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Full Length Research Paper

Heat adaptation towards improve survival of *Bifidobacterium longum* BB536 during the spray drying of Sudanese fermented *Medida*

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This study examined the survival of *Bifidobacterium longum* BB 536 following the spray drying and during the storage of *Medida*, a fermented Sudanese cereal porridge. *Medida* was produced using 225 g flour of two days malted brown rice; blended in 0.405 L distilled water and cooked in 1 L boiling water. 150 g skim milk was added followed by sterilization; and the mixture was inoculated with *B. longum* BB536 and incubated until the biomass reached approximately 9 Log CFU/ml. Spray drying inlet temperature was regulated at 160°C. The strain BB536 survivability at different outlet temperatures (95, 85, and 75ºC), powder rehydration times (15 and 30 min) and mild heat adaptations (50 and 45ºC) prior to spray drying were evaluated. The viable count at 95ºC outlet temperature recorded only 2.48 Log CFU/g but improved to 4.28 ± 0.30 log CFU due to decrease outlet temperature to 85°C. Using 45°C heat adaptation for 30 min and 85°C outlet temperature, high viability of 7.82 ± 0.30 Log CFU/g meeting the requirement of probiotic foods was attained. Moreover, the population obtained did not reveal any significant cells reduction during 60 days refrigeration storage showing survivability maintenance of 88.31%. Therefore, the current investigation demonstrated that development of fermented probiotic *Medida* powder from malted brown rice flour is possible employing suitable outlet temperature and heat adaptation.

Key words: *Bifidobacterium*, rice, adaptation, spray drying, *Medida*.

INTRODUCTION

Medida is Sudanese fermented cereal thin porridge consumed by adults and children of all ages and also used as a weaning food. Traditionally, it is prepared from spontaneously fermented dough mainly sorghum dominated by lactic acid bacteria, cooked with continuous stirring in large amount of boiling water (Dirar, 1993). Recently we developed fermented *Medida* from malted rice with *Bifidobacterium longum* BB356 and its viable count maintained relatively stable for two weeks under refrigeration storage (Kabeir et al., 2005). Utilization of *B. longum* for fermentation of *Medida* added value to the product, as the strain BB536 is believed to exert beneficial effects on human hosts (Namba et al., 2003).

Bifidobacterium tend to decline during storage of liquid fermented products (Dave and Shah, 1997). Maintaining high viable numbers has gained much interest in industrial applications of probiotics for therapeutic purposes. Spray drying technique has been used to produce powders containing viable lactic acid bacteria (To and Etzel, 1997). Main factors reported to enhance survive-ability include: microorganisms in stationary phases, heat shock stress, and presence of prebiotic ingredients (Prasad et al., 2003; Corcoran et al., 2004).

Probiotic *Bifidobacterium* is sensitive to spray drying temperature. Therefore, limited studies were carried out

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to enhance their thermotolerances to heat. In this study, we evaluated the effect of different outlet temperatures and heat adaptations on survivability of *B. longum* BB536 during spray drying of fermented *Medida*.

MATERIALS AND METHODS

Malting of rice paddy

Rice paddy variety 219 grown in Malaysia was provided by Komplex Bernas Sekinchan, Selangor, Malaysia. Cleaned paddy rice were washed and soaked in twice its volume with distilled water in 2 L beakers and placed in a temperature controlled water bath (Yihder Bu-420, Taipei, Taiwan ROC) at 30°C for 36 h. Water was renewed every 12 h during soaking period to avoid fermentation.

