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Production of Lactic Acid from Banana Peels

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ABSTRACT

The research aimed to optimize lactic acid production from banana peels, a valuable organic acid source, using Bacillus coagulans as the fermenting agent. The banana peels underwent pre-treatment, including washing, sun-drying, milling, sieving, and immersion in an Oxalic acid solution to remove impurities. The Bacillus coagulans inoculum was prepared in a YEX medium, and fermentation occurred by introducing treated banana peels, yeast extract, and the inoculum to the fermentation medium at various temperatures and times. Lactic acid, the response variable, was optimized using a Central Composite Design (CCD) with time and temperature as factors. Response surface methodology (RSM) suggested a quadratic model, and a calibration curve was constructed to determine lactic acid concentration using both titration and spectrophotometric methods. The results indicated that the optimal conditions for lactic acid production were 48 hours of fermentation time and a temperature of 35°C, resulting in a lactic acid concentration of 4.533 g/l. FTIR characterization confirmed the chemical composition of the lactic acid produced. This study demonstrates that banana peels can be a valuable source of lactic acid, and the method can be scaled up for commercial production

1. Introduction

The production of Lactic acid has become a very popular topic due to its applications in various industries, including food [1], pharmaceuticals [2]. cosmetics, and chemicals [3]. Lactic acid has been endorsed as safe for use as a food additive and can be produced by either chemical synthesis or microbial fermentation [4], [5]. It is expected that the demand for lactic acid will increase rapidly in the coming years especially due to its use as a monomer in the production of biodegradable

poly-lactic acid [6]. There are two methods for Lactic acid production; chemical and fermentation methods. While chemical synthesis of lactic acid produces a racemic mixture of D, L-lactic acid, lactic fermentation based on a biomass carbon source is more advantageous due to its low energy consumption and pure lactic acid production [7]. This fermentation method has become more successful due to the growing market demand for naturally produced lactic acid, and the utilization of low-priced agricultural residues in bioprocess serves as a substitute for expensive raw materials and resolves environmental hazards and pollution caused by these materials [8]. This study aims to produce lactic acid through solid-state fermentation, using organic waste (banana peel) and *Bacillus coagulans*. The research seeks to determine the optimal temperature and time of fermentation, compare the concentration of lactic acid produced using titration and UV-spectrophotometric methods, and characterize the lactic acid produced using Fourier Transform Infrared Spectroscopy (FTIR) analysis. The scope of the project work is limited to the production of lactic acid from banana peel using the microbial fermentation process. This study highlights a methodology for recycling, reprocessing, and eventual utilization of valueless fruit waste; specifically, banana peel for the production of the invaluable Lactic acid. Thus, reducing the amount of waste discharged to the environment, which causes detrimental effects to the environment.

Lactic acid was discovered in sour milk in 1780 by Scheele [9]. In 1839, Fremy carried out lactic acid fermentation with various carbohydrates, and in 1857 Pasteur discovered that lactic acid was a metabolite produced by certain microorganisms [4], [10]. Lactic acid demand has grown substantially in recent decades, with an estimated 714.2 kilotons demanded in 2013 and expected to reach approximately 2000 kilotons by 2025 [11] with Purac, Cargill, and Henan Jindan Lactic Acid Technology Co., Ltd said to be the top three producers of Lactic acid in the world [2].

The mass production of bananas on the other hand, started in 1834 and became more popular in the late 1880s. Central American banana trade began on land previously used for sugar but was expanded through the clearing of forests and draining of low marshland.

1.2 Banana Peels

Banana is one of the earliest crops cultivated in human agriculture and has its origin from India to Papua New Guinea, including Southeast Asia. It is the world’s second largest fruit crop with a gross production exceeding 139 million tons, and India, China, Uganda, Ecuador, Philippines, and Nigeria are the leading producers. The worldwide production of bananas has increased over the last two decades from about 70 million to around 117 million as of 2019 [12], [13]. Banana farms generate several tons of underused by-products and wastes, with the peels accounting for about 35-50% of the total mass of fruit produced [14], [12]. Without proper agricultural waste management practices, valuable untapped commodities will be lost and cause serious ecological damage. Banana by-products have been used for various purposes, including wrappings foods, clothes, and ceremonial occasions [15]. Banana peels are rich in starch, hemicellulose, cellulose etc., making them a good source for the production of industrial enzymes [12], [15]. Banana peel’s nutritional value depends on its maturity and cultivar, typically containing 15% to 40% starch, 6-9% dry matter of protein and 20-30% fiber [16]. Table 1 shows the typical nutritional component of banana peels.

