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Lactic Acid Production from Peels of *Kalanchoa thrysflora* (Terne) and *Opuntia ficus-indica* L. (Beles) using *Lactobacillus plantarum*

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ABSTRACT

Lactic acid, commonly used in food, chemical and pharmaceutical industries, has recently received much attention for the production of biodegradable materials. For the cost effective production of this organic acid, cheap raw material is one of the key inputs. Therefore, this research paper aimed in the generating of lactic acid through batch fermentation from two carbohydrate rich wastes particularly *kalanchoa thrysflora* and *Opuntia ficus-indica* L. (commonly found in northern Ethiopia, Tigray region) by using *Lactobacillus plantarum* as a starter culture. The result revealed that during the fermentation period (a duration of 6 days), the maximum lactic acid production was noticed in the 3rd day (Exponential phase) and at this stage Beles fruit peels hydrolysate produced about 8.4 g/L of lactic acid and Terne peels hydrolysate produced 5.8 g/L of lactic acid. Therefore, this research clues the procedure for the wise and environmentally friend employing ways of fruit wastes for beneficial purposes instead of disposing them to the environment.

INTRODUCTION

Lactic acid (LA) is one of the broadly known organic acids and was revealed by the Swedish chemist Scheele. It can be formed by fermentation processes and/or chemical processes. It is available in a lot of foodstuffs naturally and as the end-product of microbial fermentation. It is also the most important metabolic intermediate in the majority of living things, from the glycolytic microorganisms to higher organisms ^[1].

There are naturally two types of optical isomers of LA Lactic particularly D (-) LA and L (+) LA. L(-) type of LA is the most common form of it where it can be found in a number of foods and pharmaceuticals as D-isomer are damaging to human beings ^[2].

Some wild fruits that are traditionally consumed by many peoples of Africa (e.g. Northern Ethiopia) can be a good source of fruit based industry but at the same time these industries have their own effluent which produces huge amount of wastes that brings serious environmental pollution problems. However, these types of fruit wastes when utilized properly can fill many societal gaps. This is because these wastes have very rich amount of carbohydrate and as a result they can be used as a good source of nutrient for the growth of relevant fermentative microorganisms and the end product of this process in case of this study can produce LA in large amount.

Therefore, this study aims to assess the utilization of peel wastes of Terne and Beles fruit as a source for LA production fermentatively by using *Lactobacillus plantarum* as the starter culture and it clues the procedure for the wise and environmentally friend employing ways of fruit wastes for beneficial purposes instead of disposing them to the environment.

METHODS AND MATERIALS

Starter culture

Pure culture of 1 was acquired from Mekelle University, collage of veterinary medicine, Ethiopia. This bacterium was sub-

cultured by inoculating it in 30 ml of MRS (broth) comprised of the following: 1.0% peptone, 0.8% egg extract, 0.4% yeast extract, 2.0% glucose 0.5% sodium acetate trihydrate, 0.1% polysorbate80, (also known as Tween 80), 0.2% dipotassium hydrogen phosphate, 0.2% triammomium citrate, 0.02% magnesium sulfate heptahydrate, 0.005% manganese sulfate tetrahydrate 1.0% agar, and pH adjusted to 6.2 at 25 °C). Then, this medium was allowed to be incubated in a rotary shaker at 37 °C for duration of six days. After this, temperature, pH and salt concentration for the effective growth of *L. plantarum* were optimized. Here, the culture of *L. plantarum* strain was incubated at 37 °C for duration of five days for the three optimization processes. pH levels used for the optimization were 2, 4, 6 and 8 and the concentration of NaCl used for the optimization were 2%, 4%, 6.5% and 10% and the cell density of the bacterium during these optimizations was examined by measuring OD at 600 nm for the consecutive of five days. Finally the temperature range for optimization were set to 10 °C, 15 °C, 37 °C and 45 °C under pH of 7. The cell density of the bacterium at this stage was also examined by measuring OD at 600 nm for the five consecutive days as done by [3].

Estimation of acid formation

The acid formation of *L. plantarum* in skim milk as performed by [4] was minutely modified and the acidic activity was determined by the change in pH of milk medium (10%w/v). Using a pH meter, pH measurements was done on the inoculated milk medium at varying times particularly 0, 2, 4, and 6 hrs.

Estimation of exopolysaccharide formation

EPS formation by *L. plantarum* was performed applying the technique illustrated by [5] on LTV Agar plates and incubated at 30 °C for 48 hrs.

