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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 11 (2): 173-180, 2008
ISSN 1028-8880

Effects of Initial Air Removal Methods on Microorganisms and Characteristics of Fermented Plant Beverages

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Abstract: The effects of 3 different methods for removing the initial air on the properties of fermented plant beverages produced from phom-nang seaweed (*Gracilaria fisheri*) and wild forest noni (*Morinda coreia* Ham.) were investigated. Only method M which covered the space above the fermentation liquid with a water filled plastic bag produced no surface film of yeast, had the highest acidity and also antibacterial activity from both plants after 90 days of fermentation. However, the yeast count still exceeded the standard guidelines for plant beverages. The fermented beverage from wild forest noni showed more antibacterial activity against 3 of 4 pathogenic bacteria tested than that from the phomnang seaweed, probably for its higher levels of acidity and ethanol content. Lactic Acid Bacteria (LAB) isolated from the fermentation samples from days 1-5 using the method M from both fermented plant beverages were *Leuconostoc mesenteroides* supsp. *mesenteroides* and *Leu. mesenteroides* subsp. *dextranicum* while presence of *Lactobacilus plantarum* was only recorded at days 4-5 in the wild forest noni beverage. From days 6-14 the isolates were *Lactobacillus plantarum*, *Lactobacillus fermentum* and *Lactobacillus brevis* from wild forest noni beverage, whereas only *L. brevis* was not detected in the seaweed beverage. During days 21-45 both beverages had a similar LAB population of *L. plantarum* and *L. brevis* while *L. coryniformis* was only found in the wild forest noni beverage. Between days 60-90 in both plant beverages only *L. plantarum* and *Lactobacillius* sp. were detected.

Key words: Acidity, film yeast, initial air removal, lactic acid bacteria, water filled plastic bag seal (M)

INTRODUCTION

In Thailand, fermented plant beverages (FPB or FPBs), produced from a variety of plants by Lactic Acid Bacteria (LAB), are considered to be non-alcoholic and contain mainly organic acids, particularly lactic acid (Kantachote *et al*., 2005a). Local Thai people who consume the FPBs believe that they are healthy beverages for its high nutritional value in addition to the presence of bioactive compounds derived not only from the raw materials but also the fermentation process (Kantachote *et al*., 2005b). However, variable amounts of ethanol and methanol were detected in some FPBs (Kantachote *et al*., 2005a). Our previous study demonstrated that some FPBs were able to inhibit some pathogenic Gram-negative and Gram-positive bacteria. Their antibacterial activities depended on total acidity, potassium ions and perhaps some bioactive compounds extracted from the plants (unpublished data). Although there is not a lot of scientific information available on these fermentation processes these FPBs are still household products even though there are always serious health problems with a high contamination by yeast cells (Prachyakij *et al*., 2007). Therefore the beverage product cannot meet guidelines of microbiological quality (yeast count: not more than 100 cfu mL^{-1}) based on the Section of Food Analysis, Division of Medical Science, Ministry of Public Health, Thailand, even though these products are being produced throughout the country.

Yeast and LAB can be found in fermented plants like pickles or non alcoholic beverage because their habitats and some of their physiological properties are similar (Osuntogun and Aboaba, 2004; Okada *et al*., 2006). Under limited air conditions, both types of organisms are allowed to grow as facultative anaerobes and most pickling processes use salt to inhibit some yeast and spoilage microorganisms (Wood, 1985; Adams and Moss, 2000). However, salt is not used in producing a fermented plant beverage as it consists of plant material, sugar and water in a ratio of 3:1:10 (W/W/V) (Kantachote *et al*., 2005a). This mixture of raw materials is placed in a container to leave only a little space on top. This means that the process of plant beverage fermentation provides suitable conditions for both yeast and LAB although they favor LAB due to some yeast preferring aerobic conditions and also their lower proliferation than bacteria. The aim of this

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study was to investigate the effects of initial air removal methods on plant beverage fermentation reactions focusing on microbiological succession and the properties of the products.

