diseases, such as diabetes, cardiovascular diseases with hypercholesterolemia, osteoporosis, digestive infections, and gastrointestinal inflammation (Al-Ghazzewi *et al.*, 2007; Florowska, 2016; Slavin, 2013).

The delivery combination of probiotics with prebiotics also known as symbiotic is a novel approach for food development and typically involved encapsulation and edible film applications. Reported dehydration process for production of probiotic films includes vacuum drying, spray drying, and freeze-drying (Karla *et al*., 2012). Since effectiveness of probiotic film was related to the number of active microorganisms, survival of probiotics was a key parameter to monitor during shelf life and storage to guarantee a success in commercial applications (Falguera *et al.,* 2011) Previous studies have showed that prebiotics enhanced survival rate of probiotics, especially during drying process, by acting as a bio-protective base for probiotics (De Lacey *et al*., 2012; Sathyabama *et al*., 2014). Common prebiotics used in the edible film were inulin, polydextrose, wheat dextrin, and gluco-oligosaccharides. Soukoulis *et al*. (2014A) reported that edible film containing inulin was the most effective compared to others when stored at 4°C and had a shelf life of 100 day. Prebiotics not only provided protection to probiotic cells but they also limited access of physical and chemical interference from outside and consequently prolonged shelf life of probiotics (Kanmani and Lim, 2013; Piermaria *et al*., 2015; Soukoulis *et al*., 2014B).

This study aimed to investigate a survival rate of *L. casei* in edible films containing various prebiotics and also compare physical properties of the films during storage of 20 day at 4°C and room temperature.

2. Materials and Methods

2.1 Materials

The probiotic strain *L. casei* TISTR1465 was obtained from Thailand Institute of Scientific and Technology Research. Sodium alginate, pectin (from citrus, rapid set) and gum arabic were purchased from Wendt Chemie Company (Hamburg, Germany). Konjac flour was bought from Yok Intertrade (Chiangmai, Thailand) and inulin (90% purity from chicory) was purchased from Agency DPO Ltd., (Bangkok, Thailand). Gelatin was used as film forming aid. Glycerol analytical (87% purity, VWR Chemical, Leicestershire, UK) was used as a plasticizer. MRS broth (Titan Biotech Ltd., New Delhi, India) was used for microorganism analysis.

2.2 Film Preparation

Films were prepared according to a modified method of Soukoulis *et al.,* (2014). The investigated prebiotics were sodium alginate, gum arabic, konjac flour, pectin, or inulin. Film solution was prepared as described in Table 1. The ingredients were mixed under agitation and heated by hotplate at 80°C for 30 min to reduce the initial microbial load. The pH of each solution was measured.

Probiotic *L. casei* preparation was followed a method of Soukoulis *et al.* (2017). *L. casei* was incubated in MRS broth for 24 h. (stationary phase). A pellet of *L. casei* was collected by centrifugation of 50 mL MRS broth (3000×g, 5 min) and washed twice using a phosphate buffer (10 ppm). Three cleaned pellets were inoculated in 100 ml film solution (ca. 10 log CFU/mL minimum). Then 20 mL of film solution were transferred to aseptic round petri dishes (inner diameter 8.8 cm). Film solution was dried in hot air oven (37°C, 50% RH) for 24 h. Final prebiotic films were peeled off petri dish and kept at RH 50±2% by saturated magnesium nitrate solution for 3 day. After that moisture content, water activity, thickness, mechanical properties (strength, elongation and Young's modulus), opacity, and color characteristics were determined. Mechanical characteristics were detected using universal testing machine H1K-S (Tinius Olsen TMC, Pennsylvania, USA). Survival of *L. casei* was determined immediately after the film was formed and monitored every 5 d at 4ºC and room temperature (59±2% and 50±2% RH, respectively) for 20 day. Data were used to calculate shelf-life prediction.

Table 1 Different type of prebiotic edible film formulation

Treatments	Prebiotic (g)	Gelatin (g)	50%Glycerol (mL)	Water (mL)
Sodium alginate	4	1	2	100
Gum arabic	4	1	2	100
Konjac flour	4	1	2	100
Pectin	4	1	2	100
Inulin	4	4	2	100

2.3 Enumeration of *Lactobacillus casei*

Enumeration of probiotics in the prebiotic edible film was carried out according to modified methods of Kanmani and Lim (2013) and Altamirano-Fortoul *et al.* (2012). Film was cut into small pieces (proximately 1 mm x 1 mm). One g of film was diluted in 9 ml peptone water, hold for 10 min, then vortexed for 2 min, and analyzed for microbial count. Percent viability was calculated according to equation:

$$\% \text{ Viability} = 100 \log N / \log N_0$$

Where $\log N_0$ was initial viable *L.casei* and $\log N$ stands for the number of viable *L.casei* after drying process.

