Vol.4, No.5B, 78-83 (2013)
doi:10.4236/as.2013.45B015

Survival of encapsulated probiotics through spray drying and non-refrigerated storage for animal feeds application

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Received 2013

ABSTRACT

The objective of this study is to verify potential of various types of microorganisms during spray drying and non-refrigerated storage that can be enhanced substantially by selecting suitable protective colloids. Four selected probiotics tested are Lactbacillus plantarum B13 and B18, which are the bacteria probiotics and Kluyveromyces lactis and Saccharomyces blouradii, non-bacteria probiotics. Two levels of experiment occur starting with formulation study of encapsulation agent followed by the viability study of different probiotics after spray dry and two weeks nonrefrigerated storage. The formulation of 30% of gum Arabic, 15% of gelatin and 45% of coconut oil can homogenize well at least for two hours and can produce acceptable dried product (below 4% of moisture content) at low outlet temperature (70℃ - 75℃). K. lactis, S. blouradii gives 2.57% and 2.4% of viability percentage after spray drying process and 25.84% and 2.04% after two weeks nonrefrigerated storage respectively. The colonies of non-probiotics bacteria after both conditions are between 1010 and 106 cfu/mL which is among the accepted level for industrial application. However, the survival of probiotics in a spray-dried form during non-refrigerated storage is higher at low of moisture content compared to others.

Keywords: Probiotics; Survival Rate; Spray Drying; Nonrefrigerated Storage

1. INTRODUCTION

Probiotics have been recently defined as “live microbes which transit to the gastro-intestinal tract and having positive effects to the health for the consumers [1]. It have been applied in aquaculture for controlling diseases, enhancing the immune response, supplementing or even in some cases replacing the use of anti microbial. The use of probiotics as farm animal feed supplements dates back to the 1970' and originally incorporated into feed to increase the animal's growth and to improve its health by increasing its resistance to disease. It was assumed that the effectiveness of probiotics were related to the gastroin-testinal tract and effects on incidence of diarrhea and other gut infections. According to literatures show in **Table 1**, there are some probiotic strains which can give positive effect to various types of animals.

Some of other probiotics which used in animal feed are microscopic fungi such as strain of yeasts. There are billion strains of yeasts that are supposed to be explored in animal feed study. The strains like *Saccharomyces boulardii* and *Kluveromyces lactis* are having high potential as a probiotic that can improve animal health. Yeast seemed to facilitate increased mobilization of body reserves and to increase milk fatty acid production of ruminants. Therefore, it was possible to apply yeast’s strain as probiotics strain in powder form of animal feed in line with the effectiveness in animal health. For example camel calves can improve weight gain, average daily gain and feed utilizations after apply yeast as supplementation diet in different forms [9].

It is known that probiotics were very useful for animal but the preparation of animal feed that containing probiotic especially in powder form are not easy. To ensure the amount of probiotics culture required can achieve the target; some of the factors are to be tested first in order to increase stability or viability of the probiotic products. Encapsulation agent is the formulation of single or combination of wall material that can resist the condition along the journey. Encapsulation is a process in which tiny droplets or particles are wrapped with a protective

coating yielding capsules for countless application. Several methods of encapsulation of probiotic have been reported by [10]. The objective of the research is to demonstrate the various types of probiotics microorganisms during spray drying and storage at room temperature by selecting suitable protective colloids as an encapsulation agent.

2. MATERIALS AND METHODOLOGY

2.1. Stability of the Feed Composition

The stage one of this experiments was conducted by the conventional try and error method. The several feed formulations were choose which with high advantages in spray dryer process and animal feed industry. At the first stage, the homogenize mixture without probiotic cell was hold for 2 hours to test stability of the mixture by using homogenizer (Heidolth, DIAX 900, Germany). This is holding time of the feed formulation in the feed bottle during spray dry (Pilot Spray Drying Plant PSD-00, Hemray Enterprise, Bombay). The homogenized mixtures were proceed for drying process in the spray dryer at inlet temperature 110℃ and outlet temperature of air 70℃- 75℃ and should give the moisture content below 4%. Details of the various wall materials used are shown in **Table 2**.

Table 1. Some probitiocs strain with the benefits to animals.

