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Optimisation of Lactic Acid Fermentation from Cassava Peel by *Lactobacillus casei* (ATCC334)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The demand for lactic acid is steadily increasing due to the desire of its bioproduction over chemical synthesis. The associated cost, however, is a significant hurdle. This study reports lactic acid fermentation by *Lactobacillus casei* ATCC334 from cassava peel. It investigates the effect of unhydrolysed cassava peels, acidic, alkali hydrolysates; fermenting pH; substrate concentration; nitrogen source concentration; duration; and inoculum size. An attempt at a cheaper purification and recovery protocol relative to those currently in use was similarly performed. Acidic hydrolysate yielded 10.53%, unhydrolysed substrate gave 4.80% with alkali hydrolysate yielding 4.75%. The highest LA yield was obtained at pH 6.0, 2.0% v/v inoculum size, 25% w/v substrate concentration, 5% nitrogen source concentration. A post-optimisation combination yielded 18.3% LA suggesting that one-factor-at-a-time may be unsuitable for optimisation studies involving cassava peel and *L. casei* ATCC334. FTIR spectra of product suggests effective partial purification. Hence, an improvement in the optimization strategy for production is recommended for subsequent study.

Keywords: Lactobacillus casei; Lactic acid; cassava peel; hydrolysates; optimisation.

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1. INTRODUCTION

Lactic acid (LA), also referred to as 2-hydroxypropanoic acid is an organic acid (formula $CH_3CHOHCOOH$) with a naturally occurring organic compound that can be derived from product fermentation [1,2,3]. Lactic acid, being the simplest known hydroxyl acid [4] is a high value compound [5] with extensive applications including food, chemical, pharmaceutical, biotechnological and cosmetic industries [6,4,7,8].

This wide application is evident in the global demand for LA which is currently estimated at 1,960.1 kilo tons [9]. with a significant portion of this demand feeding the production of the biodegradable polylactic acid (PLA) which is taunted as the environmentally friendly substitute to plastics of petrochemical origin [9]. with further medical application [8]. This high demand is currently being met via chemical synthesis and microbial fermentation [10]. with microbial fermentation fulfilling around 90 % of this because it leads to (optically) pure LA as opposed to chemical synthesis that produces a racemic mixture [8]. For this reason, pure LA is considered more valuable [9]. because it eliminates the subsequent downstream process required to separate the racemic solution thus cutting down production costs. Further advantages of fermentative production are: low environmental impact, low energy and temperature requirements coupled with high purity [8].

The economics and efficacy of industrial scale fermentative production of LA depends on fermenting organism, conditions, and substrates [6,11]. Consequently, attention is being paid to substrates used in fermentative production. A little over a third of the food globally produced is lost and the economic value of this is put at $9bn [12]. It is thus logical that food wastes, instead of constituting environmental health and waste management concerns, can serve as feedstock for LA fermentation since their organic nature makes them rich in carbon, nutrients, and moisture [13,14] apart from the fact that they are abundant, cheap, and renewable without competing with food. Of these, cassava, perennial tropical root crop rich in starch [14] is a veritable substrate for LA fermentation.

During processing, the peels of cassava – which constitute around 10 % of the fresh root [2] – weight are often discarded thus constituting environmental and waste problem. The lignocellulosic nature of the peels [8] further makes LA fermentation from cassava peel attractive [2] reported the production of LA from cassava peels using cultures of *Rhizopus oligosporus*and *Lactobacillus plantarum* after acidic and alkaline hydrolysis of peels while [15] reported the effect of polysorbate (Tween) and cyclopropane synthase on LA production using glucose substrate. To the best of our knowledge, no study has explored the impact of acidic and alkali hydrolysates on LA yield by *L. casei* (ATCC334). The properties of *L.casei* makes it a favourable choice for producing lactic acid. Beyond the safe status, *L. casei* is a non-spore forming, Gram positive [16] acid-tolerant, lactic acid bacterium that is rod-shaped. *L. casei* produces predominantly L-lactic acid (95 %) as product during carbohydrate fermentation [15] Further, a reconstruction of metabolic pathways for *L. casei* (ATCC334) at the genome level performed by [16] identified 548 genes and 1,040 reactions modulated by 959 metabolites. *L. casei* (ATCC334) has also been reported to produce anticancer peptides which can be added additive to the desire product [17]. This diversity of metabolic processes is a statement of metabolic versatility and stability of the organism making it a fit model to elucidate effects of optimisation studies.

Therefore, this study seeks to determine the effect of some growth factors (growth pH, inoculum size, substrate, and nitrogen source concentrations) on lactic acid fermentation by *L. casei* (ATCC334).

2. MATERIALS AND METHODS

2.1 Microorganism

L. casei ATCC 334 was procured from VRS International Limited, Nigeria and was cultured as described by Broadbent et al. (2014) on de Man Rogosa Sharpe (MRS) broth (HiMedia, India). Stock cultures were made using the same protocol.

