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A comparative study on utilisation of citrus and mango peels for lactic acid production and optimisation by *Rhizopus oryzae* in submerged fermentation

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Abstract
The purpose of the study was to enhance the lactic acid production using fruit peels by conventional method and to optimise by Response Surface Methodology. The total sugars, cellulose, phenolics and reducing sugars were lower in citrus peel than the mango peel; and total flavonoids were higher in citrus peel than the mango peel. Lactic acid production of 0.51 and 0.9 g/L was obtained from citrus and mango peels, respectively at 3% substrate concentration. Conventional mango peel (30%) batch submerged fermentation at optimum pH 6.0 and temperature 35 ºC produced 7.0 g/L of lactic acid by *Rhizopus oryzae* NCIM 1009. The statistical analysis of factorial study showed 7.25 g/L of lactic acid with yield 0.65 g/g in 60 h at 35 ºC were the best conditions. The optimised mango peel fermentation conditions produced by six times more lactic acid and yield (g/g) based on substrate consumed.

Keywords: Lactic acid, citrus peel, mango peel, submerged fermentation, reducing sugars, *Rhizopus oryzae.*

1. Introduction

Lactic acid (LA) is one of microbial products which has great value in the global market due to its versatile applications in food, chemical, cosmetic and pharmaceutical industries as well as used in the production of biodegradable and environmentally friendly biopolymer which is a suitable substitute for petrochemical plastics. LA is produced by hydrothermal conversion of carbohydrates and also by fermentation of cellulosic materials. It has one chiral center, and it exists in two optical isomeric forms i.e. D (-) or L (+) LA [1, 2, 3]. Lignocelluloses and cellulosic biomass are the most abundant global renewable resources from agriculture, food, paper and forestry industries. Utilisation of these to avoid environmental pollution and to produce useful products is needed. The management of these materials were directed towards useful value-added products such as lactic acid production [4].

Citrus (*Citrus sinensis*) and mango (*Mangifera indica,* L.) are important and subtropical and tropical fruits produced in many countries of the world. Citrus and mango world production in 2013 is 135.76 and 43.30 million metric tonnes respectively. India is the largest producer of mango fruits, in 2013 it was18.33 million tonnes, whereas for citrus it was 10.09 million tonnes, which accounted 40% of world production (FAOSTAT 2014) [5]. Citrus processing industries by-products are the principal solid derivatives and constitute about 50% of the fresh fruit weight. They contain the peel (60-65%), internal tissues (30-35%) and seeds (0-10%), whereas mango fruit peel and stone proportions in range from 20 to 30% and 10 to 30%, respectively. Peel contributes about 15–20% of the fruit. However, a number of people consume mango with the peel especially some of the mango varieties like *Totapuri*, where the taste of the mango is not greatly affected by the presence of mango peel. If a factory is processing five tons of *Totapuri* mangoes per hour about six tons of peel would be available as waste per day of 8 h work. Approximately, 0.4 to 0.6 million tons of mango peel is generated annually in India. This waste is either used as cattle feed or dumped in open areas, where it adds to environmental pollution such as heavy odour, plenty of leacheate, as well as attracting flies and rats [6].

During fruit processing, by-products such as peel, pulp, seeds or kernel as well as whole (cull) fruits that do not meet quality requirements are generated. The edible pulp makes up 33 – 85% of the fresh fruit, while the peel and the kernel amount to 7 – 24% and 9 – 40%, respectively [7, 8]. It is good source of nutrients, which has been utilised in a variety of applications and traditionally has been used as animal feed [9].

The ensiled mango peel nutrient digestibility with rice straw increased with increasing a mixture of Leucaena *(Leucaena leucocephala)* leaves [10].

The main polysaccharides in citrus peel (CP) are first pectin and then cellulose and hemicellulose. Mango peels (MP) are rich in carbohydrates, pectin, and crude fiber and suitable for multi enzyme production, higher than that obtained with wheat bran [11, 12]. Used in increasing dietary fibre content in biscuits preparation, recently the anti-diabetic activities, antiproliferative activity, anti-oxidant activities were evaluated in mango peels [13].

The aim of the study was to explore the feasibility of citus and mango peels for lactic acid fermentation using *Rhizopus oryzae*. This is simple, cost-effective and novel approach potentially maximizes the utilisation of carbohydrate waste, which aids in minimising problems associated with pollution.

2.0 Materials and Methods

2.1 Procurement and preparation of substrate

Citrus peels (*Citrus sinensis*) were procured from the fruit juice vendors of the local market. Mango peels (*Cv. Totapuri* and *Bengaluru*) were collected from mango processing industries around Tirupati. First peels were washed with hot water and air dried and subsequently oven dried for moisture removal at 70 ˚C for 24 h. The dried peels ground into fine powder and milled to a particle size of 40 BS (British Standard) mesh in an Apex mill and it was stored at 4 ˚C until further use.

