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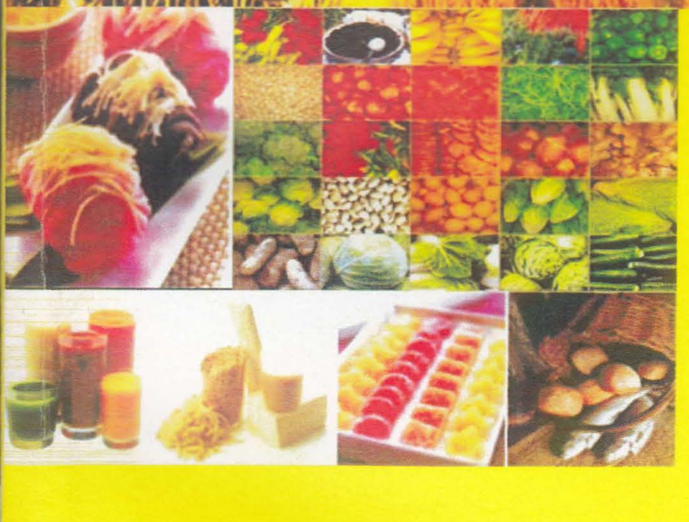

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EVALUATION ON POTENCY OF GREEN CHINESSE LEAF (*Brassica juncea L)* JUICE FERMENTED BY LACTIC ACID BACTERIA ISOLATED FROM BREAST MILK AS WET NOODLES PRESERVATIVES

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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 Chinesse 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 fermenttaion 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 chinesse leafe 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 Chinesse leaf juice was still acceptable. There was no difference in pH and a_w between noodle prepared with addiiton of fermented green Chinesse leaf and control.

Key words: *Green Chinesse 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 veariety 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 comonly 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 mousture content of about 35%, while cooked noodles is about 52% (Astawan 1999). The high moisture content of noodles made this product easily undergos 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 bacerial 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 as 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 trigerred many effort 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 Desember 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 repoted 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 maicrobial 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 ans 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 of 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 preseervatives.

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 grenn Chinesse leaf juice

The green Chinesse leaf (*Brassica juncea* L.) was cleaned and soaked in 200 ppm Na-hipoklorit 200 ppm for 2 minutes. The juice was prepared by macerating the leaf with addiiton 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 innoculated 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 againts test bacteria i.e. *E. coli*, *S. aureus* and *Bacillus cereus* using well difussion methods (Gariga *et al.*, 1993). The fermented juice was centrifuged and filter sterilised prior to evaluation of their antimicrobial activity. The test bacteria were grwon in NB for 24 h, then 0.2 ml was innoculated 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 measure and represent inhibiton 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 inculation. The fermentation and evaluation on antimicrobial activity was done similar as above.

Effect of heating of fermented Chinesse 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 Chinesse 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 chinesse leaf juice was sterilised prior to innoculation. 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 Na_2CO_3 (0.6% of the wheat flour). The noodle was prepared by mixing all ingredient, including fermented chinesse 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 Chinesse 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 chinesse leaf reached 3,4 x 10^8 cfu/ml.

The antimicrobial activity of the fermented juice is depicted in Figure 1. Isolate R21, R22 and A7 show stonger inhibition on the sterilised juice as compared to the pasteurised one. *B. Cereus* was more succeptible 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 innoculation. Heating of the juice was intended to inactivate the microorganism naturally present in the Chinesse leaf. Traditionally, Chinesse leaf was fermented by lactic acid bacteria without addition of starter culture. Control of microrganism is done by addition of salt, for example to make fermented cabbage (saurekraut), into shreded 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 (Seinkraus, 1997).

