African Journal of Food Science Vol. 4(9), pp. 558 - 562, September 2010
Available online http://www.academicjournals.org/ajfs
ISSN 1996-0794 ©2010 Academic Journals

Full Length Research Paper

Manufacture of fermented coco milk-drink containing lactic acid bacteria cultures

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Accepted 14 April, 2010

Fermented coco milk drink was manufactured to study an appropriate lactic acid bacteria and water coconut composition as well as to evaluate product stability and lactic acid bacteria (LAB) viability during storage at 5°C. *Lactobacillus acidophilus, Lactobacillus bulgaricus* and *Streptococcus thermophilus* could grow well in all of the coco milk drink prepared from mixture of coconut water and coconut milk combination with the ratio of 3:1, 3:2 and 4:1, respectively. Among the three of lactic acid bacteria, *L. acidophilus* produced the most acid with titrable acidity of 0.62% after 24 h fermentation and was the most preferable in term of the taste. Coco milk drink fermented by *L. acidophilus* was noted to have pH value of 3.79, and folic acid content of 5.42 ppm/ml after 24 h fermentation. The growth of *L. acidophilus* was increase after 4 h fermentation until it reached maximum at 20 h ranged from log 4.32 to log 9.89 cfu/ml and relatively constant afterwards. Storage of fermented coco milk drink at 5°C for 16 day could stabilize the quality of this drink with viability of *L. acidophilus* was log 10.201(log cfu/ml) and the pH values was 3.58.

Key words: Fermented coco milk drink, viability, lactic acid bacteria.

INTRODUCTION

Utilization of the coconut milk, the aqueous extract of the solid coconut endosperm in food product development production is an interesting alternative option in the regions with high coconut production. Researchers have been at the forefront of efforts to develop new dairy foods using a combination of coconut milk. Amongst others were flavored filled milk beverages, soft and blue cheeses, and low fat fruit yogurt (Davide et al., 1990), four types of yoghurts made from mixtures of cow milk and coconut milk in different combinations (Imele and Atemnkeng , 2001), soy-coconut milk and, and a butter-like product from coconut milk.

Other product that may be developed over the coconut milk is a new fermented drink prepared by mixture of coconut water and young coconut milk in different proportions and fermented by lactic acid bacteria. Seow and Gwee (1997) reported that the young coconut milk contains carbohydrates (primarily sucrose and some starch), lipid and minerals such as phosphorous, calcium, and potassium. Coconut protein is rich in lysine, methionine and tryptophan. Coconut water extracted from young coconuts has a pleasant taste, and balance of sodium, potassium, calcium and magnesium (Rumokoi, 1991). The composition of these may favor lactic acid bacteria (LAB) fermentation to produce lactic coco milk drink with healthy advantages. Besides providing mineral for the LAB growth media, presence of mineral in coconut drink is a part of fortified cultured milk itself. Mineral fortification with calcium salts and calcium content is a usual attempt in some milk cultured for example in yoghurt (Pirkul et al., 1977; Khurana and Kanawjia, 2007).

Recently there has been an increased trend to fortify milk products with lactic acid bacteria to produce probiotic fermented milks, one major segment amongst fermented milks that has tremendous potential for growth and development (Khurana and Kanawjia, 2007). Oral probiotics are living microorganisms, which upon

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ingestion in certain numbers exert health benefits beyond inherent basic nutrition (Guarner and Schaafsma, 1998). The manufacture of lactic drink containing probiotic bacteria could be an interesting way to diversify coconut product, giving them an addition of health promoting function.

Production of high-quality fermented milk products containing probiotic bacteria is a major challenge (Yeung et al., 2002; Hadadji and Bensoltane, 2006). The usual practice to manufacture the lactic beverage is to add conventional yogurt bacteria such as *Lactobacillus bulgaricus* and *Streptococcus thermophilus* as well as probiotic bacteria as *Lactobaclilllus acidophilus* to enhance the fermentation process. To succeed in manufacturing the coconut milk drink products, we had to select strains, for fermented milk production criteria does not include only the properties of intestinal effects but also the growth in coconut milk and survival in acidic coconut milk. Additionally, the cultivation of *L. bulgaricus*, *S. thermophilus*, and *L. acidophilus* in coconut milk is a not easy task compared with that in dairy milk because coconut milk is not the original medium of these nutritionally fastidious microorganisms. Therefore, the appropriate media composition is also important to be investigated. The objective of study was to compare the viability of three lactic acid bacteria on the coconut milk drink media (mixture of coconut water and coconut milk in various combination ratio), and to examine the viability of selected LAB and lactic coconut milk drink stability during storage at 5°C.