2.2 Plant Material

Cassava peel, obtained from cassava processing factory in Ido-Osun (7.782100° N, 4.549790° E), Ososgbo, Osun State, Nigeria, was dried at 180°C for 30 minutes, shred into tiny bits and blended (Kenwood), at ambience, into fine powder. This was used as substrate for fermentation.

2.3 Proximate Analysis of Cassava Peel

Dry matter content, ash content, crude protein, fat content, crude fibre, moisture content, carbohydrate content, glucose analysis and sucrose analysis of cassava peel was determined using the method of [18].

2.4 Fermentation Medium and Pre-treatment of Substrate

The fermenting medium as reported by Jawad et al. (2013), with slight modification was used. Medium component was 0.2 g $MgSO_4 \cdot 7\ H_2O$, 0.05 g $MnSO_4 \cdot 4\ H_2O$, 0.5 g sodium acetate, 1.5 g KH_2PO_4, 1.5 g K_2HPO_4 and 5 g yeast extract per litre of distilled water. Cassava peel, hydrolysed using 30 mL of 0.5 N NaOH for alkali hydrolysis and 30 mL 0.5 N HCl for acid hydrolysis, served as carbon source throughout the fermentation process. The media were autoclaved at 121°C at 15 psi for 15 min.

2.5 Submerged Fermentation for LA Production

For seed cultureof *L. casei*, a loopful of cells from pure *L. casei* culture stock was inoculated aseptically (10% v/v), into MRS broth (50 mL) after sterilization, in a plugged 250 mL Erlenmeyer flask. This was incubated stationarily at 37±1 ℃ for 48 h. To remove MRS broth, post-incubation, the inocula were centrifuged at 5000 rpm for 8 mins and the cell pellet left after decanting was suspended in sterile medium [13]. All subsequent optimisation studies were also executed under submerged fermentation condition.

2.6 Optimization of Fermentation Conditions

2.6.1 Effect of pH Variation on Lactic Acid Yield

Fermenting medium containing hydrolysates (20% w/v), at the different pH (5.5, 6.0, and 6.5) conditions, was measured into different conical flasks and autoclaved at 121°C, 15 psi for 15 minutes. Unhydrolysed substrates served as control. The set ups were inoculated with 1% v/v inoculum size.

2.6.2 Effect of Inoculum Size on Lactic Acid Yield

Hydrolysed substrate (20% w/v) was measured into different conical flask, mineral salt medium was added and autoclaved at 121°C for 15 mins at 15 psi. Suspended bacteria cell pellet was inoculated into the substrate at different concentration (0.5, 1.0, 1.5, and 2.0% v/v). The substrates were then incubated at 37±1°C for 6 days without aeration.

2.6.3 Effect of Varying Substrate Concentration on Lactic Acid Yield

Hydrolysed substrate concentration was varied at 10, 15, 20, and 25% w/v into different conical flasks and mineral salt medium was added. This was then autoclaved at 121°C, 15 psi for 15 mins. After autoclaving, the substrate was allowed to cool and 1% v/v inoculum size was introduced into the substrate for fermentation without aeration for 6 days.

2.6.4 Effect of Nitrogen source concentration on Lactic Acid Yield

Yeast extract was used as nitrogen source. This was then varied by measuring different grams of yeast extract (1, 3, 5, and 7% w/v) into different 250 mL Erlenmeyer flask containing cassava peel. The organism (1% v/v) was inoculated and then incubated at 37±1°C for 6 days without aeration.

2.7 Recovery and Partial Purification Processes of Lactic Acid

After fermentation, the fermented broth was retrieved. Of this lot, 100 mL was placed in 250 mL conical flask heated to 80-100 ℃. The broth was allowed to cool and the pH was increased to 10-11 using calcium hydroxide (20 mL) which inactivate microorganisms, coagulate proteins, solubilise calcium lactate, and degrade some residual sugars [8]. The cells and coagulated protein were filtered (Whatman No 1) to make a crude extract of lactic acid [19]. This was further processed via the following techniques.

2.8 Filtration, Carbon Treatment, and Evaporation

With the crude extract, 20 g of activated carbon was mixed decolourisation. The spent carbon was then filtered. The filtrate was evaporated under mild vacuum at moderate temperature (0.57 atm and 70±1°C) to 37% calcium lactate concentration prior to acidification with 63% sulphuric acid. The calcium sulphate precipitate produced was filtered out. A repeat decolourisation was performed before evaporation to 52% concentration [20].