Estimation of proteolysis process

24 hours old 0.1 ml broth culture of *L. plantarum* was inoculated into the milk medium, which was incubated at 37 °C for 48 hrs. After this, zone of inhibited growth of this bacterium was observed on the plates as done by [6].

Estimation of amylolysis process

24hrs old culture of *L. plantarum* was inoculated to the plates of starch agar and allowed to incubate at 30 °C for 48 hrs. Then, the plates were washed by iodine solution for 15 minutes and observed the formation of inhibited zones on the plate to ensure amylolysis process as done previously [6].

Estimation of lipolysis

24 hrs old 0.1 ml broth culture of MRS (broth) culture of *L. plantarum* was inoculated into Tributyrin agar plates and allowed to incubate at 37 °C for 48 hrs. After this, zone of inhibited growth of this bacterium was observed on the plates following the same manner with [7].

Investigation of the fermentation of carbohydrates

MRS broth which contained 0.04 g/L of phenol red, which is used as pH indicator, but devoid of glucose was first prepared. Then, 1% of all of the following constitutes (glucose, galactose, fructose, lactose, maltose, inulin and xylose) was added to the broth for the determination of the fermentation of carbohydrates.

Fermentation of peels

Two locally available peels particularly Beles fruit from market (Edagahamus) and Terne which was manually collected from the highlands of Edagahamus, Wolwallo, northern Ethiopia were first prepared. Then, these peels were used as substrates for LA production using *Lactobacillus plantarum* as starter culture.

Determination of total sugar in the peels

Anthrone method was used to determine the entire sugar composition of the peels of the two samples.

Preparation of peel hydrolysates for fermentation

10 g of each peel waste was autoclaved at 121 °C for 15 minutes. Then, 200 ml distilled (contamination free) water was added to the peel sample. It was then boiled at 80 °C for 30 minutes. After it cooled, it was filtered by a cotton cloth. Then 1% of HCl v/v was added to the filtrate and autoclaved at 121 °C for 30 min. Finally, hydrolysis process was carried out and the pH of the peels hydrolysate was attuned to 6-6.8 with CaO and the precipitation of CaSO₄ produced during this adjustment was avoided by filtration with Whatmann filter paper No.1. as done [8].

Preparation of the starter culture

The *L. plantarum* culture was cultivated in MRS broth containing the two peel hydrolysates. Then, the media were kept for incubation purpose at room temperature on rotary shaker at 180 rpm for 2 days which were used as inoculum for further studies.

Preparation of fermentation media

200 ml of filtered sterilized peel hydrolysates were added to 50 ml of sterile MRS medium in 2 conical flasks. Then, cultures of 5% *L. plantarum* were inoculated to each peel hydrolysate and allowed to be incubated at 37 °C in a rotary incubator (180 rpm) for 5 days. Then, the consumption of these hydrolysed peels by the bacterium and the production of LA were assessed daily.

Assessment of the consumption of substrates

Dinitrosalicylic Colorimetric method was used to estimate the residual reducing sugar content of the fermentation broth as described [9].

Evaluation of the production of lactic acid

LA production was principally identified by evaluating the titrable acidity of the fermentation medium daily by titrating the fermentation medium against 1 M NaOH by using phenolphthalein as indicator [3].

Recovery of lactic acid

The amount of LA available in the fermentation broth was recovered by using calcium lactate precipitation method [10]. These crystals were additionally used for verification of LA using phydroxy diphenyl method as described [11].

RESULT AND DISCUSSION

A pH of 6, a temperature of 37 °C and two percent of sodium chloride were found to be the optimal conditions for the growth of *L. plantarum* and this research strongly agreed with other previous researches done [1]. The need for the assessment of the properties of the starter culture bacterium particularly *L. plantarum* was done for the sake of its fitness to be used as a fermentative agent for the batch process of the selected peels. Based on this process, the estimation of acid formation by this bacterium was ranged from 0.46 to 0.67 after 6 hrs. The capacity of starter cultures in terms of their speed as acidifying agents are mostly chosen (like *L. plantarum*) which are potential to save time during batch fermentation process [12]. The need to determine EPS's activities was because of their importance in texturizers and stabilizers by increasing the viscosity of a final product; they also helped in proteins and micelles to strengthen the rigidity of their network [13]. The proteolysis process of *L. plantarum* strains inoculated into milk medium plates was for the purpose of ensuring the appearance of clear zone around the colonies. These activities are vital for the growth of the bacterium in protein rich substrates and are essential in the development of organoleptic characteristics of different fermented products. Before fermentation took place, some enzymatic pretreatments and hydrolysis processes are very important in case of starch based substrates as *L. plantarum* strains are incapable of degrading starch and in case of this study, amylase production has not taken place. The investigation of lypolysis process was carried out for the sake of approving that when this bacterium is used as a starter culture for foodstuffs to humans, since it has the ability to degrade lipids when taken in the form of food in small amount. It can proceed its lypolytic activity in vivo so that it helps in the dropping of cholesterol level inside the body of human beings.