For germination, the paddy was spread on aluminum dishes and incubated for 48 h at 30°C. During the germination, the paddy was turned and rinsed every 16 h with distilled water to promote aeration and prevent development of mould. The germinated paddy was dried in an oven at 50°C for 48 h. The root was removed and the remaining portion was dehusked using a Satake rice machine (Satake Engineering Co. Ltd, Tokyo, Japan) followed by grinding into flour using a hammer mill and sieved through a 335 µm screen. The flour was packed in a plastic container and kept at refrigeration temperature until used

Formulation of *Medida* porridge

225 g flour of two days malted brown rice was blended in 0.405 L distilled water using a commercial Warring blender for two min. The resulting mixture was cooked in 1 L boiling water with continuous stirring for 6 - 8 min on a hot plate. After cooling, 150 g skim milk (NZMP, New Zealand) was added to the mixture followed by sterilization at 121ºC for 15 min.

Bacterial culture

Bifidobacterium longum BB536 (Morinaga Milk Industries, Tokyo, Japan) from the stock culture of Food Biotechnology and Functional Food Laboratory, Faculty of Food Science and Technology, Universiti Putra Malaysia was used. Freeze dried culture was transferred into 10% sterilized (121 ºC for 15 min) skim milk supplemented with 0.05% (w/v) human food grade yeast extract and incubated anaerobically in aerobic jar at 37°C for 24 h. The culture was further subcultured twice in a similar sterilized skim milk for 48 h prior to use for fermentation.

Fermentation technique

Fermentation was carried out in a batch system using a 2 L Bioreactor with a temperature controlled water bath (Model Jeio Tech Desk Top, Korea) and an electronic stirrer (Model G gas-Col, Terre Haute, USA). For an anaerobic condition, oxygen free nitrogen (OFN) (Malaysia Oxygen Berhad) was used. The Bioreactor was autoclaved for 45 min at 121°C. After cooling, the sterilized *Medida* was aseptically added and agitated until its temperature equilibrated to 37°C, followed by inoculation with 13% *B. longum* BB536 culture. Fermentation was run to a final pH of 4.6.

Heat adaptation

The fermented *Medida* in glass bottles equilibrated to required temperature using a shaking water bath at constant agitation. The bottles were maintained at set temperatures for 30 min prior to use for spray drying.

Spray drying of fermented *Medida*

Spray drier used was MOBILE MINOR ™12000, model E (Niro Atomizer, Gladsaxevej, Denmark), with exhaust system (Cyclone dia, 140CHE). The inlet temperature regulated at 160°C. A peristaltic pump feed the fermented *Medida* to rotary stainless steel atomizer. The flow rate of the feed solution was used to control the spray drying outlet temperatures. The *Medida* powder was collected from the base of the cyclone. The powder was either used directly for *Bifidobacterium* enumerations or kept in sterile bottles at 4°C for storage study and further analysis. Moisture content was determined by AOAC method (1990).

Survivability of the strain during powder storage

Fermented spray dried *Medida* in glass bottles containing viable cell of BB536 (7.87 ± 0.17 log CFU/ g) were kept under refrigeration for 60 days. During the storage samples were collected every 15 days to check for BB536 count.

Enumeration of *Bifidobacterium*

The enumeration media was TPY agar (Scharlau Chemie S.A, Barcelona, Spain). 1 g of fermented spray dried *Medida* was suspended in 9 ml of sterile water and allowed to rehydrate and then serially diluted with buffered peptone water containing 0.05% L-cysteine. 0.1 ml was aseptically plated onto TPY agar and incubated anaerobically at 37°C for 48 h. The colonies appeared on the plates were counted as colony-forming units (CFU) per g.

Statistical analysis

MINITAB statistical software (2006) was used for analysis. Data were analyzed by One-way ANOVA. Probability levels of less than 0.05 were considered significant ($p < 0.05$).

RESULTS AND DISCUSSION

Using spray drying outlet temperature of 95ºC, the results on viable number of *B. longum* BB536 cultured in *Medida* to stationary phases of 10 h (Figure 1) was significantly ($p < 0.05$) higher (2.48 0.02 log CFU/ g powder) in comparison with those in early growth stages of five h incubations (1.01 0.37 log CFU/ g). The acidic intercellular reported to activate the heat thermoresistance at this stage of growth dependent on employed outlet temperatures and microbial sensitivity (Coote et al., 1991; Carcoran et al., 2004).