2.9 Analytical Method

Lactic acid yield was estimated by the titrable acidity of the fermentation medium against 1 N NaOH. Fermented medium (2 mL) was dispensed into 8 ml of distilled water in a test tube and vortexed (1800 rpm for 2 mins). The homogenised solution was then boiled for a minute to remove air. The medium was subsequently filtered with Whatman filter paper No 1 to remove the solid substrate. One drop of phenolphthalein indicator was added to the solution. Sodium hydroxide (1 N) solution was titrated with continuous shaking till the formation of pink colour. The volume of sodium hydroxide utilized in the titration was measured. The milliequivalent weight of lactic acid is given as 0.090 and the yield of lactic acid determined using the formula below [21].

$$\%\ \text{Lactic acid} = \frac{\text{Volume (ml) of NaOH} \times \text{Concentration of NaOH} \times 0.090 \times 100}{\text{Volume/Weight of the Sample}}$$

2.10 Analysis of Data

All readings were in triplicates and presented as mean standard values which were subjected to a One-way Analysis of Variance (ANOVA) and the Least Significance Difference (LSD) was carried out. Significance was accepted at $p \leq 0.05$. The results obtained were analysed using Sigma Plot 10.

2.11 Fourier Transform Infra-Red (FTIR) Spectroscopy for the Extracted Lactic Acid

FTIR Spectroscopy was used to characterize the partially purified lactic relative to a standard obtained from General Purpose Agent (GPA), England. The transmittance, with the range of 400-4000 cm^{-1}, at a resolution of 2 cm^{-1} was carried out using Thermo Scientific Nicolet iS5 FT-IR spectrometer [22].

3. RESULTS AND DISCUSSION

3.1 Proximate Composition of Cassava Peel

The economics of bio-fermentation must be amenable to easy scale-up at the least possible cost without competing with food supplies; these form the major reasons agro-wastes, with high lignocellulosic biomass available for bioconversion [5], constitute the major substrate for biotechnological application these days [6,4,11] and cassava peel is a good model for this. This utilisation is not farfetched as it is abundant, often a waste during cassava processing, and its proximate composition makes it a veritable substrate for several bio-metabolites, including LA.

Our proximate evaluation (Table 1) estimated the carbohydrate content of cassava peel at 63.62% (of which 16.94% were sugars) which can be further degraded into simple sugars to be used by fermenting organism for LA fermentation. This value is lower than 86.2% reported by [23], 91.15% reported by [24], and 93.5% reported by [25]. While these differences are not entirely surprising because different cultivars are bound to be different [26], environmental and soil conditions [27] are also factors that can contribute to the observed difference. The range, however, is wide and comparison should be done with caution. Other components were estimated at: 5.68% crude protein; 9.12% crude fibre; 4.3% fat; 3.68% ash content; and 13.60% moisture content.

3.2 Effect of Substrate Pre-treatment on Lactic Acid Yield

Apart from operating conditions, the nature and type of substrate is known to influence the type and quality of fermentation product [28] and this is also true for LA [6]. Cassava peel, a non-food carbohydrate [6], is a lignocellulosic biomass composed of a linear β-D-glucan (i.e. cellulose) enclosed in a lignin and hemicellulose (including glucose, xylose, galactose, arabinose, and mannose) matrix [8]. While the cellulosic contents are easily hydrolysable, the lignin content is recalcitrant thus inhibiting microbial fermentation [29]. To overcome this limitation and optimise for enhanced yield, the impact of substrate pre-treatment with acid and alkali, relative to unhydrolysed substrate, was investigated.

The effect of substrate hydrolysis with acid and alkali with unhydrolysed control is shown in Fig. 1. Acidic hydrolysates yielded (10.53%) the most with peak values recorded on day 5. Unhydrolysed substrate, peaking (4.80%) fast on day 2, marginally edged alkali hydrolysates which peaked (4.75%) the next day. The minimum lactic acid yield (1.8%) was observed on day 5 for alkali hydrolysed substrate. However, acid hydrolysed and unhydrolyzed substrates had minimum lactic acid on day 1.

Significantly, acidic hydrolysates yield was significantly different ($p \leq 0.05$) from unhydrolysed and alkali hydrolysates.

Pre-treatment, apart from distorting the structure and making the sugar content [30] accessible and available for utilisation, also reduces particle size with an attendant increase in surface area available for microbial deterioration for substrate bioconversion [8]. As expected, acidic hydrolysates, significantly different ($p \leq 0.05$) from unhydrolysed and alkali hydrolysates, improved LA yield corroborating previous reports and justifying its extensive usage in lignocellulosic and starchy substrate treatment [8]. Surprisingly, alkali hydrolysis gave lower yield compared to unhydrolysed substrates until day 5 suggesting a time-course effect and/or stress induction hindering bioconversion with alkali hydrolysis even though what is common is that alkali hydrolysates yield better than unhydrolysed substrates [31]. This may be corrected/enhanced by investigating different alkali concentrations to determine the optimum condition since alkali pre-treatment is known to enhance total soluble organic carbon [32], hemicellulose solubilisation, and surface area [33] which are variables that improve yield. Under our operating conditions, it was observed that high substrate concentration impeded enhanced yield [14]. The use of a different alkali can also be investigated since [11] reported that pre-treating soybean straw with ammonia resulted in increased LA yield due to nutrient supplementation and degradation and conversion of cellulose. Acidic hydrolysis was the most productive following established precedent [34,23,11].

