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FOOD WASTE MANAGEMENT- LACTIC ACID PRODUCTION BY *LACTOBACILLUS* SPECIES

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ABSTRACT
Lactic acid is widely used in the food, cosmetic, pharmaceutical, and chemical industries and has received increased attention for use as a monomer for the production of biodegradable poly (lactic acid). There have been various attempts to produce lactic acid efficiently from inexpensive raw materials. The main objective of this work was to produce lactic acid using agricultural food waste viz. peels of Potato, Green peas, Sweet corn, Orange and Mango as substrates. The bacterial strains used for the fermentation process was *Lactobacillus casei* and *Lactobacillus delbrueckii*. The highest lactic acid production, 63.33g/L was obtained for mango peels by *L. casei*, whereas for orange peels it was 54.54g/L by *L. delbrueckii*. The estimated lactic acid from the other substrates viz. peels of Potato, Corn, Green peas and Orange using the strain *L. casei* was 38.88g/L, 37.62g/L, 39.14g/L, 25.75g/L respectively, whereas from peels of Potato, Mango, Green peas and corn using the strain *L. delbrueckii* the estimated lactic acid was 13.63g/L, 15.15g/L, 15.90g/L, 13.38g/L respectively. Thus our study shows that lactic acid can be efficiently produced using agricultural food wastes at a cheaper cost and a higher rate as compared to other substrates being used.

KEY WORDS: *Lactobacillus casei*, *Lactobacillus delbrueckii*, Food waste, peels.

INTRODUCTION

Lactic acid has received a significant amount of attention as a chemical with many potential applications. There are four major categories for the current uses and applications of lactic acid: food, cosmetic, pharmaceutical, and chemical applications. Since lactic acid is classified as GRAS for use as a food additive by the US FDA(Datta, 1995) , it is widely used in almost every segment of the food industry, where it serves in a wide range of functions, such as flavoring, pH regulation, improved microbial quality, and mineral fortification.

Moreover, lactic acid is used commercially in the processed meat and poultry industries, to provide products with an increased shelf life, enhanced flavor, and better control of food-borne pathogens. Due to the mild acidic taste of lactic acid, it is also used as an acidulant in salads and dressings, baked goods, pickled vegetables, and beverages.

Lactic acid is also used in the pharmaceutical industry as an electrolyte in many parenteral/I.V. (intravenous) solutions that are intended to replenish the bodily fluids or electrolytes. Examples include Lactated Ringer's or Hartmann's solutions, CAPD (continuous ambulatory peritoneal dialysis) solution, and dialysis solution for conventional artificial kidney machines. Moreover, lactic acid is used in a wide variety of mineral preparations, which include tablets, prostheses, surgical sutures, and controlled drug delivery systems. Lactic acid and its salt are used increasingly in various types of chemical products and processes. In this category of applications, lactic acid functions as a descaling agent, pH regulator, neutralizer, chiral intermediate, solvent, cleaning agent, slow acid-release agent, metal complexing agent, antimicrobial agent, and humectants (Bulletin of the Purac, Bulletin of the Galactic).

In industry, lactic acid fermentation is performed by lactic acid bacteria. Food industry produces large volumes 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, inspite of their pollution and hazard aspects, in many cases, food processing wastes have a good potential for conversion into useful products of higher value as by-product, or even as raw material for other industries. Organic acids are examples of such valuable by-product of the fermentation of high carbohydrate containing industrial substrates. For example potato processing plants release an appreciable amount of starch in wastewater streams, additionally; potatoes, which do not fit the standard quality criterion, are discarded. They therefore could be utilized cheaply as substrate for microorganisms producing intermediate volume high value organic acids like lactic acid. In India, the annual production capacity of Lactic acid is 6000t and an estimated gap of 2300 t in supply by the year 2015 have been predicted, if the present level of production is not increased (TIFAC, 2001). Wastes containing starch generated from food processing plants may be regarded as a viable option for meeting this growing demand for lactic acid, if appropriate biotechnological interventions are used and specific sectors amongst the Indian food processing industry are targeted (World Bank Group, 2002).

Currently, lactic acid is considered the most potential feedstock monomer for chemical conversions, because it contains two reactive functional groups, a carboxylic group and a hydroxyl group. Lactic acid can undergo a variety of chemical conversions into potentially useful chemicals, such as propylene oxide (via hydrogenation), acetaldehyde (via decarboxylation), acrylic acid (via dehydration), propanoic acid (via reduction), 2,3-

pentanedione (via condensation), and dilactide (via self-esterification) (Varadarajan, 1999).