Animal	Probiotics	Functions	References
Fresh water fish	Lactobacilli spp. *Lactobacillus sakei*)	Enhance the host protection against pathogens.	[2]
Broiler chicken	*Lactobacillus reuteri* (33%) *Lactobacillus crispatus* (18.7%) *Lactobacillus salivarius* (12.3%)	To maintain balanced microbiota and to reduce potential pathogens	[3]
Pig	*Enterococcus faecium*	Great production of specific antibodies against *salmonella enteric*	[4]
Broiler chicken	*Lactobacillus acidphilus* and *Streptococcus faecium*	Reducing population of *Clostridium perfringers* which can lead to necrotic enteritis prevention	[5]
Fish	*Lactobacillus plantarum*	To promote growth and enhance immunity and resistance against *Streptococcus sp*. and an *iridovirus*	[6]
Piglets	*Lactobacillus plantarum*	Improve growth performance without affecting the gastrointestinal ecology.	[7]
Young dairy calves	*Lactobacillus acidophilus* and *Lactobacillus plantarum*	Body weight increased	[8]

Table 2. Formulation of emulsion.

Formulations	Ingredient
A	30% gum Arabic, 15% gelatin, 45% coconut oil
B	30% gum Arabic, 15% gelatin, 45% lecithin
C	30% gum Arabic, 15% lecithin, 45% coconut oil
D	15% gelatin, 30% lecithin, 45% coconut oil
E	15% gelatin, 30% maltodextrin, 45% coconut oil
F	30% maltodextrin, 15% lecithin, 45% coconut oil
G	15% gelatin, 30% maltodextrin, 45% lecithin
H	15% gum Arabic, 45% gelatin, 7.5% lecithin, 67.5% coconut oil
I	45% gum Arabic, 15% gelatin, 7.5% lecithin, 67.5% coconut oil

2.2. Survival of Different Types of Probiotics During Spray Dry

The second stage of study was focused on the viability of different type of probiotics on the spray dry condition and storage at 25℃ in two weeks. Four different types of probiotics microorganisms were chosen to investigate the viability, stability with wall material used, condition of spray dry, and two weeks non-refrigerated condition. The chosen probiotics were *Lactobacillus plantarum B13* and *B18*, *Kluyveromyces lactis* and *Saccharomyces blouradii*. The mixtures of wall materials were mixed with deionised water and autoclave a 121℃ for 15 minutes (water phase). The coconut oil was sterilized filter and then the probiotic culture was dispersed into coconut oil using ho- mogenizer at the lowest speed (3000 rpm) for 10 - 15 minutes (Zentrifugen, Hettich, D-78532,Tuttlinge n, Ger- many). Then, the coconut oil were mixed up with water phase mixture by using homogenizer at 3000 rpm for 10 minutes in sterilized condition.

2.3. Viability Analysis

1 mL of each formulation was transfer into a universal bottle and 9 mL sterilized water is added. The solution is serially diluted until dilution factor of 10^{10}. 0.01 mL is taken from all the dilution factors and transferred into the petri dish contain medium agar for each type of probiotic respectively. After that, petri dish is incubate for 24 hours at 37℃ for *Lactobacillus plantarum* and *Kluyveromyces lactis* and 30℃for 24 hours for *Saccharomyces blouradii*. After 24 hours, the number of colony forming is calculated with naked eyes. The moisture content of spray dried powders was determined by oven drying at 102℃ until constant weight was obtained.

3. RESULTS AND DISCUSSION

3.1. Formulations study of Encapsulation Agent

Try and error method carried out to get the suitable emulsion formulation in order to get the good product. Based on previous studies, four basic materials which can contribute on production of good powder product were selected. After doing some try and error formulation and stability checked, the observation were shown in **Table 3.**

After doing the observation, the stability test resulted that Formulation A and Formulation D show a highest potential in maintaining the stable characteristics after keeping for two hours. The maximum duration of spray drying is about two hours. So, at least the formulation can homogenized well at the duration time is more than enough. Lecithin was an economic an effective wall material but unfortunately it cannot homogenize well with other wall material selected. The ability of solid percentage used in the solution has been checking up after selection of the suitable emulsion formulation successful. Formulation A and Formulation D were the only two formulations that have been selected to the next part of study.

