Global Scientific JOURNALS

GSJ: Volume 9, Issue 11, November 2021, Online: ISSN 2320-9186

www.globalscientificjournal.com

COMPARATIVE STUDY ON LACTIC ACID PRODUCTION FROM PINEAPPLE AND SWEET POTATO PEELS USING *LACTOBACILLUS SPECIES*

FUNMILAYO DEBORAH IJEBOR*, HALIMA YUSUF*, BRIDGET EBU*, WADA NAFIU MUHAMMAD*, AHMAD F. UMAR**, EDIGA B. AGBO**,

***Department of Science Laboratory Technology, Federal Polytechnic Bauchi**
****Department of biological sciences, Faculty of Sciences, Abubakar Tafawa Balewa University, Bauchi**

Corresponding author: fdijebor@fptb.edu.ng +2348067907172

ABSTRACT

A comparative study on lactic acid production from pineapple and sweet potato peels waste residue was carried out using *Lactobacillus species*. Samples of fermented milk were collected from four different locations namely Gwalameji, Yelwa,Wunti and Muda lawal market. The morphology, cultural and biochemical characterization of the isolates from fermented milk (Nono) was carried out using MRS media. Species of lactic acid bacteria isolated after screening were *L .acidophilus*, *L .delbrueckii*, *L .lactis*, *L. bulgaricus*, *L. rhamnosus*, *L. salivarius*, *L. fermentus* and *L. plantarum*. From the result obtained *L .delbrueckii*, and *L. bulgaricus*, have the highest frequency of two (2) and percentage frequency of (20%) respectively. While *L. rhamnosus*, *L. salivarius*, *L. fermentus* and *L. plantarum*, *L .acidophilus, L .lactis* ,have the lowest frequency of one(1) and percentage frequency of (10%). The effect of temperature, pH, and incubation time of the medium, were optimized. The highest lactic acid yield (41.5g/L) was obtained from sweet potato peel medium at 40°C, pH of 5.0 within 48 hours, while the lowest (39.5g/L) was recorded at 45°C, pH of 4.0, also at 48 hours. Pineapple peel medium has the highest lactic acid yield of (39.3g/L) at 40°C, pH of 5.0,within 48 hours, while the lowest lactic acid of (36.3g/L) was produced at 40°C, pH of 4.5 also at 48 hours. The proximate analysis of fermented and unfermented peels of sweet potato and pineapple was carried out, the results obtained for sweet potato peels before fermentation were (moisture 8.24% carbohydrate 72.60% ,protein 4.64% ,ash 4.56%,crude fiber 3.79% and fat 2.02%),while the results recorded for the fermented sweet potato peels were (moisture 11.21%, carbohydrate 53.43%, protein 6.67%, ash 5.77 %, crude fiber 2.16% and fat 4.57%).The results from the unfermented pineapple peels has (moisture 6.78%, carbohydrate 75.83%, protein 3.69%, ash 2.61%, crude fiber 10.80% and fat 5.31%), while the results recorded from the fermented pineapple peels contained, (moisture 10.46%, carbohydrate 45.37%, protein 8.89%, ash 4.79%, crude fiber 2.49% and fat 7.62%) respectively. Analysis of the results showed that there was a significant difference between lactic acid produced from sweet potato peel and pineapple peel medium at different pH, temperature and incubation time at $p \leq 0.05$, the effects of the optimized parameters have a significant influence on the production process of lactic acid. The result obtained revealed that fermentation has a great influence and can bring a s desirable changes into product as recorded from the proximate analysis. It is recommended that both sweet potato and pineapple peels may be used as low cost substrates for lactic acid production, a well fermented sweet potato and pineapple peels could be used to solve the feed needs of animals.