MATERIALS AND METHODS

Preparation of plant beverage fermentation: Fruit of wild forest noni (*Morinda coreia* Ham) was selected for this study due to the fact that this tree is a medicinal plant and that the fermented beverages from the fruit had the highest activity to control enteropathogenic bacteria when compared to other medicinal plants according to our previous study (unpublished data). In case of phomnang seaweed (*Gracilaria fisheri*), it has been selected because it is used as edible seaweed and its bioactive compounds is a subject for many recent researches (Watanabe *et al*., 1990). Phomnang seaweed and wild forest noni were purchased from local markets, sugarcane from a supermarket and water was normal potable tap water. Unripe fruits of wild forest noni were used in this study because ripe fruits have unpleasant smell and it is likely to contain spoilage organisms. The effects of initial air removal on the beverage fermentation process were tested as follows: 1) the traditional method (T) using a plastic bucket only 4/5 full and a space under the lid, 2) the space above the fermentation liquid was filled with a water filled plastic bag to make the fermentation virtually anaerobic (M) and 3) a small gas release pipe was fitted into the lid used to seal the bucket prepared as in T except for the sealed lid (N). Each bucket (28 L) contained 6 kg of plant material, 2 kg of sugarcane and 20 L of tap water. Three replicates were conducted in each treatment and sampling occurred at days 0, 1, 2, 3, 4, 5, 6, 7, 14, 21, 30, 45, 60, 75 and 90 in order to monitor the characteristics of the FPBs and microbiological succession. Plant are commonly fermented for 90 days prior using as beverages due to people who prepare the beverages believe that is a suitable time for drinking based on their taste and benefits to consumer's health. Some chemical properties and antagonistic activities of the FPBs were also monitored.

Investigation of chemical property of FPBs: The following chemical properties were examined for all sampling times, using standard analytical methods (AOAC, 2002), pH, titratable acidity and Total Acidity (TA) was calculated as lactic acid. Total Sugar (TS) as glucose was also determined at all sampling times by the phenol sulfuric method (Dubois *et al*., 1956). Elemental composition, some organic compounds that could be involved with antibacterial activity and the antibacterial activity were investigated at 0, 30, 60 and 90 days. Elements (potassium: K and sodium: Na) were determined using inductively coupled plasma-atomic emission spectroscopy (ICP-AES) according to the instructions for the instrument. Organic acids, alcohols and acetaldehyde were determined by gas chromatography as described by Yang and Choong (2001).

Determination of antagonistic activity of FPBs: Antagonistic activity of FPBs was examined using the cup well diffusion method (Schillinger and Lucke, 1989) with test organisms *Staphylococcus aureus* PSSCMI 0004, *Escherichia coli* PSSCMI 0001, *Salmonella* sp. PSSCMI 0002 and *Vibrio parahaemolyticus* VP 4. The stock cultures were obtained from the Department of Microbiology, Faculty of Science, Prince of Songkla University, Thailand. At least two additional subcultures (24 h, 37°C) were did on fresh (TSA: tryptic soy agar or TSA plus 2% NaCl for *V. parahaemolyticus* VP 4) plates prior to use in the experiment. The turbidity of each actively growing culture was adjusted to 0.5 (McFarland standard) and then swabbed over the surface of a TSA. The FPBs were sterilized using 0.45 μm filter and 125 μL of a fermented plant beverage was introduced into each cup well.

Microbial enumeration and isolation of LAB: Standard methods using ten fold dilutions beginning with 25 mL of each sample added to 225 mL normal saline solution to obtain a 10^{-1} dilution and then appropriate dilutions were used for the pour plate counting (FDA, 2001) of LAB and Total Bacterial Counts (TBC). De Man Rogosa Shape (MRS) medium was used for LAB and Plate Count Agar (PCA) for TBC. Potato Dextrose Agar (PDA) was used for counting molds and yeasts by the spread plate technique. All plates were incubated at room temperature (28±3°C) because fermentation buckets were kept at room temperature. For each sample, representative colonies of LAB were isolated from MRS medium based on their distinct morphology. Pure cultures of each isolate that were Gram positive and catalase negative were maintained on the same medium for further identification. In addition, at the end of fermentation (90 days), the beverage yielded from all methods (M, N and T) were visually inspected to check the presence or absence of yeast film on the top of the beverage.

