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Viability of microencapsulated *Lactobacillus casei* in synbiotic mayonnaise

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Abstract

In this study, whey protein, maltodextrin and GOS (Galacto-oligosaccharides) used as microencapsulating agents to protect *Lactobacillus casei* during spray-drying and mayonnaise storage. The morphology of microcapsules, pH charges, the survival rate during mayonnaise storage as well as survival in simulated gastric fluid (SGF) and intestinal fluid (SIF) was tested in this study. The results indicated that whey protein showed a protective effect better than maltodextrin during spray-drying. The particles showed spherical shape and typical concavity of all samples and encapsulating agents were not affected by the size and surface structure of particles. The pH charges were not significantly different in all mayonnaise samples in this test. The viability of free cell *L. casei* after 6 weeks storage was significant decrease about 4 log CFU/g compared to 1.55 to 3.27 log CFU/g in the mayonnaise samples containing microcapsules in which maltodextrin showed the lowest of *L. casei* survival rate. In SGF and SIF conditions, maltodextrin act as prebiotic sufficiently which do not need adding GOS. The combination of whey protein and maltodextrin in which maltodextrin plays a role as supporting agents for the spray-drying process as well as prebiotic potential, while whey protein with high buffer property which enhancing the survival rate of *L. casie* in low pH.

1. Introduction

Probiotic has been defined as "Live microorganisms (bacteria or yeasts), which when ingested or locally applied in sufficient numbers confer one or more specified demonstrated health benefits for the host" (FAO/WHO, 2001). Because of their health benefits, incorporation of probiotic bacteria into food to enhance the therapeutic value of food products are very interesting, such as yogurt, mayonnaise. (Ali and Esam, 1998; Maryam *et al.*, 2012; Adja *et al.*, 2014). Addition, Prebiotics are non-digestible dietary ingredients that benefit the host by selectively stimulating the growth and/or activity of beneficial bacteria in the colon, whereas synergistic combinations of probiotic and prebiotics are called synbiotics (Michael *et al.*, 2008). To achieve the health benefits, however, probiotic bacteria must be stable in the product as well as survive in large numbers through the digestive tract, to the appropriate location and have beneficial effects for the host (Roy, 2005). The research on probiotic microencapsulated techniques which enhance the viability of probiotic bacteria in adverse conditions is increasing and getting a lot of attention today. The technique of microencapsulation was used commonly in the previous study include extrusion, emulsion, and spray drying techniques which improve the viability of probiotic bacteria in adverse conditions compare to free cells (Won *et al.*, 2001; Akalin *et al.*, 2007; Fabiane *et al.*, 2012; Kartheek *et al.*, 2013). In particular, spray drying technique could make particles which are small size, unaffected organoleptic and easy application on an industrial scale with low cost (Adja *et al.*, 2014). However, it is surprising that in spite of well documented of extrusion and emulsion technique to protect the probiotic bacteria in mayonnaise, there is no report in the literature on using the spray-drying technique to protect probiotic bacteria in mayonnaise. In the previous studies, whey protein, skim milk and maltodextrin showed effectiveness in improving the viability of probiotic

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bacteria (Carlise *et al.*, 2012; Fabiane *et al.*, 2012; Adja *et al.*, 2014). The survival rate of probiotic is not only affected by spray drying conditions, carrier but also affected by strain probiotic using (Paéz *et al.*, 2012). Therefore, evaluation of the role of the carrier should be carried out on the same probiotic bacteria as well as the spray drying condition. In the present study, *Lactobacillus casei* VTCC AS 186 was microencapsulated by spray-drying in which whey protein, maltodextrin and prebiotic GOS (Galacto-Oligosaccharide) as wall material. The microcapsules were evaluated the morphology of particles and adding to mayonnaise. The pH charges, the survival rate of *L. casei* during mayonnaise storage as well as survival in simulated gastric fluid (SGF) and intestinal fluid (SIF) were tested in this study.

2. Materials and methods

2.1 Bacterial strain and culture conditions

Lactobacillus casei VTCC AS 186 were harvested from 500 ml of a 20-h culture (late log phase) by centrifugation at 5000 rpm. Then, the cells were used in the microencapsulation process in the next step.