In general, the survivability of the strain BB536 at high spray drying outlet temperatures of 95°C was low (Table 1). That was due to over-drying as ravealed by the low moisture content (2.31 - 0.44%) of the final *Medida* powder. The mechanism of death or diminish at this high outlet temperature is related to inactivation of critical sites in the cell such as direct ribosome damaged, loss of magnesium and denaturation of protein (Teixeira et al., 1997).

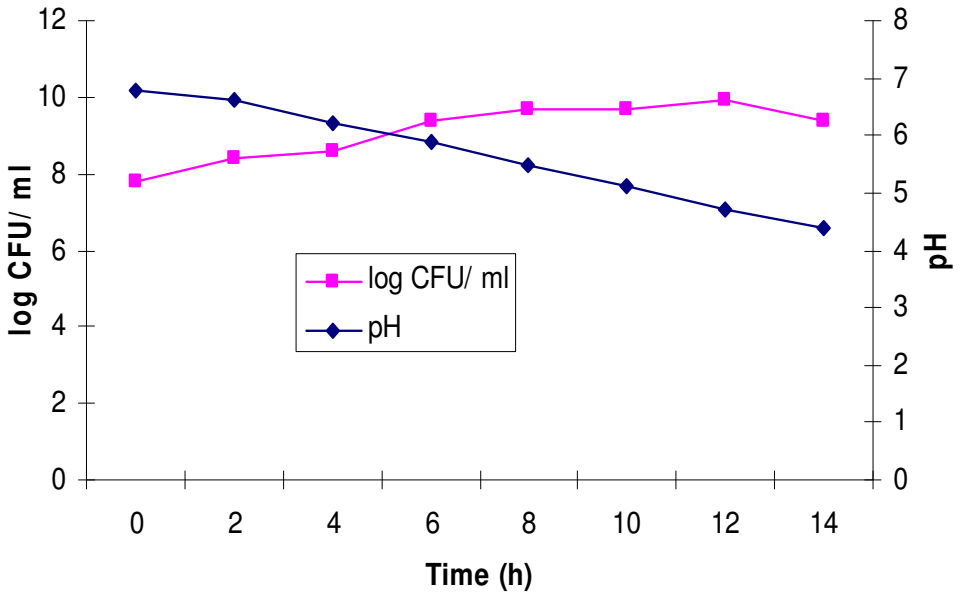

Figure 1. The growth of *B. longum* BB536 during fermentation of rice prior to the spray drying.

Table 1. Effect of spray drying outlet temperatures on the survival of *B. longum* BB536 at different rehydration periods*.

Outlet temperature	Survival (Log CFU/g powder)	
	15 min	30 min
85°C	4.28 ± 0.30[a]	5.65 ± 0.92[a]
95°C	2.48 ± 0.01[b]	2.31 ± 0.41[b]

*Data are mean ± SD of duplicates.
Values sharing a different letter in each column are significantly difference ($p < 0.05$).
Medium population before spray drying was 9.3 ± 0.28 Log CFU *B. longum* BB536/ml.

Table 2. Effect of different heat adaptation on survival of *B. longum* BB536 at rehydration period of 30 min*.

Outlet temperature	Survival (Log CFU/gram powder)	
	45°C	50°C
75°C	8.44 ± 0.64 [a]	8.38 ± 0.82 [a]
85°C	7.81 ± 0.30 [a]	6.1 ± 0.19 [a]

*Data are mean ± SD of duplicates.
Values sharing a different letter in each column are significantly difference ($p < 0.05$).
Medium population before spray drying was 9.3 ± 0.28 Log CFU *B. longum* BB536/ml.