MATERIALS AND METHODS

Microbial cultures

Three LAB commercial cultures: *L. bulgaricus* (LB), *S. thermophilus* (ST) and *L. acidophilus (LA)* were obtained from Biotechnology Laboratory, Institut Pertanian Bogor, Indonesia. After two successive transfer of the LAB in MRS broth (Difco, Detroit, Michigan,USA) and incubation for 24 h at 40°C for *S. thermophilus* and at 37°C for either *L. bulgaricus, S. thermophilus* and *L. acidophilus*, each activated culture was inoculated into sterile media containing 5% defatted milk and 5% glucose and incubated for 24 h for getting working inocula.

Lactic coconut milk fermentation

The lactic coconut milk were prepared by mixing coconut water and undiluted coconut milk in the ratio of 3:1; 3:2 and 4:1 (v/v) in 100 ml bottle flask and standardized with 3% skim milk, 9% sugar and 2% lecithin. Each mixture was then submitted to thermal treatment at 90°C during 10 min and cooled to 4°C in ice bath. The flasks were inoculated with 3% (v/v) of *S. thermophilus* and 3% (v/v) of either *L. bulgaricus* or *L. acidophilus* at the appropriate fermentation temperature (42°C for *S. thermophilus* and 37°C for either *L. bulgaricus* or *L. acidophilus*) for 24 h. Each fermentation was performed in 3 replicates and monitored for the pH, LAB count, acidity, folic acid total and sensory analysis.

Preparation of cultured coco milk-drink for sensory analysis

The cultured coco- milk drinks for sensory analysis were prepared by mixing fermented coconut milk with an appropriate amount of sterile sucrose solution (60%) to obtain a total solid content of about 15ºB. The fermented coconut drinks were then subjected to 18 panelists for aroma, and taste through hedonic test.

Growth behavior of selected LAB during fermentation of coconut milk

A 100 ml of sterile coconut milk drink (60% coconut water and 40% coconut milk) was placed in a 150 ml-Erlenmeyer flask and was inoculated with 1% of selected LAB (*L. acidophilus*). In this experiment, the initial population of organism in coconut milk drink was between 3 and 4 log cfu ml^{-1}. Inoculated coconut milk drink was incubated at 37°C for 28 h. During that period, samples were taken at intervals to determine the pH, titrable acidity and the number of LAB in the coconut milk drink.

Viability of LAB during storage

Lactic coconut milk drink were prepared by mixing 60% coconut water and 40% coconut milk (the best ratio of previous experiment) and was inoculated with *L. acidophilus*) after being standardized and thermally treated as above procedure. Incubation was performed at 37°C and analyzed after 0, 4, 8, 12 and 16 days of storage at 5°C. The acidity was determined by pH measurement and titration method. The viability of LAB was measured by pour plate method using MRS agar.

Microbiological and chemical analysis

Rogosa agar (Oxoid) was used for enumeration of *L. bulgaricus), S. thermophilus* and *L. acidophilus*. One milliliter of appropriate serial dilutions of each sample was pour plate onto the sterile media. After 24 h incubation at 37°C, the colonies that appeared on the plates were counted and the cfu ml^{-1} was calculated. The titrable acidity (TA) was determined by the AOAC method and expressed as % lactic acid (AOAC, 2000). The pH values of the samples were measured using a pH electrode and meter. The folic acid total was measured using spectrophotometer method (AOAC, 2000).

Statistical analysis

The mean values and the standard deviations were calculated from the data obtained with triplicate trials. Means were separated using LSD (least significant difference).

RESULTS AND DISCUSSION

Coconut milk drink with different coconut water compositions and various LAB

TA, pH and sensory of fermented coconut milk drink with various ratio of coconut water and three LAB are summarized in Table 1. Among the LAB, *L. acidophilus* produced the most acid with TA of 0.62% after 24 h fermentation and the highest folate acid content and the

Table 1. pH, titrable acidity, aroma and taste, as well as folic acid total of coconut milk after fermenting with various LAB for 24 h.

