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＊ **機能性民族風味發酵乳之開發** ＊

＊ Development of national flavor fermented milk with ＊

＊ physiological functionality ＊

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Microencapsulation of probiotics and chiu-niang extract in Kou Woan Lao (an oriental style dairy product) by spray-drying

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Key words: probiotics, lao-chao, Kou Woan Lao, microencapsulation
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ABSTRACT

This objective was to combine the physiological functionality of probiotics and the unique flavor and milk-clotting activity of culture filtrate from lao-chao for the development of a new dairy product so different from the commercial yogurt. Two probiotic strains, *Lactobacillus acidophilus* and *Bifidobacterium longum* were applied to skim milk before acid-curd formation, and then culture filtrates were added for the formation of milk curd. The higher percentage of SNF, the stronger curd firmness ($P<0.05$). The percentage of syneresis decreases significantly when the level of SNF rising ($P<0.05$). As the SNF increasing from 10% to 18%, the percentage of syneresis decreases from 12.9% to 4.3%. The pH value and probiotic counts of 1% *L. acidophilus* group did not significantly decrease during 14 days storage at 4 ℃. The counts of probiotics decrease about 1 log CFU/ml after spray-drying process at 50℃ outlet temperature. The final total probiotic counts are about 6.58 log CFU/ml. Regarding resuspension methods, it is found that there are no significant difference of using stomacher or vortex to release probiotics ($P>0.05$). The counts of probiotics significantly decrease when the outlet temperature getting higher. Therefore, when the spray-drying outlet temperature cools down, the survival percentage of probiotics

increases. In order to increase the probiotic counts of Kou Woan Lao, it is suggested to choose the lower temperature of microencapsulation. In the same time, the product quality and storage period could be effected due to the spray-drying process. It will be a challengeable task to adjust different production procedure in order to meet market needs.

INTRODUCTION

The use of *Lactobacillus acidophilus* and/or *Bifidobacterium* spp. in fermented or culture-containing dairy products became popular by the end of the 1970s as a result of the increase in knowledge encompassing the properties of bifidobacteria. The nutritional benefits of probiotics have been mostly studied in milk-based products fermented with lactobacilli and bifidobacteria. They are characterized by a lower level of residual lactose and higher levels of free amino acids and certain vitamins than non-fermented milks. Furthermore, they preferentially contain L(＋)-lactic acid (that is more easily metabolized by human beings than D(－)-lactic acid) produced by bifidobacteria in addition to acetic acid. Moreover, the L(＋)-lactic acid absorbed in the intestine is used as energy source, with an energy yield of 15 kJ/g, that compares well with 16 kJ/g for lactose(Gurr, 1987). During the past years, numerous healthy and nutritional benefits have been claimed including maintenance of normal intestinal microflora, alleviation of lactose intolerance, reduction of serum cholesterol levels, potential antitumor activity, and some therapeutic effects on intestinal disturbances and intestinal infections. The intact intestinal epithelium, together with the normal intestinal microflora represents a barrier to movement of pathogenic bacteria, antigens and other noxious substances from the gut lumen to the blood. In healthy subjects this barrier is stable, thus protecting the host and assuring normal intestinal function. When either the normal microflora or the epithelial cells are disturbed, as triggered by dietary antigens, pathogens, chemicals or radiation, defects in the barrier mechanisms become apparent; altered permeability facilitates blood invasion by pathogens, foreign antigens and other harmful substances (Gomes and Malcata, 1999). Experiments performed in animal models showed that a few strains of *L. acidophilus* and *Bifidobacterium* spp. are able to decrease the levels of enzymes responsible for activation of some procarcinogens, and consequently decrease the risk of tumor development. (Mital and Garg, 1992). A number of studies indicate that administration of bifidobacteria or lactobacilli alone or with fermentable carbohydrate (defined as a prebiotic) can alter colonic microflora populations and decrease the development of early preneoplastic lesions and tumors. Many of the antitumor activities attributed to lactic cultures have been suggested to involve an enhanced function of the immune response (Hirayama and Rafter, 2000). The anticarcinogenic, antitumorigenic, and antimutagenic activities of these probiotic bacteria have been proposed to occur via some modification of mutagens *in vitro* (Vorobeva *et al*., 1995), reduction in carcinogen-generating fecal enzymes *in vivo* (Kulkarni and Reddy, 1994), and suppression of precancerous lesion or tumor formation (Reddy and Rivenson, 1993).

A new oriental-style dairy product coagulated with culture filtrates from lao-chao (chiu-niang), a fermented rice product well known in China, has been developed to meet consumer preferences for a low-acid or non-sour tasting yogurt-like product (Kuo *et al*., 1996 ; Lin and Chen, 1996). Culture filtrates from lao-chao, which is produced through fermentation by inoculating steamed glutinous rice with commercial starter (chiu-yao) or fungal cultures, have been used as both milk-clotting agents and flavoring agents. The resultant yogurt-like products with soft curd appearance are characterized by a sweet, fruity and slightly alcoholic flavor. Therefore, this objective was to combine the physiological functionality of probiotics and the unique flavor and milk-clotting activity of culture filtrate from lao-chao for the development of a new dairy product so different from the commercial yogurt.