Citrus peels were pretreated with steam (hydro) distillation at 150 – 160 °C for 10 min and centrifuged at 3000 rpm, and pellet was collected and stored at 4 ˚C was used as substrate in the fermentation [14, 15] and mango peels were ground and autoclaved at 121 °C for 15 mins [16].

2.2 Microorganism and culture media

Pure culture of *Rhizopus oryzae* NCIM 1009 was procured from the National Collection of Industrial Microorganisms (NCIM), Pune, India. Spores of *Rhizopus oryzae* NCIM 1009 were grown and maintained on Potato Dextrose Agar (PDA). Spores were first cultivated in an Erlenmeyer flask of 250 ml capacity containing 50 mL of Potato Dextrose Broth (PDB) at 37 ºC for 4 days, under stationary conditions for the development of fungal spores. This spore suspension was inoculated on to PDA slants and were incubated at 37 ºC for 4-5 days and then stored at 4 ºC for further use.

Spores were suspended in sterile distilled water at concentration of 1×10^7 spores/mL and inoculated into fermentation medium. The seeding medium (g/L) contained starch 50, $(NH_4)_2SO_4$ 2.0, KH_2PO_4 0.25, $MgSO_4$ 0.50 and $ZnSO_4$ 0.10 [17, 18].

2.3 Spore harvesting

The spores were harvested from 14 day old Petri plate culture, as this condition to allow the culture to be fully sporulate. Petri dish was first flooded with a mixture of 5 mL sterile distilled water and 5 mL of 0.05% Tween 80 and then scraped the culture gently with a sterile bent glass rod to release the spores. The subsequent spore suspension was filtered into a 100 mL Erlenmeyer flask through sterilized glass wool positioned in a funnel. This procedure was repeated once and the glass wool was finally washed with 5 mL sterile distilled water.

2.4 Preparation of spore suspensions

Fourteen day old spore suspension was transferred directly to 50 mL sterile centrifuge tubes and centrifuged for 5 min at 8000 rpm at 4 °C. After centrifugation, the supernatant was discarded and the pellet re-suspended in 10 mL sterile distilled water containing 0·1% (v/v) Tween 80, and vortexed for 30 sec to suspend it completely. This process was repeated for two more times. This process produced a high-density suspension of more than 1.0×10^7 spores/mL and the number of spores in the suspension was counted using haemocytometer [18].

2.5 Batch fermentation

Fermentation medium (g/100mL) contained citrus or mango peel 1 or 2, yeast extract 2.0, KH_2PO_4 0.025, $MgSO_4$ 0.050 and $ZnSO_4$ 0.010 of medium for 4 days at 35 °C at 200 rpm agitation, and then 5% concentration of 10^7 spores/mL was transferred to fermentation medium having pH around 5.0-6.0 by adding 1% (w/v) $CaCO_3$ at 34 °C for 60 h. Samples are withdrawn periodically for 72 to 96 h. In varied substrate concentration (10-40 g/L) and the effect of other variables nitrogen source, pH, and temperature were studied in the batch fermentation [17, 18].

2.6 Analytical methods

Phenol sulphuric acid method was used to measure the total sugars [19]. Sample (1 mL) was mixed with 5% phenol (1 mL) and 96% H_2SO_4 (5 mL). The above mixture was kept for 10 - 15 min at room temperature. The optical density (OD) values were checked at 470 nm. Reducing sugar concentration was carried out by using 3, 5-dinitrosalicylic acid (DNS) method [20]. Sample (1 mL) was mixed with DNS (3 mL) and boiled for 10 min. The OD values were checked at 540 nm. Cellulose was estimated by the method of by Updegroff 1969 [21]. Singleton and Rossi (1965) method was used to measure total phenolics [22] and Careri *et al*. (2000) method was used to measure total flavonoids [23]. Method of Kimberley and Taylor (1996) [24] was used for quantification of LA. Samples were withdrawn at desired intervals and made protein-free filtrate, treated and diluted with 1M H_2SO_4 for oxidation of LA to acetaldehyde in a strong acidic medium, then treated with 0.1 mL of p-hydroxy biphenyl at 30 ˚C for 30 min. The OD values were checked at 560 nm. Biomass was determined by weighing the mass after washing with 4 M HCl followed by distilled water to remove the residual calcium carbonate and finally drying at 60 °C for 36 h [2].

2.7 Optimizations of nutritional and physical parameters

In the conventional scale up approach, required nutritional and physical parameters were optimized to maintain all factors at constant levels in the fermentation medium, except the one under study. Each subsequent factor was considered after it was tested in batch fermentation characteristics.

2.8 Experimental design and optimisation by RSM

Based upon the results of one variable at time approach, Design-Expert version 9.0 (State-Ease Inc., Mineeapolis, U.S.A) was used for the experimental design (Central composite Design (CCD), five independent minimum and maximum ranges of variables namely mango peel (carbon source g/L): (10 - 40: X_1), $(NH_4)_2SO_4$ (g/L): (1.0 - 3.0: X_2), pH (4.0 - 6.5 X_3), Temperature (˚C) (20 – 40 ˚C: X_4) and Incubation period(h): (24 - 72: X_5) are presented in Table 3.