Identification of lactic acid bacteria in FPBs: Only representative colonies isolated from the fermentation method M were identified because this method seemed to be the most appropriate method with which to persevere. Sixty representative isolates (30+30) of LAB which collected at various fermentation times from the method M of each FPB were identified. The identification of LAB was performed according to morphological characters, arrangement of cells, biochemical tests as well as

physiological properties as described in Bergey's Manual of Systematic Bacteriology,(Kandler and Weiss, 1986) and Lactic Acid Bacteria (Axelsson, 2004). Gas production from glucose was tested for identification to genus level in coccobacilli LAB. In order to identify to species level, carbohydrate fermentation profiles of all isolates of coccobacilli LAB was conducted in MRS fermentation broth where glucose was replaced by 2% (W/V) of one of the following sugars (arabinose, fructose, galactose glucose, lactose, maltose, mannitol, raffinose, ribose, sucrose and trehalose). In addition, growth in 4 and 6% NaCl and hydrolysis of esculin were examined. The lactobacilli were identified to species level by testing ability of gas production from glucose utilization, ability to ferment the following 17 carbohydrate compounds (arabinose, cellobiose, fructose, galactose, lactose, maltose, mannitol, mannose, melezitose, raffinose, rhamnose, sorbitol, sucrose, glucose, ribose, xylose and trehalose) were tested to identify bacilli LAB species.

Statistical analysis: To analyze the effects of initial air removal on the characteristics of a fermented plant beverage and their microbial populations two ways ANOVA was used by the SPSS version 12 for Windows. Means and Standard Deviation (SD) are presented. p-value results considered as non significant ($p>0.05$) and significant p from 0.00 to 0.05.

RESULTS

Effect of initial air removal methods on characteristic of FPBs and film yeast: Significant differences were observed in the chemical properties and microbial populations among the beverages yielded from the different fermentation processes carried out using different methods of air removal (Table 1). For the fermented beverages produced from phomnang seaweed, each fermentation method gave significant differences for values of most of the monitored parameters (TA, TS, K, acetic acid, lactic acid, ethanol, methanol, acetaldehyde, LAB and yeast), but no significant difference was found for the following parameters: Na, pH and TBC. For the fermented wild forest noni beverages all parameters, except TS and K, were significantly different. At the end of fermentation (90 days) potassium levels in each beverage produced from wild forest noni (800-1000 mg L^{-1}) were higher than in phomnang seaweed (120-180 mg L^{-1}), whereas the amounts of Na were similar in the range of 20-30 mg L^{-1} in both FPBs. After 90 days of fermentation, only the method M produced no film of yeast at the surface in both fermented plant beverages (Fig. 1). Therefore as yeast is a non preferred microbe, further results and discussion are focused on the results obtained by the method M.

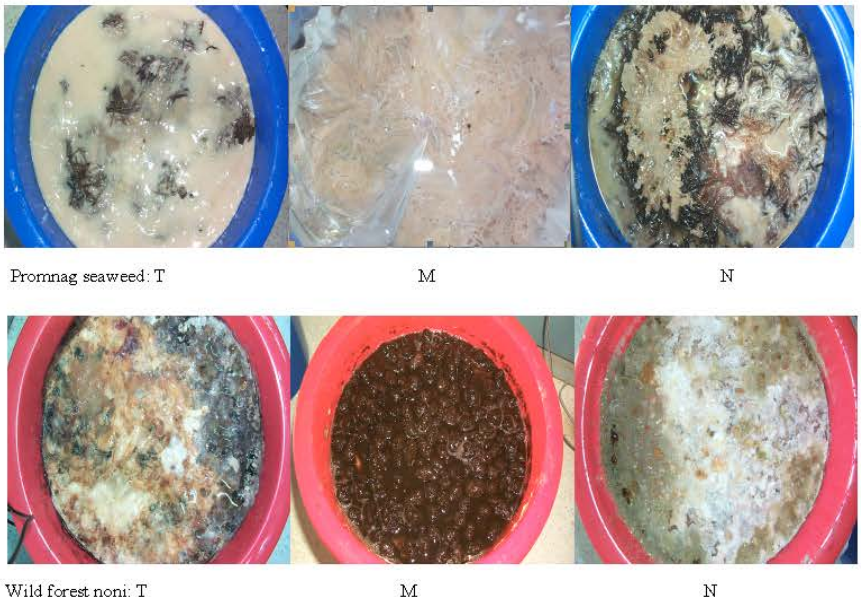

Fig. 1: Presentation of film yeast occurrence at the end of fermentation in traditional method (T) for plant beverage fermentation and two modified methods (water filled plastic bag to fill space under the lid (M) and small air release pipe with sealed lid (N) to withdraw initial air at the start of fermentation