Microencapsulation is a technique in which a membrane encloses small particles of solid, liquid, or gas, with the objective of offering protection to the sensitive core material from adverse environmental conditions such as undesirable effects of light, moisture, and oxygen, thus contributing to an increase in the shelf life of the product and promoting a controlled liberation of the encapsulate (Shahidi and Han, 1993). Many methods for preparing microcapsules have been developed and improved significantly. They can briefly classify as mechanical and chemical processes (Thies, 1996). Spray-drying is the microencapsulation technique most widely used in the chemical, pharmaceutical, and food industries (Gibbs, 1999). Spray-drying emulsions is a particularly effective means of microencapsulating chemically reactive oils, volatile oils, and flavor compounds. Spray-dried powder particles contain the encapsulated material as minute droplets embedded within its wall (McNamee *et al.*, 1998).

Gum arabic (gum *Acacia*) is a hydrocolloid produced by the natural excudation of arabic trees and is an effective encapsulation agent due to its high water solubility, the low viscosity of concentrated solutions relative to other hydrocolloid gums, and its ability to act as an oil-in-water emulsifier. Gum arabic is composed of a highly branched arrangement of the simple sugars galactose, arabinose, rhamnose, and glucuronic acids (Street and Anderson, 1983) and also contains a protein component (~2%w/w) covalently bound within its molecular arrangement (Anderson *et al.*, 1985). The protein fraction plays a crucial role in determining the functional properties of gum arabic (Randall *et al.*, 1988).

Maltodextrins and chemically modified starches have been investigated as replacers for gum arabic in spray-dried emulsions (Amandaraman and Reineccius, 1987). To wholly or partially replace gum arabic as an encapsulating agent.

MATERIALS & METHODS

Preparation of inocula

The two strains of fungal cultures, *Rhizopus javanicus* (CCRC 30288) and *Saccharomyces cerevisiae* (CCRC 21685), from the Culture Collection and Research Center, Taiwan, R. O. C., were used to inoculate steamed glutinous rice. Before use, *R. javanicus* and *S. cerevisiae* were transferred to slants of YM agar and those of potato dextrose agar, respectively, and incubated at 30±1 ℃ for 6-8 days. In addition, the probiotics strains, *Lactobacillus acidophilus* (CCRC 14079) and *Bifidobacterium longum* (CCRC 14605) were transferred to Lactobacilli MRS (deMan, Rogosa and Sharpe) and MGL (modified Garche’s lithium-chloride) media. Fungal spore suspensions and yeast cell suspensions for inoculation were prepared by adding sterilized distilled water containing 0.1 gL^{-1} Tween 80 to slants and shaking the cultures vigorously for 1 min.

Preparation of fermented rice (lao-chao) and culture filtrates

Glutinous rice was purchased from a local market (Taipei, Taiwan, R.O.C.). One hundred grams of glutinous rice, which had been washed with distilled water and drained, was soaked in 75 ml distilled water at room temperature (20-25℃) for 12 hr, sterilized at 121℃ for 15 min, and then cooled to 35℃. The steamed glutinous rice was inoculated with 5 ml suspensions containing 10^7 cells of *S. cerevisiae* and 10^7 spores of *R.*

javanicus and mixed completely, followed by static incubation at 30±1 ℃ for the designated period. After fermentation, the culture filtrates were obtained by filtering the fermented rice through 4 layers of cheesecloth. The sediment in the culture filtrate was eliminated by centrifuging at 1,480×g for 30 min (Kubota, KR-20000T, Japan).The culture filtrates were stored at 4 ℃ for further analysis.

Preparation of Kou Woan Loa and determination of viable probiotics

Pasteurized skimmilk contains various ratios of SNF was inoculated with probiotics and then incubated at 37 ℃. The viable probiotics were determined during the periods of incubation and storage (4℃) by according to the method of Teraguchi *et al*.(1982).

Determination of the firmness of product

The firmness, as measured by the breaking force of the Kou Woan Loa product, was determined by using a rheometer (Fudoh NRM-2010J-CW, Tokyo 141, Japan) with a rheoplotter (Rikadenki Kigyo, FR 801, Tokyo 113, Japan). Adaptor No.4 (20 mm diam.) of the rheometer was used and table speed was 50 mm min^{-1}.

Viscosity of Kou Woan Loa

The apparent viscosity was determined by using a Viscometer (Brookfield, LVDV-II＋Viscometer, U. S. A.) with a No. 3 disc spindle at a speed of 0.3rpm.

Encapsulation procedure

Preparation of probiotics and culture filtrates emulsion

All glassware, and solution used in the protocols were sterilized at 121 ℃ for 15 min. Gum arabic obtained from Hayashi Pure Chemical Industries, Ltd. (Osaka, Japan). Maltodextrins and chemically modified starches obtained from Gemfont Co. (Taipei, Taiwan, R.O.C.). Emulsion with different gum arabic ratios were prepared and agitated with magnetic stirrer bars for 30 min.

Spray-Drying the Emulsion

The emulsions were spray-dried using a laboratory mini spray drier (model B-190, Buchi Co., Flawil, Switzerland) by control inlet air temperature, compressed air for spray-flow, compressed air consumption and feed rate to obtain outlet temperatures ranging from 50 to 60 ℃. The residence time of the product was very low. The dried powders were collected and analysis immediately or subsequently stored in sealed glass bottles at 4 ℃.

Determination of probiotic viability in spray-dried powders

We assessed the viability of the probiotics in the inoculated emulsions preparations before spray drying and in the resulting powders by examining MRS and MGL pour plates after 72 hrs of incubation at 37°C (aerobically or anaerobically). To 1g of powder, 9ml of diluents were added (1:10 dilution); the powders were allowed to rehydrate by stomacher (Stomacher 400 Lab Blender, Sewad Medical, London, UK) at normal speed (230 rpm 65%) for 1 min or by mixing with a vortex (Maxi Mix II, Barnstead Thermolyne, Iowa, U.S.A.) for 1 min to release microencapsulated probiotics and then diluted further with diluent, and appropriate dilutions were pour plated.