A 2^5 factorial design (five level half factorial) was employed with 32 experiments [25, 26]. The full experimental designs with respect to their coded values are shown in the Table 4. The RSM was used to analyse response variable was fitted by a first and higher order effects of each factor and their interactions on lactic acid yield ($Y_{p/s}$). The second-order polynomial equations were used to estimate the response of the independent variable, i.e. lactic acid production. The behaviour of the system was explained by the following second-order polynomial equation.

$$Y = \beta_0 + \sum \beta_i X_i + \sum \beta_{ii} Xi^2 + \sum \beta_{ij} XiXj \quad (1)$$

Where, Y is predicted response which is dependant variable i.e. lactic acid, β_0 is an off set term (model constant), β_i is a linear coefficient, β_{ii} is a quadratic effect and β_{ij} is the interaction coefficient. When i = j and interaction effect when i < j, B*ii* is a squared term, X*i* is the *i*th variable, which are called as an independent variables.
The statistical analysis of the model was performed in the form of analysis of variance (ANOVA). Design expert, version 9.0.1 was used for all the statistical analysis and the response surface plotting.

2.9 Statistical analysis
The experimental results were expressed as mean ± SD (n=3). The software package SPSS was to analyze the data variance (ANOVA) and Duncan's Range test was performed to determine the differences among various groups ($p<0.05$).

3. Results and Discussion
3.1 The physico-chemical characteristics
Total sugars, cellulose, reducing sugars and total phenolics were lower in citrus peel (CP) than mango peel (MP) and flavonoids were greater in citrus peel than MP as shown in Table 1. Citrus peel pH (3.0) was lower than mango peel (3.9) as reported by earlier authors [11] and some other authors reported pH of CP as 4.3 - 4.4 [14], total sugars and reducing sugars were 172 mg/g, 68.6 mg/g respectively. Other authors reported total sugars in CP were 500 -700 g/kg [27] and reducing sugars were 249 mg/g of WUOP (Water Unextractable Orange Peels) [12]. Mausambi peel (*C. sinensis*) contained 14.96% of cellulose and 14.24% of total sugars in dried citrus peel in its proximate analysis. Cellulose content in mango peel was 13.2% and total sugars were 20.73% [28]. *Totapuri* mango peel contained high amounts of reducing sugars (45%) of nutrients. Similarly the cellulose and lignin content in dried mango peel was 23% and reducing sugars were 30% [29].
Total phenolics and total flavonoids were 20.5 and 0.62 mg/g respectively in the present study, whereas other authors have reported 132 mg GAE/g of TPC, 7.2 mg QE/g of *C. sinensis* [30] as well as authors reported TPC in CP varieties the range of 9.0 - 44.4 mg GAE/g, TFC in the range of 0.2 to 3.25 mg QE/g [31]. Unfermented citrus peel the TPC content was 79.3 and 132.3 mg GAE/g at 100 and 150 ºC, respectively [32]. This variation might be due to the variety of citrus fruit and its peel content, the extraction methods of the compounds.
In the present study, phenolic content was 26.3 mg GAE/g; total flavonoids were 0.48 mg QE/g in mango peel. The polyphenol content was found to be significantly higher in the raw peels than dried peels, TPC content of the ripe peels was 54.67 mg GAE/g which is may be due to phenolic acids were present in the form of esters of glycosides and are free, soluble and insoluble bound phenol acids [33]. Bound flavonoids in the range of 10.29 and 52.73 µg/g kaempferol and 56.83 and 18.58 µg/g quecertin were the major flavonoids identified in the *Raspuri* and *Badami* peels respectively. The present study in *Totapuri* peels contains 48 µg/g of flavonioid in the dried peel [34]. This variation might be due to the variety of mango fruit peel and the extraction methods.

3.2 Batch fermentation
The effects of fruit peels as carbon source were screened for feasibility of lactic acid production with yeast extract as nitrogen source in submerged fermentation as shown in Fig 1 and 2. The fermentation resulted in more reducing sugars formation in MP than CP. In 1% fruit peel fermentation of CP and MP separately 31.6 and 45.6 mg /100 mL LA was formed (i.e. 0.32 and 0.45 g/L from 10 g/L) respectively. At 2% peel fermentation of CP and MP 41.2 and 78.9 mg/100 mL of LA was produced respectively (i.e. 0.40 and 0.80 g/L). At 3% peel fermentation of CP and MP have 50.8 and 90.3 mg/100 mL of LA respectively (i.e. 0.51 and 0.9 g/L). More LA titer was produced in MP than CP in the growth phase could be attributed to insufficient reducing sugars liberation and cell (biomass) growth in the latter. The kinetics of LA, reducing sugars and biomass production in *R. oryzae* fermentation are shown in Table 2. The mango fruit peels were significantly higher in LA and RS production than citrus peel. Hence, mango peel was used in further LA fermentation characteristics.
Other authors reported that the maximum 9.3 g/L LA was produced in 3 days of fermentation and 17.48 g/L of LA in 6 days of fermentation at pH 10 at 35 ºC with un-specified substrate concentration and microorganism from golden dragon variety mango peels [16]. Some other author has reported 6.39 and 7.11 g/100 mL of LA in 96 and 144 h respectively by *Lactobacillus delbruckii* in a fermentation medium containing 230 mL of citrus peel juice (the total sugars 7.5 g /100 mL and reducing sugars were 0.20 g /100 mL) and 50 mL of yeast autolysate in a total volume of 500 mL [35]. Another author reported a 10.3 mg/g of LA from 10 g potato pulp (dry matter 20.8%) and inoculated with10^5 spores/mL for 6 days by strain *Rhizopus oryzae* IFO 4707. The fungal cell seems to be secreting the enzymes that degraded the structural components of the potato pulp with the acidification-less buffered environment [36].