2.2 Spray-drying of L. casei

Microencapsulated *L. casei* was carried out as assay previously described (Adja *et al.*, 2014) with slight modifications. Briefly, whey protein (Meggle, Germany), maltodextrin (Roquette, France), GOS (Galacto-oligosaccharides; PureBulk, USA) were used as protective agents. The samples, including W (whey protein 10% ww/v), M (maltodextrin 10% w/v), WM (whey protein 8% w/v and maltodextrin 2% w/v), WG (whey protein 8% w/v and GOS 2% w/v) and MG (maltodextrin 8% w/v and GOS 2% w/v) were evaluated in this study.

The spray-drying of cell suspensions was performed in a laboratory scale spray dryer (Mini spray dryer SD-06AG, Labplant UK), using the following process parameters: inlet temperature = 110^{o}C, outlet temperature = 60-65^{o}C, spraying rate = 4.5 ml/min. The survival rate of *L. casei* after spray-drying was evaluated by the following equation:

$$Survival\ (\%) = \frac{\sum logCFU_{after\ spray-drying}}{\sum logCFU_{before\ spray-drying}} \times 100\%$$

The preparations after spray-drying were visualized with a scanning electron microscope (SEM). Microencapsulating particles size were evaluated by HORIBA LA-920 machine

2.3 Mayonnaise preparation

Mayonnaise was prepared using the following formula: soybean oil 74%, egg 14%, vinegar (5% w/v) 10%, salt 1%, sugar 1% and white pepper 0.2%. Sugar and vinegar mixed together and then all other ingredients except oil were added and stirred homogeneously. The oil was added very slowly, while stirring homogeneously. Finally, preparations were added into mayonnaise and storage at 4^{o}C.

The pH of mayonnaise samples and the survival rate of *L. casei* during storage were determined after storing immediately as well as the end of every 7 days until 42 days of storage at 4^{o}C.

2.4 Survival rate of free and microencapsulated bacteria in simulated gastric condition

The survival rate of free and microencapsulated bacteria incorporated into mayonnaise was tested for acid and bile salts tolerance after 42 days of storage. Simulated gastric fluid (SGF) consisted of 9 g/l of sodium chloride containing 3 g/l of pepsin (Himedia) was adjusted to pH 2.5 with 5.0M HCl. Simulated intestinal fluid (SIF) consisted of 9 g/l sodium chloride containing 3ml/l of bile salts was adjusted to pH 6.5 with 5.0M NaOH. The viability of *L. casei* in mayonnaise samples was evaluated after 2 hours incubated in SGF and 4 hours incubated in SIF. The samples containing free *L. casei* were used as a control. The survival rate of *L. casei* was evaluated by the following equation:

$$Survival\ (\%) = \frac{\sum logCFU_{after\ incubation}}{\sum logCFU_{before\ incubation}} \times 100\%$$

2.5 Statistical analysis

The data were subjected to analysis of variance (ANOVA) using Statgraphics 15 followed by Tukey test to compare means, with a significance level of 5% when the significant difference between treatments was noted. All tests were performed in triplicate and the data expressed as means ± standard deviation.

3. Results and discussion

3.1 The effect of spray-drying process on the viability of L. casei

The viability of *L. casei* before and after spray drying is shown in Table 1. The results showed that the survival rate of *L. casei* after spray drying in five samples including W, M, WM, WG, and MG was 82.88%; 76.07%; 82.11%; 80.68% and 77.83%. The viability of *L. casei* in M sample and MG sample were lower compared to the other samples in this test. Figure 1

shows the SEM micrographs of *L. casei* preparations with different microencapsulating agents. The particles showed spherical shape and typical concavity of all samples. Microcapsules from all samples are various sizes from 3÷12 µm and an average size of 6.2÷6.5 µm.