Statistical analysis

Data were analyzed using the general linear model procedure of the SAS software package (SAS/STAT, 1987), and Duncan's multiple range test (Montgomery, 1991) were used to detect differences between treatment means, statistical significance was tested at the 5% level. All experiments were replicated three times.

RESULTS & DISCUSSIONS

Two probiotic strains, *Lactobacillus acidophilus* (CCRC 14079) and *Bifidobacterium longum* (CCRC 14605) were applied to skim milk before acid-curd formation. Different culture ratios on total probiotic counts during incubation period examined are shown in Fig. 1. In the beginning of incubation period, the counts of 1% *L. acidophilus* was lower than the other three treatments significantly ($P < 0.05$). When the single starter treatment compares with the mix of 1% *L. acidophilus* and 1% *B. longum* treatments, the counts of *L. acidophilus* was lower than the counts of *B. longum* significantly ($P < 0.05$). Synergistic growth-promoting effects between *L. acidophilus* and *B. longum* are occur. *L. acidophilus* is beneficial to *B. longum* as the former organism acts as an oxygen scavenger.

According preliminary screening tests for suitable fungal and yeast cultures, *Rhizopus javanicus* (CCRC 30288) and *Saccharomyces cerevisiae* (CCRC 21685) were selected as starters of lao-chao and the resultant culture filtrates were used as the milk-clotting agents and flavoring agents. Effects of various ratios of culture filtrate on pH value and titratable acidity in Kou Woan Lao during incubation period are shown in Fig. 2. As the incubation period increasing, the pH value decreases and titratable acidity rising gradually since the number of probiotics increase. However, when the percentage of culture filtrate increases, the growth of probiotics is inhibited. As long as the percentage of culture filtrate getting higher, the increasing level of the titratable acidity decreases. If the percentage of culture filtrate was controlled in 40%, the titratable acidity was keeping in the same level. The acidity increases significantly during the 30th to 36th incubation hours.

During the 18th –36th incubation hour, there are no significant differences among the four SNF treatments on probiotic counts of Kou Woan Lao (Fig. 3).

Fig. 4 shows the higher percentage of SNF, the stronger curd firmness ($P<0.05$). When the SNF of skim milk increasing from 10% to 16%, the curd firmness grows from 18.9g to 44.6g. In the mean time, the viscosity significantly increases from 12200cps to 29800cps ($P<0.05$). In Fig. 5, the percentage of syneresis decreases significantly when the level of SNF rising ($P<0.05$). As the SNF increasing from 10% to 18%, the percentage of syneresis decreases from 12.9% to 4.3%.

These results are similar to the finding of Parnell-clunies *et al.* (1988). According to Parnell-clunies *et al*. (1988), the curd firmness and viscosity depend on if the structure of casein micelle is complete or not. They also indicate the curd firmness has positive relationship with the viscosity.

In order to produce better quality of Kou Woan Lao, the increase of SNF will help to improve the curd firmness and viscosity. It is also helpful to decrease the percentage of syneresis. However, in order to meet consumer flavor expectation and lower the production cost, it is suggested to keep SNF between 12 and 14%.

After adding culture filtrate, the total probiotics counts in other three treatment groups decrease significantly ($P<0.05$), except the 1% *L. acidophilus* group (Fig. 6). The counts of 1% *B. longum* group decrease from 6.3 log CFU/ml to 6.0 log CFU/ml. The 1% *B. longum* group has dramatically decreased when compared with other groups. The pH value and probiotic counts of 1% *L. acidophilus* group did not significantly decrease during 14 days storage at 4 ℃.

In Table 1, the counts of probiotics decrease about 1 log CFU/ml after spray-drying process at 50℃ outlet temperature. The final total probiotic counts are about 6.58 log CFU/ml. Regarding resuspension methods, it is found that there are no significant difference of using stomacher or vortex to release probiotics ($P>0.05$). It is attribute to the high solubility of gum arabic (about 50 %).

The viability of probiotics differs in various spray-drying outlet temperature showed as Fig. 7. The counts of probiotics significantly decrease when the outlet temperature getting higher. Therefore, when the spray-drying outlet temperature cools down, the survival percentage of probiotics increases. However, it may cause the poor condition of spray-drying process, which will decrease the efficiency of microencapsulation and the stability of storge.

There are four separate phases of spray-drying methods : 1. Nebulization of the solution in the form of an aerosol. 2. Contact of the nebulized solution with the warm air. 3. Drying of the aerosol. 4. Separation of the dried product and the air charged with the solvent. During these process, the probiotics are easily vanished. In order to increase the probiotic counts of Kou Woan Lao, it is suggested to choose the lower temperature of microencapsulation. In the same time, the product quality and storage period could be effected due to the spray-drying process. It will be a challengeable task to adjust different production procedure in order to meet market needs.