3.3 Optimum conditions of mango peel fermentation for LA production
The 30 g/L substrate concentration produced 7.1 g/L LA production as shown in Fig 3. Increase in the substrate concentration from 30 to 40 g/L did not result significant increase in the yield of LA could be due to polyphenolics content that may inhibit the biomass growth and residual sugars utilisation [37, 38]. Further LA fermentation was done with 30 g/L of mango peel with nitrogen sources such as yeast extract, peptone and ammonium sulphate and analysed for LA production as shown in Fig 4. Inorganic nitrogen source $(NH_4)_2SO_4$ produced more LA (5 g/L) than the other organic nitrogen sources YE and Peptone as the better produced higher biomass than $(NH_4)_2SO_4$. It could be due to C/N ratio and C/P ratio factor that contribute to LA production. However, the present study results are in good agreement with the earlier reports on nitrogen source selection [39, 40, 41]. The optimum pH was found to be 6.0

which gave LA 4.8 g/L (Fig 5) and optimum temperature was 35 ºC which gave 6.5 g/L of LA as shown in Fig 6.

3.4 Experimental design

The present study a five level half factorial central composite design (CCD) was evaluated for maximum LA titre 7.25 g/L (yield 0.65 g/g) from 30 g/L of mango peel substrate, pH 6.0, temperature 35 ºC for 60 h. The correlation coefficient value (R^2=0.9596) was considered satisfactory for this experiment. The adjusted R^2 value 0.8860 showed that model to be significant. The ANOVA data obtained for lactic acid production are shown in Table 5. The Co-efficient of variation (C.V) is a measure of residual variation. The resulting regression equation fitted to the experimental data was:

$$Y_{LA\ (g/g)} = 0.58 - 0.023\ X_1 + 0.026\ X_2 + 0.036\ X_3 + 0.058\ X_4 + 0.081\ X_5 - 0.080\ X_1^2 - 0.044\ X_2^2 - 9.773\ X_3^2 - 0.015\ X_4^2 - 4.773\ X_5^2 + 0.014\ X_1 X_2 - 9.375\ X_1 X_3 - 0.019\ X_1 X_4 - 9.375\ X_1 X_5 + 0.011\ X_2 X_3 + 0.033\ X_2 X_4 + 0.011\ X_2 X_5 - 8.125\ X_3 X_4 + 0.034\ X_3 X_5 + 0.039\ X_4 X_5.$$

Where X_1, X_2, X_3, X_4 and X_5 are independent variables the coded level for the factors (Table 3) the interactions of the selected variables on LA production were shown in Fig 7 (b-f).

Earlier the authors reported that mango peel fermentation on LA production was thought to be influenced by three factors namely initial medium pH, process temperature, and incubation time by unspecified microorganisms and a 13-run factorial design was developed to describe the relationship between selected factors exhibited a significant effect on two responses. However, three factors do not work independently and the effect of each factor depends on the levels of other factors. The value of correlation coefficient determination R^2 for LA production was 0.998 [16].

Some other authors reported 2^{5-1} five experimental factors (wood hydrolysate substrate concentration, C/N ratio, C/P ratio, agitation level and added $CaCO_3$) at two levels design were used to optimize the culture conditions for the LA production and higher LA productivity.

A 3^2 factorial design, two factors (wood hydrolysate substrate concentration and C/N ratio) at three levels response surface was used to optimize the culture conditions for best LA production. The correlation coefficient value (R^2 is 0.90684) was considered satisfactory for this experiment. The author reported that at highest substrate concentration (90 g/L) there was no fungal growth (*R. oryzae* NRRL 395) and the LA production reached a maximum (19.13 g/L) only at 86 g/L of substrate concentration, C/N ratio 40.0 after 120 h fermentation [42].

In another report three level four factors (temperature, pH, inoculam volume and substrate (ziziphus fruit powder) CCD design employed by *Lb. amylophilus* GV6 for maximum LA production. the determination coefficient (R^2 = 83.15%) and the adjusted determination coefficient (adjusted R^2=76.76%) showed that model was significant. At pH 6.5, the bacterium was able to convert 75.98% of starch to LA [43]. Further investigations will be focussed on increasing the LA production by pre-treating the substrates and optimising the sugar consumption.