Table 3. Stability of different of encapsulation agent formulations.

Formulations	Ingredients	Stability Observations
A	30% gum Arabic, 15% gelatin, 45% coconut oil	After homogenize, milky white and stable solution produced
B	30% gum Arabic, 15% gelatin, 45% lecithin	After homogenize, 70 percent from solution precipitated.
C	30% gum Arabic, 15% lecithin, 45% coconut oil	After homogenize, 50 percent from solution precipitated.
D	15% gelatin, 30% lecithin, 45% coconut oil	After homogenize, milky yellow and stable solution produced
E	15% gelatin, 30% maltodextrin, 45% coconut oil	After homogenize, milky white and unstable solution produced
F	30% maltodextrin, 15% lecithin, 45% coconut oil	After homogenize, 70 percent from solution precipitated
G	15% gelatin, 30% maltodextrin, 45% lecithin	After homogenize, 50 percent from solution precipitated.
H	15% gum Arabic, 45% gelatin, 7.5% lecithin, 67.5% coconut oil	After homogenize, 30 percent from solution precipitated.
I	45% gum Arabic, 15% gelatin, 7.5% lecithin, 67.5% coconut oil	2 layer of milky and plain solution produced

3.2. The Effect of Feed Formulation on Moisture Content

Formulations A and D were the only two emulsions that can be homogenize well in the long time given. After spray drying process, Formulation A give more positive result than Formulation D but still cannot achieve the target. According to Desmond *et al*.,[11], the level of moisture content required for prolonged powder storage life and stability are at least four percent. Different of solid percentage selected and being tested to improve the dried dairy product and the parameters used also can contribute to the improvement of dried product quality.

By increasing the solid percentage used in the solution, it shows the positive result for formulation A. The moisture content decreased to a very stable value. Because of the suitable outlet temperature have been proven by Meng *et al*.,[12] which the best value to dry the probiotic culture optimally is around 70℃ - 75℃. Inlet temperature have been controlled based on the outlet temperature selected. The inlet temperature was maintained at 110℃ in order to obtain an outlet air temperature in the range of 70℃ - 75℃. Therefore, the only parameters that can be manipulated here is feed flowrate. Based on previous part of study, wet product produced if using 120 rpm of feed rate. It preferred to decrease the feed rate to produce the better form of powder. By using 100 rpm of feed rate, the product form gives a very low moisture content percentage that was below four percent.

For formulation D, when the amount of the solid particle in the formulation increases, the moisture content was increased up to 15%. The high stickiness of the powder on the walls of drying chamber was observed. The results were unreliable and the formulation D disqualified to the next part of study automatically.

3.3. Spray Drying of Different Types of Probiotic Microorganisms

Different types of microorganisms selected based on the priority in animal health enhancement. Other researcher has been proved that *Lactobacillus* strain and yeast strain can improve animal health. Encapsulations of the entire microorganisms by the best-selected wall material and the best-feed flowrate from the previous part of study have been done. The objective of this part of the study is to check the viability of different probiotics such as *L. plantarum* strain 13 and 18, *K.lactis* and *S.blouradii.*

Figure 1 shows the viability of the different types of microorganisms through spray dryer condition and two weeks of non-refrigerated storage at 25℃. It directly shows the yeast strain of *Kluyveromyces lactis* give highest survival rate on both situation. The recommended minimum numbers of viable microorganisms in

probiotic food for efficacy are 10^6 cfu/g [13]. The retention of high viability during drying and storage presents particular challenged and can be classified as a major problem in commercial probiotic production [12]. In this research, the inlet, outlet temperature and feed rate was constant at 110℃, 75℃ and 100 rpm respectively. Generally, it was found that percentage of survival of *Lactobacillus plantarum B18* strain was higher than others during spray dry with the similar carrier. However, it gives lowest viability during storage. It was believed that better survival of *Lactobacillus plantarum B18* may be attributed to less sensitivity of this organism when exposed to heat. Only *Kluyveromyces lactis* can gives a stable survival characteristic than others.