© GSJ

Introductions

Lactic acid or 2-hydroxypropanoic acid, is a water soluble and highly hygroscopic organic acid with ubiquitous distribution in nature. Lactic acid was discovered in (1780) by Scheele in sour milk, and in 1881 Fermi obtained this compound by fermentation, resulting in its industrial production (Galina *et al*., 2017). Lactic acid is widely used in food, pharmaceutical, cosmetic and other manufacturing sectors. In the chemical industry, lactic acid is treated as a raw material for production of lactate ester, propylene glycol, 2,3- pentanedione, propanoic acid, acrylic acid, acetaldehyde and dilactide. It can also be used for fabrication of polylactic acid (PLA), sustainable bioplastic material mainly applied in packaging. Lactic acid functions as a descaling agent, pH regulator, neutralizer, chiral intermediate, solvent, and humectant, cleaning aid, slow acid-release, metal complexing and antimicrobial agents (Galina *et al*., 2017). Technical-grade lactic acid is used in leather tanning industry as an acidulant for deliming hides. Besides moisturizing and pH adjusting effect, the substance is characterized by antimicrobial activity, skin lightening and hydrating action in cosmetic industry. In medicine, lactic acid is applied in tableting, prostheses, surgical sutures, controlled drug delivery systems and electroslyte solutions (Wee *et al*., 2006). However, food industry is the main consumer of lactic acid. Food and food-related applications account for approximately 85% of lactic acid demand, whereas the other industrial sectors cover the remaining 15% (John *et al*., 2007). Lactic acid and its salts are used as antimicrobials, flavor enhancers, stabilizers, thickeners, humectants, emulsifiers, firming and leavening agents and so on (Wee *et al*., 2006). Lactic acid is applied in a wide variety of foodstuffs, such as candies, bread and bakery products, soft drinks, soups, sherbets, dairy products, beer, jams and jellies, mayonnaise and processed eggs (Reddy *et al*., 2013). The global lactic acid demand estimated to be 714.2 kilo tons in 2013 is expected to reach 1960.1 kilo tons by 2020 (Galina *et al*., 2017). Substrates for lactic acid production should be characterized by cheapness, low contamination level, year-round availability, rapid fermentation rate and high yields of lactic acid from fermentation. A major problem facing most developing nations of the World is to increase agricultural production without degrading the environment. Food is a basic human need and producing enough to feed the growing population of developing nations is one of the biggest challenges facing a large proportion of nations. Hence, there should be a greater intervention in form of environment friendly science and technology in food production (USDA, 2010). One of such environment friendly intervention is effective management of wastes, particularly as it concerns agricultural and food processing wastes. The quality of the total environment and health status of the inhabitants are related to the quality and quantity of wastes generated in those areas, as partly defined by the nature of activities carried out by the populace. This environment-health relationship dynamics are particularly evident in most tropical environments where various environmental media are laden with sundry pollutants most of which are often furnished by wastes (Tobias *et al*., 2012). Food waste is a growing problem with around 1.3 billion tons produced globally (Gustavsson *et al*., 2011). Generally, these organic food wastes are composted and applied to cropland or used as animal feed (Nelson, 2010). Furthermore, these wastes are also land filled which have associated environmental and societal impacts (Gunders, 2012).Lactic acid (LA) is produced biosynthetically (90%) by fermentation of sugars (such as glucose) using pure cultures of lactic acid producing bacteria (Sonomoto & Yokota, 2011). Lactic acid is natural and used extensively in the food industry (85%) as an acidulant, flavoring or preservative agent. Fermentations are highly expensive with commercial media for certain type of microorganism, the use of inexpensive raw materials (for example, sweet potato and pineapple peel waste) leads to a profitable process. These wastes contain compounds for bacteria growth to produce bioproducts (John *et al*., 2007). The food and agricultural industries generate excessive volumes of agro-industrial wastes worldwide. These wastes pose a serious problem in their disposal and environmental pollution (Rodríguez-Couto, 2008). In the last few years, various environmental-friendly and cost-effective technologies have been developed with the efforts made both at industrial and academic levels. These technologies aim at utilizing the waste for the development of value-added products; thereby reducing the environmental pollution and solving the issues associated with their disposal (Ezejiofor *et al*., 2014). To conduct a comparative study on lactic acid production from wastes of sweet potato and pineapple peels using *Lactobacillus species*.

i. To isolate and identify *Lactobacillus species* from fermented milk (nono) sold in Bauchi metropolis.
ii. To optimize the parameters for the production of lactic acid from the substrates.
iii. To analyze the substrate that will give the highest yield of lactic acid.

iv. To determine the proximate composition of the substrate.

Source and Isolation of *Lactobacillus species*.

The isolation of *Lactobacillus species was* carried out following a method described by (Somnath *et al*.,2017). 1ml of the milk sample was suspended in a test tube containing 9ml of sterile distilled water to make a milk suspension and ten-fold serial dilution was made by transferring one ml of the milk suspension to another test tube containing 9ml of sterile distilled water. These steps were repeated to obtain a dilution of 10^{-5}. From these test tubes, 10^{-4}, and10^{-5} were selected ,0.5 ml of the aliquot was spread on nutrient agar plates and was incubated at 37°C for 18 to 24hours. The bacterial isolate was sub-cultured on nutrient agar slant and kept at 4^{0}C until required.

Collection of Pineapple and Sweet potato peels

Sweet potato peels and Pineapple peels were collected from Gwallamaji, Yelwa Tudu, Wunti and Muda Lawal markets within Bauchi metropolis in a clean polythene bag, cow milk sample for the isolation of *Lactobacillus spp*. was also collected in a sterile container and transferred to the Microbiology Laboratory of Department of Science Laboratory Technology, Federal Polytechnic Bauchi.