Table 1: Physico-chemical characteristics of fruit peel powders

Parameter	Citrus peel*	Mango peel*
pH	3.0	3.9
Total sugars mg/g	172±0.05	210±0.08
Cellulose mg/g	642±0.1	685±0.2
Reducing sugars mg/g	68.6±0.02	116.2±0.05
Total phenolics mg GAE/g	20.5±0.52	26.3±0.64
Total flavonoids mg QE/g	0.62±0.34	0.48±0.25

*The above composition is on dry weight basis

Table 2: Kinetics of LA, RS and BM on mango peel fermentation by *R. oryzae* NCIM 1009

S. No	Citrus peel fermentation characteristics												
	Parameter	48 h				60 h				72 h			
	Substrate	1%	2%	3%	4%	1%	2%	3%	4%	1%	2%	3%	4%
1.	LA	15.4 ±0.3	18.6 ±0.4	25.4 ±0.24	26.8 ±0.5	27 ±0.4	32.6 ±0.3	35.6 ±0.2	30.1 ±0.3	31.6±0.4	40.2±0.2	43.8±0.3	38.5 ±0.2
2.	RS	225.8 ±1.6	258 ±1.4	324 ±1.8	438 ±.1.6	178.±1.5	390± 1.0	482 ±1.8	256 ±1.9	61.5±0.8	153 ±1.2	169 ±1.5	107 ±1.6
3.	BM	---	----	---	---	--	----	---	---	0.3	0.7	1.3	1.0
4.	SU	---	---	----	----	--	----	---	----	238	320	358	360
5.	Yield	---	----	---	---	--	----	---	---	0.14	0.12	0.12	0.10
	Mango peel fermentation characteristics												
1.	LA	18.1 ±0.4	38.1 ±0.2	46.3 ±0.3	36.4 ±0.5	35.3 ±0.2	57.5 ±0.3	75.6 ±0.4	63.7 ±0.4	45.6 ±0.5	78.9±0.5	120 ±0.6	82.4 ±0.5
2.	RS	294.6 ±1.4	351 ±1.5	483 ±1.6	526 ±1.5	104 ±1.6	510 ±1.5	785 ±1.2	627 ±1.8	66.1 ±1.4	346 ±1.3	568 ±1.5	435 ±1.2
3.	BM	---	----	---	---	--	----	---	---	0.40	1.0	1.6	1.2
4.	SU	---	---	----	----	--	----	---	----	282	635	846	518
5.	Yield	---	----	---	---	--	----	---	---	0.16	0.12	0.14	0.16

Table 3: Levels of independent variables in the experimental plan

Variables	Coded values				
	-2	-1	0	1	2
Mango peel(g/L): X_1	10	20	25	30	40
$(NH_4)_2SO_4$ (g/L): X_2	05	10	10	15	20
pH: X_3	4.5	5.0	5.5	6.0	6.5
Temperature (˚C): X_4	20	25	30	35	40
Incubation Period (h): X_5	24	36	48	60	72

LA: Lactic acid (mg/100 mL); RS: Reducing sugars (mg/100 mL); BM: Biomass (g/100 mL); SU: Substrate utilized (mg/100 mL); Production: LA (mg)/total substrate concentration; Yield (g/g) = LA production (g)/substrate utilized (g).

Table 4: Experimental design used to optimize lactic acid production from mango peel hydrolysate by *R. oryzae* NCIM 1009

