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A comparative study on Lactic acid production from Canistel fruit using *Lactobacillus species*

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Abstract
The high production cost involved in the chemical synthesis of Lactic acid along with its ever increasing demand due to wide spread application calls for new approaches to screen and produce LA from cheap and inexpensive raw material. The main focus of the research work is to produce LA from an under-utilized tropical fruit canistel (*Pouteria campechiana*) by employing three species of lactobacillus. The analysis of nutritive content revealed an appreciable amount of carbohydrates (36.1%) and reducing sugars (19.8%) in canistel pulp. The highest amount of LA was produced by *L. casei* (20.07g/L) followed by *L. acidophilus* (18.9g/L) and *L. plantarum* (18.9g/L).

Keywords: Lactic Acid, Canistel, Hydrolysate, Lactobacillus.

1. Introduction
Lactic acid (LA), a three carbon organic acid, is a widely used industrially important carboxylic acid. It could be produced chemically (chemical synthesis) using acetaldehyde, hydrogen cyanide, sulphuric acid and methanol or by microbial (carbohydrate) fermentation. Nowadays, carbohydrate fermentation is mostly preferred for its production, as chemical synthesis leads to a racemic mixture, where recovery of stereo specific acid [L (+) or D (-) lactic acid] is problematic.
Lactic acid is used as acidulant/flavouring/pH buffering agent or inhibitor of bacterial spoilage in a wide variety of processed foods (Niju *et al.,* 2004) [1]. It is also widely used for textile, pharmaceutical and cosmetic applications, as it is considered as GRAS by FDA. These valuable applications of lactic acid have opened the potential of lactic acid producing microorganisms and their commercial use in various industries. Both bacteria (*Lactobacillus*, *Lactococcus*) and fungi (*Rhizopus oryzae*) can produce lactic acid through carbohydrate fermentation. The choice of the microorganism used for the fermentation mainly depends on the sugar being used. Stereo specific lactic acid is produced, depending upon the strain being used for fermentation.
Over the decades a variety of raw materials rich in sugar content has been screened for their potential ability to produce lactic acid in high quantities using various strains of lactic acid bacteria. Canistel (*Pouteria campechiana*) is an ever green tree native to Central American Countries. They produce orange yellow fruits also called 'Yellow Sapote' that are usually 7cm large and fleshy in texture. The fruits could be eaten raw or used for preparation of variety of deserts. Canistel flesh is sweet and has close resemblance with hard-boiled egg yolk as reflected by its colloquial name "egg fruit". Inspite of its wider climatic adaptability and high nutritive content the fruit remains underutilized in most of the American and Asian countries. This often results in unintentional discharge of the ripened fruits that paves way for a wide variety of problems. Presence of high moisture content and carbohydrate content makes them liable for easy microbial contamination and spoilage unless proper precautionary measures were taken to increase its shelf life. In spite of its enormous potential to be utilized as a feed stock for various fermentation process, very few research has been employed so far on this particular fruit for its commercial utilization as a raw material for industrial fermentation process. The current research work exploits the possibilities of utilizing canistel pulp as a raw material for lactic acid production using lactobacillus. The research work thus highlights a new perspective for utilization of underutilized fruits rather than their careless discharge which could bring serious environmental concerns.

2. Materials and Methods
2.1. Collection of raw material
Canistel (*Pouteria campechiana)* fruit used in the current study was collected from Palakkad, Kerala (Figure 1). It was washed with water several times for the removal of dirt and then grinded into pulp and stored in air tight container in room temperature until further analysis

2.2. Analysis of nutritive parameters
Moisture content and Crude fiber content of Canistel fruit was analyzed using standard A.P.H.A, 2005 [2] protocol. Lipid content and crude fiber content of the fruit was analyzed using A.O.A.C, 2005 [3] method. Protein content of the fruit was assessed using Lowry *et al*., [4] method. The total sugar content after hydrolysate preparation was done using Anthrone method (Hedge *et al.,* 1962) [5]. The reducing sugar content post acid hydrolysis was analyzed using DNS method (Miller, 1959) [6].

2.3. Starter culture
Lyophilized pure culture of *Lactobacillus plantarum, Lactobacillus acidophilus and Lactobacillus casei* were 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. The activated

culture was further sub cultured in to MRS agar plates and used for further studies.

2.4. Preparation of hydro lysates for fermentation

Steam explosion

The modified method of Pumiput *et al.,* (2008) [7] was used for substrate hydrolysate preparation. About 8gram of canistel pulp 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. Later the hydrolysate was recovered by filtration with cheese cloth.

