DETERMINATION OF SUGAR UTILISATION BY *Pediococcus pentosaceus* IN FERMENTATION OF MADATHAWALU

N. D. Wijemanna[1,2], D. M. W. D. Divisekera*[1], I. G. N. Hewajulige[1], M. M. N. P. Gunasekera[1], J. Goonaratne[1] and R. A. U. J. Marapana[2]

[1]Food Technology Section, Industrial Technology Institute (ITI), Colombo 7, Sri Lanka
[2] Department of Food Science, University of Sri Jayewardenepura, Sri Lanka

ABSTRACT

In order to study the kinetics of the Lactic acid bacteria in indigenous rice based media, it is essential to investigate sugar utilization pattern by the isolate. Therefore, the objective of the study was to determine the sugar utilization ability of *Pediococccus pentosaceus* in *Madathawalu* based fermentation media. *Madathawalu* rice samples were inoculated with the known concentration of *Pediococccus pentosaceus* and after the fermentation, the sugar concentration was determined by using high performance liquid chromatography (HPLC) and the results show the sugar utilization pattern by the isolate.

Key words: P. pentosaceus, Madathawalu, Fermentation

1. INTRODUCTION

Bio-preservation refers to extended shelf life and enhanced safety of foods using microorganisms and/or their metabolites. Lactic Acid Bacteria (LAB) is generally employed in bio-preservation due to of their significant contribution to enhance the flavor, texture and, the nutritional value of the food products. LAB is used as natural or selected starters in food fermentations. Therefore LAB performs an essential role in the preservation and production of wholesome foods. Potential probiotic LAB, *Pediococcus pentosaceus* has been isolated, characterized and identified from fermented *Madathawalu* an indigenous rice variety of Sri Lanka at Industrial Technology Institute, Sri Lanka.

The genus *Pediococcus* belongs to the family *Lactobacillaceae* in the order *Lactobacillales*. They are coccus shape microbes, Gram-positive, non-motile, non-spore forming and are spherical bacteria. Since the end product of them is lactic acid, *Pediococcus pentosaceus* are tolerant to acid and bile [1]. *Pediococcus pentosaceus* can be cultured at 35 – 40 ^{0}C but are unable to grow at 50 ^{0}C. They are able to grow in pH values between 4.5 and 8.0 [2]. *Pediococcus pentosaceus* has been intensively investigated and widely employed for food preservation due to its ability to produce antimicrobial agents. Strains of *Pediococcus pentosaceus* produce antimicrobial inhibitory compounds known as bacteriocins and it is important LAB involved as starter culture in meat, vegetable and dairy fermentation causing characteristic flavor changes, improving hygienic quality and extending the shelf life of several products [3]. They are largely found in fermented foods that are rich in sugar content and ferment glucose to produce lactic acid [4] and they are widely distributed in beverages. One of the reasons for the increasing interest in fermented foods is its ability to promote the functions of the human digestive system in a number of positive ways. This particular contribution is called probiotic effect [5]. *Madathawalu* is red rice with dark, fine grain and it is highly recommended as an ayurvedic treatment to boost the immune system [6]. The indigenous rice rich have high amount of Glutamic acid, high concentration of vitamins, rich in fiber and they have low Glycemic index [6].

The substrates from the environment that are utilized for bacterial growth are called nutrients. During anabolism, nutrients are taken up and are changed into cell constituents in an energy depending process. Lactic acid bacteria have numerous nutritional requirements for growth, especially an organic compound as their carbon source including carbohydrates, peptides or amino acids, fatty acids, organic acids, nitrogen bases and aromatic compounds [7].

According to the Charalampopoulos [8] all microbes attained high maximum cell populations when there is the sufficient availability of total fermentable sugars likes maltose, sucrose, glucose, fructose and free amino nitrogen in the fermentation media. Substrate deficiency in sugars and free amino nitrogen contributed to growth limitation. As well as the deficiency in specific vitamins or minerals also could contribute to growth limitation [9].

2.1 Substrate media preparation

Commercially available variety of indigenous rice namely *Madathawalu* was purchased. Rice grains were ground in a Fritsch mill with a sieve of size 0.5 mm. A sample of the flour obtained was mixed with water (1:8). The resulting slurry was shook well in shaker incubator for 3 h with 150 rpm. The resulting slurry was centrifuged at 10 000 rpm for 30 min. at room temperature. The supernatant fluid was collected and immediately sterilized at 121 ^{0} C for 20 min in the autoclave and the substrate media were stored under refrigeration condition until it use.

2.2 Fermentation

Experiment (in flasks) was carried out as batch fermentation. Since, Sugar is low in the fermentation media, to initiate the bacterial activity; (1% w/v) lactose was added to the media before the fermentation. Samples were inoculated with a 10 % (v/v) of *Pediococcus pentosaceus* and incubated at 37 ^{0}C for 30h. Samples were collected every 2h for the first 10h and then at 24 and 30h for the analysis.