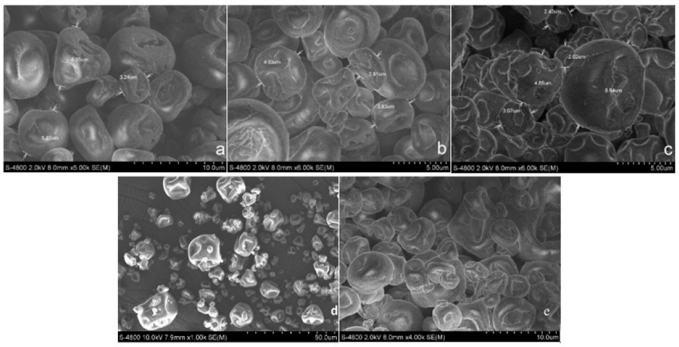

Figure 1. Scanning electron micrograph and surface morphology of preparations (Figure 1a, 1b, 1c, 1d and 1e: WM, W, M, MG and WG samples)

The phenomenon of the concave surface of the particle has been reported in previous studies. This phenomenon depends on spray-drying temperature and normal drying process, causing dents on the surface and the surface structure of microcapsules is not affected by the microencapsulating agents (Carlise *et al.*, 2012). In a study by O'Riordan *et al.* (2001) showed that the sizes of the microcapsules which modified starch used as the wall material were approximately 5 µm on average. The microcapsules size about 5.6 ÷ 5.9 µm with natural starch as wall material was also reported by Sandra *et al.* (2014). The research team indicated that the size of the microcapsules was not affected by the wall material concentration and inlet temperature during the spray-drying process (Sandra *et al.*, 2014). In the present study showed that different encapsulating agents were not affected to the size and surface structure of microcapsules (Figure 1). The size of microcapsules less than 10 µm is an ideal size to not affect organoleptic when adding to food (O'Riordan *et al.*, 2001).

The survival rate of probiotic bacteria plays an important role. The amounts of microcapsules which need to add into food products would be decreased when the viability of probiotic during spray-drying is high. In the spray drying, the high inlet and outlet temperature affect significantly to the viability of probiotic bacteria (Sandra *et al.*, 2014). The survival rate of probiotic bacteria reduced nearly 55% when the inlet temperature was 130^{o}C (Kartheek *et al.*, 2013) and up to 80% when the inlet temperature was 150^{o}C (Sandra *et al.*, 2014) but the survival rate would be up to 81% when the inlet temperature was 100^{o}C (Kartheek *et al.*, 2013). Adja *et al.* (2014) who found that higher spray drying temperatures leading to reduce the viability of *B. animalis* BI-07 and darker color products (Adja *et al.*, 2014). Similarly, the outlet temperature increase from 70^{o}C to 100^{o}C leading to the survival rate of *L. rhamnosus* reduce from 70% to 10% (Ananta, 2005). In the present study, an inlet temperature of 110^{o}C showed good protective effect during spray drying (Table 1). The low inlet temperature (below 100^{o}C) makes the product yield that was not dry due to high moisture (O'Riordan *et al.*, 2001). To limit the impact caused by the high temperature of the spray drying process, the choice of microencapsulating agents is very important. Sandra *et al.* (2014) reported that aggregation of natural starch in spray drying process improved the survival rate of *L. rhamnosus* better than inulin (Sandra *et al.*, 2014). Similar, spray drying process resulted in denaturation and aggregation of whey protein, leading to formed probiotic protecting walls during storage condition (Millqvist, 2001). According to Kartheek *et al.* (2013) maltodextrin showed protective effect which can reduce the caking and stickiness to the spray dryer's walls (Kartheek *et al.*, 2013). Adja *et al.* (2014) indicated that maltodextrin, milk protein and fat use as microencapsulating agents may have protected the microorganisms during the drying process (Adja *et al.*, 2014). In the present study, the viability of *L. casei* in M sample was lower than W and WM samples. This result revealed that whey protein showed protective effect better than maltodextrin (Table 1). The amount of powder from WM was slightly higher than the W sample (data not shown). However, the survival rate of *L.casei* was not significantly different between two groups (p>0.05) (Table 1).