Acid hydrolysis

Acid post hydrolysis of hydrolysate was carried out to cleave the oligosaccharides in to monomeric sugars by autoclaving at 121 °C with concentration of 1% HCl v/v for 30min (Pumiput, 2008) [7].

pH adjustment

The hydrolysate from acid post hydrolysis was adjusted with NaOH to pH 6-6.8 and the precipitate was removed by filtration with Whatmann filter paper No.1 (Pumiput, 2008) [7].

2.5. Inoculum preparation

The *Lactobacillus* cultures were cultivated in modified MRS broth containing canistel 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.

2.6. Media and fermentation conditions

To screen for lactic acid production from canistel hydrosylates , fermentation media containing fruit hydrolysate was prepared in 250ml capacity conical flask by adding components of synthetic medium (Cheng 1991) [8] (0.034g $FeSO_4$, 1.0g Sodium acetate, 1.23g $MgSO_4.7H_2O$, 0.034g $MnSO_4.H_2O$, 0.65g KH_2PO_4, 30g Yeast extract) in 100ml of hydrolysate instead of distilled water. These flasks were inoculated with 5% *L. plantarum, L. casei and L. acidophillus* cultures precultivated in s fruit hydrolysate based media and incubated at 37 °C in a shaker incubator (120rpm) for 5 days. The product (lactic acid) formation was estimated on daily basis.

2.7. 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.

$$\text{Amount of LA (g/L)} = \frac{\text{Vol. of NaOH x Gram Eq.wt of LA x Normality of NaOH}}{\text{Vol. of fermentation broth used for titration}}$$

2.8. Lactic acid downstream processing and confirmation

Lactic acid present in the fermentation broth was separated using the conventional Calcium lactate precipitation method for the recovery of lactic acid. These crystals were further used for confirmation of lactic acid using p-hydroxy diphenyl method as described by Barnett (1951) [9]. The estimation of L(+) and D(-) lactic acid content was done using NAD+ linked L- lactate dehydrogenase assay (using analytical kit made by Randox labortries,UK).

3. Results

3.1. Analysis of nutritive parameters in canistel pulp

Nutritive composition of canistel pulp was tabulated (Table 1)

Table 1: Nutritive parameters in Canistel fruit pulp

Serial No.	Nutritive Parameters	Obtained Value (%)
1.	Moisture Content	66.3 ± 0.21
2.	Ash Content	16.5 ± 0.32
3.	Protein Content	1.65 ± 0.12
4.	Fiber Content	0.92 ± 0.06
5.	Lipid Content	0.16 ± 0.04
7.	Total Sugars	36.1 ± 0.06
8.	Reducing Sugars	19.8 ± 0.14

Data represents the mean of triplicates ± standard deviation

3.2. Preparation of hydrolysates for fermentation

The high amount of carbohydrates present in the canistel pulp were extracted in to the hydrolysate (Figure 2) through steam explosion method as suggested by Puimput *et al.,* (2008) [7]. Acid hydrolysis with concentrated HCl (1%) was done to convert complex sugars in to easily fermentable monomeric (Figure 3) residues.

Fig 1: Canistel fruit

Fig 2: Canistel Hydrolysate

Fig 3: Acid Hydrolysate

3.3. Estimation of lactic acid production

In the present study, the highest amount of LA was produced during 4th day (96 hrs) of fermentation irrespective of the type of strain employed for fermentation. From the results it is evident that *L. casei* produced the highest amount of LA (20.07g/L) followed by *L. acidophilus* (19.84g/L) and *L. plantarum* (18.91g/L) as illustrated in Figure 4. The LA content gradually decreased after 4th day of fermentation possibly due to the reduction in the amount of available reducing sugars left out for fermentation. The LA content of the fermented medium during various days of fermentation is depicted in Table 2.

Table 2: Amount of Lactic Acid produced using Canistel Hydrolysate Medium (CHM)

Lactobacillus Strain	Amount of Lactic Acid (g/L)				
	24hrs	48hrs	72hrs	96hrs	120hrs
L. plantarum	10.08 ± 0.03	14.42 ± 0.11	16.21 ± 0.06	18.91 ± 0.05	12.61 ± 0.13
L. casei	12.87 ± 0.02	17.13 ± 0.06	18.01 ± 0.17	20.79 ± 0.18	17.32 ± 0.09
L. acidophilus	11.52 ± 0.06	13.54 ± 0.02	18.12 ± 0.07	19.84 ± 0.27	15.36 ± 0.17