3.3 Optimisation Study

3.3.1 Effect of medium Ph

At pH 6.5, highest lactic acid (11.7%) yield was reported for unhydrolysed substrates; alkali and acidic hydrolysates, on the other hand, yielded the maximum at pH 6.0 (Fig. 2). Since lactic acid bacteria are acidophilic, the effect of medium pH was studied. Among the levels investigated (5.5, 6.0, and 6.5), 6.0 gave the highest LA yield (Fig. 2). This reiterates previous reports [35,5] that acidic conditions give the best yield as it is often the condition at which consumption of residual sugar is optimum [3]. Apart from higher yield at low pH conditions, LA recovery at this condition is also at its most efficient [15,17] . Though [15] reported an optimum pH of 3.8 for *L. casei* (ATCC 334) even though cells are known to be stressed at low pH conditions [4,36] reports that for optimal *L*. *rhamnosus* ATCC 746 survival, pH should be kept above 5 with [3] reporting an optimum of 6.5 for LA fermentation by *Bacillus coagulans* HL-5 from corn flour hydrolysates. Results from [7] supports this assertion as low initial pH was found to be detrimental to LA production from mango peels though [4] argues that initial pH does not influence LA production in the presence of a neutralising agent. These seem to suggest that for LA yield, there appear to be a threshold pH at which there is no significant difference in yield even though higher production rate may be observed [15]. Consequently, the range of 5.0 and 7.0 is regarded as optimum [8] for the various categories of LA producing organisms. Significantly, also, the ability to survive below 3.8 appears to be tied to the activity of cyclopropane synthase (cfa), at least in *L. casei*, as *cfa* knockout mutants of *L. casei* (ATCC334) did not increase LA yield at this condition as reported by [15]. Across all conditions, acidic hydrolysates yielded the most for reasons earlier propounded. Control of medium pH is essential as accumulation of LA reduces medium pH with an attendant inhibition of cell growth and ultimately product formation [6,11].

3.3.2 Effect of Inoculum size

At 0.5% v/v inoculum size, yield followed the order: acidic>alkali>unhydrolysed. The highest lactic acid yield was recorded with 2.0% v/v. Inoculum load was related to yield at a dose-dependent rate (Fig. 3).

Since LA production dynamics is intrinsically coupled with cell growth, the inoculum size is bound to influence it. Our results show that there is a dose-dependent relationship (Fig. 3), which is hardly surprising, between yield and inoculum size with acidic hydrolysates outperforming both alkali hydrolysates and the unhydrolysed substrate. Like other variables, increasing inoculum size led to increased productivity. This observation has been reported by others too [37,38,11]. This observation can be attributed to a shortened lag phase (and overall duration of fermentation [11] thus hastening the log growth phase at which LA is produced [37]. Some studies [4,11] suggest 10% v/v as optimal inoculum size which imply that in spite of progressive increase in yield with increasing inoculum size, there is a peak value [37] beyond which yield may only undergo marginal increase (Wang et al. 2014) or reduction [37]. While [8] report an optimum of 2 to 4% for *L. casei* NBIMCC 1013, in this study, 2% v/v yielded the most though we did not test the impact of higher values. [11], on the other hand reported a peak inoculum size of 10% for soybean straw pre-treated with ammonia.

Table 1. Proximate Composition of Cassava Peel

Crude protein (%)	Crude fibre (%)	Fat content (%)	Ash content (%)	Moisture content (%)	Carbohydrate (%)
5.68	9.12	4.3	3.68	13.60	63.62

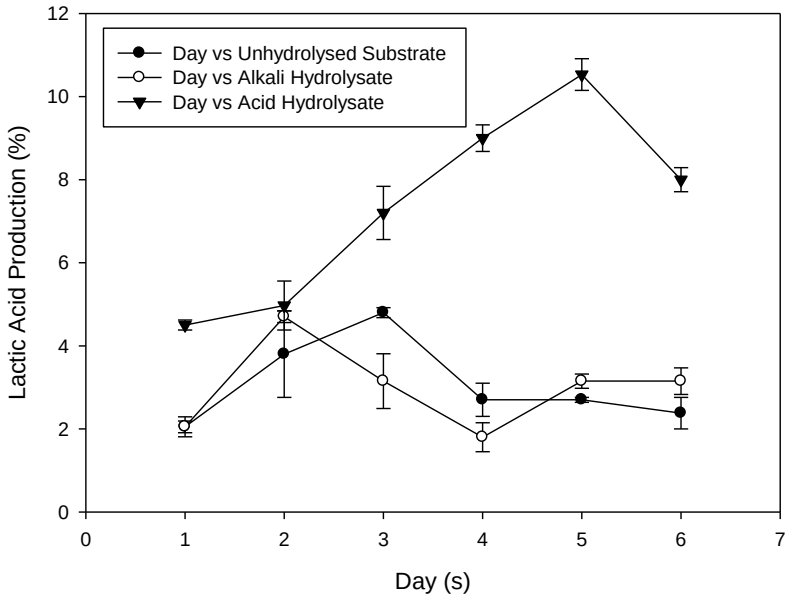

Fig. 1. Influence of varying substrate treatment on Lactic acid yield (mean ± standard error at n=3)