Figure 1 proved the low potential of *Lactobacillus plantarum B13* strain through spray dry and two weeks storage. The number of cells reduced from 1.28×10^8 cfu/ ml to 2.1×10^6 cfu/ml and finally to 3.0×10^5 cfu/ml. This type of bacteria not effective and not suitable for comercial used with the wall material of gum Arabic, gelatin and coconut oil. The result will be better with the other mixture as an encapsulation agent. The resistance was very low even only in the spray dry condition. It was not applicable to select this type of bacteria to improve the animal feed if the carrier cannot match well with the bacteria.

Lactobacillus plantarum B18 resulted the best viability after spray drying and storage at room temperature with combination of gum Arabic, gelatin and coconut oil as a wall material. Therefore, it was only reliable in spray dry condition not during storage. The figure also shows how the cells drastically decrease just after spray dry and after two weeks of storage compared to before. The number of cells reduced from 3.25×10^7 cfu/ml to 2.15×10^7 cfu/ml and after two weeks storage, the total decreased to 4×10^2 cfu/mL. The viability of the bacteria along the storage depends on the sensibility of the bacteria to oxygen and the ability of carrier material as a protector. This is because of the low moisture content of the powder about to affect the viability of the bacteria of *Lactobacillus Plantarum B18* to be decreased drastically.

The survival of *Kluyveromyces lactis* comparing with the other bacteria, it gives the most reliable where the rate of survival of the cell during spray dry and even storage were higher. Number of cells reduced from 2.99×10^9 cfu/ml to 7.4×10^7 cfu/ml and 1.9×10^7 cfu/mL after two weeks storage at 25℃. The ability of this cell with the carrier which consists of gum Arabic, gelatin and coconut oil can be commercialized. Different from human, a probiotic dose between the ranges of 10^6 to 10^7 cfu/g in the animal feeds is more than enough [13]. Because of that, the value resulted for *Kluyveromyces lactis* shows the ability to improve the probiotic in the animal feeds. The other strain of yeast name *Saccharomyces blouradii* also gives positive result for this study. The **Figure 1** proved the amount of cell culture that can maintained in heat and storage. The value of cell culture can give good impact on animal feed industry which were reduced from 2.1×10^{10} cfu/ml to 3.9×10^8 cfu/ml and after two weeks of storage at 25℃, it becomes 7.82×10^6 cfu/ml.

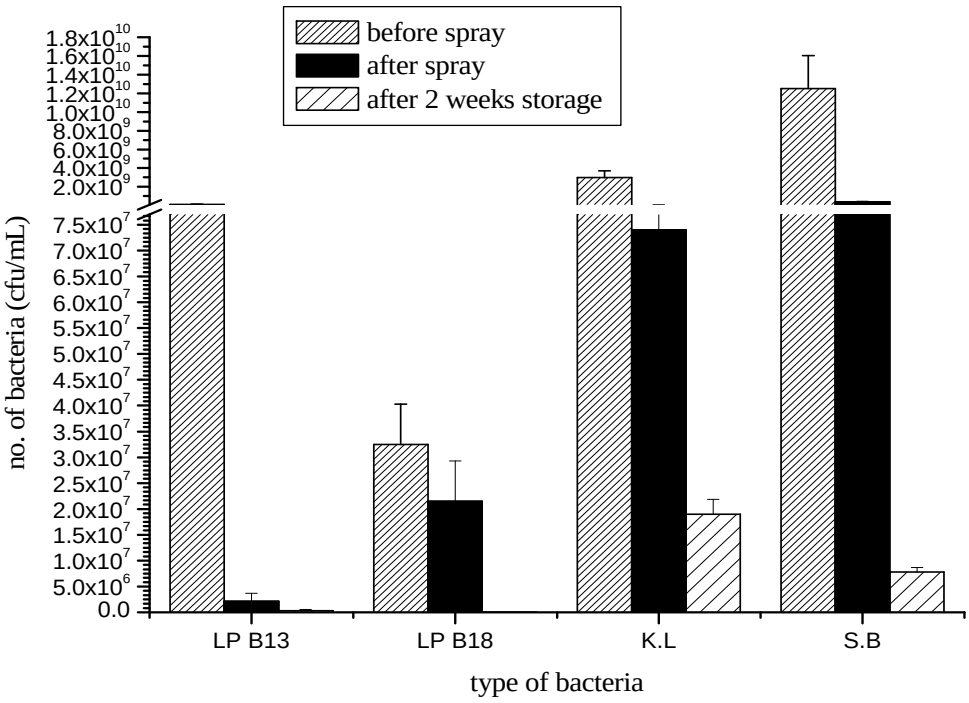

Figure 1. The colony of different probiotics after spray dry and two weeks storage.