Table 1: Nutritional components of banana peel

Nutritional Component	Average Content %
Starch	3.5 - 6.3
Resistant Starch	2.3 - 2.5
Dietary Fat	47 - 53
Crude Fat	2.24 - 11.6
Crude Protein	5.5 - 7.87
Ash	9 - 11
Carbohydrate	59.51 - 76.58

1.3 Lactic Acid

Lactic acid is a popular carboxylic acid also known as 2- hydroxypropanoic acid [14]. It has two optical isomers, S-lactic acid and R-lactic acid. it is used widely across various industries due to some of its unique properties. Lactic acid is used extensively in; biotechnology [14], cosmetics, pharmaceutical, and chemical industries. It is used commercially in food products and beverages for pH regulation, improvement of microbial quality, and mineral fortification. Due to its water-holding, antimicrobial and rejuvenating capacity, it is used in the cosmetics industry as a moisturizer, pH regulator, antimicrobial agent and skin lightener [17]). Furthermore, lactic acid is a significant precursor for valuable compounds such as acrylic polymers, propylene glycol, bio-solvents, pyruvic acid, and esters in the chemical industry [18]. In conclusion, the versatility of lactic acid as a chemical and its multifunctional properties make it an essential component in various industrial applications. Lactic acid is available commercially in 20 to 90 wt% aqueous solutions, this solution contains a fraction of oligomers due to its esterification tendency [19]. Its hygroscopicity and condensation make obtaining the pure compound on a commercial scale very difficult and its production is only feasible via laborious crystallization. A cyclic ester of two lactic acid molecules, called lactide, is the most important building block in producing polylactic acid (PLA) and is found only in trace amounts in aqueous lactic acid solutions due to its instability in water [20]. The major properties of the carboxylic acid are summarized in Table 2.

Table 2: Physical and chemical properties of lactic acid [21]

Property	Unit (Conditions)	Isomer or Conc.	Reported range
Melting point	^oC	L or D racemic	16.4 to 18.0
Boiling point	^oC (at 1.87 kPa)	L or D racemic	103
Solid density	gml^{-1} (at 20 ^oC)		1.33
Liquid density of aq. solution	gml^{-1} (at 20 ^oC)	20 wt%	1.057
		88 wt%	1.201
pKa	N/A	L or	3.79 – 3.86
		D racemic	3.37

1.3.2 Production process of lactic acid (production technology)

Lactic acid is found naturally in certain plants, microorganisms and animals. It can be produced industrially through fermentation or chemical synthesis [4], [14]. Lactic acid production by chemical synthesis has more disadvantages. It is expensive and dependent on fossil fuels. The process produces a racemic mixture of lactic acid [21]. Although there are many possible routes for the production of lactic acid by chemical synthesis, none of these routes is technically and economically feasible [17], except for the routes that use lactonitrile as the raw material and which involve a series of reactions. First Hydrogen cyanide (HCN) is added to liquid acetaldehyde

(CH3CHO) in the presence of a base catalyst under high pressure where lactonitrile is produced. The lactonitrile is then recovered, purified by distillation and hydrolysed using sulfuric acid (H2SO4) to obtain lactic acid (CH3CHOHCOOH) and ammonium salt (NH4SO4). The lactic acid is then esterified with methanol and the methyl lactate produced is recovered, purified by distillation and hydrolysed with acidified water to produce lactic acid and methanol. The Methanol is then separated by distillation and recycled. The problems of expensive raw materials, impurity of the product, and dependence on other industries for raw materials make production by fermentation a much better option for producing lactic acid [21], [7].
Approximately 90% of lactic acid produced in the world is produced from microbial fermentation [22]. This method is preferred because of its numerous advantages over chemical synthesis. It produces pure isomers and utilizes renewable resources as fermentation substrates. Several microorganisms and raw materials can be used in the production of lactic acid. A fermentation product with high purity is obtained when a pure substrate is used, such as sucrose from sugarcane and sugar beet, which results in a reduction in the cost of purification.

1. 4 Microorganism Used in the Fermentation

Microorganisms play a pivotal role in the production of lactic acid and they must be readily available and cheap. Lactic acid-producing microorganisms are classified into bacteria, fungi, and yeast. Most of the lactic acid production is done industrially by the use of lactic acid-producing bacteria [23]. The fungal species of Rhizopus can also convert starch to L(+) lactic acid through their amylolytic enzyme activity [24]. Using these fungi has some advantages; low-cost downstream processing, low nutrient requirements and production of valuable fungal biomass as they make use of glucose aerobically to produce lactic acid, but the production rate of fungal fermentation is low due to mass transfer limitations and the production of by-products. Genetic engineering techniques are exploited to improve the lactic acid yield and optical purity by various microbial producers [25].