Lactic acid has recently received a great deal of attention as a feedstock monomer for the production of PLA, which serves as a biodegradable commodity plastic. The optically pure lactic acid can be polymerized into high molecular mass PLA through the serial reactions of polycondensation, depolymerization, and ring-opening polymerization (Södergård, 2002). The resultant polymer, PLA, has numerous uses in a wide range of applications, such as protective clothing, food packaging, mulch film, trash bags, rigid containers, shrink wrap, and short shelf-life trays (Drumright, 2000 and Vink 2003). The recent huge growth of the PLA market will stimulate future demands on lactic acid considerably(Datta, 1995 and Lunt, 1998).

In this study, lactic acid was produced using agricultural food wastes such as vegetables and fruit peels i.e. peels of Mango, Banana, Orange, Potato, Green peas, Sweet corn. The bacterial strains used were *Lactobacillus casei* and *Lactobacillus delbrueckii.* The obtained lactic acid was then further tested for application based study like its antimicrobial activity against gram positive and negative organisms and its antioxidant activity.

This study mainly focuses on use of food wastes which can be a cheaper source of raw material as compared to others for the commercial production of lactic acid.

MATERIALS AND METHODS:

Raw material processing:-

A total of 5 different food wastes viz. peels of potato, mango, corn, orange and green peas; were screened for the amount of sugar content by preparing their hydrolysate and used for lactic acid production by *Lactobacillus casei and Lactobacillus delbrueckii*.

Preparation of hydrolysates

Steam explosion

The modified method of Pumiput *et al*. was used for substrate hydrolysate preparation (Pumiput, 2008). 40gram of each food waste substrate was steam-exploded in 100 L capacity autoclave at 121°C for 20min. Water was added to the wet pretreated material to make up the volume of 1 L 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 into monomeric sugars by autoclaving at 121°C with concentration of 1% HCl v/v for 30 min (Pumiput, 2008).

pH adjustment

The hydrolysate from acid post hydrolysis was adjusted with CaO to pH 6- 6.8 and the $CaSO_4$ precipitates were removed by filtration with Whatmann filter paper No.1(Pumiput, 2008).

Fermentation (batch culture):

Microbial strain

The pure culture of *Lactobacillus casei* NCIM 2360 and *Lactobacillus delbrueckii* NCIM 2025 were grown on solid synthetic MRS medium (deMan, 1960) (HIMEDIA).

Inoculum preparation

The scraped growth was subcultured in liquid synthetic MRS medium & different test hydrolysates separately. The culture media containing hydrolysate were prepared in 250 mL capacity conical flasks by adding components of synthetic medium (Cheng, 1991) (0.034 g FeSO4, 1.0 g Sodium acetate 1.23 g $MgSO_4 \cdot 7H_2O$, 0.034 g $MnSO_4 \cdot H_2O$, 0.65 g $K_2HPO_4 \cdot 3H_2O$, 0.5 g KH_2PO_4, 30 g yeast extract) in 100 mL of each hydrolysate 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 in further studies.

Media and fermentation conditions

To screen for lactic acid production from food waste hydrolysates, 5% of *L. casei & L. delbrueckii inoculum* was added separately to 3L of fermentation media in 5L capacity chemostat bioreactor and incubated at ambient temperature at 120rpm for 6 days. The fermentation medium was similar to the inoculum medium for each test hydrolysate. The substrate consumption and also product (lactic acid) formation was examined on daily basis.

RESULTS AND DISCUSSION

Screening of different raw materials for reducing sugar content

A total of 5 biomass wastes were screened for their reducing sugar content by DNSA method (Miller, 1959). The acid, conc. HCl at concentration of 1% was observed to give good sugar yield from biomass after hydrolysis. (Table.1).

TABLE 1. Reducing sugar content in raw material

Substrate (peels)	Reducing sugar content (gm/L)
Mango	52
Orange	51
Green peas	46
Potato	29
Corn	26