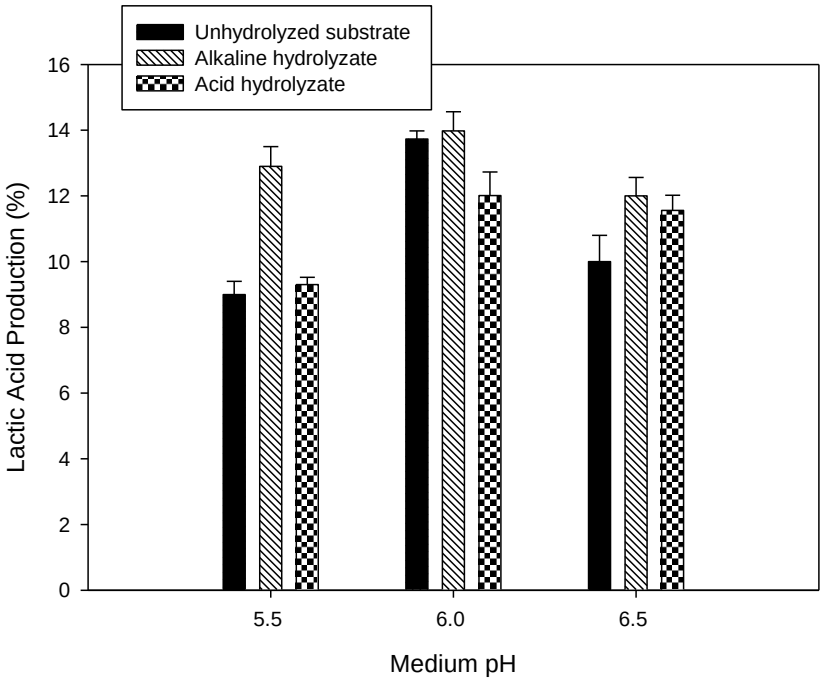

Fig. 2. Influence of substrate pH on Lactic acid yield (mean ± standard error at n=3)

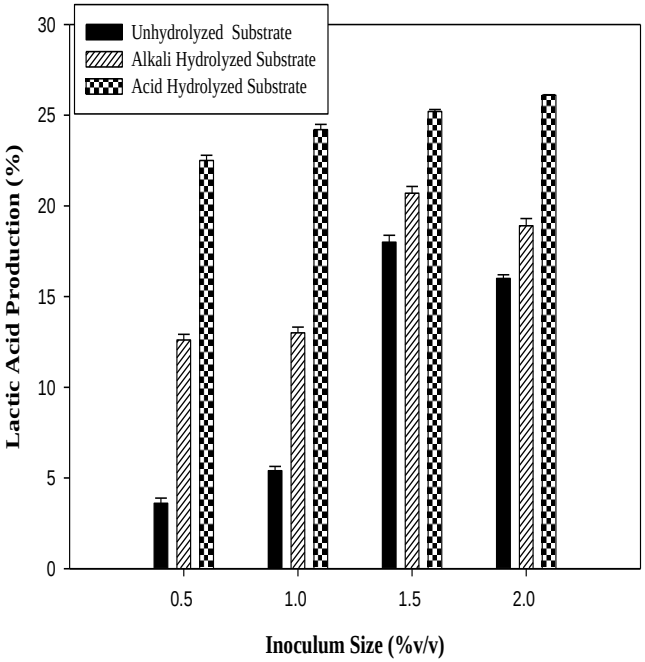

Fig. 3. Influence of variation in Inoculum Size on Lactic acid yield (mean ± standard error at n=3)

3.3.3 Effect of Varying Substrate Concentration

Substrate concentration was relative to lactic acid yield at a dose-dependent rate (Fig. 4). At 10 % w/v substrate concentration, 19.8%, 5.83% and 20.6% lactic acid was produced in unhydrolyzed substrate, alkali and acid hydrolyzed substrate respectively. Peak lactic acid yield was observed at 25% w/v substrate concentration irrespective of substrate treatment though acidic hydrolysates yielded the most at this concentration.