Fermentation requires abundant nutrient supply, with yeast often used as a key nutrient source for lactic acid production. Yeast's advantages include pH tolerance and growth in mineral media, which reduces neutralization costs. Wild-type yeast yields low amounts of lactic acid. Genetically modified yeasts such as *Saccharomyces, Candida, Zygosaccharomyces,* and *Pichia* are now used to produce high yields of lactic acid. Although using yeast as a nutrient source increases production costs, alternatives like corn-steep liquor, rice bran, and wheat bran can be used [26]. Bacteria are the better alternative to yeasts because of some of the disadvantages of using yeasts such as lower lactic acid yield, slower fermentation rates, nutrients requirements etc., affecting the overall efficiency of the process.

1.4.1 Bacteria

There are four main categories of lactic acid-producing bacteria, which are, lactic acid bacteria (LAB), *Escherichiacoli*, *Corynebacterium glutamicum*, and Bacillus strains [27]. Out of these, lactic acid bacteria are the most commonly exploited. Choosing a proper strain is very important because factors such as yield, productivity, purity and nutrition requirements are dependent on it [28]. Some of the limitations of lactic acid production by bacteria include low yield due to the formation of byproducts, the requirement of nutrient- rich medium, the high risk of cell lysis, the necessity of mixed strains for the development of phage-resistant strains to prevent bacterio-phage infection [28]. Lactic acid bacteria can produce lactic acid by anaerobic glycolysis with high yield

and productivity. They are present in dairy products, meat, and in plants. Different bacteria grow under different conditions. In general, the optimal pH range for the growth of bacteria is 3.5–9.6 and the optimal temperature is 5–45° C [28]. Lactic acid bacteria are classified according to fermentation end product either as homofermentative or heterofermentative.

1.4.2 Bacillus coagulans

Bacillus coagulans is a lactic acid producing bacteria that belongs to the Bacillus genus unlike the LABs. *Bacillus coagulans* is an example of a probiotic bacillus. Probiotics are defined by the World Health Organization as “live microorganisms that, when administered in adequate amounts, confer a health benefit on the host” [29]. It was first isolated from spoiled canned milk by Hammer in 1915 [30]. As a kind of lactic acid-producing bacteria, some B. coagulans strains were mislabeled as *Lactobacillus sporogenes* [31]. Subsequent taxonomic studies demonstrated that this specie should be classified within the genus Bacillus [32]. B. coagulans is a lactic acid producing, spore-forming, catalase positive and facultative anaerobic bacterium [33]. Its optimum growth temperature is 35–50 ^{o}C and its optimum pH is close to 6. During the growth process, *B. coagulans* can decompose sugars to produce L-lactic acid. Biotin and thiamine are major growth factors that promote the growth of B. coagulans [24]. Some studies have shown that B. coagulans differs from other Bacillus probiotics in several physiological characteristics, including microscopic observations, growth conditions, biochemical reactions and carbon sources utilization [34]. For instance, the compostion of B. coagulans cell wall is different from similar organisms since the teichoic acid from B. coagulans cell has a higher lipid content (12.6%) than many Gram-positive bacteria (1–2%). Moreover, compared with other Bacillus probiotics, the teichoic acid from B. coagulans lacks amino acid substituents [35]. Bacillus coagulans has advantages over other LABs due to its heat stability, long shelf life and fermentation efficiency.

1.5 Fermentation Methods for Lactic Acid Production

The production of lactic acid through fermentation is dependent on several factors, such as the raw materials used, nutrients present in the media, and the microorganisms employed. Three methods of fermentation are commonly practiced, which are batch fermentation, fed-batch fermentation, and continuous fermentation. In batch fermentation, all required materials are added before the fermentation process, making it the simplest process, and it prevents contamination to a good extent compared to other methods (Hofvendahl, et al., 2000). However, it has low productivity and yields low cell concentrations [26]. Fed-batch fermentation involves adding raw materials during the fermentation process at regular intervals, which reduces substrate inhibition and is more efficient than other methods [36]. Continuous fermentation involves continuously adding fresh medium to the fermenter while withdrawing the already existing broth at the same rate, and it has the advantage of preventing end-product inhibition and less decrease in productivity during the lag phase, but it suffers from contamination, requires an expert operator, and is expensive to perform [37].

1.6 Current Fermentation Production

Over 90% of commercial lactic acid production is carried out through fermentation, mainly using carbohydrates derived from corn syrups, molasses, beet extracts, whey, and starches. Anaerobic fermentation of sugar to lactic acid is usually carried out by homo-fermentative bacteria, such as *Lactobacillus delbrueckii*, under a pH range of 5 to 7, with a lactate yield of up to 90% with

dextrose. However, this fermentation process has two major drawbacks. Firstly, the bacteria have their optimum productivity in a specific pH range, which requires careful pH control. Secondly, the production of lactic acid through fermentation produces equal amounts of lactic acid and water, which makes the process inefficient. Nevertheless, researchers are investigating non-edible cellulose as a key substrate to produce lactic acid in the future [38].