Substrate utilization assay

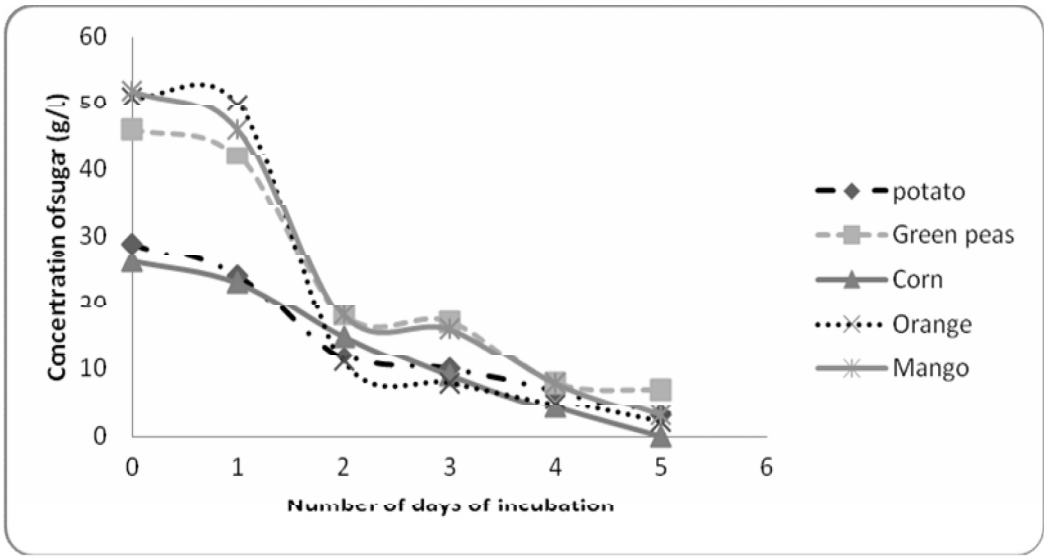
This experiment was performed to analyze the comparative substrate utilization efficiency by *L. casei & L. delbrueckii* for product formation. The substrate utilization assay was carried for a period of seven days. Maximum substrate utilization was observed approximately after 4 days incubation, for all the substrates in the study. However, for substrate utilization by *L. delbrueckii,* an initial increase in substrate concentration was observed after 24hrs of incubation. This could be probably because of simultaneous saccharification of the substrate by *L. delbrueckii* (Yáñez, 2003). (Graph.1,2).

Lactic acid production

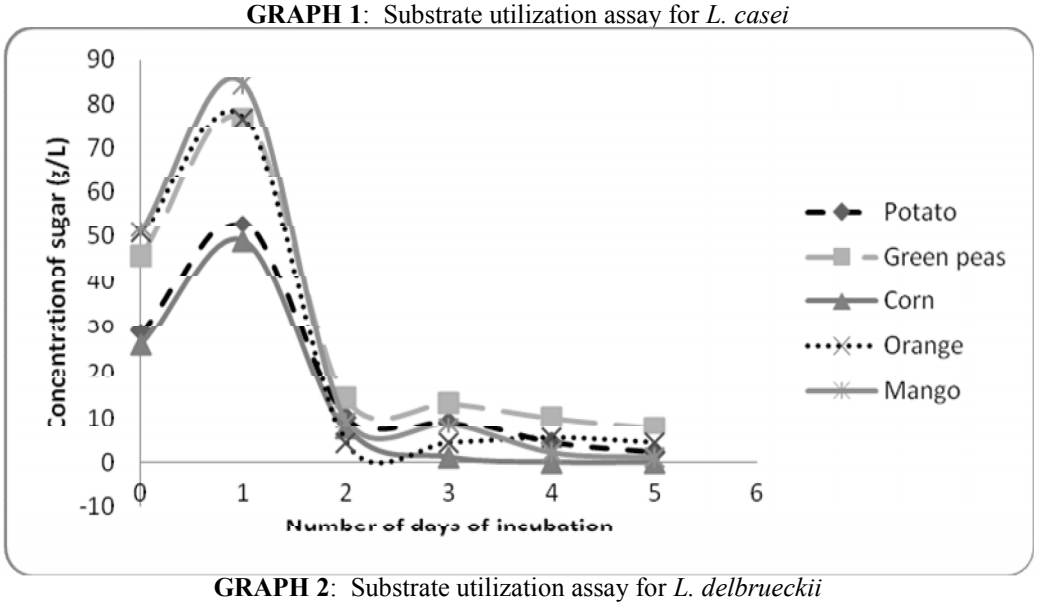
Titrable acidity

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 1N NaOH. The titrable acidity was observed to be highest on the fifth day of fermentation for *L. casei*. However, the titrable acidity for *L. delbrueckii* with peels of potato, orange and green pea substrate was observed to be highest on the fifth day of fermentation, whereas for