Survival of *L. casei* during storage was reported as a rate of relative viability N/N_0 over time. First order reaction kinetic model was used to predict survival of viable bacterial as described by equation:

$$\log N_t = \log N_0 - K_T t$$

where N_0, stands for initial number of the viable bacteria, N_t stand for survival number of bacteria after a specific time of storage (CFU/g) and k_T is a coefficient reduction rate at a storage temperature (d^{-1}) and t is the storage time (d) (Bevilacqua *et al*., 2015).

2.4 Moisture content and water activity

After preconditioning at 52 %RH for 3 d, film moisture content and water activity were measured by moisture analyzer balance (Precisa XM-60, Switzerland) and water activity analyzer (AquaLab 4, Meter Group, Washington, USA), respectively. Measurements were performed in triplicate and reported as mean±standard deviation.

2.5 Thickness and mechanical characteristics

The thickness of probiotic films was measured in micrometer with sensitivity of 0.01 mm (Mitutoyo JTC Tool-1MIT-103-137, Tokyo, Japan) and reported as an average of 5 measurements per sample. Tensile strength, elongation of film (%E) and Young's modulus

were analyzed according to a modified method of Yuki *et al*. (1999). Samples were cut in rectangular shape (20 mm × 80 mm). Measurement conditions were 50 mm grip length, 1000 N force, and 10 mm/min speed using Universal testing machine H1K-S (Tinius Olsen TMC, Pennsylvania, USA). Data was calculated by equation:

$$TS\ (Stress) = F_{max} / Area$$

$$\%\ E\ (strain) = 100 \times (\Delta L/L_0)$$

$$Young\ modulus = TS / E\ or\ (Stress/Strain)$$

where: F_{max}= force at break (N), area of film (mm^2), L_0= original length of film (mm), ΔL= extension of film length at break.

2.6 Opacity and color characteristics

Opacity of probiotic film were evaluated according to Núñez-Flores *et al*. (2012). Samples were cut into a rectangular shape (0.7 mm × 1.5 mm) and used to coat a cuvette surface (use an empty cuvette for blank). Absorbance was measured using a UV–VIS spectrophotometer (Thermal Scientific, New York, USA) at 550 nm and calculated according to the equation:

$$Opacity = A_{550}/Thickness$$

Color characteristics of the probiotics film were measured by Chroma meter CR-400 Ver.1.01 (Konica Minolta, Chiyoda, Japan) and reported in CIELab color system (L* a* b*).

2.7 Statistical analysis

The effect of prebiotics, storage time and temperature were analyzed for significance (p<0.05) using one-way analysis of variance (ANOVA) and Duncan's post hoc test. All statistical analysis was performed using statistical software SPSS Ver.17 (Chicago, Illinois, USA).

3. Results and Discussion

3.1 Effect of prebiotics on film characteristic

Moisture content and water activity of prebiotic films (shown in Table 2) were significantly different (P<0.05). This may be due to a water-holding capacity of individual biopolymer and interaction with plasticizer. Konjac film had the highest water activity (0.59) and moisture content (18.3%), which was in agreement with a previous study by Rhim and Wang (2013). Its higher water holding capacity in this study might be due to the hydrophilic structure of konjac polysaccharide (Rhim and Wang. 2013).

All prebiotic films had a similar thickness, except konjac film (Table 3). However, tensile strength and film elongation were significantly different (P<0.05). Films with the highest tensile strength and elongation property were alginate film (25.9 MPa) and inulin film (92.9%),

respectively. Mechanical characteristics usually depended on film composition and the nature of polysaccharides (Vieira *et al.,* 2011; Cazón *et al*., 2017). In this case, it was presumably due to individual matrix of prebiotic films and plasticizer.

The opacity and color characteristics of edible film were significantly affected by prebiotics used (p<0.05). Edible film containing pectin was duller and had more yellow shade than the other films (raw material color). The films opacity of alginate and pectin were significantly different with inulin, konjac flour, and gum arabic (Table 4).