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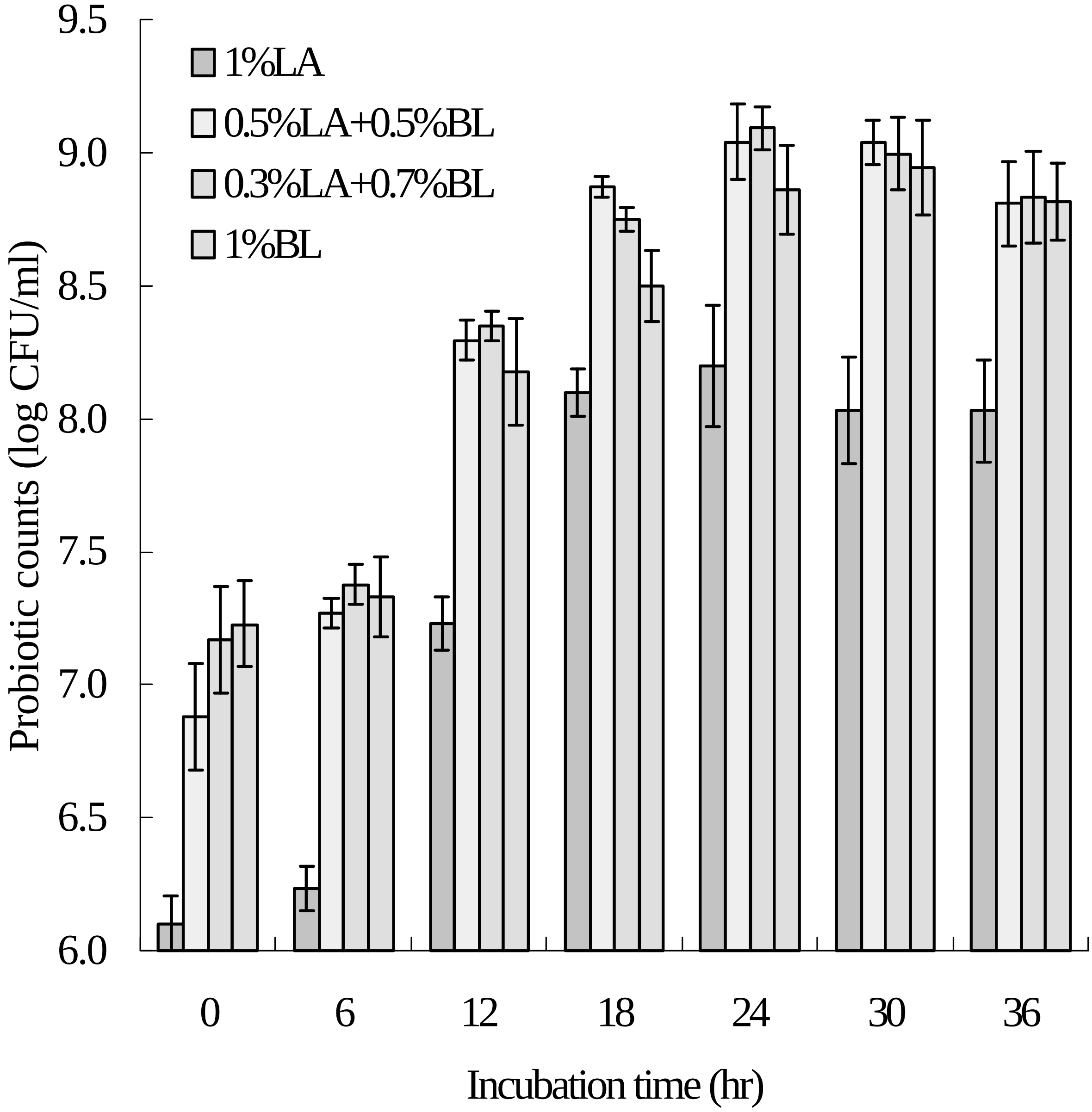

Fig. 1. Effects of different culture ratios on total probiotic counts in skim milk during incubation period (n = 9 ; error bars indicate 95% confidence intervals).
LA : *L. acidophilus.* BL : *B. longum.*