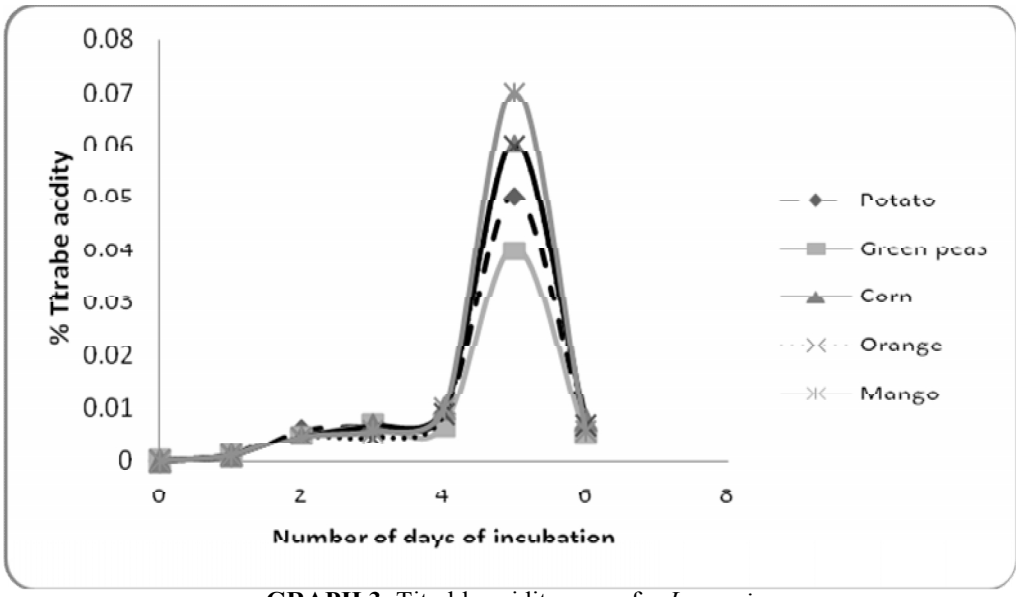
mango and corn peels, the titrable acidity was highest on the fourth day of fermentation. (Graph 3,4).



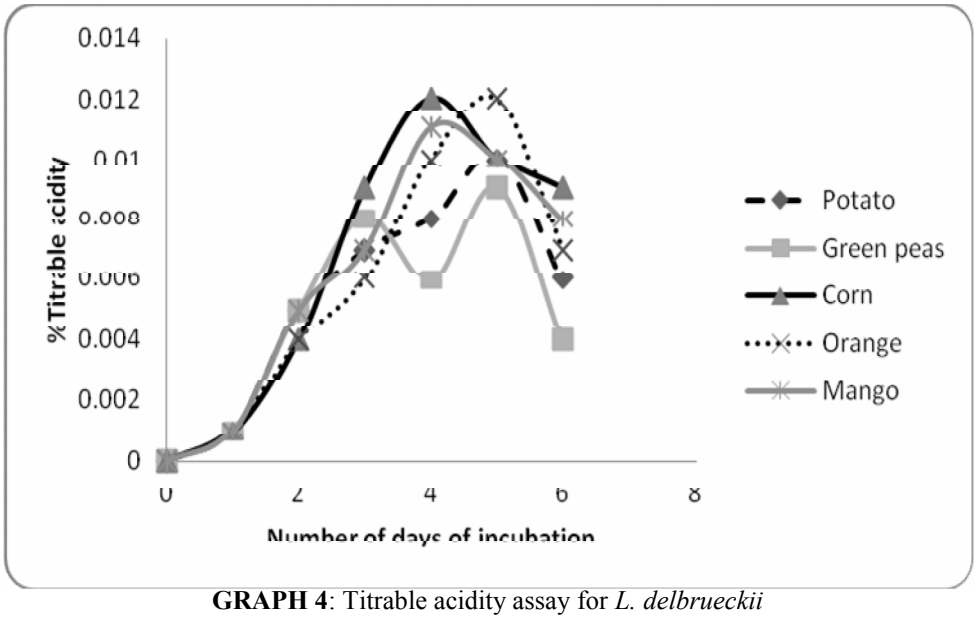
GRAPH 1: Substrate utilization assay for *L. casei*



GRAPH 2: Substrate utilization assay for *L. delbrueckii*



GRAPH 3: Titrable acidity assay for *L. casei*



GRAPH 4: Titrable acidity assay for *L. delbrueckii*

Lactic acid downstream processing

Lactic acid was purified and the crystals were obtained from the fermentation medium after completion of 4-5days of incubation, by the method described by Sodeck, (1981). The crystals obtained were then used for confirmatory test for lactic acid estimation.

Lactic acid estimation

Lactobacillus species are also known to produce other organic acid from food waste substrate (Zalán, 2010). Hence, for the confirmation and quantitation of lactic acid, its estimation by the p-hydroxy diphenyl was performed (Barnett, 1951). The concentration of lactic acid was observed to be the highest in mango peel substrate (63.33g/L) by *L. casei* and highest for Orange peel substrate (54.54g/L) by *L. delbrueckii*. However, lowest lactic acid production was observed with corn peels substrate (Table 2).