Substrate and YE concentration affects carbon to nitrogen (C:N) ratio which is known to influence LA yield [4,39,30,40]. Where the substrate serves as major carbon source, YE serve the role of nitrogen, minerals, and vitamins source [8]. Results in this study show that LA yield remain virtually similar at 10, 15, and 20% substrate concentration before a sharp increase in the yield from hydrolysed substrates at 25%. Significantly, unhydrolysed substrates at 10, 15, and 20% yielded better than alkali hydrolysates an observation that can be attributed to the effect of time-course relationship and/or stress induction under alkali conditions [31] suggesting that NaOH may not be suitable for the hydrolysis of cassava peel. While there was no decrease in LA yield, other reports seem to suggest a threshold exist which may be caused by decreased conversion rate, product inhibition among other factors [39]. This threshold is a function of the equilibrium between the organism's water activity and plasma membrane stability [41] reported that beyond 30%, LA yield from cassava powder dropped while [4] suggests 18% as optimum substrate concentration for LA fermentation. Substrate concentration, similarly, affects initial sugar concentration [4,41] since the degradation of the lignocellulosic substrates yields the component sugars [14] which are then converted into products.

3.2.4 Effect of Varying Concentration of Nitrogen

Lactic acid yield was dose dependent on nitrogen concentration until a threshold of 5% when diminishing returns set in (Fig. 5). The best yield (12.6%) was reported in acidic hydrolysates at all concentrations with unhydrolysed substrate generally being the least productive except at 3 % concentration where it was more productive relative to alkali hydrolysates. The difference between unhydrolysed and alkali hydrolysates was not generally wide save for 3% nitrogen concentration as opposed to the wide difference

between acidic hydrolysates yield and other substrates.

Like substrate concentration, increasing yeast extract concentration resulted in a corresponding increase in LA yield corroborating earlier reports [18,28,42]. This observation is similarly true for D-LA [43]. As opposed to substrate concentration, however, a threshold dose was observed at 5% which is relatively higher than some reports. [43] reported 7 g/L as optimum in their study using *Lactobacillus coryniformis*; [37] reporting 3 g/L as peak dose for *Lactobacillus rhamnosus*. [44] seem to suggest that peak concentration for optimum LA yield is 1% of the fermentation broth with [16] touting 7.5% as optimum for industrial production. The significance of YE supplementation is tied to the fastidious nature of LAB and YE as a growth factor and nitrogen source [4,45] while also providing optimum C:N ratio that facilitates enhanced LA yield [8]. The drawback to YE supplementation of medium, however, is that it increases production cost by up to 30% and 38% consequently leading to suggestions of using cheaper alternatives like corn steep liquor silkworm larvae, extract of wheat bran, fish waste hydrolysates [35], tryptone, $(NH_4)_2SO_4$ [37], peanut meal [43] amongst others.

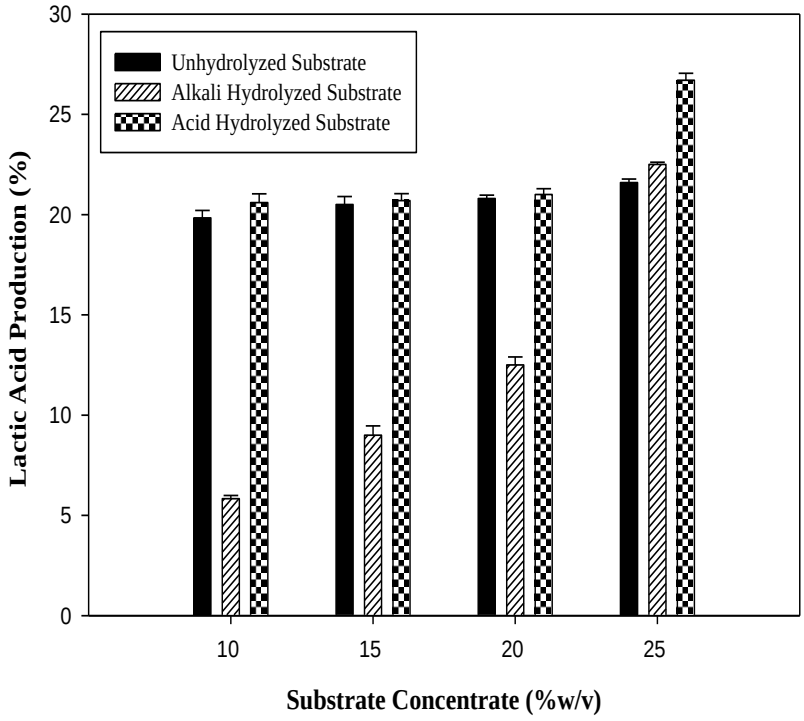

Fig. 4. Influence of varying substrate concentration on Lactic acid yield (mean ± standard error at n=3)

Table 2. Post optimisation fermentation

Days	% Lactic acid Production
1	8.28±0.14
2	11.07±0.09
3	15.28±0.19
4	16.23±0.19
5	17.18±0.22
6	18.53±0.20
7	12.10±0.32