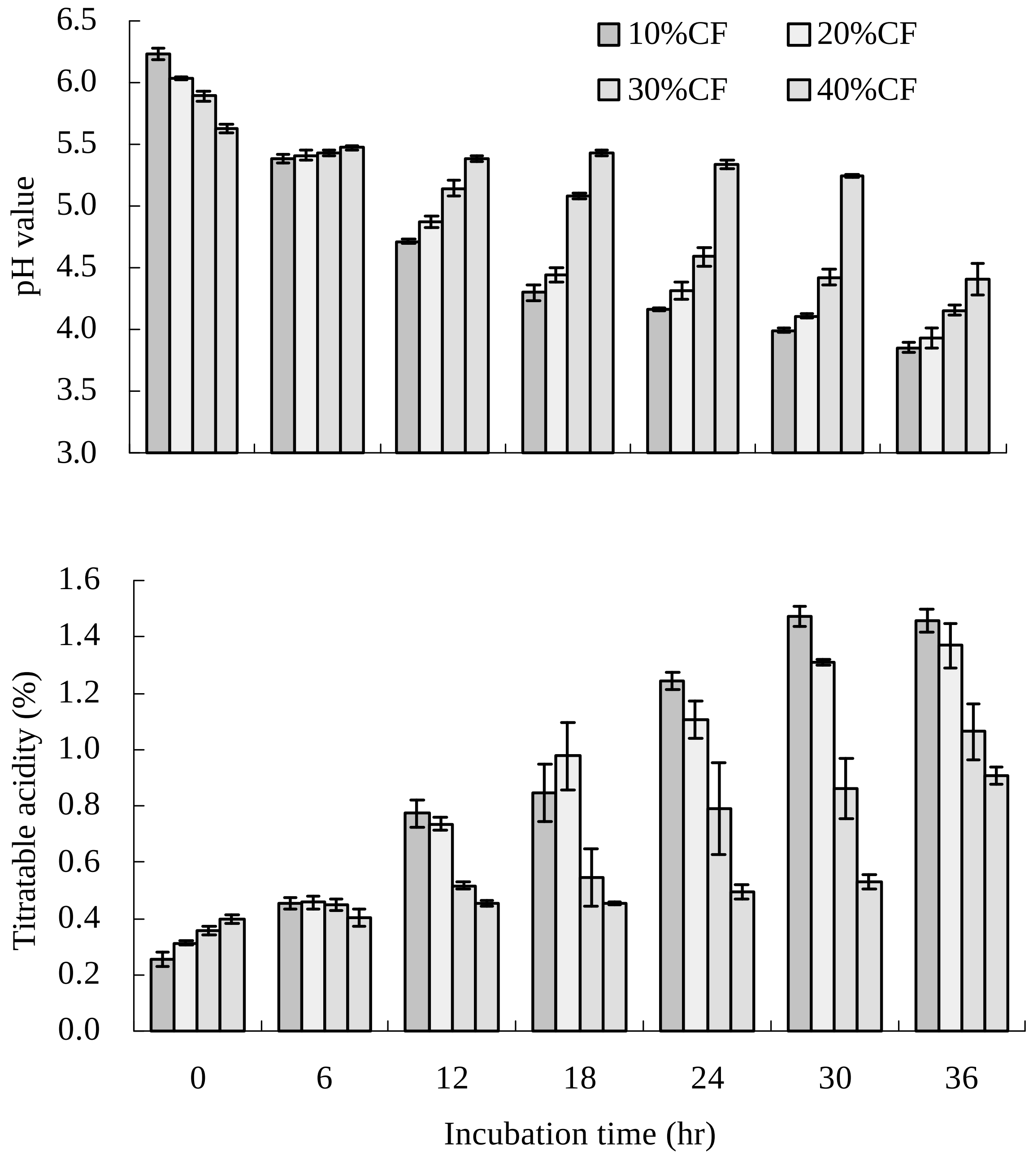

Fig. 2. Effects of culture filtrate on pH value and titratable acidity in Kou Woan Lao during incubation period (n = 9 ; error bars indicate 95% confidence intervals).

*CF : culture filtrate.