S. No	X_1 (g/L) Mango peel	X_2 (g/L) $(NH_4)_2SO_4$	X_3 pH	X_4 (°C) Temp	X_5 (h) Time	LA titer (g/L)	Response Y_{LA} (g/g)
1	20 (-1)	10 (-1)	5 (-1)	25(-1)	60 (1)	4.60	0.31
2	30 (1)	10 (-1)	5 (-1)	25 (-1)	36 (-1)	2.24	0.50
3	20 (-1)	15 (1)	5 (-1)	25 (-1)	36 (-1)	3.00	0.35
4	30 (1)	15 (1)	5 (-1)	25(-1)	60 (1)	5.20	0.28
5	20 (-1)	10 (-1)	6.0 (1)	25 (-1)	36 (-1)	2.86	0.54
6	30 (1)	10 (-1)	6.0 (1)	25 (-1)	60 (1)	5.54	0.62
7	20 (-1)	15 (1)	6.0 (1)	25 (-1)	60 (1)	3.49	0.36
8	30 (1)	15 (1)	6.0 (1)	25 (-1)	36 (-1)	2.94	0.5
9	20 (-1)	10 (-1)	5 (-1)	35 (1)	36 (-1)	2.26	0.32
10	30 (1)	10 (-1)	5 (-1)	35 (1)	60 (1)	4.86	0.54
11	20 (-1)	15 (1)	5 (-1)	35 (1)	60 (1)	5.08	0.4
12	30(1)	15 (1)	5 (-1)	35 (1)	36 (-1)	3.14	0.3
13	20(-1)	10 (-1)	6.0 (1)	35 (1)	60 (1)	3.02	0.32
14	30 (1)	10 (-1)	6.0 (1)	35 (1)	36 (-1)	2.93	0.3
15	20 (-1)	15 (1)	6.0 (1)	35 (1)	36 (-1)	2.10	0.35
16	30 (1)	15 (1)	6.0 (1)	35 (1)	60 (1)	7.25	0.65
17	10 (-2)	10 (0)	5.5 (0)	30 (0)	48 (0)	1.56	0.4
18	40 (2)	10 (0)	5.5 (0)	30 (0)	48 (0)	2.64	0.32
19	25 (0)	05 (-2)	5.5 (0)	30 (0)	48 (0)	2.03	0.25
20	25 (0)	20 (2)	5.5 (0)	30 (0)	48 (0)	2.15	0.34
21	25 (0)	10 (0)	4.5 (-2)	30 (0)	48 (0)	0. 72	0.42
22	25 (0)	10 (0)	6.5 (2)	30 (0)	48 (0)	2.68	0.57
23	25 (0)	10 (0)	5.5 (0)	20 (-2)	48 (0)	2.46	0.19
24	25 (0)	10 (0)	5.5 (0)	40 (2)	48 (0)	2.84	0.46
25	25 (0)	10 (0)	5.5 (0)	30 (0)	24 (-2)	0.08	0.15
26	25 (0)	10 (0)	5.5 (0)	30 (0)	72 (2)	2.98	0.56
27	25 (0)	10 (0)	5.5 (0)	30 (0)	48 (0)	2.47	0.46
28	25 (0)	10 (0)	5.5 (0)	30 (0)	48 (0)	2.35	0.40
29	25 (0)	10 (0)	5.5 (0)	30 (0)	48 (0)	2.28	0.38
30	25 (0)	10 (0)	5.5 (0)	30 (0)	48 (0)	2.36	0.35
31	25 (0)	10 (0)	5.5 (0)	30 (0)	48 (0)	2.45	0.35
32	25 (0)	10 (0)	5.5 (0)	30 (0)	48 (0)	2.57	0.41

Table 5: ANOVA for lactic acid produced response from the optimization design

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F
Model	1.30	20	0.065	13.05	< 0.0001
X_1-Mangopeel (g/100 mL)	0.53	1	0.53	106.78	< 0.0001
X_2-$(NH_4)_2SO_4$ (g/100 mL)	0.098	1	0.098	19.53	0.0010
X_3-pH	0.093	1	0.093	18.54	< 0.0012
X_4-Temp (°C)	9.800	1	9.800	1.96	0.1886
X_5-Time (h)	0.070	1	0.070	14.09	0.0032
$X_1 X_2$	5.625	1	5.625	0.011	0.9173
$X_1 X_3$	0.059	1	0.059	11.79	0.0056
$X_1 X_4$	0.12	1	0.12	24.21	0.0005
$X_1 X_5$	0.11	1	0.11	22.16	0.0006
$X_2 X_3$	0.011	1	0.011	2.11	0.1746
X_2X_4	7.562	1	7.562	0.15	0.7044
$X_2 X_5$	7.656	1	7.656	1.53	0.2412
X_3X_4	0.015	1	0.015	3.01	0.1107
X_3X_5	7.656	1	7.656	1.53	0.2412

X_4X_5	3.062	1	3.062	0.061	0.08089
X_1^2	1.690	1	1.690	0.34	< 0.0001
X_2^2	0.044	1	0.044	8.78	< 0.0012
X_3^2	0.031	1	0.031	6.16	0.0305
X_4^2	8.162	1	8.162	1.64	0.0227
X_5^2	4.72	1	4.72	0.95	0.0512
Residual	0.055	11	4.988		
Lack of Fit	0.031	4	7.834	2.33	0.1550
Pure Error	0.024	7	3.362		
Cor Total	1.36	31			
R^2	0.9596				
Adj- R^2	0.8860				
C.V	13.89%				