Table 2 Moisture content and water activity of prebiotic edible films

Prebiotic films	**Moisture content (%)**	**Water activity**
Sodium alginate	17.9 ^{a} ± 1.4	0.56 ^{c} ± 0.01
Gum Arabic	13.7 ^{b} ± 0.7	0.57 ^{bc} ± 0.01
Konjac flour	18.3 ^{a} ± 0.4	0.59 ^{a} ± 0.00
Pectin	16.2 ^{a} ± 0.7	0.58 ^{ab} ± 0.01
Inulin	11.3 ^{c} ± 0.9	0.58 ^{ab} ± 0.01

Note: The different superscript letters are significantly different at column (p < 0.05).

Table 3 Thickness and mechanical characteristics of prebiotic films

Prebiotic films	**Thickness (mm)**	**Tensile strength (MPa)**	**Elongation (%)**	**Young's Modulus (MPa)**
Sodium alginate	0.12 ^{a} ± 0.01	25.9 ^{a} ± 5.31	7.1 ^{c} ± 2.57	3.95 ^{a} ± 1.45
Gum arabic	0.12 ^{a} ± 0.01	3.63 ^{d} ± 0.31	23.2 ^{bc} ± 4.55	0.16 ^{b} ± 0.03
Konjac flour	0.09 ^{b} ± 0.01	14.9 ^{b} ± 3.35	29.6 ^{b} ± 6.51	0.50 ^{b} ± 0.06
Pectin	0.11 ^{a} ± 0.01	7.13 ^{dc} ± 2.24	17.1 ^{bc} ± 1.95	0.41 ^{b} ± 0.11
Inulin	0.12 ^{a} ± 0.02	9.34 ^{c} ± 3.89	92.9 ^{a} ± 27.06	0.09 ^{b} ± 0.02

Note: The different superscript letters are significantly different at column (p < 0.05).

Table 4 Optical and color characteristics of the edible films

Prebiotic films	**L***	**a***	**b***	**Opacity**
Sodium alginate	85.05 ^{b} ± 0.34	-0.61 ^{b} ± 0.04	5.28 ^{a} ± 0.45	1.36 ^{b} ± 0.26
Gum arabic	84.54 ^{b} ± 0.75	-0.63 ^{b} ± 0.04	5.64 a ^{b} ± 0.29	0.64 ^{a} ± 0.09
Konjac flour	84.36 ^{b} ± 0.27	-0.68 ^{b} ± 0.02	6.33 ^{b} ± 0.05	0.93 ^{a} ± 0.06
Pectin	82.10 ^{a} ± 0.21	-0.39 ^{c} ± 0.08	12.12 ^{c} ± 0.77	1.36 ^{b} ± 0.32
Inulin	84.36 ^{b} ± 0.27	-0.83 ^{a} ± 0.04	6.33 ^{b} ± 0.05	0.90 ^{a} ± 0.14

Note: The different superscript letters are significantly different at column (p < 0.05).

3.2 Effect of prebiotics on *L. casei* survival

Drying process involving high temperature and change in osmotic pressure typically generated stress condition for microorganisms, caused microbial cell injury, and limited their survival (Soukoulis *et al*., 2014). In this study, each prebiotic film offered a different level of protection for *L. casei* n (Figure 1). Inulin film had the highest microbial survival (87.5%) followed by alginate film (83.6%), gum arabic film (80.3%), konjac film (80.0%), and pectin film (47.6%). Viable cultures in alginate, gum arabic, and konjac films were not significantly different (p>0.05). Burgain *et al*. (2014) reported that addition of bio-polymer shielded viable cells from osmotic force, water decrease, and oxidative stress. Therefore, polymer structure and conformation may be the key to the degree of protection. Glass transition phenomena of individual films was also another possible explanation for the improved survival of *L. casei* in prebiotic films during drying process (Fritzen-Freire *et al.,* 2012; Soukoulis *et al*., 2014).

Furthermore, pH of the film solution may also play an important role on microorganism reduction. Brink *et al.* (2006) performed a study on Lactic acid bacteria and found that solution with acidic pH (lower than optimum) had less microbial cell survival. Since the optimum pH range of *Lactobacillus* species was 5.5–6.0 (Tripathi and Giri, 2014), this might explain the lower survival of *L casei* in pectin film solution (pH 3.2) compared to other prebiotic film solutions (pH 5.0-5.6).