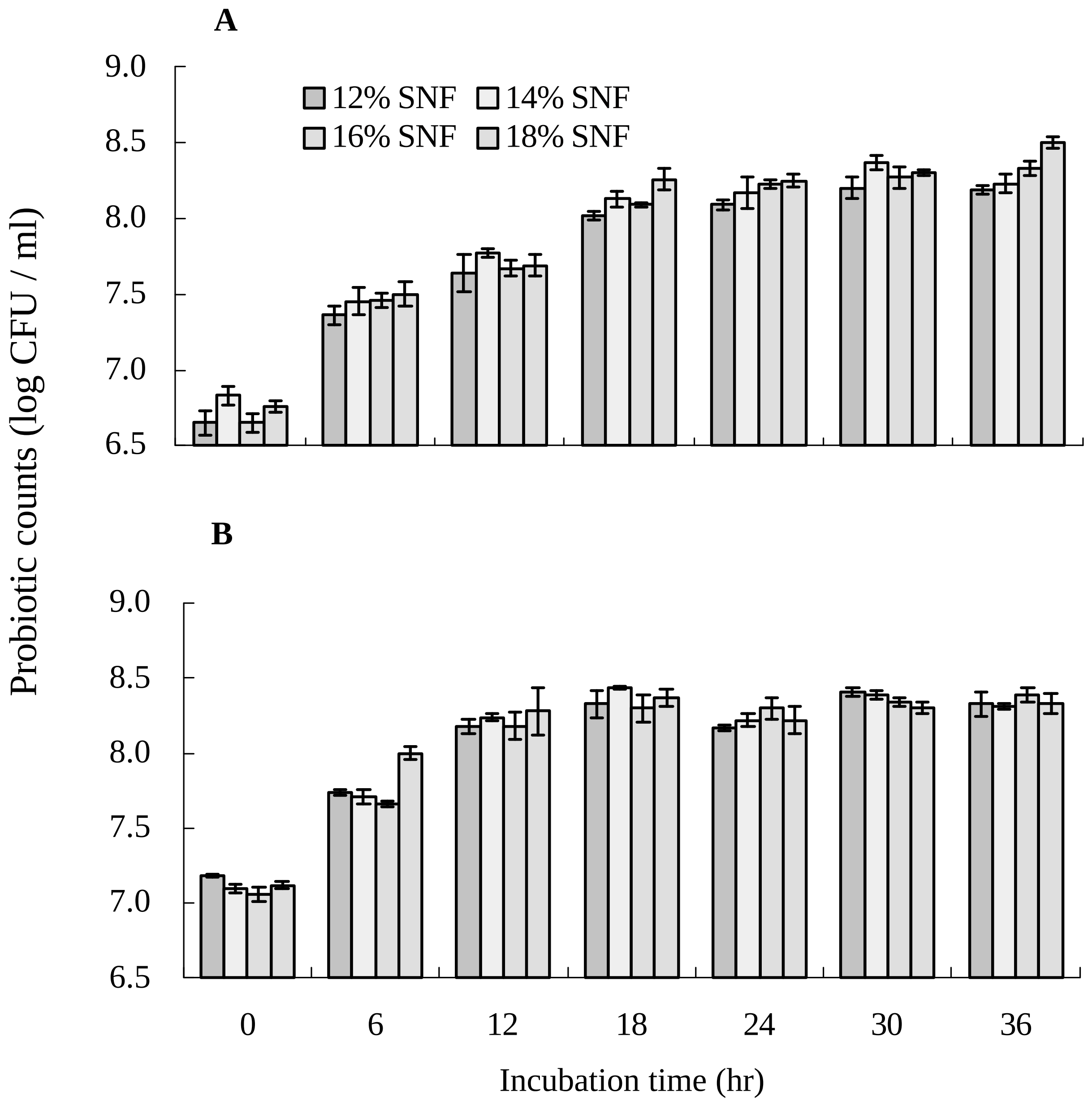

Fig. 3. Effects of solids-not-fat on probiotic counts in Kou Woan Lao during incubation period (n = 9 ; error bars indicate 95% confidence intervals).

*A : *L. acidophilus* .

B : *B. longum* .

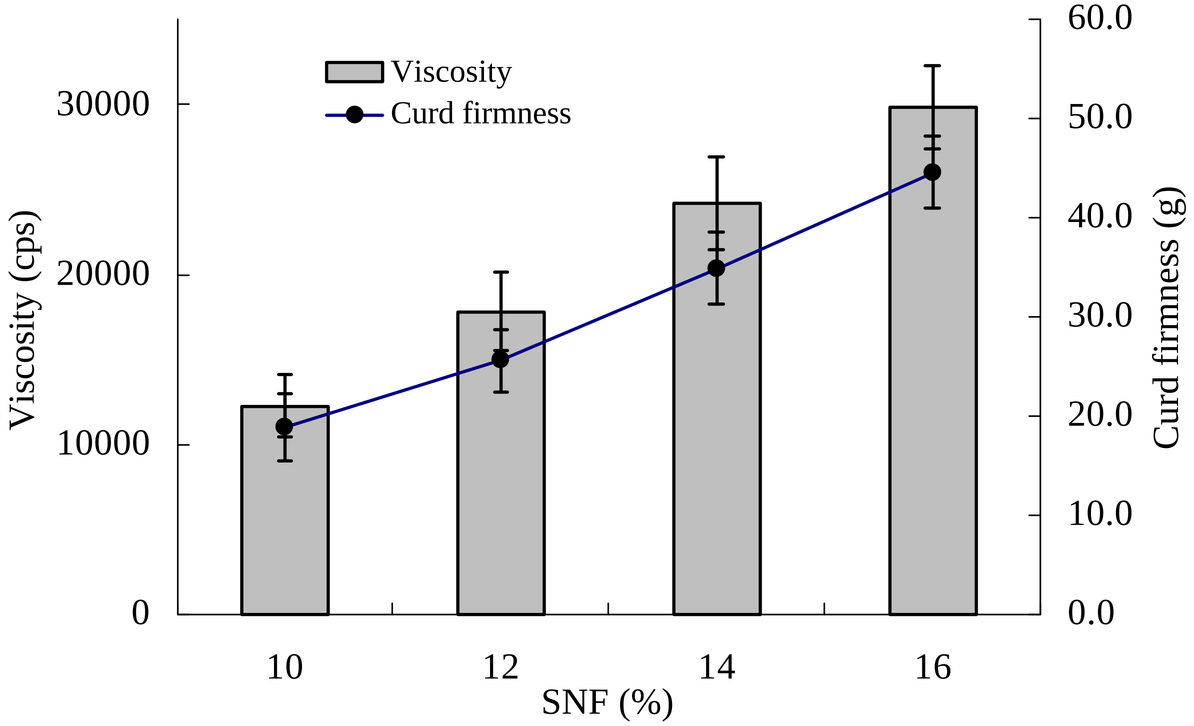

Fig. 4. Effect of SNF content on curd firmness and viscosity of curd (n = 5 ; error bars indicate 95% confidence intervals).