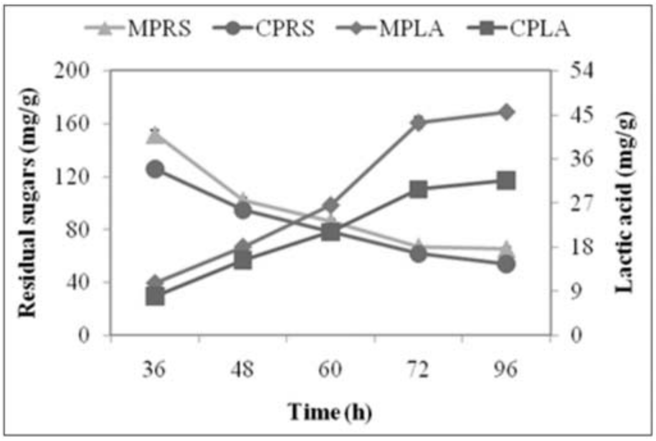

Fig 1: Production of residual sugars and lactic acid in 1g/100 mL

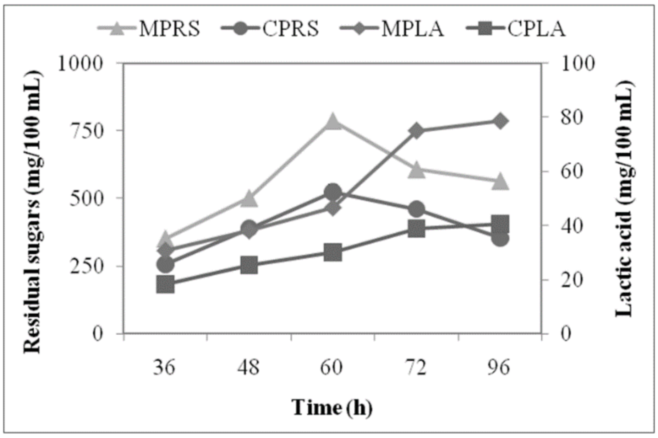

Fig 2: Production of residual sugars and lactic acid in 2 g/100 mL

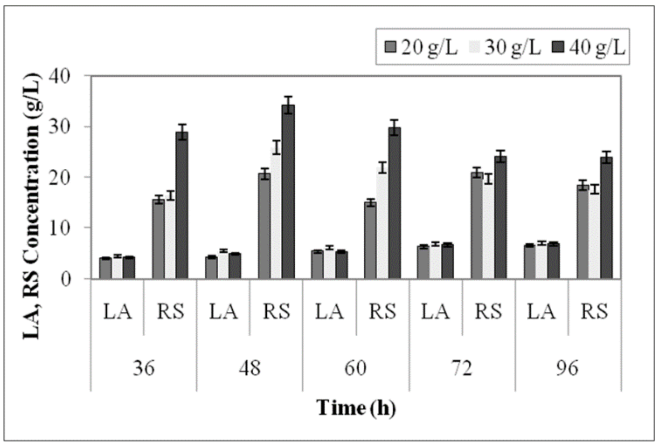

Fig 3: Effect of substrate concentration on LA production

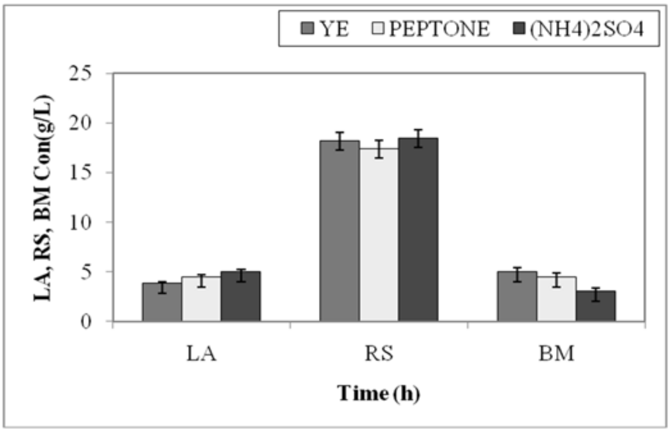

Fig 4: Effect of nitrogen source at 2% (w/v) level on LA production

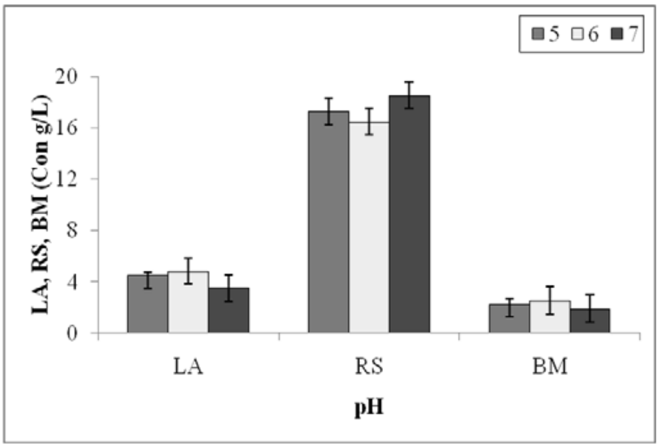

Fig 5: Effect of pH on LA at 3% (w/v) substrate concentration

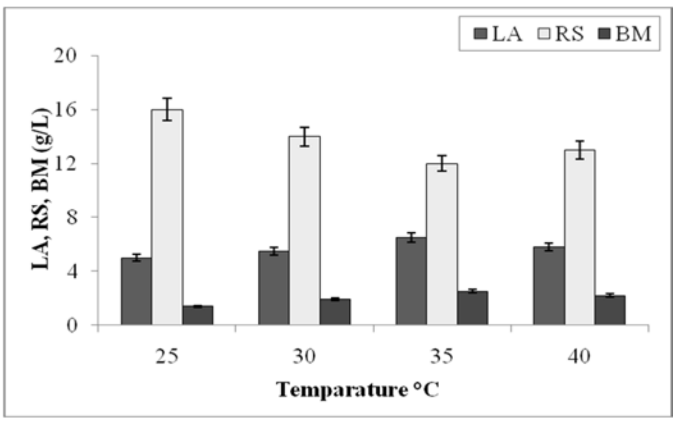

Fig 6: Effect of temperature on LA at 3% (w/v) substrate concentration.

