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Fermentative Utilization of Fruit Peel Waste for Lactic Acid Production by Lactobacillus plantarum

KEYWORDS: Laclobacillus plantarum, fruit peels, hydrolysate, lactic acid (LA).

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ABSTRACT *The high production cost involved in the chemical synthesis of lactic acid (LA) along with its increased demand calls for attempts to produce LA efficiently from inexpensive raw materials. The main focus of this research work was to carry out the fermentative production of LA from various fruit peel waste (mango, orange, banana and pineapple) as substrates by employing Lactoctobacillus plantarum as the starter culture. The optimal conditions, in terms of pH, temperature and salt concentration, for lactic acid production were determined. The technological properties such as acidification activity, exopolysaccharide (EPS) production, enzymatic activities and carbohydrate fermentation pattern were studied following standard procedures. The highest lactic acid production was obtained from mango peels (10.08 g/L) , whereas the other substrates viz. orange peels, banana peels and pineapple peels produced 5.74 g/L, 4.68 g/L and 4.68 g/L of lactic acid respectively..*

INTRODUCTION

Lactic acid production has received significant attention due to its potential applications in food, cosmetics, pharmaceutical and chemical industries. Lactic acid, classified as GRAS (generally regarded as safe) for use as food additive by the US FDA (Food and Drug Administration) can be produced by either microbial fermentation or chemical synthesis. In recent years, fermentation approach has become more successful because of increasing market demand for naturally produced lactic acid. Moreover this method pave way for the sustainable utilization of inexpensive agricultural residues in bioprocess and thus serves as an alternative way to replace costly raw materials and bulk use of such materials will solve environmental hazards.

Fruit based industry produces large volume of wastes, both solids and liquids; these wastes pose increasing disposal and pollution (high BOD or COD) problems and represent a loss of valuable biomass and nutrients. However these carbohydrate rich wastes can be tuned as valuable substrates for the commercial production of organic acids like lactic acid and thus can be regarded as a viable option for meeting the growing demand for LA.

This research work evaluates the fermentative utilization of fruit peel wastes (mango, orange, banana and pineapple) as substrates for lactic acid production by employing Lactoctobacillus plantarum as the starter culture. Thus the present study highlights a methodology for recycling, reprocessing and eventual utilization of fruit waste for beneficial uses rather than their discharge to the environment which might cause detrimental environmental effects.

MATERIALS AND METHODS

Starter culture

Lyophilized pure culture of L.plantarum was procured from National Dairy Research Institute, Karnal and was activated by inoculating it in 30ml of MRS broth and incubated in a rotory shaker at 37°C for 7 days.

Optimization of temperature, pH and salt concentration for the effective growth of L.plantarum.

L.plantarum culture was cultivated in MRS broth medium of varying pH (2, 4, 6 and 8) and NaCl concentration (2%, 4%, 6.5% and 10%) at 37 °C for 5days and cell density was monitored by measuring OD at 600nm on daily basis. For temperature optimization , the strain was cultivated in MRS broth medium (pH 7) and incubated in varying temperature range (10°C , 15°C , 37°C ,45°C) for 5days and cell density was monitored by measuring OD at 600nm on daily basis.

Assessment of Acidification activity

Acidification activity of L.plantarum was measured by change in pH during time in Skim milk medium (10%w/v) (Ayad et. al., 2004).The pH of the inoculated skimmed milk medium was measured at 0, 2, 4, and 6 hrs using a pH meter.

Assessment of Exopolysaccharide activity

EPSs production by L.plantarum was carried out using the method described by Guiraud (1998) on to LTV Agar plates and incubated at 30°C for 48hrs.

Assessment of proteolytic activity

Overnight broth culture of L.plantarum (0.1ml) was spread plated on to Skim milk agar plates and incubated at 37°C for 48hrs. Plates were observed for zone of clearance around the colonies. (Gordon et .al., 1973).