Table 1: Significant differences of some chemical properties and microbial populations in fermented beverage from phomnang seaweed and wild forest noni using 3 different methods for removing air

	Phomnang seaweed		Wild forest noni	
Parameters	Significance	p-value	Significance	p-value
Na	NS	0.26	S	0.00
K	S	0.00	NS	0.17
TS (Total sugar)	S	0.02	NS	0.13
TA (Total acidity)	S	0.03	S	0.01
pH	NS	0.24	S	0.01
Acetic acid	S	0.00	S	0.01
Lactic acid	S	0.00	S	0.00
Ethanol	S	0.00	S	0.00
Methanol	S	0.00	S	0.00
Acetaldehyde	S	0.00	S	0.00
TBC (Total bacterial count)	NS	0.55	S	0.00
LAB (Lactic acid bacteria)	S	0.00	S	0.00
Yeast	S	0.01	S	0.00

S = Significance; NS = Non Significance

Changes of chemical properties in FPBs: Amongst the 3 simple fermentation methods used, method M gave the highest TA. Changes of pH, TA and TS showed similar tendencies in both FPBs under the method M with decreasing amounts of TS corresponding with increasing amounts of TA and the TA values were inversely related to the pH values (Fig. 2a-b). In both FPBs, the initial TS (roughly 13%) sharply decreased to about 9% after only 1 day of fermentation with a corresponding decrease of pH from 6.1 to 3.9 in phomnang seaweed and from 4.1 to 3.5 in wild forest noni and then gradually decreased to around 3% TS at the end of fermentation (90 days). However, the increase in TA of fermenting phomnang seaweed was slower than with wild forest noni and at the end of fermentation, the former beverage had only 1.0% TA with a pH of 2.7 and the latter beverage had 1.5% TA with a pH of 3.1.

Organic acids and alcohols in FPBs: Both FPBs under the method M after 90 days of fermentation contained acetic acid, lactic acid, ethanol and methanol (Fig. 3). The major products detected in the phomnang seaweed beverage were lactic acid (3.9 g L^{-1}) and acetic acid (2.3 g L^{-1}), with only a small amount of each alcohol. The main product in the wild forest noni was ethanol (9.9 g L^{-1}), followed by acetic acid (3.7 g L^{-1}), lactic acid (3.2 g L^{-1}) and methanol (2.3 g L^{-1}). Acetaldehyde was also detected in very small amounts in both FPBs (0.001 g L^{-1}).

Antibacterial activities of FPBs against pathogenic bacteria: Among the 3 simple methods of plant beverage fermentation, both FPBs gave similar antibacterial activity results with the least activity found in method T (control set) while the other 2 methods gave similar results (data not shown). In addition, the maximum inhibition of