2.3 Determination of sugar concentration

Sugar concentration was determined by HPLC. for the sugar analysis , samples were prepared by centrifuging 5 ml of each fermented rice sample at 6000 rpm for 10 min , filtered and injected for to the HPLC system. The properties and operating conditions of HPLC system are given in below table.

2. RESULTS

Madathawalu medium supported well the growth of *Pediococccus pentosaceus* which showed increases in their cell populations during 8 hours of fermentation. This could be due to the presence of organic compound as their carbon source including carbohydrates, peptides or amino acids, fatty acids, organic acids, nitrogen bases and aromatic compounds [7].

Table 1: The properties and operating conditions of HPLC system

Properties	Specifications
Column	Agilent Carbohydrate / NH_2 column
Column length	250 mm
Column diameter	4.6 mm
Particle size	5µm
Guard cartridge	Agilent NH_2
Column cleaning solvent	2- Propanol
Mobile phase	78:22 ; Acetonitrile : H_2O
Flow rate	1.2 ml/ min
Temperature	30.0 ^{0}C
Detector	Refractive index
Elution type	Isocratic

Since the objective of the study was to determine sugar utilization pattern by the *Pediococccus pentosaceus* in *Madathawalu* based fermentation media. It was observed that the initial concentration of lactose sugar 8.58 g/l was rapidly decreased during 8 hours of fermentation period up to 5.62 g/l. This may be due to rapid consumption of lactose sugar by the starter for multiplication and increment of cell mass during 8 hours of fermentation.

Results showed (figure 1) the isolate demonstrated a specific preference for sugars during the first 8 hours of fermentation which is usually given the exponential phase of lactic acid bacteria, while giving the maximum cell growth [8]. By drawing a growth curve, it was observed that the starter reached the exponential phase in 8 hours of fermentation. The preference of Lactic acid bacteria towards the sugars has also been suggested by many studies. [10, 11].

After 8 hours, Sugar content gradually decreased. It decreased up to 5.45 g/l, at the end of 24 hours of fermentation period. The reason for the reduction of sugar utilization by the starter culture after 8 hours could be due to deceleration of cell growth and cell division as well as depletion of essential nutrients, and/or accumulation of toxic by-products [8]. The amounts of the available sugars consumed during the exponential phase by isolate was 35% and Passos [12] also has demonstrated a 45% reduction in sugar content during the *Lactobacillus plantarum* exponential phase (10 h) in cucumber juice, when growth ceased.

This fermentation performed in *Madathawalu* medium without pH control. The given 35 % of sugar consumption could be an incomplete

consumption of the available sugars in medium. In agreement with the above, Elizete [13] suggested the incomplete consumption of sugar by *Lactobacillus reuteri* after 26 hours of fermentation in cane sugar based medium with uncontrolled pH condition while having rapid and almost complete consumption of sugar during 26 hours of fermentation time with controlled pH. Venkatesh [13] also reported an incomplete consumption of the available sugars (17%) and a fast cessation of *Lactobacillus bulgaricus* growth (approximately 10 h) in fermentations performed in synthetic media without pH control and at constant pH (5.6) and at controlled pH a 90% reduction in sugar and a longer exponential phase (approximately 18 h) were observed.

Charalampopoulos [8] reported that sugar content of the malt medium is not the decisive growth limiting factor when small amounts of the available sugars consumed during the exponential phase by LAB (19%, 17%). when isolate shows the high consumption of sugar, here it gives the higher production of acid specially lactic acid, and this rapid production of acid cause to progressively decrement of pH of media during fermentation [8]. These organic acids can inhibit microbial growth in their un-dissociated form, dissociated form or indirectly by the protons (H+) [14]. Without the optimum pH of isolate to grow, it could be caused to deceleration of cell growth and division [13].

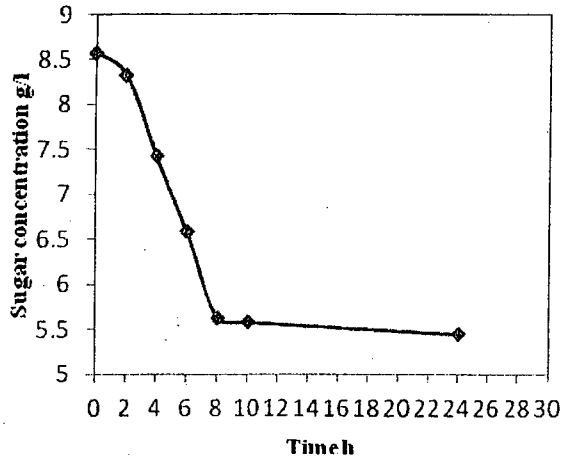

Figure 1: Change of Lactose sugar concentration with fermentation time

3. CONCLUSION

P.pentosaceus can be used as a starter culture to develop indigenous rice based value added food products.

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