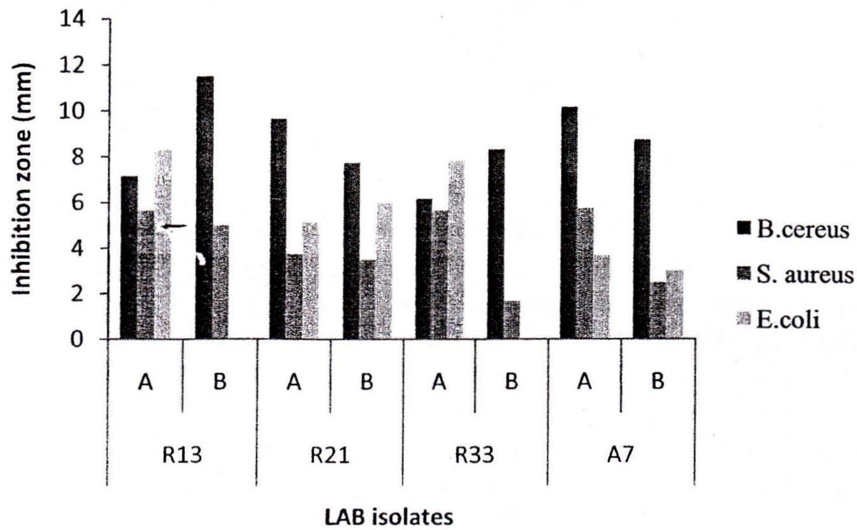

Figure 1. Antimicrobial activity of fermented juice of green Chinese leaf that has been sterilised (A) and pasteurised (B) prior to fermentation by the LAB isolates.

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 (Tabel 1). This indicate that the sugar available in the juice was sufficient to support the growth of lactic acid bacteria. Green Chinesse leaf also contains (per 100 g) 6460 IU Vitamin A, 102 mg Vit B, 0.09 mg Vit C, 220 mg Calsium 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

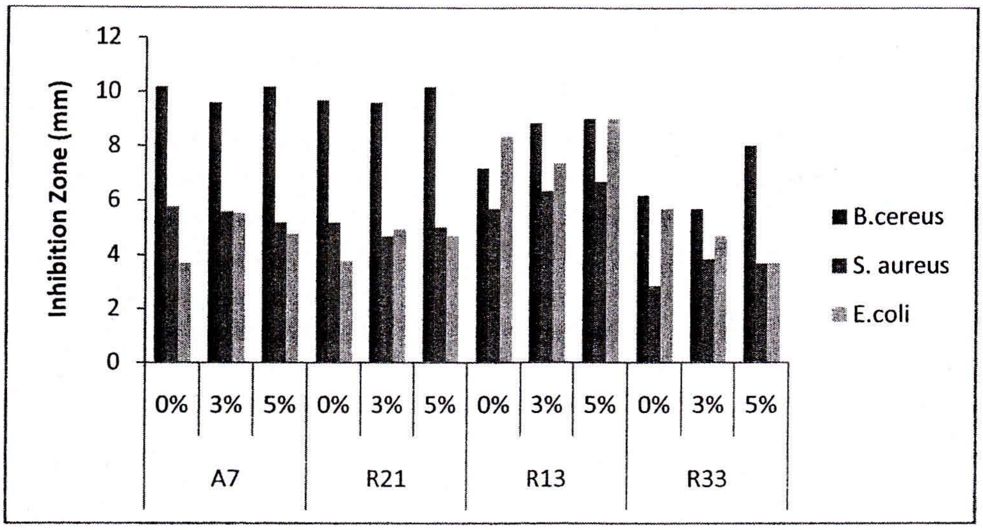

Figure 2. Effect of addition of sucrose into juice of green Chinese leaf prior to fermentetion on antimicrobial activity.

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

Sugar concentration	LAB count (cfu/ml)	
	R33	R13
0%	$9.1x10^8$	$1,19 \times 10^9$
3%	$1.4x10^9$	$1,2 \times 10^9$
5%	$6.5x10^8$	$1,19 \times 10^9$