TABLE 2. Concentration of lactic acid

Substrate (peels)	Lactic acid concentration (g/L)	
	L. casei	*L. delbrueckii*
Mango	63.33	15.15
Green peas	39.14	15.90
Potato	38.88	13.63
Corn	37.62	13.38
Orange	25.75	54.54

CONCLUSION

This study records highest production of lactic acid from corn (37.62g/L, 13.38g/L) and potato (38.88g/L, 13.63g/L) substrates as compared to previous studies which reports around 10.1g/L for corn and 4.2g/L for potato (Wee, 2006). Also the study shows novel use of food wastes like mango, orange and green peas peels as use for substrate in lactic acid production. Among the two different strains of Lactobacillus experimented, *L. casei* proves to be a better producer of lactic acid in case of substrates like peels of mango, green peas, corn and potato. However, *L. delbrueckii* is an efficient producer in case of orange peel substrate. The inference drawn from this study aims for the use of food wastes for commercial and economic production of application based products by fermentative bioconversions. Further aspects could include media optimization studies for higher and efficient production of lactic acid.

REFERENCES

Barnett, A.J.G. (1951), The colorimetric determination of Lactic acid in silage, *Biochem J*. Sep 49(4):527-529.

Bulletin of the Galactic (*http://www.lactic.com/*).

Bulletin of the Purac (*http://www.purac.com/*).

Cheng, P., R. Mueller, S. Jaeger, R. Bajpai, and G. Iannotti (1991), Lactic acid production from enzyme thinned corn starch using, *Lactobacillus amylovorus*. *J. Ind. Microbiol*. 7:27-34.

Datta, R., Tsai, S.P., Bonsignore, P., Moon, S.H., and Frank, J.R. (1995), Technological and economic potential of poly(lactic acid) and lactic acid derivatives, *FEMS Microbiol. Rev.* 16:221–231.

deMan, J. C., M. Rogosa, and M. E. Sharpe (1960), A medium for the cultivation of lactobacilli, *J. Bacteriol*. 23**:**130.

Drumright, R.E., Gruber, P.R., and Henton, D.E. (2000), Polylactic acid technology, *Adv. Mater.* 12:1841–1846.

Lunt, J. (1998), Large-scale production, properties and commercial applications of polylactic acid polymers, *Polym. Degrad. Stabil.* 59:145–152.

Miller and Gail, L (1959), Use of dinitrosalicylic acid reagent for determination of reducing sugar, *Anal. Chem*. 31(3):426-428.

Pumiput. P, Chuntranuluck. S, Kitpreechavanich. V, Punsuron. V and Vaithanomsat, P (2008). Production

process of hydrolysate from Steam Explosion of oil Palm Trunk for Xylitol Fermentation, *Kasetsart J. (Nat.Sci)* 42:73-78.

Sodeck, G., Modi, J., Kominek, J. and Salzbrunn, G (1981) Production of citric acid according to the submerged fermentation. *Process Biochem*.16(6):9-11

Södergård, A., and Stolt, M. (2002) Properties of lactic acid based polymers and their correlation with composition, *Prog. Polym. Sci.* 27:1123–1163.

TIFAC. Vision 2020 reports. (2001) Technology Linked Business Opportunity Publications, Code No. TMS157, Vol. 4.

Varadarajan, S., and Miller, D.J. (1999) Catalytic upgrading of fermentation-derived organic acids, *Biotechnol. Progr.* 15:845–854.

Vink, E.T.H., Rábago, K.R., Glassner, D.A., and Gruber, P.R. (2003) Applications of life cycle assessment to NatureWorks™ polylactide (PLA) production, *Polym. Degrad. Stabil.* 80:403–419.

Wee, Y. J., Kim, J-N., and Ryu, H-W. (2006) Biotechnological Production of Lactic Acid, *Food Technol. Biotechnol.* 44(2):163–172

World Bank Group. (2002) Markets and Agro enterprises. Accessed: 21.5.05.

Yáñez, R., Moldes, A. B., Alonso, J. L., and Parajó, J. C. (2003) Production of D(-)-lactic acid from cellulose by simultaneous saccharification and fermentation using *Lactobacillus coryniformis subsp. Torquens*, *Biotechnology Letters* 25 (14):1161-1164.

Zalán, Z., Hudáček, J., Štětina, J., Chumchalová, J. and Halász, A. (2010) Production of organic acids by Lactobacillus strains in three different media, *European Food Research and Technology*. 230(3):395-404.