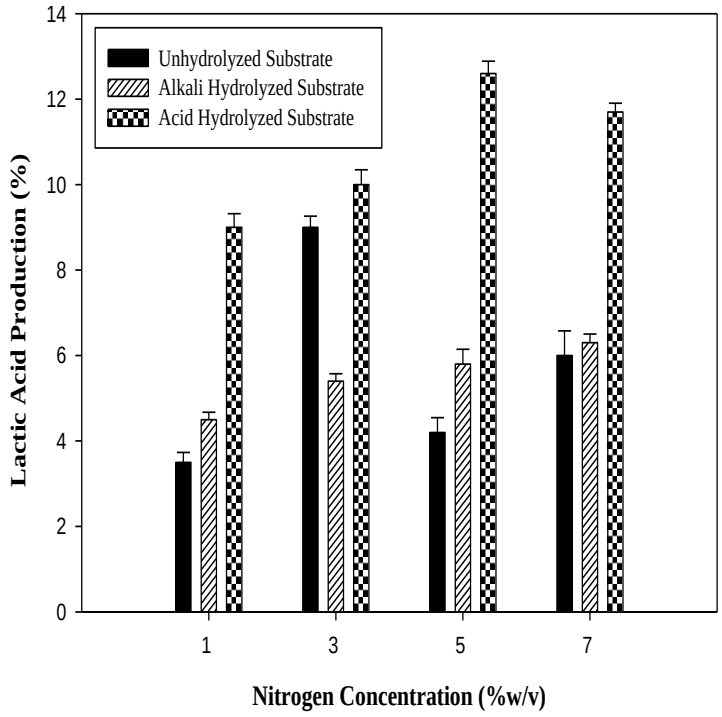

Fig. 5. Influence of varying nitrogen concentration on Lactic acid yield (mean ± standard error at n=3)

3.3 Post Optimization Experiment for Production of Lactic Acid from Cassava Peel by *L. casei* (ATCC 334)

The optimum conditions in the parameters earlier investigated were combined for lactic acid fermentation from cassava peel (Table 2). The best conditions were earlier determined as: 25% w/v substrate concentration, 5% w/v yeast extract, 2 mL inocula size, pH 6 for 5 days at 37±1 ℃. At day 1, *L. casei* (ATCC 334) produced 8.28% lactic acid. Lactic acid yield increased progressively from day 2 to day 5. Maximum lactic acid was obtained at day 6 with a yield of 18.3%. However, by day 7, the production of lactic acid decreased to 12.1% (Table 3). The combination of all optimised variables yielded 18% LA suggesting that there is a complex and systems relationship between the variables during fermentation by *L. casei* (ATCC 334). Consequently, one factor at a time (OFAT) optimisation scheme may be unsuitable for optimisation study of LA fermentation by *L. casei* (ATCC 334) using cassava peel hydrolysates. This limitation, majorly due to its inability to measure the effect of multiple interactions at the same time, has been suggested by [46]. Other drawbacks are that the method is difficult and time consuming [47]. Hence, in our subsequent study, we shall compare the impact of other statistical optimization strategies to ascertain optimum conditions.

Apart from substrate exhaustion [36] and accumulation of toxic waste products, post-threshold reduction in yield can be ascribed to the conversion of LA into pyruvate and/or volatile fatty acids (VFAs), which sadly our study did not measure. The crux of our subsequent study was to monitor, real-time, the production and disappearance of these products. Under mixed culture condition, via the acrylate pathway (which generates propionic acid) or a reverse β-oxidation pathway that yields acetic acid, LA can be catalysed into pyruvate by the enzyme NAD-independent lactate dehydrogenase (iLDH) which in turn generates VFAs by chain elongation [48]. Since LA fermentation was, however, done using monoculture, it is plausible that *L. casei* (ATCC334) possess genes regulating these pathways thus it is capable of producing the products after all 548 genes coding for 1,040

reactions mediated by 959 metabolites [47] are attributed to *L. casei* (ATCC334). High initial sugar concentration can also raise osmotic pressure beyond threshold to create a negative feedback effect [41]. To overcome this, fed-batch fermentation has been touted as a solution [39,30].