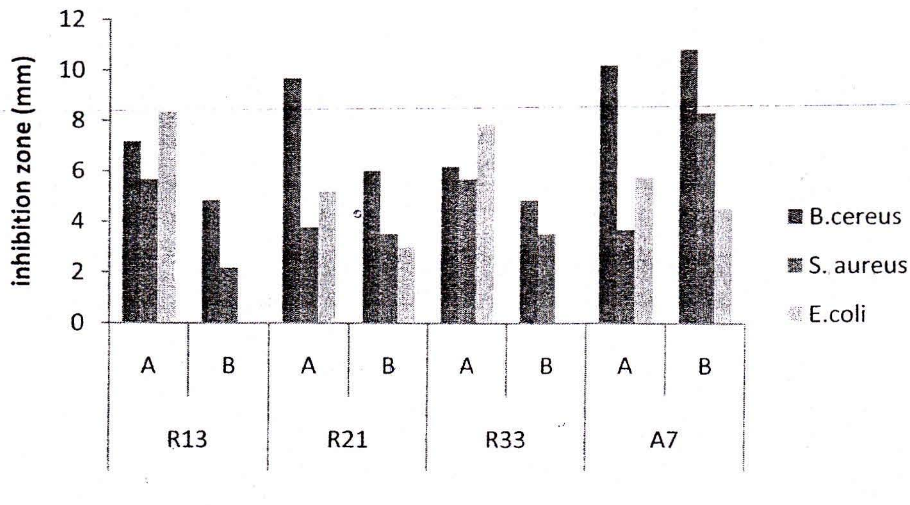

Figure 3. Effect of heating of fermented green Chinese leaf juice on antimicrobial activity. (A) unheated, (B) heated at 96°C for 2 minutes (pasteurised). The juice was sterilised prior to fermentation.

Effect of heating of fermented Chinesse leaf juice on its antimicrobial activity

Heating the fermented green Chinesse 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. Resrach 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 Chinesse leaf juice for noodle preservation

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

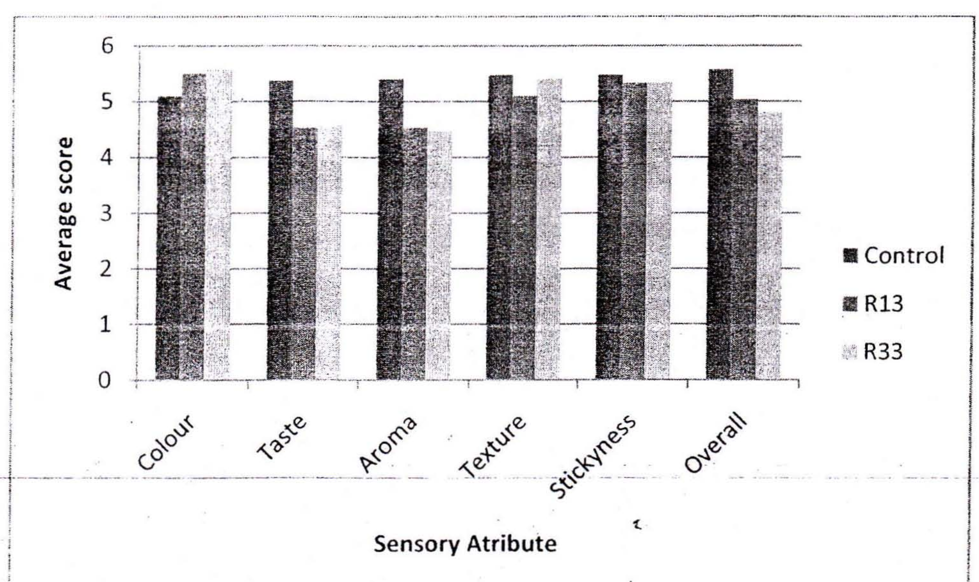

Figure 4. Effect of addition of fermented green Chinesse leaf juice on sensory characteristic of noodle

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 noodel 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 Chinesse leaf juice and control, i.e. in the range of 8.93 – 9.03. this indicate that addition of fermented green Chinesse 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 ain the range of There was no differnce between control and noodle with addition of green Chinesse leaf juice. 8.9-9.7 (Figure 5)