Data represents the mean of triplicates ± standard deviation

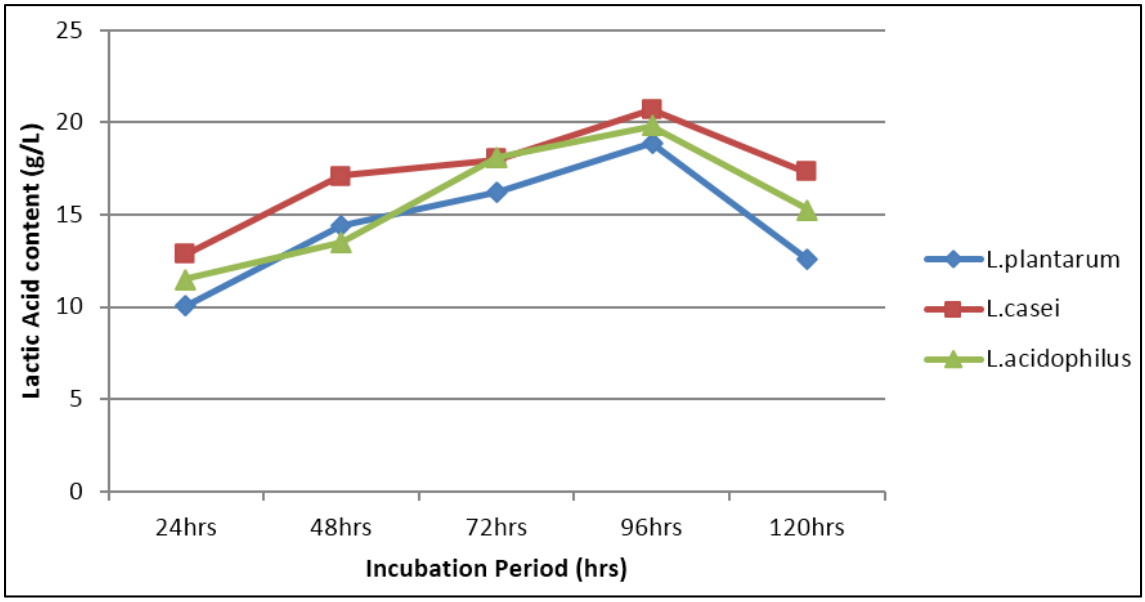

Fig 4: Amount of Lactic Acid produced using Canistel Hydrolysate Medium (CHM)

3.4. Lactic acid downstream processing and confirmation

Lactic acid produced in the fermentation broth was crystalised using conventional calcium precipitation method and analysis of crystals through p-hydroxy diphenyl method as described by Barnett (1951) [9] revealed that irrespective of lactobacillus strain type in all the CHM L-Lactate contributes to 20% of DL mixture approximately in other words the bacteria are producing D: L isomers in 4:1 ratio (Table 3).

Table 3: Amount of Lactic Acid Isomers (D/L- Lactate) in CHM

Lactobacillus Strain	D-Lactate (g/L)	L-Lactate (g/L)
L. plantarum	15.12	3.78
L. casei	16.63	4.15
L. acidophilus	15.87	3.96

4. Discusssion

Evaluation of nutritive parameters in fully ripened canistel fruit pulp was essential to analyze its feasibility to be used as the substrate for any fermentation process. These were in correlation with the results obtained by previous researchers working with canistel fruit (Morton (1987 [10]; 1992 [11]); Pushpa-kumara (2007) [12]. The carbohydrate and crude protein content in the canistel fruit pulp is similar to the result obtained by Attapattu *et al.*, (2014) [13]. The acid hydrolysis method suggested by Pumiput *et al.*, (2008) [7] working with lactic acid production from fruit waste was successful in producing and extracting reducing sugars from canistel fruit pulp. The gradual increase in the amount of LA produced from first day to fourth day irrespective of the strain type is due to the gradual utilization of the reducing sugars in the CHM with simultaneous production of LA in the medium. These results were in correlation with the work done by Pushparani *et al.*, (2012) [14] using fruit peels as substrate for LA production. The substantial decrease in the LA quantity after fourth day of fermentation may be attributed to the decrease in the amount of available reducing sugars left over

for fermentation along with the reduction in the number of bacteria due to increasing acidity of the CHM.

5. Conclusion

Many unexplored indigenous fruit species have not received much attention by people inspite of its potential nutritional or phytochemical components (Kong *et al*., 1980) [15]. The present study highlights a methodology for eventual utilization, recycling and reprocessing of an under-utilized fruit for the production of a commercially important organic acid. This could reduce the overall economics of production and further commercialization of the process to industrial scale can save the environment from the detrimental effects offered by careless discharge of fruit waste.

6. References

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