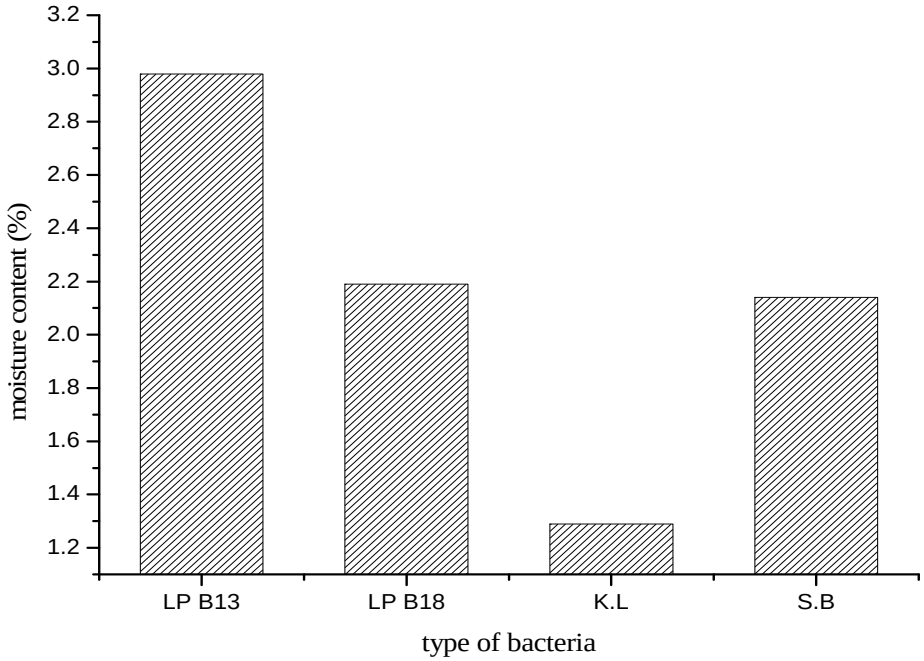

Figure 2. Moisture percentage of powder form of different probiotics after drying using spray dry.

3.4. Moisture Content of Different Spray-Dried Probiotics

The carrier used during spray drying of probiotics is known to have an influence on storage ability. Because of this study deal with the different types of probiotics, the ability of the encapsulation agent to protect probiotic microorganisms during drying is important characteristic. **Figure 2** had shown the graph of moisture content percentage in the different type of dried form probiotics. Moisture content values give the big impact to viability of probiotic microorganisms during storage. Spraydried cultures retain viability for longer at low temperature; however, refrigeration is expensive to supplier and retailer of product. There is a need to produce the probiotic bacteria cultures that are stable in ambient temperature [11].

4. CONCLUSIONS

This study demonstrated the possibility of producing dry probiotic that suitable as an animal feed using spray drying. Using combination of gum Arabic, gelatin and coconut oil with inlet and outlet temperature, feed rate and solid percentage in the solution is 110℃, 75℃, 100 rpm and 13.5% respectively give the best powder form of product which is below 4%. A bacterial survival rate during both spray dry and two weeks non-refrigerated storage condition have a wide range of percentages. *Kluyveromyces lactis* and *Saccharomyces blouradii* gives the big potential result for this study, which is suitable to use in animal feed industry. Survival of probiotic in a spray-dried form during storage is higher at lower moisture content.

5. ACKNOWLEDGEMENTS

The Universiti Teknologi Malaysia is thankful for the grant given to Dr. S.H. Mohd-Setapar's project (Q.JI3000.7125.00H02) under Research University Grant Scheme, part of which enabled this review article to be prepared. We are grateful for the contribution of all people involved in this paper preparation.

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