Table 1. The average particle size and viability of *Lactobacillus casei* during spray-drying

Wall material (w/v)	Total probiotic counts Log CFU		Average particle size (µm)
	Before spray-drying	After spray-drying	
W	13.55 ± 0.18^{a}	11.23 ± 0.11^{a}	6.5
M	13.73 ± 0.13^{a}	10.53 ± 0.17^{b}	6.3
WM	13.64 ± 0.10^{a}	11.21 ± 0.12^{a}	6.2
WG	13.51±0.16^{a}	10.90±0.18^{b}	6.3
MG	13.49±0.18^{a}	10.50±0.17^{b}	6.2

Results are expressed as mean ± SD; (n = 3)

^{ab} Means in the same column followed by different superscripts are significantly different (p < 0.05). W: whey protein 10%; M: maltodextrin 10%; WM: whey protein 8% + maltodextrin 2%; WG: whey protein 8% + GOS 2%; MG: maltodextrin 8% + GOS 2%

3.2 pH changes and viability of L. casei in mayonnaise during storage

The change in the population of non-microencapsulated versus micro-encapsulated *L. casei* is shown in Figure 2. It was observed that, the viability of free cell *L. casei* after 6 weeks of storage was significant decreased about 4 log CFU/g compared to 1.55 to 3.27 log CFU/g in the mayonnaise samples containing microcapsules in which viable *L. casei* in M sample was lowest and there were no significant differences (p>0.05) among W, WG and WM samples (Figure 2).

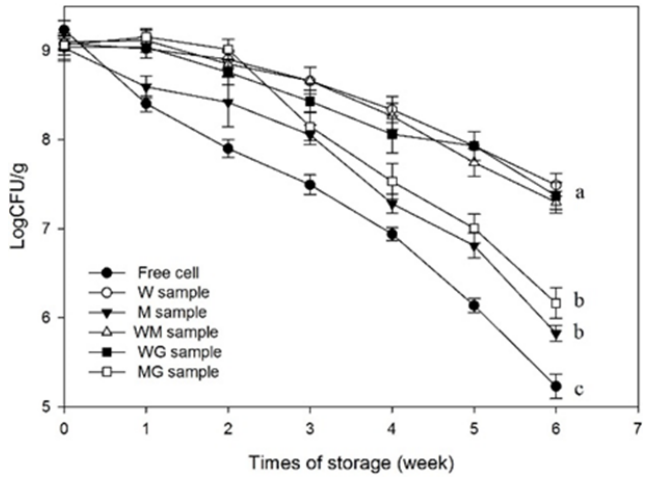

Figure 2. The viability of *L. casei* in mayonnaise during storage ([abc] Means in the same column followed by different superscripts are significantly different (p < 0.05)). W sample: whey protein 10% (w/v); M sample: maltodextrin 10% (w/v); WM sample: whey protein 8% (w/v) + maltodextrin 2% (w/v); WG sample: whey protein 8% (w/v) + GOS 2% (w/v); MG sample: maltodextrin 8% (w/v) + GOS 2% (w/v)

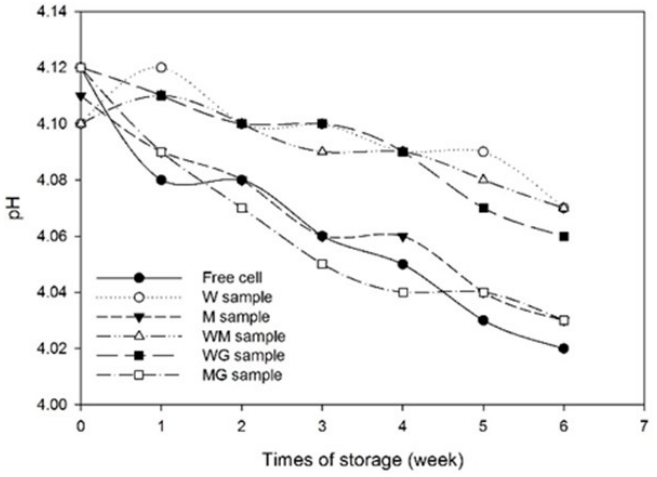

Figure 3. The pH changes during storage
W sample: whey protein 10% (w/v); M sample: maltodextrin 10% (w/v); WM sample: whey protein 8% (w/v) + maltodextrin 2% (w/v); WG sample: whey protein 8% (w/v) + GOS 2% (w/v); MG sample: maltodextrin 8% (w/v) + GOS 2% (w/v)

The pH changes during storage are shown in Figure 3. The initial pH values for all the samples were about 4.10 to 4.12 and there was a decrease in pH during storage for all the samples. After 7 weeks of storage, the final pH of mayonnaise were 4.07; 4.03; 4.07; 4.06; 4.02 and 4.03 respectively for W; M; WM; WG; MG and free cell samples. The results showed that whey protein could help maintain the pH of the mayonnaise during storage better than maltodextrin.