Assessment of amylolytic activity

Surface dried plates of starch agar was inoculated with 24hrs old culture of L.plantarum in the centre and incubated at 30°C for 48hrs. The plates were then flooded with iodine solution for 15min and examined for clear zones around the growth for the assessment of amylolytic activity (Gordon et .al., 1973).

Assessment of lipolytic activity

About 0.1ml of 24hour old MRS broth culture of L.plantarum was spread plated on to Tributyrin agar plates and incubated at 37°C for 48hrs. Plates were observed for zone of clearance around the colonies. (Leuschner et. al, 1997).

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Analysis of carbohydrate fermentation pattern
The fermentation of carbohydrates was determined in modified MRS broth (glucose omitted) containing 0.04g/L of phenol red (pH indicator), and supplemented with 1% of the following carbohydrates; glucose, galactose, fructose, lactose, maltose, inulin and xylose.

Raw material for fermentation
A total of four different fruit waste substrates (mango, orange, banana and pineapple) were collected from fruit markets and used for lactic acid production by Lactobacillus plantarum

Estimation of total sugars in the fruit peels
Total sugar content in the fruit peels were determined by Anthrone method.

Preparation of hydrolysates for fermentation
The modified method of Puimput et. al., 2008 was used for substrate hydrolysate preparation. About 8gram of each fruit peel waste was steam exploded in an autoclave at 121°C for 20min .Sterile water was added to the wet pretreated material to make the volume of 200ml and boiled at 80°C for 30 min followed by filtration with cheese cloth. Acid hydrolysis of filtrate was carried out by autoclaving at 121°C with concentration of 1% HCl v/v for 30min. The pH of the hydrolysate after hydrolysis was adjusted with CaO to 6-6.8 and the CaSO4 precipitate formed was removed by filtration with Whatmann filter paper No.1.

Inoculum preparation
The L.plantarum culture was cultivated in modified MRS broth containing different fruit peel hydrolysates instead of distilled water. These media were kept for incubation at ambient temperature on rotary shaker at 120 rpm for 3 days. These were used as inocula for further studies.

Media and fermentation conditions
Fermentation media was prepared by adding 200ml of filter sterilized fruit peel hydrolysates to 50ml of sterile MRS medium in separate 500ml conical flasks. These flasks were inoculated with 5% L.plantarum cultures precultivated in specific fruit hydrolysate based media and incubated at 37°C in a shaker incubator (120rpm) for 6 days. The substrate consumption (reducing sugar content) and also product (LA formation) was estimated on daily basis.

Estimation of reducing sugars (analysis of substrate consumption)
The residual reducing sugar content of the fermentation broth was estimated on regular basis spectrophotometrically using DNSA (dinitrosalicylic acid) method as described by Miller (1959).

Estimation of lactic acid production
The production of lactic acid was primarily detected by estimating the titrable acidity of the fermentation medium on daily basis, by titrating the fermentation medium against 1M NaOH using phenolphthalein as indicator.

Amount of lactic acid (g/L) =

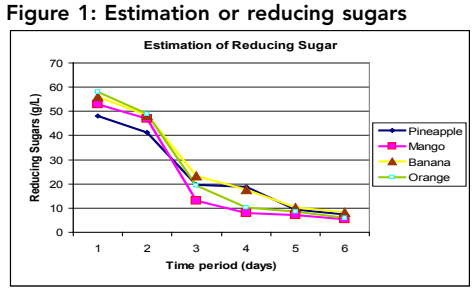
$$\frac{\text{Volume of NaOH consumed for titration} \times \text{Gram Eq.wt of LA} \times \text{Normality of NaOH}}{\text{Volume of fermentation broth used for titration}}$$

Lactic acid downstream processing and confirmation
Lactic acid present in the fermentation broth was separated using the conventional Calcium lactate precipitation method (Sodeck, 1981) for the recovery of LA. These crystals were further used for confirmation of LA using p-hydroxy diphenyl method as described by Barnett (1951). The estimation of L(+) and D(-) lactic acid content was done using NAD+ linked L- lactate dehydrogenase assay.