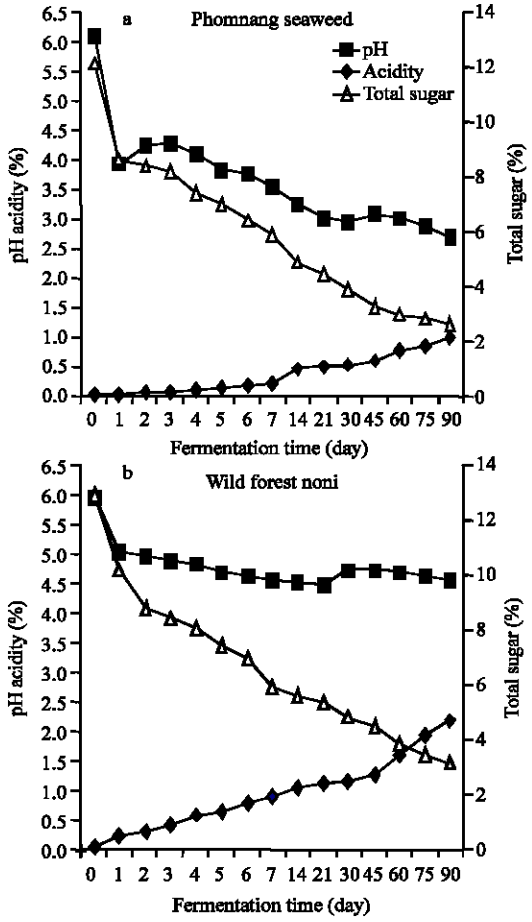

Fig. 2: Changes of chemical properties in fermenting plant beverages under a modified method using a water filled plastic bag cover at the top of the liquid

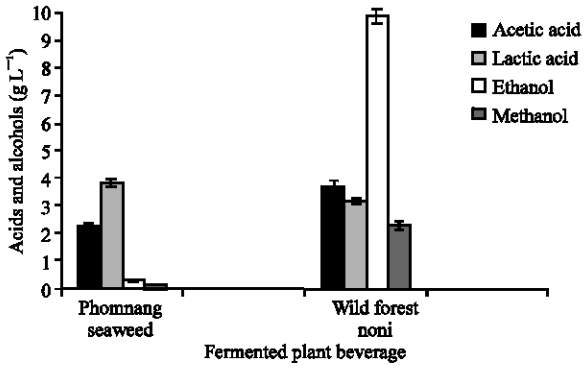

Fig. 3: Organic acids and alcohols contents of fermented plant beverages after 90 days of fermentation collected from the method M

test organisms was found at day 90, hence only the results of the inhibition obtained with method M at

Table 2: Inhibitory effects of 125 μL of 90 days fermented plant beverages collected from the method M

	Inhibition zone in mm (mean±SD)	
Food borne pathogenic bacteria	Phomnang seaweed	Wild forest noni
Staphylococcus aureus PSSCMI 0004	9.0±0.15	10.0±0.17
Escherichia coli PSSCMI 0001	6.5±0.13	4.2±0.14
Salmonella sp. PSSCMI 0002	10.0±0.20	12.0±0.22
Vibrio parahaemolyticus VP 4	13.8±0.23	16.0±0.27

90 days of fermentation are presented in Table 2. The zones of inhibition from the fermented wild forest noni beverage were generally larger than from the fermented phomnang seaweed beverage for test organisms *S. aureus* PSSCMI 0004, *Salmonella* sp. PSSCMI 0002 and *V. parahaemolyticus* VP 4, but not *E. coli* PSSCMI 0001. *V. parahaemolyticus* VP 4 was the most sensitive to both FPBs (inhibition zone: 13.8-16 mm), followed by *Salmonella* sp. PSSCMI 0002 (inhibition zone: 10-12 mm) and *S. aureus* PSSCMI 0004 (9-10 mm). *E. coli* PSSCMI 0001 was the least sensitive to both FPBs and in this case the inhibitory effect of the phomnang beverage (inhibition zone: 6.5 mm) was higher than for the wild forest noni (4.2 mm).

Changes of microbial populations in FPBs: Molds were not detected in any sample of the plant beverages throughout the monitoring period. The initial TBC of the fermented plant beverage using method M and phomnang seaweed was about 1 log cfu mL^{-1} higher than with wild forest noni (Fig. 4a-b). However, both FPBs reached their maximum TBC at day 7, 7.3 log cfu mL^{-1} in the phomnang seaweed and 8.6 log cfu mL^{-1} in the wild forest noni beverage. In both situations TBC's then declined over the 90 days to 4.2 log cfu mL^{-1} for the phomnang seaweed and 4.6 log cfu mL^{-1} for the wild forest noni. No yeasts were detected in either preparation at the start of the fermentation but increased until day 7 to between 7-7.3 log cfu mL^{-1} then sharply decreased to between 2.5-2.8 log cfu mL^{-1} at the end of fermentation (Fig. 4a,b). There were significant differences between the growth of LAB in the 2 preparations. In the phomnang seaweed beverage LAB increased slowly to about 2.5 log cfu mL^{-1} until about day 6 then rapidly to 6.2 log cfu mL^{-1} at about day 14 (Fig. 4a). With the wild forest noni preparation, growth of LAB mirrored the TBC reaching a maximum of 8.7 log cfu mL^{-1} during days 6-7 (Fig. 4b). In both cases populations of LAB's then declined until the end of fermentation to 3.7 and 4.5 log cfu mL^{-1}, respectively (Fig. 4a,b).