1.7 Separation and Purification of Lactic Acid

The separation and purification of lactic acid are crucial steps in its production process, as they account for a significant portion of the operating and capital costs [4]. Despite the significant difference between the boiling points of lactic acid and water, it is difficult to obtain pure crystalline lactic acid due to its high affinity for water and the formation of lactate dimers. Classical downstream treatments such as precipitation, filtration, and crystallization have been used, but these methods have limitations in terms of cost, product purity, and environmental impact. Membrane separation processes like reverse osmosis, electrodialysis, and ultrafiltration have been developed as promising alternatives, but they also have their own drawbacks, including high equipment cost and membrane fouling. Non-traditional distillation separations such as reactive and molecular distillation and direct fermentation of organic lactates are also being explored to address these challenges

1.8 Safety Guidelines for Handling Lactic Acid

When handling lactic acid, it is important to take precautionary measures such as having medical advice available, keeping it out of reach of children, and wearing protective clothing. First-aid measures should also be taken into consideration, including removing a person to fresh air if they have inhaled the acid, washing the skin with soap and water if it comes into contact with the acid, and seeking medical attention if symptoms occur. Proper handling and storage can greatly reduce risks, and this can be achieved by providing adequate ventilation, washing hands before and after work, keeping the container tightly closed, and storing it according to the product insert instructions, while using local and general ventilation. The recommended temperature range for storage is 15 - 25 ^oC.

1.9 Design Expert

Design Expert is a software designed to help with the design and interpretation of multi-factor experiments. In polymer processing, we might use the software to help us design an experiment to see how a property such as tensile strength varies with changes in the processing conditions - e.g. changes in rotor speed or ram pressure. The software offers a wide range of designs, including factorials, fractional factorials and composite designs. It can handle both process variables, such as rotor speed, and also mixture variables, such as the proportion of resin in a plastic compound. Design expert offers computer-generated D-optimal designs for cases where standard designs are not applicable, or where we wish to augment an existing design - for example, to fit a more flexible model [39].

1.9.1 Statistical terms and concepts

The factors that we vary in an experiment can be divided into “process variables”, such as the speed of an engine or the thickness of an adhesive layer, and “mixture variables”, such as the proportion of resin in a plastic compound, or the proportion of fat in a chicken feed. With a mixture variable, the effect depends on the proportion of a constituent in the mixture, rather than on the

absolute amount. We fit models relating a response or quality characteristic to a set of controllable variables. For continuous control variables, we often use a linear, factorial or quadratic model [40]. A full factorial design is one where the experiment uses all combinations of the levels of factors. Many designs involve running only a small fraction of a full factorial. This makes our experiments more economical, but results in what is known as aliasing between different effects. If two effects are aliased together, we can estimate their combined effect, but cannot separate out the size of each individual effect. The resolution of a design gives an indication of the degree to which we're going to be able to separate out individual effects.

1.9.2 Steps in running design expert

Running Design Expert is a simple process with three main steps once the type of design to be used has been selected. The first step is constructing the design which involves specifying variables and their ranges as well as the degree of replication. Design Expert provides the design layout, showing the list of experimental settings to be used for each of the runs. The second step is evaluating the design by assessing the Alias pattern to check whether the effects of interest can be estimated, and the precision of the fitted model to predict the likely precision. If the required precision cannot be achieved, a larger experiment may be necessary. The final step is modelling and interpreting the experimental data using a wide range of analytical and graphical techniques, including the use of Normal probability plots for highlighting active factors in the analysis of 2-level Factorial designs [39];

2. Materials and Methodology

2.1. Equipment

The laboratory equipment used for the study Include: Measuring Cylinder, Beaker, Conical flask, Autoclave, Oven, pH Meter, Filter paper, Clock, Cheese cloth, Sieve.

