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EVALUATION ON POTENCY OF GREEN CHINESE LEAF (Brassica juncea L) JUICE FERMENTED BY LACTIC ACID BACTERIA ISOLATED FROM BREAST MILK AS WET NOODLES PRESERVATIVES

Lilis Nuraida¹,², Suliantari¹,², Fitri Syawaliyah², Yessica Sinaga², Vanya A. Muthia² and Ulfa Nurmaidã²

¹Southeast Asian Food and Agricultural Science and Technology (SEAFAST) Center, and Department of Food ²Science and Technology, Bogor Agricultural University – Indonesia, Bogor Agricultural University

*Corresponding author: lilis@seafast.org

ABSTRACT

Wet noodles generally produced by home industries and small scale industries. Due to chemical characteristics of wet noodles, this product undergoes spoilage quickly. This has stimulated the use of preservatives to prolong shelf life of wet noodles. Unfortunately, the lack of knowledge and awareness of wet noodles producers has caused the use of illegal substances to preserve wet noodles. The purpose of this study was to evaluate the antimicrobial activity of green Chinese leaf fermented by lactic acid bacteria isolated from breast milk and its potency as wet noodles preservatives. Four lactic acid bacteria isolated from breast milk, namely Lactobacillus rhamnosus R21, L. rhamnosus R33, L. pentosus R13 and L. pentosus A7 were used in this study. The media use to grow the lactic acid bacteria was juice of green Chinese leaf (Brassica juncea L.). The juice were sterilised or pasteurised prior to inoculation. The results revealed that the best preparation of the media was sterilisation prior to fermentation to produce metabolite as shown by antimicrobial activity against B. cereus, S. aureus and E.coli. Addition of sugar did not increased antimicrobial activity of chinesse leaf juice. Heating the fermented juices reduced the antimicrobial activity. Application of fermented chinese leaf juice to substitute 40% of water in wet noodles preparation, slightly reduced the total microbial count of wet noodles kept at refrigerator but no effect when it was stored at room temperature. Sensory characteristic of noodle prepared with addition of fermented Chinese leaf juice was still acceptable. There was no difference in pH and aw between noodle prepared with addition of fermented green Chinesse leaf and control.

Key words: Green Chinese Leaf (Brassica juncea L.), fresh noodles, preservatives, Lactobacillus, breast milk

INTRODUCTION

Noodles is one of populer food in Asia especially in East and Southeast Asia, including Indonesia. In Indonesia, noodle has been included in variety of meals and dishes, and is consumed as second staple after rice or consumed as a snack. Variety of food served by food vendors and restaurant used wet noodles. There are two type of wet noodles, i.e, raw noodle which is further processed into meals such as chicke noodle, and cooked noodle which is comomly used for meatball noodle.

Wet noodle are produced mostly by home and small scale industries. Both raw and cooked noodles have short shelf-life, which are less than 48 h at tropical conditions. Raw noodle has a moisture content of about 35%, while cooked noodles is about 52% (Astawan 1999). The high moisture content of noodles made this
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product easily undergoes spoilage. The spoilage of cooked noodles kept at room temperature occurs after 48 h indicating by the growth of molds (Hoseney 1998). Noodles spoilage is also indicated by changes in color, off-flavor, acid taste and slimy surface. Slimy surface and off-flavor indicate bacterial growth (Hoseney 1998).

Limited technology and poor hygiene and sanitation practices during production as well as product handling and distribution also contribute to product spoilage at faster rate. Refrigeration is one of best choice for noodle preservation, however such technology can only be accessed by supermarket and restaurants. Home and small scale industries in general do not have access to refrigerators.

Limited shelf life of wet noodles has triggered many efforts to extend the shelf life, such as incorporation of preservatives into noodles formulation. Unfortunately, this also includes dangerous substance such as formalin that is prohibited to be used in food. Investigation in Bandar Lampung, Jakarta, Bandung, Semarang, Yogyakarta, Surabaya, Mataram, and Makasar in December 2005 done by National Agency for Drug and Food Control (NADFC) showed that 64.32% of wet noodles contained formalin (Astawan, 2006). In addition, NADFC also reported that 24 samples taken from markets in Bandung contain formalin and borax (Astawan 2006). Borax is used to improve texture of noodles, however this substance is also prohibited to be used in food.