The **Figure 1** below illustrates that the evaluation of reducing sugars according to the dinitrosalicylic colorimetric method. This brings a slow diminishing of the sugar content due to the consumption of the sugars by the starter culture bacterium as carbohydrates are the main supply for the fabrication of LA.

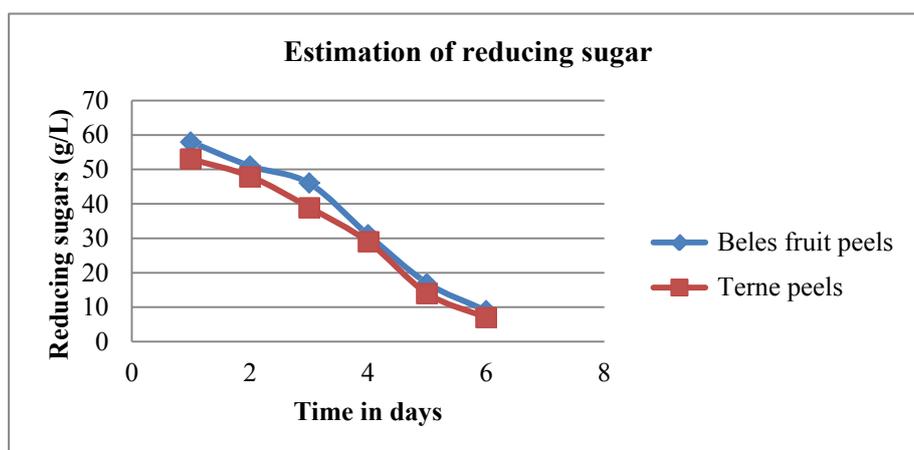


Figure 1. Estimation of reducing sugars.

Evaluation of the production of lactic acid

The present study reveals that *L. plantarum* was used as a starter culture for the fermentation of the two peels (for the duration of 6 days) and the maximum acid production was noticed in the third day (Exponential phase) of fermentation as represented in the figure below (**Figure 2**). From the two peels, Beles fruit peels hydrolysate produced about 8.4 g/L of LA and Terne peels hydrolysate produced 5.8 g/L of LA. The immense reduction of LA content after 3rd day was because of the limiting amount of reducing sugars in the batch medium and it was an indication of the arrival of the bacterium in the stationary and decline phase.

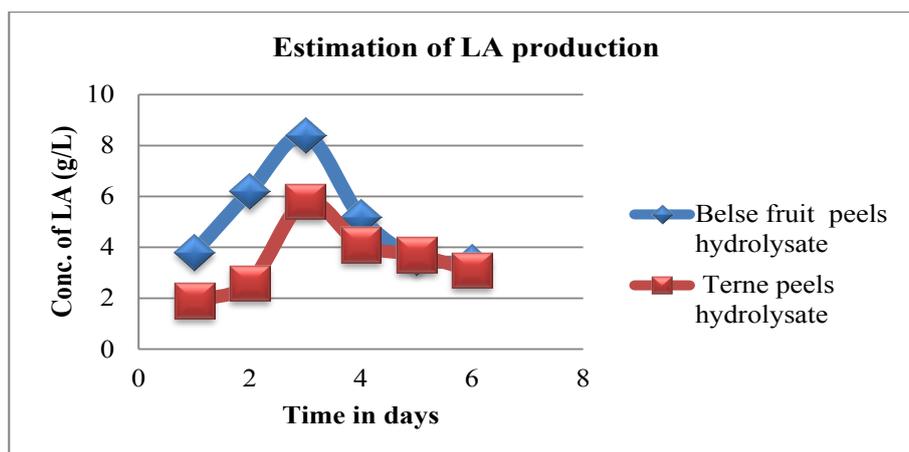


Figure 2. Estimation of LA production.

CONCLUSION

The maximum LA fabrication was gained from the Beles Fruit peels hydrolysate (8.4 g/L), whereas the Terne peels hydrolysate produced 5.8 g/L of LA. Therefore, this research clues the procedure for the wise and environmentally friend employing ways of fruit wastes for beneficial purposes instead of disposing them to the environment.

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