The pH changing during storage was reported in previous studies. Maryam *et al.* (2012) reported that pH changing of mayonnaise containing free cells or encapsulated cell were not significantly different (p>0.05) after 30 days of storage (Maryam *et al.*, 2012). In the present study, however, we are in good agreement with Khalil *et al*. (1998) and Ding *et al.* (2008), who concluded that the final pH of mayonnaise containing encapsulated particles was higher than samples containing free cell (Ali *et al.*, 1998; Ding *et al.*, 2008). In low pH condition, free probiotic bacteria could still utilize carbohydrates and produce small amounts of organic acids leading to lowering the pH of the product during storage (Ding *et al.*, 2008). Encapsulation technique with the gel matrix may reduce metabolic activity of probiotic in mayonnaise and yogurt products, thereby pH was higher than the pH of the sample with non-encapsulated cells (Akalin *et al.*, 2007; Ding *et al.*, 2008).

In previous studies, Probiotic bacteria have also shown no ability to survive in mayonnaise (Maryam *et al.*, 2012; Ali *et al.*, 2014). It is due to intrinsic properties of mayonnaise such as low pH, refrigerated (4°C) storage. These factors have caused cell death during storage. Khalil *et al.* (1998) showed that none of non-encapsulated *B. bifidum* in mayonnaise survived after 2 weeks of storage, whereas the amount of encapsulated cells by emulsion technique was only reduced by about 1.4 log CFU/g after 6 weeks of storage (Ali *et al.*, 2014). Similarly, the viability of encapsulated cell of emulsion technique showed higher than non-encapsulated cells in mayonnaise containing *L. casei* and *B. bifidum* about 2 log and 5 log cycles respectively after 30 days of storage (Maryam *et al.*, 2012).

In the present study, the viability of *L. casei* in microcapsules was 0.8 to 2.41 log CFU higher than the free cell (Figure 2). The result indicated that *L. casei* could resist the low pH and the viability of probiotic bacteria in mayonnaise was depend on the type of strain and microencapsulated technique. Emulsion technique with bigger size particles which can protect probiotic bacteria from the penetration of ion H^+ better than spray

drying technique. However, microencapsulating agents play an important role in protecting probiotics from adverse conditions. In the present study showed that the amount of microencapsulated *L. casei* in W and WM samples were above recommended for probiotic food after 6 weeks storage which equal to or greater 6 log CFU/g (Roy, 2005). This suggests that whey protein could improve the viability of probiotic during storage.

3.3 Viability of L. casei in SGF and SIF after 6 weeks of storage

After 6 weeks of storage, the mayonnaise samples were tested for its survival in simulated gastric conditions. The effect of SGF (pH 2.5) and SIF on the viability of free cell and microencapsulated *L. casei* were shown in Table 2. There were significant differences ($p<0.05$) between the free cell and microencapsulated *L. casei*. The viable cell numbers of free *L. casei* rapidly decreased from 100% to 49.87% after 4 hours incubated in SIF and none of the free cells survived after 2-hour incubation in SGF (Table 2). In W, M; WM; WG; MG samples, the viability *of L. casei* remained 75.74%; 69.65%; 78.41%; 75.35%; 75.63% after 4 hours incubation in SIF and 55.20%; 33.92%; 68.83%; 67.35%; 45.70% after 2 hours incubation in SGF respectively (Table 2).