Identification of the dominant LAB in FPBs: As LAB were the most abundant microorganisms detected during the fermentation their activities probably play the most

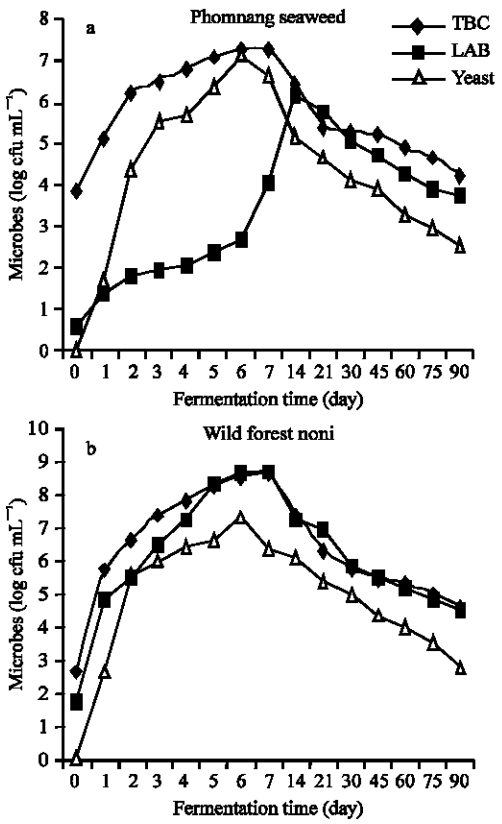

Fig. 4: Microbiological changes in fermented plant beverages using a modified method with a water filled plastic bag placed at the top of the liquid

important role in the characteristics of the fermented beverages. It was of interest to identify the LAB species present during the fermentation processes to establish if there was any correlation between the characteristics of the beverages and the identity of the LAB. During the early stages of fermentation, in both FPBs *Leu. mesenteroides* subsp. *mesenteroides* and *Leu. mesenteroides* subsp. *dextranicum* were the main species detected. However, at days 4-5 in the wild forest noni fermentation the LAB had changed from cocci to rods with *Lactobacillus plantarum* and *Lactobacillus* sp. predominating (Table 3). *Lactobacillus fermentum* was present at days 6-7 of the fermented wild forest noni beverage and later at day 14 in the phomnang seaweed preparation, in which *L. plantarum* had predominated at days 6-7. During days 14-45, similar species of lactobacilli (i.e., *L. brevis*, *L. plantarum* and *Lactobacillus* sp.) were detected in both FPBs, except that *L. coryniformis* was found only in the wild forest noni fermentation at day 21. During days 60-90, *Lactobacillus* sp. and *L. plantarum* were the only LAB found in both fermented plants.

Table 3: Identification of lactic acid bacteria isolated from the method M in beverages of phomnang seaweed and wild forest noni at varying days of fermentation

Days	Phomnang seaweed (P)	Wild forest noni (W)
1-3	*Leuconostoc mesenteroides* subsp. *mesenteroides* P1 and P2	*Leu. mesenteroides* subsp. *mesenteroides* W1 and W2
	Leuconostoc mesenteroides subsp. *dextranicum* P3	*Leu. mesenteroides* subsp. *dextranicum* W3
4-5	*Leu. mesenteroides* subsp. *dextranicum* P4A, P4B and P5	*Lactobacillus plantarum* W4
		Lactobacillus sp. W5A and 5B
6-7	*L. plantarum* P6, P7A and P7B	*Lactobacillus fermentum* W6A, W6B and W7
14	*L. fermentum* P14,	*Lactobacillus brevis* W14
	L. plantarum P14A and P14B	*L. plantarum* W14A and W14B
21	*L. plantarum* P21A and P21B	*L. plantarum* W21A and W21B
	L. brevis P21	*Lactobacillus coryniformis* W21
30	*Lactobacillus* sp. P30,	*L. brevis* W30,
	L. plantarum P30A and P30B	*L. plantarum* W30A and W30B
45	*L. plantarum* P45A and P45B,	*L. plantarum* W45A and W45B,
	L. brevis P45	*L. brevis* W45
60	*L. plantarum* P60A and P60B,	*L. plantarum* W60A and W60B,
	Lactobacillus sp. P60	*Lactobacillus* sp. W60
75	*L. plantarum* P75A, P75B and P75C	*Lactobacillus* sp. W75,
		L. plantarum W75A and W75B
90	*L. plantarum* P90A ad P90B,	*L. plantarum* W90A, W90B and W90C
	Lactobacillus sp. P90	