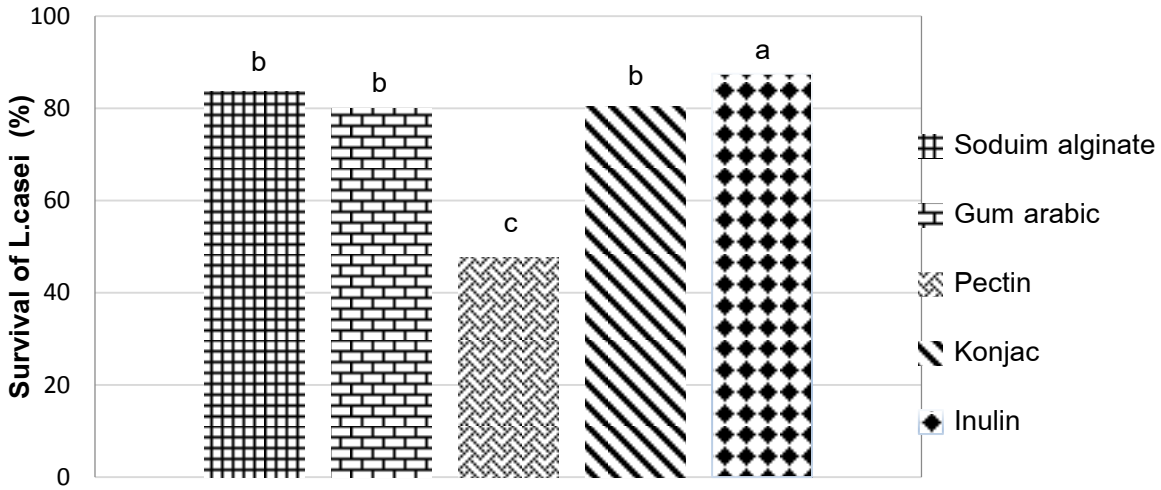

Figure 1 Percent survival of *L.casei* in dried films containing different prebiotics

3.3 Effect of storage temperature on survival rate of *L. casei*

According to FAO/WHO (2002), viable cultures of more than 10^6 CFU/g were required for probiotic efficiency. Therefore, in this study the same level was used for screening films for storage trials. All prebiotic films passed, except for pectin film (5.12 log CFU/g). Figure 2 shows a reduction in *L. casei* survival rate in the selected prebiotic films during a storage temperature of 4°C and room temperature for 20 day.

The stability of *L.casei* was affected by type of prebiotic and storage temperature. At room temperature, gum arabic film had the highest reduction rate followed by alginate film, inulin film, and konjac film, respectively. Shelf-life prediction was estimated to be 5 day or lower for all prebiotic films (Table 5). Betoret *et al.,* (2012) and Dong *et al.,* (2013) reported that moisture content and water activity influenced microbial cell lethality by controlling cell-structure and cell-water permeability. The lower moisture content and water activity led to lower chance of *L. casei* survival. At 4°C storage, konjac film had the highest reduction rate followed by inulin film, alginate film, and gum arabic film, respectively. The shelf life predictions were more than 100 day for all films, except konjac film. Soukoulis *et al*. (2017) reported chilling storage retarded both enzyme and biochemical reactions, including lipid-oxidation. At this temperature, alginate film was best in retaining *L. casei*.

Table 5 Survival of *L.casei* during storage at chilling and room temperature at controlled relative humidity and estimated shelf life (d) R^2 displays correlation coefficient

Prebiotic films	Chilling temperature (4°C)			Room temperature (28±2°C)		
	K (d^{-1})	Shelf life	R^2	K (d^{-1})	Shelf life	R^2
Alginate	0.03^{b} ± 0.01	130	0.87	0.98^{ab} ± 0.01	4.55	0.95
Gum arabic	0.03^{b} ± 0.01	116	0.87	1.01^{a} ± 0.01	5.04	0.90
Konjac	0.05^{a} ± 0.01	67	0.90	0.96^{b} ± 0.01	4.20	0.98
Inulin	0.04^{ab} ± 0.01	103	0.97	0.99^{ab} ± 0.01	4.54	0.96

Note: The different superscript letters are significantly different at column (p<0.05).