Table 2. Viability of *Lactobacillus casei* in SGF and SIF after 6 weeks of storage

Mayonnaise Sample	*Lactobacillus casei* cell counts in mayonnaise (log CFU/g)				
	Initial	After incubation in SIF	Survival rate (%)	After incubated in SGF	Survival rate (%)
W	7.49 ± 0.12	5.67 ± 0.12	75.74 ± 0.69[a]	4.13 ± 0.14	55.20 ± 2.35[a]
M	5.82 ± 0.09	4.05 ± 0.15	69.65 ± 1.62[b]	1.98 ± 0.16	33.92 ± 2.26[b]
WM	7.30 ± 0.12	5.72 ± 0.12	78.41 ± 2.3[a]	5.02 ± 0.14	68.83 ± 0.90[c]
WG	7.37±0.15	5.55± 0.13	75.35± 0.81[a]	4.96±0.15	67.35±1.19[c]
MG	6.16±0.17	4.66± 0.24	67.35± 1.19[b]	2.81±0.17	45.71±2.68[b]
Free cell	5.23 ± 0.14	2.61 ± 0.18	49.87 ± 2.06[c]	0	0[d]

Results are expressed as mean ± SD; (n = 3)

[abc] Means in the same column followed by different superscripts are significantly different ($p < 0.05$)

W sample: whey protein 10% (w/v); M sample: maltodextrin 10% (w/v); WM sample: whey protein 8% (w/v) + maltodextrin 2% (w/v); WG sample: whey protein 8% (w/v) + GOS 2% (w/v); MG sample: maltodextrin 8% (w/v) + GOS 2% (w/v)

O'Riordan *et al.* (2001) indicated that microencapsulated *B. bifidum* by spray drying with starch (10% w/v) as wall materials was not improved the viability of *B. bifidum* in SGF and SIF compared to the free cell (O'Riordan *et al.*, 2001). However, Fabiane *et al.* (2012) showed that the protective effect of whey protein which used as microencapsulating agents on *Bifidobacterium* in SGF condition better than non-microencapsulated. Skim milk and whey protein have the ability to limit the influence of H^+ ions due to high buffer properties (Won *et al.*, 2001; Akalin *et al.*, 2007). Combination of alginate 3% (w/v) and skim milk 0.6% (w/v) maintained pH of preparations (above 4) for 20 minutes incubating in pH 1.5 (Won *et al.*, 2001). Similar, Akalin *et al.* (2007) indicated that high buffer properties of whey protein concentration which adding to reduced-fat yogurts could make pH value higher than control samples during 28 days of storage (Akalin *et al.*, 2007). This suggests that the viability of probiotic bacteria depends on the type of microencapsulated agents.

In the present study, it is interesting to note that the viability of microencapsulated *L. casei* in WM, W and WG samples were no significant difference ($p>0.05$) during storage (Figure 2), but the survival rate of *L. casei* in W sample was significantly lower ($p<0.05$) than WM and WG samples after 2 hours incubation in SGF (Table 2). Karrtheek *et al.* (2013) suggested that maltodextrin plays a role as supporting agents for the spray-drying process as well as prebiotic potential (Kartheek *et al.*, 2013). According to Iyer *et al.* (2005), probiotic bacteria have an efficient metabolic mechanism to prebiotic than simple sugar (Iyer and Kailasapathy, 2005). It's suggested that, maltodextrin act as prebiotic sufficiently which do not need to add GOS. The viability of *L. casei* during spray-drying in which maltodextrin act as wall material showed better than GOS (Table 1). Therefore, the combination of whey protein and maltodextrin as wall materials, eventually leading to has dual efficiency: First, the denaturation and aggregation of whey protein leading to formed probiotic protecting walls during spray-drying process and storage condition; Second, maltodextrin's role as prebiotic potential and high buffer properties of whey protein may improve the viability of *L. casei* in SGF and SIF conditions.

4. Conclusion

In conclusion, the present study indicated that the viability of *L. casei* was affected significantly by microencapsulated agents during spray-drying, storage and in SGF and SIF conditions. The size and surface structure of microcapsules were not affected by

microencapsulating agents. The viable counts of *L. casei* with whey protein as wall material was higher than maltodextrin. Prebiotic enhanced significantly the viability of *L. casei* in SGF and SIF in which maltodextrin act as prebiotic sufficiently which do not need adding GOS. Maltodextrin's role not only as a wall material in microencapsulation but also as a prebiotic potential, eventually leading to a combination of whey protein and maltodextrin provided an excellent protection of *L. casei* cells from adverse conditions.