DISCUSSION

Effect of initial air removal on the characteristics of FPBs and film yeast: In order to establish the most appropriate method to produce fermented plant beverages that might assist local communities, tests were conducted and comparisons made between the traditional method (control set) and two simple modified methods. The most significant result was that one method, M, produced no surface film yeast whereas there were plenty of film yeast in the other two methods. The method M, allowed for the immediate removal of oxygen and retention of CO_2 produced byheterofermentative LAB (Table 3), providing conditions, long known to prevent the growth of film yeast (Wood, 1985; Jay, 2000) and stimulate the growth of many lactobacilli that produce a high TA (Table 3, Fig. 2a, b). In the 3 different methods used for the phomnang seaweed beverage fermentation, TBC were not significantly different and as a consequence neither was the pH. Also in these cases, bacteria other than LAB were the predominant bacterial population although obviously some LAB would grow with PCA (Fig. 4a). In contrast with the wild forest noni fermentation, the only non significant differences between the 3 processes were with the K and TS levels. The K content is obviously derived from the plant tissue and does not undergo microbial induced transformations

Effect of microbes on the chemical properties of FPBs: Both plant fermentation processes using method M were initiated by *Leu. mesenteroides* supsp. *mesenteroides* and *Leu. mesenteroides* supsp. *dextranicum* (Table 3). *Leuconostoc* species are obligate heterofermentative cocci that can tolerate fairly high concentrations of sugar (up to 50%) (Battcock and Azam-Ali, 1998; Okada *et al*., 2006), therefore they grew well in the plant beverage fermentation which had an initial TS of about 13% (Fig. 2a, b). Heterofermenting LAB metabolize glucose via the 6-phosphogluconate/phosphoketolase pathway. They produced carbon dioxide and acids (lactic acid and acetic acid) which rapidly lower the pH; however, they do not tolerate high acidity (Axelsson, 2004; Bergqvist *et al*., 2005) so they were detected only during the initial stages of fermentation (days 1-5). After the initial rapid removal of TS to about 8% that occurred over the first few days *Lactobacillus fermentum*, *L. brevis* and *L. plantarum* became the dominant LAB. Both *L. fermentum* and *L. brevis* are also obligate heterofermenters (Hammes and Vogel, 1995; Battcock and Azam-Ali, 1998) and they produce intermediate amounts of acid. Interestingly, the facultative heterofermenters such as *L. plantarum* were detected from day 5 until the end of the fermentation and it was among the most lactobacilli in both FPBs. Hence, it would be useful to use this bacterium as a starter culture as it can utilize sugar by either the Embden-Meyerhof pathway or phosphoketolase pathway depending on the conditions of growth (Hammes and Vogel, 1995). Normal conditions required for the glycolytic pathway by lactic acid bacteria are excess sugar and limited oxygen (Battcock and Azam-Ali, 1998; Jay, 2000). Another facultative heterofermenter (*L. coryniformis*) was found only in the wild forest noni fermentation (Table 3). Similar observations on microbial succession have been made with sauerkraut fermentations (Wood, 1985; Jay, 2000). It has long been recognized that *Lactobacillus* spp. are the most frequently detected in fermented vegetables (McDonald *et al*., 1990; Djeghri-Hocine *et al*., 2007) and it is normally detected at later stages of many vegetable fermentations prevail over that of *Leu. mesenteroides* due to its higher acid tolerance (McDonald *et al*., 1990; Battcock and Azam-Ali, 1998).