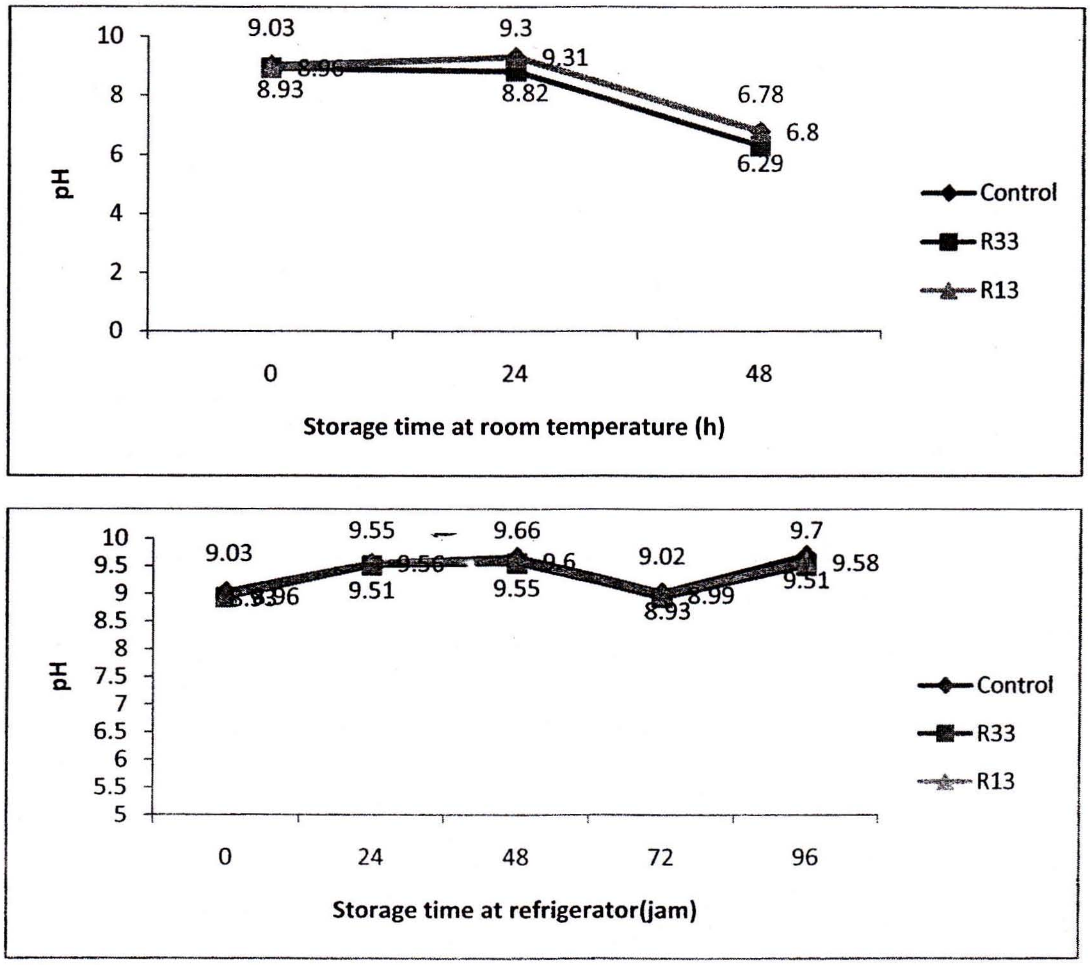

Figure 5. Effect of addition of fermented green Chinesse leaf juice on the pH during storage

The results revealed that addition of fermentee vegetable did not improved 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 extend for noodle added fermented vegetable of *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 Chinesse 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).