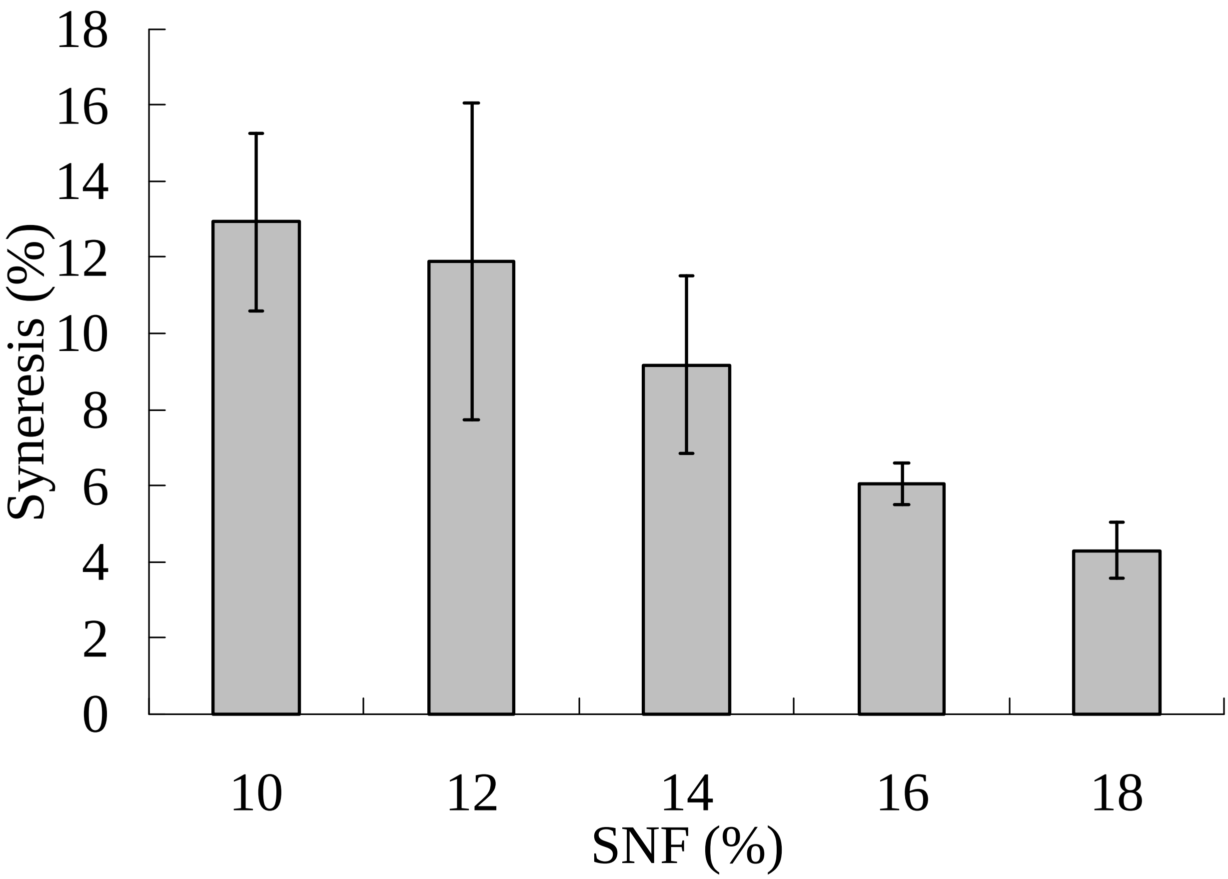

Fig. 5. Effect of SNF content on syneresis of curd (n = 5 ; error bars indicate 95% confidence intervals).

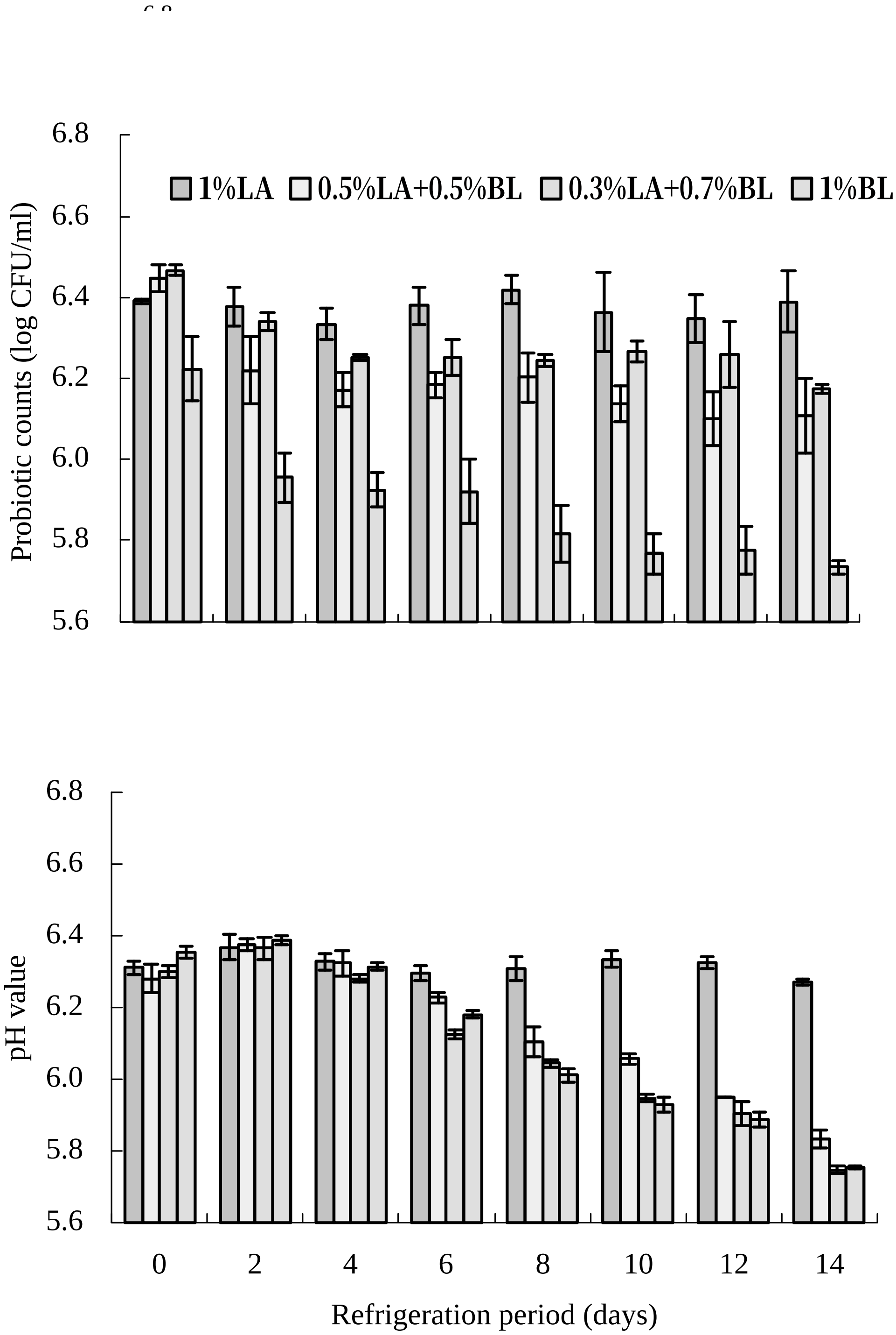

Fig. 6. Effects of different culture ratios on total probiotic counts and pH value in Kou Woan Lao during refrigeration period (n = 9 ; error bars indicate 95% confidence intervals).

