PRODUCTION OF L-(+)-LACTIC ACID FOR THE HEALTH-CARE INDUSTRY USING *Lactobacillus casei* AND *Lactobacillus delbrueckii* WITH CASSAVA AS THE RAW MATERIAL

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Introduction

Lactic acid is one of the functional and valued compounds used in many industries such as food, pharmaceutical and chemical industries. The industrial production of lactic acid is obtained either by chemical synthesis or by microbial fermentation. Currently lactic acid is produced as a biodegradable and renewable raw material via poly lactic acid (PLA) polymers. Lactic acid has two optical isomers, L-(+)-lactic acid and D-(−)-lactic acid. It is classified as GRAS (generally recognized as safe) for use as a food additive, although D-(-)-lactic acid can be harmful to human metabolism due to metabolic activity such as acidosis. In recent years the (L)-PLA is used as a representation of the orthopedic metal implants as it is strong and non-toxic to humans. The imported metal plate implants are expensive and could be removed only by surgery after the wound is healed. Therefore, replacement of (L)-PLA made metal implants would cause a major uplift in surgeries for both the patient and the hospital.

Cassava (*Manihot esculenta*), also known as manioc, tapioca or yuca, is one of the most important food crops specially in humid tropics, due to its ability to grow in low nutrient soil and to resist drought. The research is conducted with cassava, which is used as a low cost raw material instead of Dextrose, in the fermentation to produce L-(+)-lactic acid. Microbial fermentation further reduces the cost compared to synthetic lactic acid production. Microorganisms such as *Lactobacillus casei* and *Lactobacillus delbrueckii* produce L-(+)-lactic acid. The main objective in this study is to produce a high yield of L-Lactic acid using *L. casei* and *L. delbrueckii* with Cassava as the main energy source and to determine a low cost medium, which uses minimum number of nutrients.

Methodology

Media Preparation of Pilot Scale Fermentation:

The L-(+)-lactic acid fermentation was carried out separately with 100 mL of medium A, B, C, D, and E enriched with cassava and other nutrient sources (Table 1). Each medium was neutralized using 2% HCl and 2 N NaOH (pH 5.5) and fermented with *L. casei* (ATCC: 393) and *L. delbrueckii* (ATCC: 15808) separately. The fermentation was carried out in a shaking incubator for 48 hours (hrs) at 37 °C for 150 rpm.

Detection of L-(+)-lactic acid using High Performance Liquid Chromatography (HPLC):

The media were centrifuged initially at 4000 rounds per minute (rpm) for 20 minutes at 4 °C and supernatant was re-centrifuged at 12000 rpm for 15 min. at 4 °C in order to remove impurities. The High Performance Liquid Chromatography was conducted with Ultra Violet (UV) wavelength 210 nm and Refractive Index Signal (RIS). A volume of 5 *µl* of the supernatant was filtered and injected into the pump of HPLC. Phenomenex Rezex ROA H^+ (300 x 7.8 mm, 8 µm) was used as cation exchange column and deionized water was used as the mobile phase. A temperature of 30 °C was provided with a flow rate of 0.4 mL/min. The peak area corresponded to the L-(+)-lactic acid was used to quantify the amount of L-(+)-Lactic Acid. The medium which was given the high yield of L-(+)-lactic acid was identified for large scale fermentation.

Table 1. Composition of different media used.

Medium	Composition
A	Cassava- 5 g, Yeast extract-1.5 g, K_2HPO_4-0.41 g, KH_2PO_4- 0.56 g
B	Cassava- 5 g, Yeast extract- 1.5 g, $MgSO_4.7H_2SO_4$- 1 g, $(NH_4)_2SO_4$- 1 g
C	Cassava- 5 g, Yeast extract- 5 g, K_2HPO_4- 2 g, NaOAc- 5 g, $MgSO_4.7H_2SO_4$- 0.02 g, $MnSO_4.4H_2O$- 0.05 g
D	Cassava- 5 g, Yeast extract- 1.0 g, K_2HPO_4- 0.41 g, $MgSO_4.7H_2SO_4$- 1 g, $(NH_4)_2SO_4$- 1 g
E	Cassava crumble- 5 g, Yeast extract- 1.0 g, K_2HPO_4- 0.41 g, $MgSO_4.7H_2SO_4$- 1 g, $(NH_4)_2SO_4$- 1 g

Media Preparation for Large Scale Fermentation:

Large scale fermentation was conducted after analyzing the HPLC results. The medium D (4 L) was prepared by adding 200 g of cassava powder which was acid hydrolyzed using 2 L of HCl, 40 g of yeast extract, 16.4 g of K_2HPO_4, 20 g of $MgSO_4.7H_2SO_4$ and $(NH_4)_2SO_4$. The prepared medium was transferred to New Brunswick™ BioFlo® 415 SIP Fermenter, inoculated with *L. casei* and was fermented for 5 continuous days. A sample with duplicates was withdrawn each day at the same time for 5 days. The fermentation was repeated for two times and the quantity of the L-(+)-lactic acid produced in each day was detected using the HPLC as described above.

Results and Discussion

Among all the starchy sources, cassava gives higher carbohydrate production which is about 40% higher than rice and 25% more than maize (Tonukari, 2004). Therefore, cassava was used in this study as the low cost raw material which is a good energy source.

The results obtained for the pilot scale fermentation via HPLC are given in Table 2. The highest milli Absorbance Units per second (mAU*s) was observed from the C and D media fermented with *L. casei* bacterial strain. However, some constituents in the MRS medium are costly. Therefore, it is not suitable to use as a low cost fermentation medium. Hence, the medium D was identified as the suitable medium. All media which fermented with *L. delbrueckii* were resulted low mAU*s values when compared to *L. casei* (Table 2).

Factors such as colour, viscosity and turbidity were increased with increasing time. No foam was observed. The spectrophotometer shows light absorption of compounds found in the negative control sample which contains no growth. The UV 210 nm was used to detect linear molecules like lactic acid in HPLC. The HPLC reading confirmed the presence of L-(+)-lactic acid. According to the HPLC reading, till Day-04 the production of L-(+)-lactic acid has increased gradually, and on Day-05 it has reduced. Therefore, the highest production was obtained on Day-04 (Table 3). The production of L-(+)-lactic acid decreased after day 5, which explains the microorganisms entering stationary phase and the maximum fermentation process occurs in 4 days.

Table 2. Results of pilot scale fermentation of different media using 210 nm.

	milli Absorbance Units per second (mAU*s) by each bacterial strain	
	L. casei	*L. delbrueckii*
Medium	225.1	225.1
A	454.0	99.5
B	374.5	32.7
C	1683.7	738.3
D	1076.5	635.5
E	483.8	103.6

With the results obtained from the pilot scale fermentation, it was evident that the D media produced higher amount of L-(+)-Lactic acid using *L. casei*. Thus, the production of lactic acid using large scale fermentation of D medium with *L.casei* is shown in Table 3.

Table 3. Results of large-scale fermentation of D medium with *L. casei* using HPLC at 210 nm

Sampling day	milli Absorbance Units per second at 210 nm
01	604.4
02	1076.5
03	1522.4
04	8657.3
05	6526.5

Conclusions and Recommendations

Medium D was identified as the effective medium for large scale fermentation to produce lactic acid with *L. casei*. Further analysis should be performed to validate the results obtained from HPLC analysis.

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