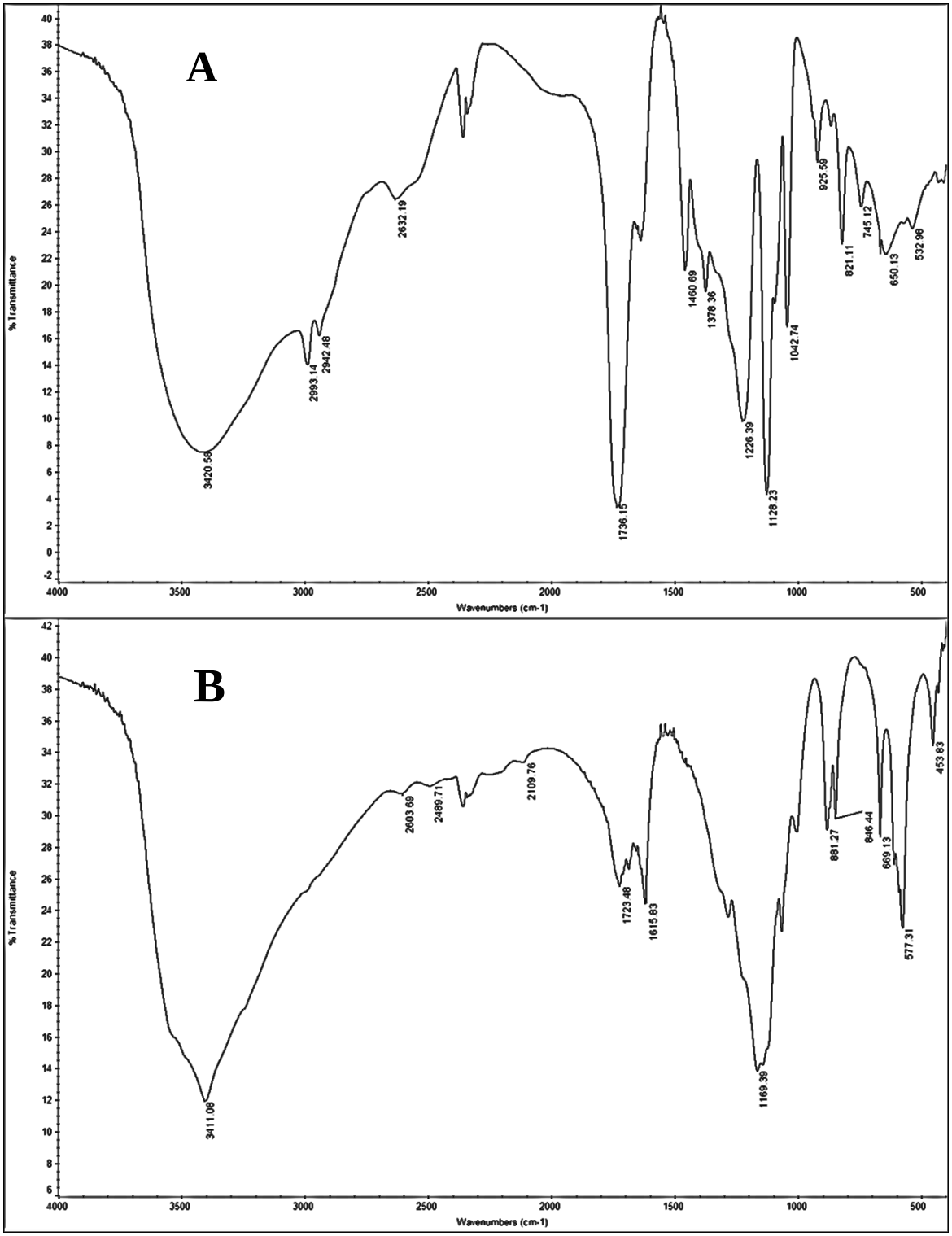

Fig. 6. FTIR spectroscopy for standard lactic acid (A) and partially purified lactic acid (B)

3.4 Recovery and Partial Purification of Lactic Acid

We attempted a multistep purification process including filtration, carbon treatment, evaporation, and crystallisation which are easy and relatively cheap after fermentation. Recovery yield was estimated at a marginal 1.4%. Currently in use are methods like ion exchange chromatography [4,49], high performance liquid chromatography (HPLC) [42], dialysis [50], esterification [51,26], evaporation [52] among others which are resource intensive, require technical expertise, and costly.

3.5 Fourier Transform Infra-red (FTIR) Spectroscopy for Lactic Acid

Infrared spectrum of the partially purified lactic acid was compared with the standard spectrum of lactic acid with the transmittance presented as demonstrated by [53]. The main characteristic peaks in A (standard lactic acid) curve, are: around 1128 cm^{-1} indicative of C–O–C; bands are 1736 cm^{-1} representative of C=O; the peak at 2993 cm^{-1} is assigned to CH_3 with 3420cm^{-1} hinting at the OH functional group. In curve B (the partially purified lactic acid product), the main characteristic bands were 1169cm^{-1}(C-O), 1723cm^{-1} (C=O) and 3411cm^{-1} (OH group) (Fig. 6). The FTIR spectra of the partially purified product relative to a commercial standard LA solution showed peaks within striking distance of each other (Figure 6) indicative of C–O–C, C=O, CH_3, and OH stretches as reported by [53]. This suggests that the partial purification was efficient and further purification step may yield a pure product [54,55].

4. CONCLUSION

This investigation provides a novel approach for lactic acid production by Lactobacilli through fermentation using cassava peels supplemented with appropriate media that enhanced the growth and lactic acid production. Different parameters analysed in the cause of the research to determine an optimised fermentation condition produced lactic acid with a post-optimization combination at 18.3% yield. The cumulative effect of optimising fermentation, should not be lost upon purification and recovery hence, a holistic approach to LA fermentation, purification, and recovery is necessitated.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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