The frequent exposure of formalin through food into human body will endanger human health. Therefore it is important to explore food additives as wet noodle preservative. Interest has raised on the use of natural preservatives, including the use of microbial base preservative. Lactic acid bacteria (LAB) has been known to be able to suppress the growth of pathogenic and spoilage bacteria. The ability of the LAB to inhibit the growth of pathogenic bacteria spoilage has been attributed to the production of acid and other inhibitory substances such as bacteriocin. Nuraida et al. (2007) has shown some LAB isolated from breast milk were capable inhibiting the growth of Escherichia coli, Bacillus cereus, Salmonella typhimurium, dan Staphylococcus aureus. Hence, those LAB are necessary to be evaluated for their potency as noodles preservative. Some vegetables have been known to be a good substrate for the growth of lactic acid bacteria. Fermentation of vegetable by lactic acid bacteria can be done by two procedures, i.e. the vegetable is fermented in a usual way and then it is processed by pressing the juice or the vegetable is processed to mash or raw juice at first and it is consecutively fermented (Karovicova and Kohajdova, 2003). Therefore the aim of the present research was to evaluate the potency of lactic acid bacteria isolated from breast milk to ferment green Chinesse leaf and to evaluate its antimicrobial activity as well as the potency of fermented green Chinesse leaf as wet noodle preservative.

MATERIALS AND METHODS

Bacterial cultures

Four lactic acid bacteria (LAB) i.e. Lactobacillus rhamnosus R21, L. rhamnosus R33, L. pentosus R13 and L. pentosus A7 previously isolated from breast milk were studied. Pathogenic bacteria used for evaluation of antimicrobial activity were Escherichia coli, Staphylococcus aureus and Bacillus cereus. All cultures were obtained from SEAFAST Center, Bogor Agricultural University. All cultures were maintained in stock culture kept in 20% glycerol at -20°C. When required the lactobacilli were grown in MRS broth while pathogenic bacteria in NB at 37°C.
Preparation of the green Chinese leaf juice
The green Chinese leaf (Brassica juncea L.) was cleaned and soaked in 200 ppm Na-hypochlorite for 2 minutes. The juice was prepared by macerating the leaf with addition of water in the ratio 1:1. The juice was filtered using filtering cloth. A part of the filtrate was then pasteurised (heating at 96°C for 2 minutes) and the other part was sterilised. The juice was then inoculated with 1% LAB isolate and incubated at 37°C for 48 h. The number of LAB was enumerated and the fermented juice was evaluated for their antimicrobial activity against test bacteria i.e. E. coli, S. aureus and Bacillus cereus using well diffusion methods (Gariga et al., 1993). The fermented juice was centrifuged and filter sterilised prior to evaluation of their antimicrobial activity. The test bacteria were grown in NB for 24 h, then 0.2 ml was inoculated into 100 ml melting NA, and about 20 ml pour into sterile petri dish. The agar was allowed to set. Well of 6 mm diameter was made on the agar seed with the test bacteria. The fermented chinesees juice was pale in the well at about 30 µl. The dish was incubated at 37 °C for 24 h. Clear zone around the well was measured and represent inhibition zone.

Effect of addition of sugar on the juice on their antimicrobial activity
The juice was prepared as above, but with addition of sugar at 0, 3 and 5%. The juice was sterilised prior to inoculation. The fermentation and evaluation on antimicrobial activity was done similar as above.

Effect of heating of fermented Chinese leaf juice on its antimicrobial activity
The vegetable juice was prepared as above but without addition of sugar (sucrose). The fermented juice was then heated at 96°C for 2 minutes. The antimicrobial activity of the juice was evaluated using well diffusion method (Gariga et al., 1993).