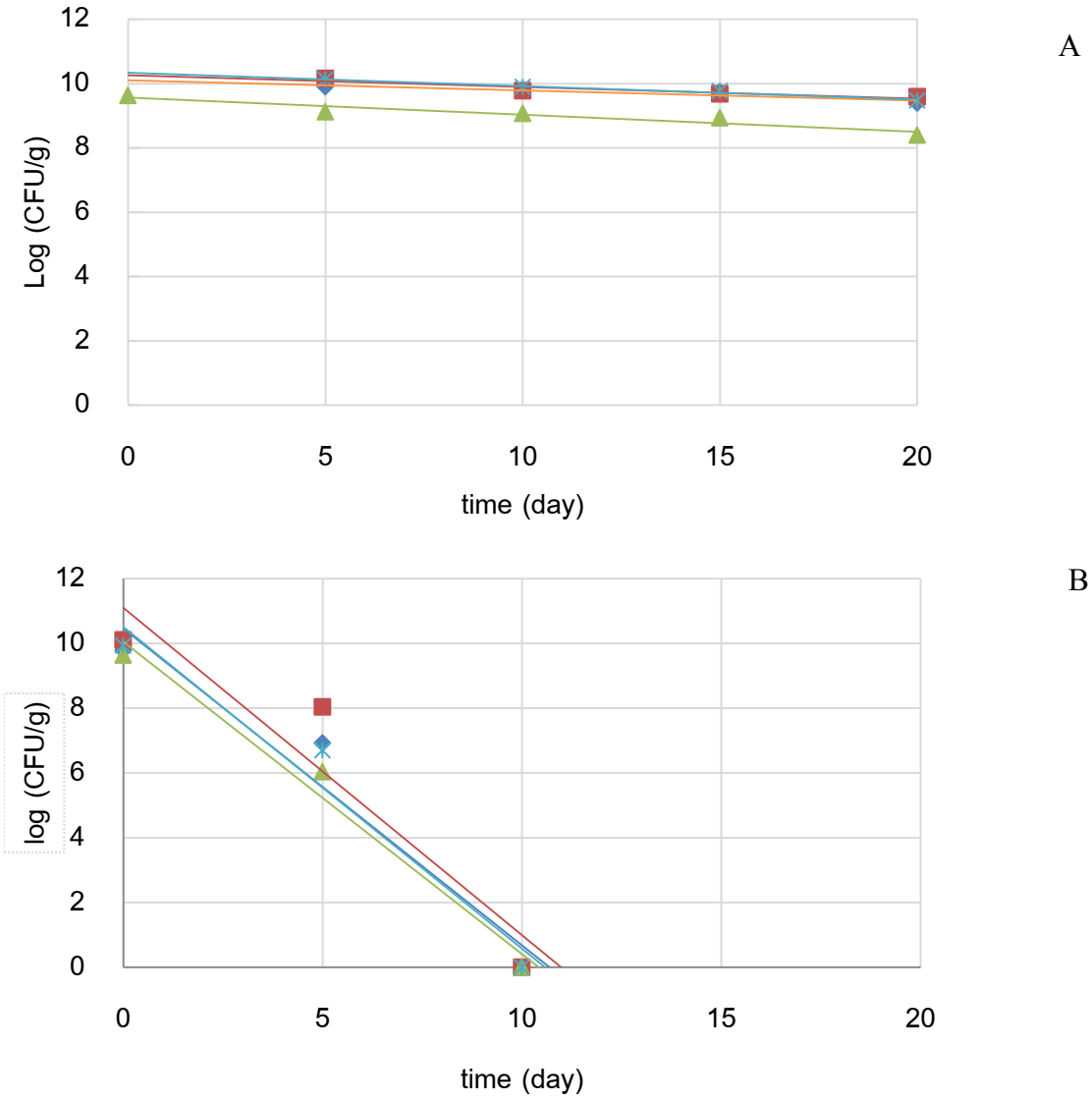

Figure 2 Reduction rate of *L. casei* in prebiotic films during storage A: chilling (4°C) and B: room temperature (28°C±2) ♦: alginate ▲: gum arabic ■: konjac ¥: Inulin

4. Conclusion

Type of prebiotics influenced both film characteristics and survival of probiotic *L. casei* in edible films. Overall alginate film had the best performance. Storage temperature greatly affected the number of viable probiotics in tested prebiotic films. At room temperature, all prebiotic films were able to maintain sufficient concentration of *L casei* to perform the function of probiotics (more than 10^6 CFU/g) up to 5 day. While lowering storage temperature to 4°C extended probiotic shelf life 16–28 times longer. This study also confirmed the benefits of symbiotic edible films and offered potential prebiotic options which could be further applied to food applications.

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