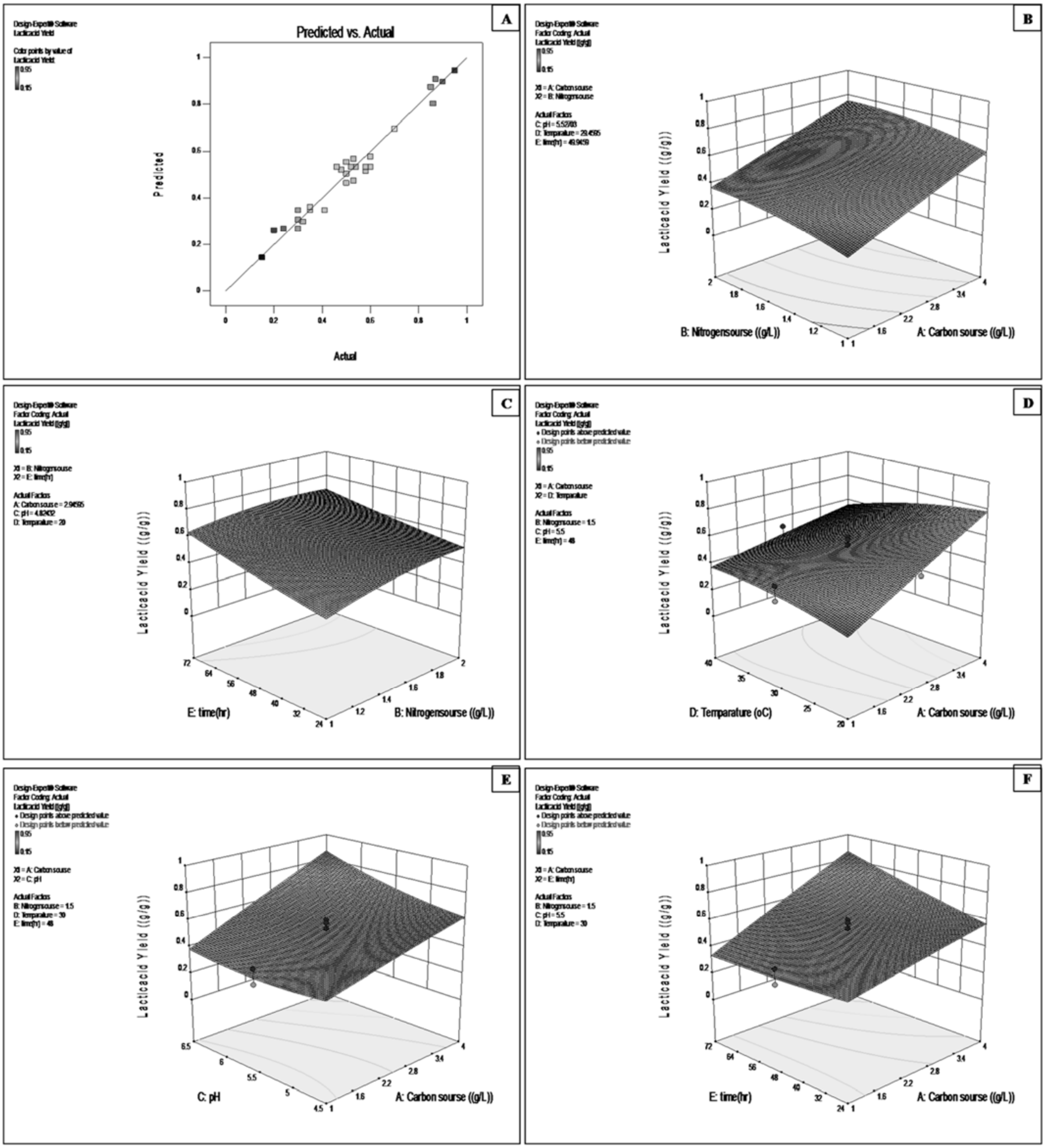

Fig 7: The interactions of the actual *Vs* predicted variables (A); Selected fermentative variables interactions on LA production were shown as carbon *Vs* nitrogen (B); Nitrogen *Vs* time (C); Carbon *Vs* temp (D); Carbon *Vs* pH (E); Carbon *Vs* time (F).

4. Conclusion

Attempts have been made in the past to utilise mango peel (MP) in one or other form but the problem of its full utilisation still persists. This study led to the successful development of a simple fermentation process for conversion of MP by *Rhizopus oryzae* to maximum yields of LA. This fermentation process is a value added approach to generate bio-products from food and agricultural residues using bio-refinery model. Huge quantity of mango peel produced suggested that the preparation of single product would not be economically feasible and production of alternative products should also be explored.

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