Application of Fermented Chinese leaf juice for noodle preservation
For the application in noodle, the fermented juice was used to substituted 40% of water in wet noodle formula. The chinesees leaf juice was sterilised prior to inoculation. The noodle formula used in this experiment were as follows: wheat flour (100%), water (35% of the wheat flour), NaCl (1% of the wheat flour) and Na2CO3 (0.6% of the wheat flour). The noodle was prepared by mixing all ingredient, including fermented chinesees leaf, sheeting, cutting and boiling with addition of vegetable oil. The noodle was pack in plastic bag and kept at room and refrigerator. Analyses was done for total aerobic count, mold and yeast, pH, aw, and sensory by hedonic.

RESULTS AND DISCUSSION

Preparation of the Chinese leaf juice
Lactic acid bacteria isolate grew well on the vegetable juice with the count ranged between $10^8 - 10^9$ cfu/ml. The result was in accordance with previous work done by Purwono (1995) showed that L. lactis subsp. cremoris grown in green chinese leaf reached $3.4 \times 10^5$ cfu/ml.

The antimicrobial activity of the fermented juice is depicted in Figure 1. Isolate R21, R22 and A7 show stronger inhibition on the sterilised juice as compared to the pasteurised one. B. Cereus was more susceptible compared to the other bacteria as shown by high inhibition zone. The results revealed there was no trend if sterilisation or pasteurisation gave better results. However for further evaluation the juice was sterilised prior to inoculation. Heating of the juice was intended to inactivate the microorganism naturally present in the Chinese leaf. Traditionally, Chinese leaf was fermented by lactic acid bacteria without addition of starter culture. Control of microorganism is done by addition of salt, for example to make
fermented cabbage (saurekraut), into shredded fresh cabbage was added 2.25% salt (Steinkraus, 1997). There are 3 types of fermentation of vegetable juices: spontaneous fermentation by natural microflora, fermentation by starter cultures that are added into raw materials and fermentation of heat-treated materials by starter cultures (Hammes, 1990). Natural fermentation of vegetables such as kimchi and saurekraut involved mix lactic acid bacteria grow sequentially (Steinkraus, 1997).

Figure 1. Antimicrobial activity of fermented juice of green Chinese leaf that has been sterilised (A) and pasteurised (B) prior to fermentation by the LAB isolates.

Effect of addition of sugar on the juice on their antimicrobial activity
Addition of sugar into the juice did not enhanced their antimicrobial activity as shown in Figure 2. Addition of sugar did not enhance production of antimicrobial substances whish mainly-acid. The lactic acid bacteria count was also not affected by addition of sugar (Table 1). This indicate that the sugar available in the juice was sufficient to support the growth of lactic acid bacteria. Green Chinese leaf also contains (per 100 g) 6460 IU Vitamin A, 102 mg Vit B, 0.09 mg Vit C, 220 mg Calcium dan kalium (Arief 1990).

Chinesse leaf/cabbage, cabbage, carrot and spinach media gave relatively higher fermentability that other vegetables because they have more fermentable saccharides than other vegetables (Kim et al., 2000).

Figure 2 indicates isolate L.pentosus R13 shows relative high antimicrobial activity against the test bacteria. Therefore, in the next step this isolate was chosen to be applied in noodle. L. rhamnosus R33 was also chosen for application as representative of L. rhamnosus strain.
Figure 2. Effect of addition of sucrose into juice of green Chinese leaf prior to fermentation on antimicrobial activity.