2.2 Materials

The materials used include: banana peel. The identification of the banana peel was investigated by Mr. Namadi Sunusi at the Herbarium of the Department of Botany, Ahmadu Bello University as Musa Sapientum with a voucher number ABU0304 belonging to the family Musaceae. Bacillus coagulans was acquired as freeze-dried stock at the Department of Microbiology, Ahmadu Bello University, Zaria. The Bacillus coagulans used was also acquired as freeze-dried stock at the Department of Microbiology, Ahmadu Bello University, Zaria. Some other materials used include: Distilled water, Sodium Hydroxide (NaOH), Hydrochloric acid, Xylose, Yeast extract, Calcium Oxide (CaO)

2.3. Pre-treatment

The banana peel was washed with water to remove impurities followed by sun drying for 3 days. The banana peel was then milled and sieved using a 0.60 mm mesh. 30 g of banana peel was immersed in 300 ml of Oxalic acid solution with a concentration of 5 wt% in a 500 ml conical flask. Subsequently, the solution was autoclaved at 121 ^{o}C for 15 min. The solution was allowed to cool and then the solid was filtered using a muslin cloth. The solid residue was washed with excess distilled water until the solid residue attains a neutral concentration

2.4 Micro-organism and inoculum preparation

Bacillus coagulans was acquired as freeze-dried stock at the Department of Microbiology, Ahmadu Bello University, Zaria. A strain of the isolate was sub-culture in a Nutrient Agar slant at a temperature of 50 ^{o}C for 24 hrs. A strain of the subculture; *Bacillus coagulans* was immersed in 5ml sterile water. *Bacillus coagulans* inoculum (seed culture) was prepared in YEX medium containing 10 g/l xylose and 10 g/l yeast extract at a pH of 6.0, and a temperature of 50 ^{o}C for a time span of 24 hr.

2.5. Fermentation of treated banana

Fermentation was carried out by introducing a 4-gram mass of hydrochloric acid treated banana peel, 10ml of 10 g/l yeast extract and 10% (v/v) of the inoculum to 100 ml of the fermentation medium (50 g/l glucose and 10g/l yeast extract) in 150 ml Erlenmeyer flask. The fermentation was accomplished in an Incubator Shaker Water Bath for each run at a different temperature and time as gotten from the design expert, agitated at 100 r.p.m. The pH of the fermentation broth was adjusted to a pH of 6.0 by charging 10 M NaOH solution

2.6 Experimental design

This was carried out on design-expert version 13.0.1.0 software. The design was with Central Composite Design (CCD) and a study-type; Response Surface Methodology (RSM) was adopted. Two factors (variables) and a response were considered. The factors were A (Time) in hours and B (Temperature) in ^{o}C. The response was the yield of banana peel in percentage (%). The lower and upper bounds for the time were 12 and 48 hours respectively. While for the temperature, 35 and 50 ^{o}C were the lower and upper limits respectively. Eventually, 10 numbers of experimental runs were generated.

2.2.5 Construction of calibration curve

To construct the calibration curve, a stock solution of lactic acid was prepared at a concentration of 89g/l. A series of solutions with decreasing concentrations were then prepared through dilution. Iron (III) chloride solution (0.2%) was also prepared. The colored solutions obtained from the reaction between the lactic acid solutions and the iron (III) chloride solution were measured for absorbance at 390nm. The absorbance values obtained were used to calculate the parameters for a linear equation that corresponds to the range of the calibration curve. Microsoft Excel was used to construct the calibration curve.

2.6. Spectrophotometric determination of lactic acid

A 50 mL test solution containing lactic acid was mixed with 2 ml of a 0.2% iron (III) chloride solution, stirred, and the absorbance was measured at 390nm against a reference solution (2 ml of a 0.2% $FeCl_3$ solution). The reaction and measurements were conducted at room temperature, with the solution colour remaining stable for 15 minutes.

2.7. Characterization of lactic acid produced

The lactic acid produced was characterized using SHIMADZU FTIR-8400S by documenting the spectra between 4000 and 650 cm-1 at a resolution of 8 cm-1. Additionally, Kelling's test using iron (III) chloride was employed to confirm the presence of lactic acid.

3. Results and Discussion

The following are the results of the various analyses carried out for the optimization of lactic acid from banana peel. The analytical method (titration method) and UV spectrophotometric methods were used in determining the concentration of lactic acid produced.

3.1 Titration Result

Ten (10) fermentation runs were carried out at different conditions as obtained from the Design expert. Table 3 shows the average titre value of the NaOH used for the titration. 25ml of the sample was titrated against 0.5 M NaOH solution and phenolphthalein was used to determine the end point of the titration.