RESULT AND DISCUSSION
The optimal growth temperature, pH and NaCl concentration for the growth of L.plantarum was found to be 37°C, pH 6 and 2% NaCl. Analysis of the technological properties of the culture was primarily done to evaluate its feasibility to be employed as a starter culture for industrial fermentation. The acidification activity analyses showed that the isolated L.plantarum strains exhibited medium acidification range of 0.65 to 0.71 ΔpH (change in pH) after 6 hrs. A moderate to fast acidifying strains are good candidates for industrial fermentation process as primary starter culture while poor acidification strains can be used as adjunct cultures depending on their properties (Ayad et.al., 2004). The formation of exopolysaccharides has been reported to function as viscosifying agents, sabilizers, gelling agents or water binding agents (De Vuyst et.al. 2001). L.plantarum strains inoculated on skim milk agar plates produced clear zone of hydrolysis around their colonies indicating that the strains exhibit proteolytic activity. The proteolytic activity of L.plantarum strains is essential for the growth of the organisms in protein based substrates and is involved in the development of organoleptic properties of different fermented products. The inability of L.plantarum strains to hydrolyze starch suggested the absence of amylase production and calls for the need of pretreatments like acid hydrolysis or enzymatic treatment of the starch based substrates prior to fermentation. The ability of L.plantarum to show lipolytic activity in vitro is very promising. It is assumed that such activity can be manifested by the culture in vivo which will lead to the reduction of cholesterol level in humans if used as starter culture or as adjunct culture.

Reducing sugar analysis according to DNSA method revealed a gradual decrease in the residual reducing sugar content irrespective of the media type as depicted in the Figure 1. This is due to the utilization of the reducing sugars by L.plantarum as carbohydrate source for the production of LA.

Figure 1: Estimation or reducing sugars



Estimation of lactic acid production
In the present study using L.plantarum during the fermentation period (6days) the highest acidity was observed in the 3rd day of fermentation as depicted in the figure 2. The highest concentration of LA was produced from mango hydrolysate (10.08g/L), where as the other three hydrolysates contributed to moderate level (5.74 g/L, 4.68 g/L and 4.68 g/L) of LA production. The LAcontent after 3rd day of fermentation reduced greatly. This is because of the decrease in the amount of the reducing sugars left in the fermentation broth

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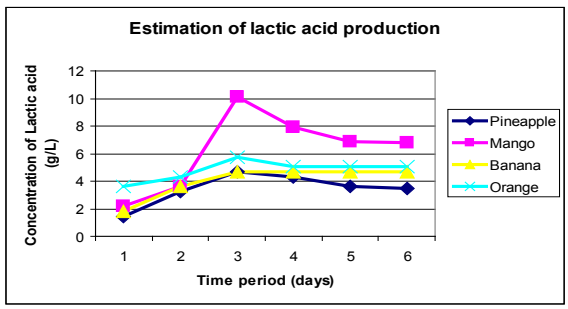
Figure 2: Estimation of lactic acid production



The confirmation of LA production was done using NAD+ linked L-Lactate dehydrogenase assay. Analysis of all the four fermentation broths revealed that DL mixture of lactic acid was produced by L.plantarum irrespective of the fermentation broth type. In all the fermentation broth L-Lactate contribute to 20% of DL mixture approximately. In other words the bacteria are producing D: L isomers in 4:1 ratio.

Table 3: Lactic acid Isomers (g/L)

Type of Lactic acid	Pineapple hydrolysate	Mango hydrolysate	Banana hydrolysate	Orange hydrolysate
D-Lactate(g/L)	3.744	8.071	3.743	4.592
L-Lactate(g/L)	0.936	2.017	0.935	1.148

CONCLUSION

The highest lactic acid production was obtained from the mango peels (10.08 g/L) , whereas the other substrates viz. orange, banana and pineapple peels produced 5.74 g/L, 4.68 g/L and 4.68 g/L of lactic acid respectively. Thus the present study highlights a methodology for recycling, re-processing and eventual utilization of fruit waste for beneficial uses rather than their discharge to the environment which might cause detrimental environmental effects

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