Table 1. Effect of addition of sugar on the lactic acid bacteria count in fermented of juice of green Chinese leaf

<table>
<thead>
<tr>
<th>Sugar concentration</th>
<th>LAB count (cfu/ml)</th>
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<tbody>
<tr>
<td></td>
<td>R33</td>
</tr>
<tr>
<td>0%</td>
<td>9.1x10^8</td>
</tr>
<tr>
<td>3%</td>
<td>1.4x10^9</td>
</tr>
<tr>
<td>5%</td>
<td>6.5x10^8</td>
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Figure 3. Effect of heating of fermented green Chinese leaf juice on antimicrobial activity. (A) unheated, (B) heated at 96°C for 2 minutes (pasteurised). The juice was sterilised prior to fermentation.
Effect of heating of fermented Chinese leaf juice on its antimicrobial activity
Heating the fermented green Chinese leaf juice decreased antimicrobial activity of L. pentosus R13, L. rhamnosus R21 and R33 as shown in Figure 3. This indicate that the antimicrobial substances by those isolates were heat sensitive. Different figures shows by L. pentosus A7 that was not affected by heating. Research done by Setyaningsih (2010) revealed that the neutralised metabolites of L. pentosus R13 still exhibited antimicrobial activity toward pathogenic bacteria. This indicated that the strain may produce antimicrobial substances other than organic acid. Further research is still needed to elucidate the antimicrobial substance produced by this strain. For application to preserve noodle, the fermented vegetables was not heated.

Application of Fermented Chinese leaf juice for noodle preservation
The unheated fermented green Chinese leaf juice was incorporated to noodle formula substituted 40% of water. Sensory characteristic of noodle prepared with addition of fermented vegetables is presented in Figure 4. Higher score indicate more acceptability of noodle. The colour of noodle was preferred than control, however lower core was given for taste, aroma and overall. There was no difference in texture and stickyness of noodle observed by sensory evaluation. Those figures means that the noodle prepared with addition of green Chinese leaf were still acceptable.

Figure 4. Effect of addition of fermented green Chinese leaf juice on sensory characteristic of noodle

The water activity (a_w) of noodle processed with addition of fermented vegetable juice was not different with control i.e. 0.98 for noodle with R13, 0.97 for noodle with R33 and 0.98 for control. No significant changes in water activity during storage. There is also no difference between pH of noodles prepared with addition of fermented green Chinese leaf juice and control, i.e. in the range of 8.93 – 9.03. This indicate that addition of fermented green Chinese leaf did not acidify the noodle. During storage at room temperature the pH dropped to 6.29-6.8, while the pH of noodle kept at refrigerator was quite stable in the range of 8.9-9.7 (Figure 5)
The results revealed that addition of fermented vegetable did not improve shelf life of noodle stored at room temperature (Figure 6). In 24 h the maximum bacterial load set by Indonesia National Standard (SNI) has been exceeded. The SNI set the maximum bacterial count is $10^6$ cfu/g. Preservation effect was observed to some extent for noodle added fermented vegetable of \textit{L. rhamnosus} R33 stored at refrigerator (Figure 7). After 168 h the maximum microbial count set by SNI has not been reached, while the noodle control (no added fermented green Chinese leaf) has reached the maximum microbial load. Mold was only detected in noodle stored at room temperature (Figure 8), however the number was still below the maximum number set by the SNI ($10^4$ cfu/g).
Figure 6. Effect of addition of fermented green Chinese leaf juice on total microbial count of noodle stored at room temperature.

Figure 7. Effect of addition of fermented green Chinese leaf juice on total microbial count of noodle stored at refrigerator.

Figure 8. Effect of addition of fermented green Chinese leaf juice on mold count of noodle stored at refrigerator.
CONCLUSION

The best preparation of the juice was sterilization prior to fermentation by LAB isolates. The sugar and the nutrient in green Chinese leaf juice was sufficient to support the growth of lactic acid bacteria isolated from breast milk. Heating of fermented green Chinese leaf juices in general reduced its antimicrobial activity, indicating the isolates may produce antimicrobial substance other than organic acid. Addition of fermented green Chinese leaf did not affect significantly sensory characteristic and did not affect pH and \( a_w \) values of the noodle. Application of fermented green Chinese leaf juice did not reduce the total microbial count of wet noodle stored at room temperature, but slightly reduce the total microbial count at refrigerator as compared to the control. Hence application of the fermented application alone is not sufficient to preserve wet noodles.

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