Organisms	TA* (%)	pH	Aroma score	Taste score	Folate content (ppm)
L. acidophilus	0.62 c	3.79 a	4.40b	4.32b	5.42
L. bulgaricus	0.50 a	3.94 c	4.22b	4.11a	4.82
S. thermophilus	0.55 b	3.83 b	3.85a	4.01a	4.95
Composition of coconut water: Pure coconut milk (v/v)					
3 : 1	0.58A	3.87A	4.35A	4.10A	
3 : 2	0.54A	3.87A	4.13A	4.32B	
4 : 1	0.56A	3.82A	4.01A	4.08A	

*Titrable acidity expressed as percent of lactic acid, **Values in the same column with different superscript are significantly different ($P < 0.05$), ***Aroma and taste score of 4 = like moderately, 1= do not like and 7 = like extremely.

most preferable for the taste as well as produced high score of aroma.

With regard to the composition of coconut milk, there was no significant difference of either pH or TA among three compositions. However, there was little impact of the taste where the composition of 3:2 ratio had taste score higher than that of others composition. The data indicates that all the compositions with the best of 3:2 (v/v) combination would be great material for making the coconut drink fermented due to their component contents could support the growth of the three LAB organisms tested and resulted in good sensory. The coconut milk contains sugars (primarily sucrose), some starch and the minerals (phosphorous, calcium, and potassium), while coconut water extracted from young coconuts contains a balance of sodium, potassium, calcium and magnesium, (Seow and Gwee,1997; Rumokoi, 1991) This is the fact that coconut milk is a very rich medium which can support the growth of all the lactic acid bacteria tested.

Nevertheless, there were differences in the responses of the three lactic acid bacteria inoculated to the media of coconut milk drink and *L. acidophilus* appear to have best growth amongst the LAB. This may be attributed to ability of LAB to effectively utilize sources of carbon in the coconut milk media that was specific species. The coconut milk has sucrose in appreciable quantities in the coconut milk drink. *L. bulgaricus* was reported to have low ability to utilize sucrose, the main sugar in soy milk (Mital and Steinkraus, 1975, Pinthong et al., 1980) and in peanut extracts (Schaffner, 1986). This may be probably also true for *L. bulagaricus* metabolism in the coconut milk media so it produced less acid than did *L. acidophilus* but higher that did in *S. thermopilus.* Besides, fermented coconut milk with *L. acidophilus* was noted to have better folate acid content. This indicated that LA can be used as folate synthesizing lactic cultures. Generally, LAB recognized as a folate producers are *S. thermophilus, lactobacillus plantarum, L. bulgaricus, Lactococcus lactis, Leuconostoc lactis, Bifidobacterium longum* and *Propionibacteria* (Lin and Young, 2000; Holasova et al, 2005).

According to Gibson and Roberfroid (1995), the technological application of probiotic organisms in fermented dairy products aims to combine the potential health benefits of the bacteria with their ability to grow in milk, resulting in a nutritionally healthy and desirable product for the consumers. They must be able to be incorporated into foods without producing off-flavors and they should be viable. Therefore, all lactic acid bacteria tested in this experiment have the potential to ferment coconut milk drink and *L. acidophilus* has the highest potential.

Growth behavior of selected LAB (*L. acidophilus*) during fermentation of coconut milk

As *L. acidophilus* is the best appropriate of LAB for manufacturing the fermented coco milk drink, this bacteria was selected for this experiment and its growth behavior during fermentation is shown in Figure 1. In the present study, we found that *L. acidophilus* was noted to have good ability to produce fermented coco milk drink with desired characteristic after 20 – 24 h fermentation. *L. acidophilus* was able to produce enough acid to cause noticeable stimulation of pH. The growth of *L. acidophilus* was increase after 4 h fermentation until it reached maximum at 20 h ranged from log 4.32 to log 9.89 cfu/ml and relatively constant afterwards. The lactic acid content (TA) was increasing from 4 - 28 h fermentation with the highest TA content up to 0.6% reached after 20 h fermentation. The increase in TA with the fermentation time is proportional with the decreases in pH that has value of 3.87 at the end of fermentation.