As similar species of LAB were found in both plant beverage fermentations, the changes of pH, TS and TA were also similar. However, the amount of TA in the beverage produced from the wild forest noni was higher than from the phomnang seaweed (Fig. 2a, b). This may be caused by higher amount of LAB in the former beverage (Fig. 4a, b). Higher amounts of ethanol (1%) are also found in the wild forest noni beverage perhaps due to there being roughly 0.5-1 log more yeast cfu mL^{-1} during the whole fermentation period (Fig. 3, Fig. 4a, b). Therefore to reduce the amount of yeast in the finished product, it may be necessary to provide an inoculum do

so. A small amount of methanol (0.25%) was also detected in the wild forest noni beverage because unripe fruits were used and their pectin was probably being modified by a pectin methylesterase that cleaved methoxyl groups and produced methanol, poly-garacturonate and H^+ (Whittaker, 1990; Moat and Foster, 1995).

Effect of FPBs on antibacterial activity: The sensitivity of the test bacteria to both FPB's which collected from the method M was *V. parahaemolyticus* VP 4> *Salmonella* sp. PSSCMI 0002> *S. aureus* PSSCMI 0004 and *E. coli* PSSCMI 0001 and the fermented wild forest noni beverage gave a stronger inhibition to all test organisms, except *E. coli* PSSCMI 0001, than did the phomnang seaweed beverage (Table 2). Fermented wild forest noni beverage as stated earlier contained higher levels of TA, acetic acid, ethanol, methanol and potassium (Fig. 2a, b, 3). The results indicate that inhibition of the food borne pathogenic bacteria by FPBs was mainly dependent on the amounts of organic acids and alcohols (Fig. 3, Table 2) although the amount of alcohols was very low in the beverage of phomnang seaweed. Therefore, no doubt that the phomnang seaweed beverage had antibacterial activity less than that found in the wild forest noni beverage. Many research reports have concluded that inhibition of some enteropathogens was mainly dependent on organic acids and antibiotic-like substances (Gonzales *et al*., 1993; Ivanova *et al*., 2000; Soomro *et al*., 2002; Cadirci and Citak, 2005). However, in this study alcohols showed a synergistic inhibitory action with the organic acids and this is expected as the alcohols actually can act as disinfectants (Pelczar *et al*., 1986). In addition, it is possible that alcohols may help to extract some bioactive compounds from the fruit of wild forest noni. The 6 times higher amount of K in fermented beverage produced from wild forest noni than in phomnang seaweed might also assist in the antibacterial activities. A similar result was found by Stella and Burgos (2001) who reported that potassium ion increased the susceptibility of *Saccharomyces cerevisiae* strain S288c to fluconazole. In contrast, *E. coli* PSSCMI 0001 seemed to be more sensitive to fermented phomnang seaweed beverage than fermented wild forest noni even though it had lower levels of those compounds. It means that *E. coli* PSSCMI 0001 was sensitive to some extracted compounds from phomnang seaweed or some bioactive compounds from indigenous LAB in phomnang seaweed fermentation. The results are supported by Chung and Yousef (2005) who demonstrated that *Lactobacillus curvatus* isolated from a fermented food produced a bacteriocin-like agent against *Salmonella enterica* serovar *Enteritidis* and *E. coli* 0157: H7. On the other hand, the seaweed itself may contain some compounds that inhibited *E. coli* PSSCMI 0001.

To conclude, the present study highlights the possibility to prevent film yeast, it is necessary to immediately remove air at the start of the plant beverage fermentation process. Over the 90 days of natural fermentation the numbers of yeast exceeded standard guideline for plant beverage although the amounts of LAB were higher than yeast. Therefore, the use of a selected LAB inoculant may be necessary to meet the standard guideline and it is currently conducted. Antibacterial activity increased with increasing fermentation time and antibacterial activities of FPBs mainly related to their organic acids and alcohols contents.

ACKNOWLEDGMENTS

The authors gratefully acknowledged National Science and Technology Development Agency (NSTDA), Thailand for providing funding for this work (CO-B-22-2C-18-601. Dr. Brian Hodgson was gratefully acknowledged for his critical reading.

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