Table 3: Titration table

Runs	T(hr)	Temp(^oC)	Titre (1)	Titre (2)	Average
1	55.5	42.5	2.40	2.50	2.45
2	12	50	1.60	1.50	1.55
3	30	42.5	2.50	2.50	2.50
4	48	35	3.40	3.50	3.45
5	30	53.1	1.10	1.10	1.10
6	30	42.5	2.50	2.50	2.50
7	48	50	2.20	2.20	2.20
8	4.5	42.5	0.60	0.50	0.55
9	12	35	1.80	1.80	1.80
10	30	31.9	1.30	1.30	1.30

3.2. Total Titratable Acidity of Lactic Acid

The total titratable acidity of lactic acid was calculated using the relation in equation 1 below

$$T = \frac{ml_{NaOH} \times N_{NaOH} \times M.E}{Vol_{sample}} \quad (1)$$

Where,
T is the total titrable acidity of lactic acid (g/l)
M.E is the mass equivalent of lactic acid which is equal to 90.08 g
N_{NaOH} is the Molarity of NaOH used

ml_{NaOH} is the volume of NaOH used for neutralization

Table 4: Concentration of Lactic acid from titration method

Runs	t(hr)	T (^oC)	Average (ml)	Conc. (g/l)
1	55.5	42.5	2.26	4.716
2	12	50	1.09	1.962
3	30	42.5	1.94	3.492

4	48	35	2.79	5.026
5	30	53.1	1.60	2.880
6	30	42.5	2.25	4.056
7	48	50	2.30	4.140
8	4.5	42.5	0.80	1.441
9	12	35	1.20	2.160
10	30	31.9	1.30	2.342

3.2 Design Expert Optimization of the Titration Result

3.2.1 Actual Design of Experiment

The response (concentration of lactic acid) was fed into the design expert version 13.0.1.0, a response surface study type and central composite design was adopted for the optimization process. Table 5 below shows the three factors from the design of the experiment

Table 5: Three factors from the design of the experiment.

Std	Runs	Factor 1 A: t(hr)	Factor 2 B: T (°C)	Factor 3 LA_{conc} (g/l)
6	1	55.5	42.5	4.716
3	2	12	50.1	1.962
10	3	30	42.5	3.492
2	4	48	35	5.026
8	5	30	53.1	2.880
9	6	30	42.5	4.056
4	7	48	50	4.140
5	8	4.5	42.5	1.441
1	9	12	35	2.160
7	10	30	31.9	2.570

3.2.2 Fit summary table and statistics

Table 6 gives the fit summary of the date analyzed, RSM suggests a quadratic model for the experiment. This model was adopted for further analysis.

Table 6: Fit summary table

Source	Sp-value	LF p-vlaue	AdjR2	PredR2
Linear	0.008	0.5295	0.8318	0.7508
2FI	0.5362	0.5020	0.8169	0.7198
Quadratic	**0.2490**	**0.5410**	**0.88630**	**0.6039**
Cubic	0.6214	0.765	0.8297	-0.7163

Table 7 shows the fit statistics of the analysis showing the R^2 values.

Table 7: Fit Statistics

Std Dev.	0.5027	**R^2**	0.8692
Mean	3.24	**Adjusted R^2**	0.8318
C.V. %	15.49	**Predicted R^2**	0.7508
		Adeq. Precision	12.4348

Where,

$S_{p-value}$= Sequential p-value
$LF_{p-value}$ = Lack of Fit(p-value)
Adj_R2 = Adjusted R^2

$Pred_R2$ = Predicted R^2

The **Predicted R²** of 0.7508 is in reasonable agreement with the **Adjusted R²** of 0.8318; i.e., the difference is less than 0.2. **Adequate Precision** of 12.434 was achieved from the model. This indicates a high signal-to-noise ratio. A ratio greater than 4 is desirable (RSM manual). The ratio of 12.435 indicates an adequate signal and allows easy navigation of the design space.

3.2.3 ANOVA Result of the Study from Design Expert

Table 8 shows the analysis of variance results for a quadratic model of the response (lactic acid concentration)

Table 8: ANOVA results for a quadratic model

Source	Sum of Squares	df	Mean Square	F-value	p-value
Model	12.70	5	2.54	12.34	0.0152
A: Time	11.70	1	11.70	56.84	0.0017
B: Temp	0.0522	1	0.0522	0.2534	0.6411
AB	0.1183	1	0.1183	0.5748	0.4906
$A2$	0.2702	1	0.2702	1.31	0.3158
$B2$	0.8046	1	0.8046	3.91	0.1192
Residual	0.8235	4	0.2059		
Lack of fit	0.6645	3	0.2215	1.39	0.5410
Pure error	0.1590	1	0.1590		
Cor Total	13.52	9			

Factor coding is **Coded**
Sum of squares is **Type III - Partial**

From the ANOVA result, the model F-value and P-value are 12.34 and 0.0152 respectively. This indicates that the model is significant as stated in (RSM manual) "P-values less than 0.05 indicates model term is significant". Lack of fit F-value of 1.39 indicates a non-significant lack of fit. No significant lack of fit is a good result since the model is expected to be fit.