The growth of LA in this experiment appears similar to that in study of several workers. The growth of *L.*

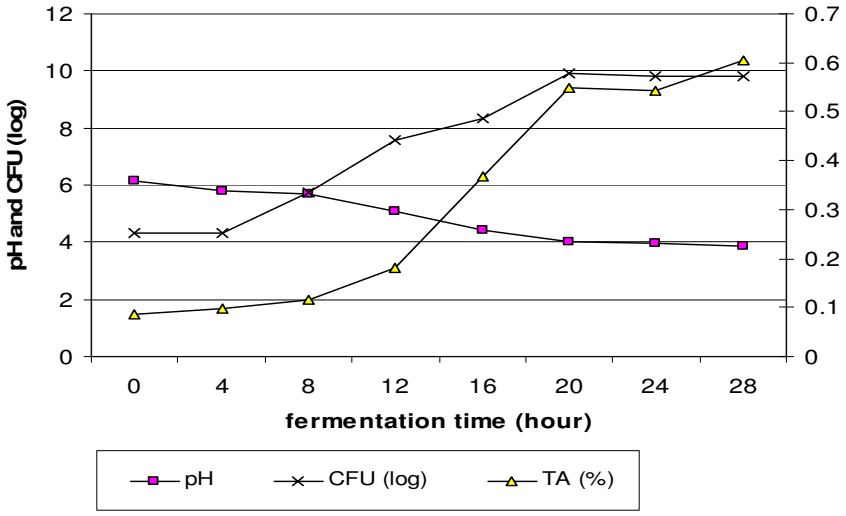

Figure 1. Growth of *L. acidophilus* and pH, TA changes in a cultured coconut milk.

Table 2. Changes of TA, pH and CFU of *L. acidophilus* during 16 days of storage at 5°C.

Storage time (day)	TA (%)	pH	CFU/ml (log)
0	0.524 a	3,79 a	8.347 b
4	0.554 ab	3,75 a	10.313 a
8	0.606 bc	3.76 a	10.116 a
12	0.735 cd	3.69 b	10.166 a
16	0.742 d	3.58 c	10.201 a

*Titrable acidity expressed as percent of lactic acid, **Values in the same column with different superscript are significantly different (P < 0.05), ***Aroma and taste score of 4 = like moderately, 1 = do not like and 7 = like extremely'.

acidophilus in milk was quite variable, however, after approximately 14 h (Hughes and Hoover, 1995) and 24 h (Hadadji and Bensoltane, 2006) growth in skim milk reached the stationary phase.

Viability of LAB and stability of fermented coconut milk drink during storage

The survival of *L. acidophilus*, pH and TA changes in the fermented coconut milk drink during storage of the drinks at 5°C was investigated and summarized in Table 2. The pH values of the drinks change little, likewise the TA of the drinks increase when the drinks were held at 5°C after 12 days storage. There was also an increase in the number of *L. acidophilus* from day 0 - 4 but it was constant afterwards. When the fermentation was terminated, the number of *L. acidophilus* was log of 8.347 cfu/ml. It was supposed that *L. acidophilus* still continue its growth metabolism during 4 days of storage due probably to its end of logarithmic phase has not reached yet and the sucrose in the coconut milk drink was still available. In this experiment, we put sucrose in appreciable amount of 9% (g/v) before fermentation. This pattern differed when *L. acidophilus* inoculated into soymilk where no marked change in the number of this microorganism was observed during 10 days of storage of 5°C even though soymilks supplemented with sucrose (Wang et al., 2002).

Table 2 shows the number of *L. acidophilus* in the coconut milk drink fermented by this organism during storage at 5°C. To exert beneficial effect in the host, it is essential that lactic acid bacteria be alive and abundant in the product at the time of consumption. Therefore survival of *L. acidophilus* in the fermented coconut milk drink during storage was important. Rybka and Kailasapathy (1995) argued that before probiotic strains can be delivered to consumers, they must first be able to be manufactured under industrial conditions, and then survive and retain their functionality during storage. Schluer-Malyyoth et al. (1986) indicated that to be some dietetic-therapeutic benefit, a cultured milk should contain at least 10^6 cfu/ml when consumed. Oliveira et al. (2002) indicated that the fermented milk should contain at

least 10^7 cfu/g of bacteria to obtain health benefit. It is usually considered that the acceptable final population of the probiotic organisms in yoghurt at the end of the products shelf life, should be anywhere between 5 and 8 log cfug-1 (Svensson, 1999). Growth promoters are sometimes added to improve viability of probiotic in fermented milk (Chen et al., 2003; Bruno et al., 2002).

It is concluded that mixture of coconut water: coconut milk in the ratio of 3:2 (v/v) fermented by *L. acidophilus* offer great potential for production of fermented milk-like drink products. The viability of *L. acidophilus* and the stability of this product could be maintained at 5ºC for 16 days storage.

ACKNOWLEDGEMENTS

The authors are indeed very grateful to DIKTI-Indonesia through Hibah Bersaing XV/2007 for financial support of this work

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