Reduce spray drying outlet temperature to 85°C significantly ($p < 0.05$) improved viability of strain BB536 by 1.8 log CFU recording viable count of 4.28 0.30 CFU/g *Medida* powder. Therefore, only this outlet temperature was used for further study. However, the viable count obtained is still below the required to presence in probiotic foods.

Rehydration time is important for enumeration of probiotic in spray dried powder. It ensures appropriate release of entrapped microorganisms from dried microparticles. The results presented in Table 1 shows that extending rehydration period to 30 min prior plating increased viability recovery by 32%. Although the increase was not significant ($p > 0.05$), the observation support employing adequate rehydration time to overcome low estimation related to poor recovery of microbial cells from dried products. Nevertheless, the procedure is not effective in microorganisms liable to heat sensitivity. Since viable cells reduction of strain BB536 in samples spray dried at higher outlet temperature of 95ºC was high 6.85% (Table 1) as a result of extending rehydration period to 30 min.

To further improve viability of strain BB536 during the spray drying of fermented *Medida*, heat conditioning or adaptations prior to the spray drying was tested. 50°C was used for 30 min and found to improve viability by 0.45 log CFU/g. Moreover, five folds viability improvement (2.16 log CFU BB536/ g) was recorded at adaptation of 45°C. The viable cell of strain BB536 in the latter (Tables 2) met the number required to presence in probiotic products which 7 log CFU live microorganism per g or ml at the time of product consumption (Ishibashi and Shimamura, 1993). Sub lethal heat shock of microorganisms above the normal temperature range was reported to increase their heat thermoresistance (Mackey and Derrick, 1986).

At 75°C outlet temperature higher viable cell was attained (Table 2) but the equivalent moisture content was not suitable for powder storage (Table 3). Suitable moisture of around 4% together with storage temperature

Table 3. The moisture content of fermented *Medida* rice spray dried at different outlet temperature*.

Outlet temperature (°C)	Moisture (%)
95	2.31 ± 0.44[a]
85	4.30 ± 0.42 [b]
75	6.52 ± 1.03 [b]

*Data are mean ± SD of duplicates.
Values sharing a different letter in the column are significantly difference ($p < 0.05$).
Medium population before spray drying was 9.3 ± 0.28 Log CFU *B. longum* BB536/ml.

Table 4. Survival of *B. longum* BB 536 in fermented spray dried *Medida* during refrigeration storage*.

Days of storage	Log CFU /g
0	7.87 ± 0.17[a]
15	7.67 ± 0.37 [a]
30	7.65 ± 0.35 [a]
45	7.56 ± 0.22 [a]
60	6.95 ± 0.10 [a]

*Data are mean ± SD of duplicates.
Values sharing a different letter in the column are significantly difference ($p < 0.05$).

is necessary for maintaining high cells viability throughout the projected shelf life of the spray dried product (Master, 1985).

As shown in Table 4, the viable numbers of strain BB536 remained relatively stable in the first 30 days of the storage; with only 0.22 log CFU/ g population reduction and 97.20% survivability maintenance. Overall reduction by the end of the 60 days was 0.92 log CFU/ g with maintenance of 88.31% viable cells in final spray dried powder. The findings of Simpson et al. (2005) were in support of our results. They found no significant reduction in viability of *Bifidobacterium* species after 30 days storage at 4ºC for powder samples with moisture content of approximately 4.2%.

Conclusion

The survival of strain BB536 during spray drying of fermented *Medida* varies with different outlet temperatures employed, rehydration times and growth phase. Improved cells viability was shown due to decrease outlet temperature, prior heat adaptation. To attain high viability with stable shelf life, outlet temperature of 85°C and prior heat adaptation at 45°C for 30 min should be employed to produce *Medida* powder having moisture content of 4.3%. Although further long-term storage studies are required, the result of the present investigation indicates that under refrigeration storage, maintaining the viability of *B. longum* BB536 in *Medida* powder is possible. However, further studies are needed to enhance strain BB536 thermotolerance.

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