3.2.4 Equation of the model

Equation 2 represents the final equation of the model in terms of coded factors

$$Conc_{LA} = 3.77 + 1.21A - 0.0808B - 0.1720AB - 0.2425A^2 - 0.4201B^2 \quad (2)$$

Where,
$Conc_{LA}$ = Lactic acid concentration
A = Time in hours
B = Temperature in °C

The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1 and the low levels are coded as -1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.

3.2.5 Numerical Optimization of the Model

Table 9 shows the numerical optimization solution of the model. 12 solutions were achieved

Table 9: Numerical optimization solution

Number	t(hr)	T (ºC)	LA concentration	Desirability
1	**48.000**	**35.000**	**4.533**	**0.863**
2	48.000	35.111	4.532	0.862
3	47.828	35.000	4.522	0.859
4	47.800	35.000	4.520	0.859
5	48.000	36.560	4.517	0.858
6	48.000	36.980	4.512	0.857
7	48.000	37.166	4.510	0.856
8	48.000	37.499	4.507	0.855
9	48.000	41.988	4.458	0.842
10	48.000	42.906	4.448	0.839
11	48.000	43.573	4.441	0.837
12	48.000	44.576	4.430	0.834

From the numerical optimization solution, the optimal conditions are between 47.80 to 48.00 hours and temperature between 35 ºC to 44.576 ºC. at temperatures above 45 ºC, low concentration or desirability of lactic acid is recorded. The selected optimal condition with the highest desirability yields a concentration of 4.533 g/L at temperature and time of 35 ºC and 48 hours respectively.

3.2.6 Graphical representation of the model

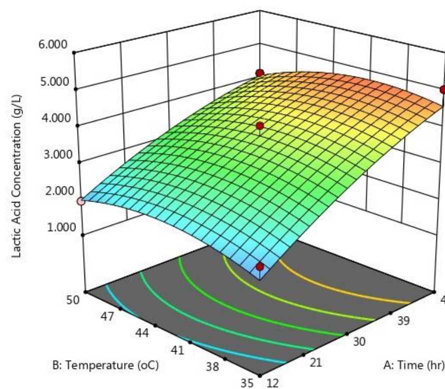


Figure 1. The 3D graphical representation of the model

Figure 2 shows the parity plots for the pre-treatment. This compares the actual experimental values with the values predicted by Design expert software. An R^2 value of 1 means the model perfectly describes the process. The R^2 value for the model is 0.938, this means the model perfectly describes the process.

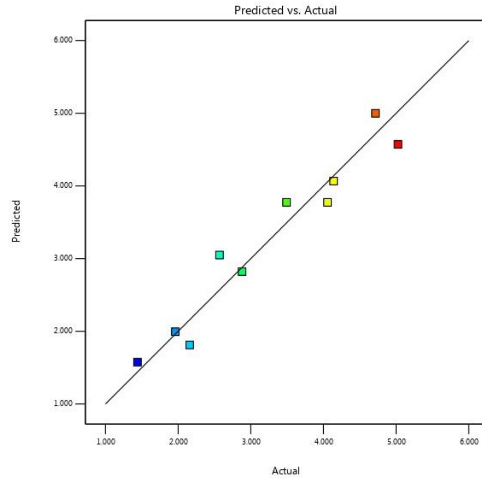


Figure 2. Pretreatment parity plots

3.3 UV- Spectrophotometric Determination of Lactic Acid Concentration

UV- spectrophotometer was also used for the determination of lactic acid concertation, by measuring the absorbance of the lactic acid.

3.3.1 Calibration curve

Table 10 shows the calibration table of the standard lactic acid

Table 10. Calibration Table

Absorbance	**Concentration (g/l)**
0.282	8.5
0.52	17
0.719	25.5
0.96	34
1.206	43

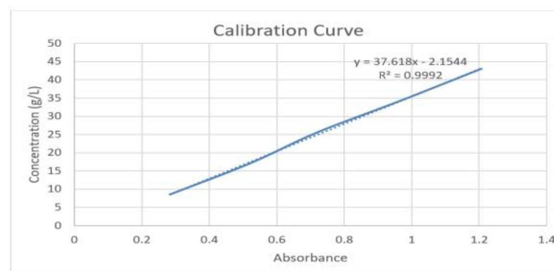


Figure 3. Calibration curve

The absorbance of lactic acid solution (x) is proportional to the concentration of lactic acid (y) in the range from 8.5 to 43 g/L. The equation of the calibration curve formed from Excel is given by equation 3.

$$y = 37.618x - 2.1544 \quad (3)$$

with a correlation of 0.9992.