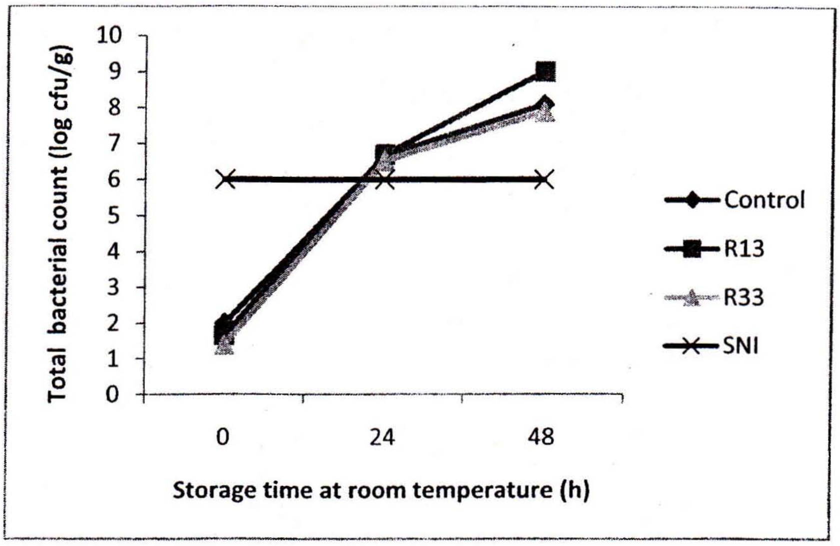

Figure 6. Effect of addition of fermented green Chinesse leaf juice on total microbial count of noodle stored at room temperature.

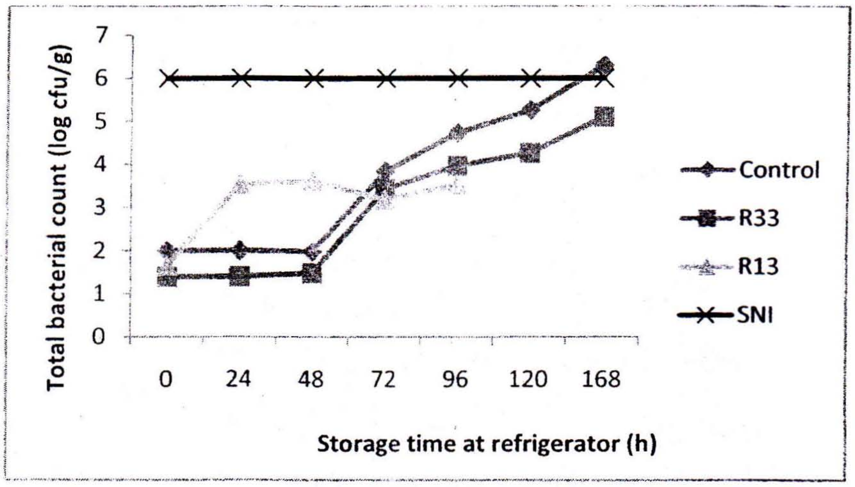

Figure 7. Effect of addition of fermented green Chinesse leaf juice on total microbial count of noodle stored at refrigerator.

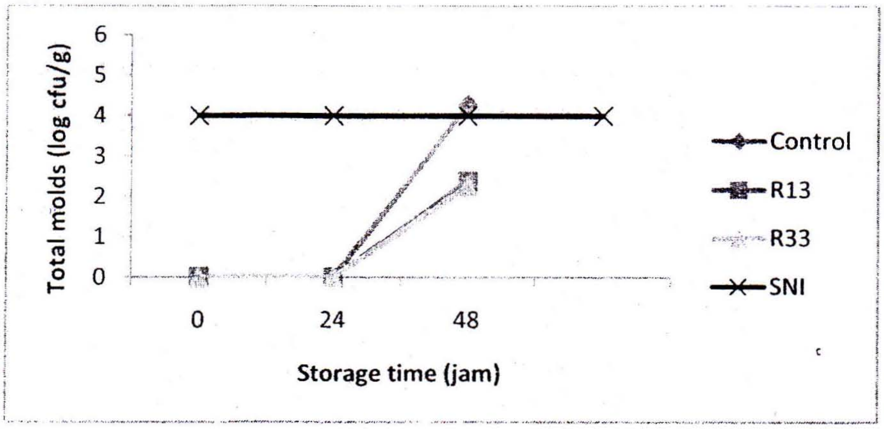

Figure 8. Effect of addition of fermented green Chinesse leaf juice on mold count of noodle stored at refrigerator.

CONCLUSION

The best preparation of the juice was sterilization prior to fermentation by LAB isolates. The sugar and the nutrient in green Chineese leaf juice was sufficient to support the growth of lactic acid bacteria isolated from breast milk. Heating of fermented green Chinèsse leaf juices in general reduced its antimicrobial activity, indicating the isolates may produce antimicrobial substance other than organic acid. Addiiton of fermented green Chinesse leaf did not affect signifinactly sensory characterisc and did not affect pH and a_w values of the noodle. Application of fermented green Chinesse 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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