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References

Adja, C.L.M., Marcelo, T., Alexandre, U., Roberta, T.P.C. and Carmen, S.F.T. (2014). Structural characterisation and cell viability of a spray dried probiotic yoghurt produced with goats' milk and *Bifidobacterium animalissubsp*. lactis (BI-07). *International Dairy Journal*, 39, 71–77.

Akalin, A.S., Gönç, S., Unal, G. and Fenderya, S. (2007). Effects of fructooligosaccharide and whey protein concentrate on the viability of starter culture in reduced-fat probiotic yogurt during storage. *Journal of Food Science*, 72, 222-227.

Ali, H.K. and Esam, H.M. (1998). Alginate Encapsulated *Bifidobacteria* Survival in Mayonnaise. *Journal of Food Science*, 63(4), 702-705.

Ananta, E., Volkert, M. and Knorr, D. (2005). Cellular injuries and storage stability of spray-dried *Lactobacillus rhamnosus* GG. *International Dairy Journal*, 15, 399–409.

Carlise, B.F., Elane, S.P., Renata, D.M.C. A., Stephanie, S.P., Aureanna, N.N. and Fabio, S.M. (2012). Microencapsulation of Bifidobacteria by spray drying in the presence of prebiotics. *Food Research International*, 45, 306–312.

Ding, W.K. and Shah, N.P. (2008). Survival of Free and Microencapsulated Probiotic Bacteria in Orange and Apple Juices. *International Food Research Journal*, 15, 219-232.

FAO/WHO. 2001. Experts' Report. Health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria. Rome: FAO

Fabiane, P.D.C.C., Carina, D.R.E.S., Carlise, B.F.F., Juliana, G.L. and Ernani, S.S.A. (2012). *Bifidobacterium* Bb-12 microencapsulated by spray drying with whey: Survival under simulated gastrointestinal conditions, tolerance to NaCl, and viability during storage. *Journal of Food Engineering,* 113, 186–193.

Iyer, C. and Kailasapathy, K. (2005). Effect of co-encapsulation of probiotics with prebiotics on increasing the viability of encapsulated bacteria under in vitro acidic and bile salt conditions and in yogurt. *Journal of Food Science,* 70, M18–M23.

Kartheek, A. and Valérie, O. (2013). Optimization of microencapsulation of probiotics in raspberry juice by spray drying. *LWT - Food Science and Technology*, 50, 17-24.

Maryam, F., Nima, M., Hamed, A., Mohammad, A.K.Z., Fariba, Z.H. and Kasra, B. (2012). Effect of microencapsulation plus resistant starch on survival of *Lactobacillus casei* and *Bifidobacterium bifidum* in mayonnaise sauce. *African Journal of Microbiology Research*, 6(40), 6853-6858.

Michael, D.V. and Schrezenmeir, J. (2008). Probiotics, Prebiotics, and Synbiotics. *Advance in Biochemical Engineering/Biotechnology*, 111, 1–66

Millqvist, F., Ulla, E. and Björn, B. (2001). Surface composition of spray-dried milk protein-stabilised emulsions in relation to pre-heat treatment of proteins. *Colloids and Surfaces B: Biointerfaces*, 21, 47-58.

O'Riordan, K., Andrews, D., Buckle, K. and Conway, P. (2001). Evaluation of microencapsulation of a Bifidobacterium strain with starch as an approach to prolonging viability during storage. *Journal of Applied Microbiology*, 91, 1059–1066.

Roy, D. (2005). Technological aspects related to the use of bifidobacteria in dairy products. *Lait*, 85, 39–56.

Sandra, V., Avila-Reyes., Francisco, J.G.S., María, T.J., María, F.S.M.G. and Luis, A. B.P. (2014). Protection of *L. rhamnosus* by spray-drying using two prebiotics colloids to enhance the viability. *Carbohydrate Polymers*, 102, 423–430.

Won, K.Y., Yim, T.B., Lee, K.Y. and Heo, T.R. (2001). Effect of Skim Milk-Alginate Beads on Survival Rate of Bifidobacteria. *Biotechnology Bioprocess Engineering*, 6, 133-138.