Table 1. The viability of probiotics during the spray-drying process (outlet temperature : 50 ℃)

	Viability		
	L. acidophilus	*B. longum*	Total probiotic counts
	Log (CFU / ml)		
Emulsions (before spray-drying)	7.46^{a}±0.05	6.99^{a}±0.06	7.59^{a}±0.04
After spray-drying — stomacher	6.45^{b}±0.03	5.99^{b}±0.01	6.58^{b}±0.02
After spray-drying — vortex	6.46^{b}±0.02	5.98^{b}±0.03	6.58^{b}±0.02

*Values in the same column with different letters were significantly different. ($P<0.05$) ($n=4$)

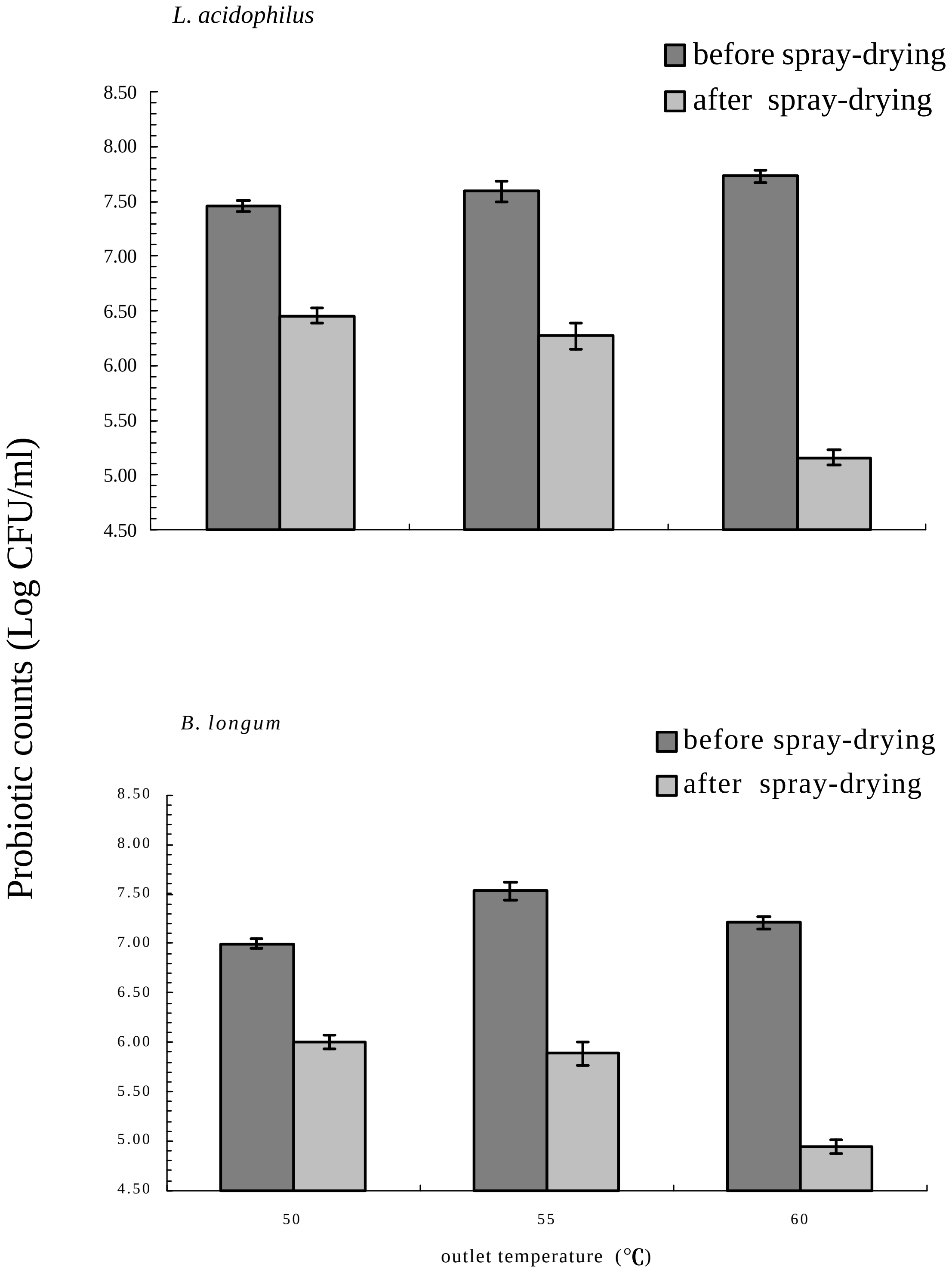

Fig. 7. Effect of outlet temperature on probiotic counts during the spray drying (n = 4 ; error bars indicate 95% confidence intervals).