The calibration curve was used to estimate the concentration of the lactic acid by measuring the absorbance of each sample for each run. Table 11 shows the concentration of each sample using UV-spectrophotometer

Table 11. Concentration of Lactic acid via UV-spectrophotometer

Runs	T(hr)	Temp (°C)	Absorbance	Conc.(g/l)
1	55.5	42.5	0.3450	10.82
2	12	50	0.2058	5.59
3	30	42.5	0.3222	9.97
4	48	35	0.3770	12.03
5	30	53.1	0.2642	7.78
6	30	42.5	42.5	0.3222
7	48	50	0.2876	8.66
8	4.5	42.5	0.1684	4.18
9	12	35	0.2648	7.81
10	30	31.9	0.2344	6.66

3.4 Comparison Between the Concentration of Lactic Acid Produced via Titration and UV Spectrophotometer Methods.

Figure 4 shows a graphical relationship between the concentration of lactic acid calculated via titration of the lactic acid with a base and the UV-spectrophotometer analysis by measuring the absorbance of the analyte.

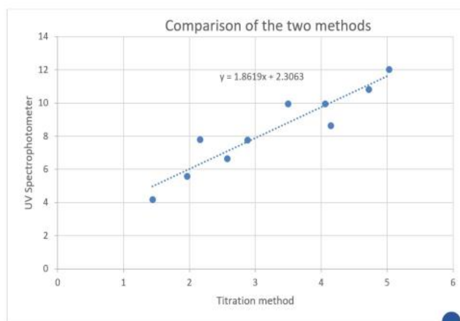
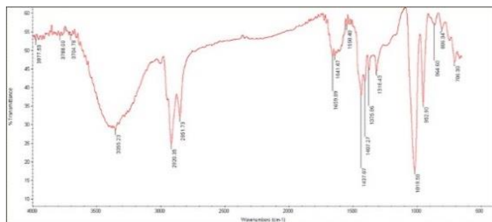


Figure 4. Comparison of lactic acid concentration from titration and UV-spectrophotometer

3.5 Fourier Transform Infrared Spectroscopy (FTIR)

Fourier transforms infrared (FTIR) spectroscopy is one of the most important and emerging tools used for analyzing functional groups present in test samples. Figure 5 and Figure 6 show the spectrum of standard lactic acid and the analyzed sample.



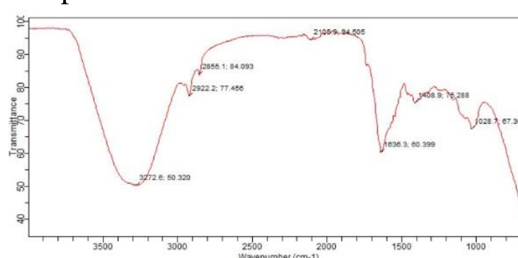


Figure 5. FTIR spectrum for lactic acid standard Figure. 6 FTIR Analyzed Lactic acid

The spectral resolution was 4 cm-1 and 30 scans were accumulated for each spectrum. The assignment of functional groups corresponding to the vibration modes was based on the identification of the spectrum peaks and matching the frequency with the chemical groups that absorb in the IR region. The C=O stretching band at 1730 cm-1 is characteristic of carboxylic acid groups. The peaks in the region 2500–3000 $cm^{-}1$ are due to the O–H stretching of the acid component, while the bands in the region 1200–950 cm-1 are explained by stretching modes of C–C and C–O functional groups. The lower range of the region below 1200 $cm^{-}1$ generally represents different kinds of C-H, C-O, and CH3 vibrations (rocking, deformation, stretching). As compared to the standard lactic spectrum, more of the lactic acid bands were identified in the lactic acid produced from banana peels. The spectral difference in the sample and the standard lactic acid is due to the formation of other acids (like acetic acid) that can be identified from advanced studies.

4. Conclusion

In this paper, lactic acid was produced from banana peel using a model strain of *Bacillus coagulans*. The optimal condition for the fermentation of lactic was achieved using design expert to be at a temperature of 35 ^{O}C and a time of 48 hours with a concentration of 4.533 g/l. The concentration of lactic acid measured using a UV-spectrophotometer is higher than that calculated using the titration method. A linear trend was established for the variation in the concentration figure 2. The FTIR result of the lactic acid produced was compared with the standard lactic acid and the carboxylic acid, alkane and ketone functional group band was noted on the spectrum.

Nomenclature

T	Total titrable acidity of lactic acid (g/l)
M.E	The mass equivalent of lactic acid (90.08g)
N_{NaOH}	Molarity of NAOH
ml_{NaOH}	The volume of NaOH used for the neutralization
DOE	Design of Experiment
LA_{conc}	Lactic Acid concentration
$S_{p-value}$	Sequential p-value
$LF_{p-value}$	Lack of Fit(p-value)
Adj_R2	Adjusted R^2
$Pred_R2$	Predicted R^2

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