Pretreatment of Substrates

Sample of sweet potato peels and pineapple peels were washed under running tap water, sliced into pieces, spread on the trays, shade dried and chopped into fine pieces. The samples were sorted out and were stored in polyethylene jar at room temperature for further study. This procedure was carried out separately for both sweet potato and pineapple peel sample (Parimala & Muthusamy, 2017).

Biochemical Characterization of the Isolated Bacterial Isolates

Identification of the isolated bacteria as *Lactobacillus species* was performed according to their morphological, cultural, physiological and biochemical characteristics. The tests carried out were Gram staining, SIM test, production of catalase, and milk coagulation activities as described by (Cheesbrough, 2002).

Gram staining

With the aid of a wire lope, colonies were picked from each plate and smears were made on a slide. The prepared slide was allowed to air dry and was heat fixed. Primary stain (crystal violet) was applied for one minute, two drops of lugols iodine was added and allowed for 30 sec. The smear was decolourized with acetone and washed with running water. The slide was counter stained with safranin for 30 seconds, washed and dried. It was examined microscopically using oil immersion objective lens and gram reaction and morphology were observed (Cheesbrough, 2002).

©GSJ

Motility test

SIM medium was use to detect motility by observing the growth from the initial stab, the growth that radiate out from the initial stab means that the bacterial are motile while growth that remain in a straight line around the stab means that the bacterial are not motile. (HPA, 2010).

Indole test

Kovac's reagent was added to the SIM medium to test for indole the appearance of a red ring on the surface of the medium after adding Kovac reagent indicated a positive indole test while the absence of red ring indicated a negative result, (Cheesbrough, 2002).

Hydrogen sulphide test

The test organism was inoculated into the test tube containing SIM medium by means of stab inoculation, the tube was then incubated at 37◦C for 24 – 48hrs, the tube was the observed for the formation of black precipitate on the medium, the presence of black precipitate indicated a positive test while lack of black precipitate shows a negative test, (Pundir *et al*., 2013).

Catalase Test

The test was done by placing a drop of hydrogen peroxide on a clean slide and then some colonies of the test organism were immersed in the hydrogen peroxide solution. The presence of bubbles (Oxygen) indicated catalase positive (Cheesbrough, 2002)

Lactose Utilization

For this experiment, media was prepared using Peptone- 10g, NaCl 15g, Phenol Red 0.018 g, Lactose 5g in 1 litter distilled water, controlling pH 7, a colony of the organism was inoculated and kept at 35°C, for 48 hours in rotary incubator. Change of colour to red from yellow, concluded as positive result (Pundir *et al*., 2013).

Fermentation of Agro Waste Residues Using *Lactobacillus Species*. Production of lactic acid using positive isolate

Inoculation of medium with positive isolate suspension was done according to (Galina *et al.,* 2017). In Solid state fermentation (SSF), 10g each of the sweet potato and pineapple peel waste residues were taken separately into 250mL Erlenmeyer flasks moistened with 5 ml of sterile distilled water, the media were fortified using lactose 0.5g, MgSo4 0.02g and yeast extract 0.5g, the pH was also adjusted to different ranges (using HCL) of 4, 4.5, 5 and 5.5 respectively, the media were then sterilized by autoclaving at 121°C for 15 min. After cooling at room temperature, The flasks were inoculated with the isolated Lactobacillus *spp*; an amount of 5 ml of sterile distilled water was added to the bacterial pure culture to make a suspension and was then transferred to the sweet potato and pineapple peel medium respectively and was allowed to ferment for incubation time of 12, 24, 48, and 72 hours. For lactic acid extraction, distilled water was added to the solid medium and the mixture was shaken in a rotary shaker at (100rpm) for 1 hr. The extract was squeezed through a Whitman filter paper and clarified by centrifugation at 10,000 × g for 20 min. The crude lactic acid was used for the estimation of lactic acid (Parimala & Muthusamy, 2017).

Optimization of Cultural Condition for Maximum Lactic Acid Yield Optimization of cultural parameters for solid state fermentation (SSF) to achieve maximum lactic acid production was studied at different temperature, pH and fermentation time (Parimala & Muthusamy, 2017).

Effect of temperature

To study the effect of temperature on lactic acid production, the solid-state fermentation was carried out at different temperatures of 35°C, 40°C, 45°C and 50° C.

Effect of pH

For determination of suitable pH range for lactic acid production, the fermentation medium was prepared by varying the pH values from 4.0, 4.5, 5.0 and 5.5 respectively.

Effect of incubation time:

The fermentation medium was inoculated with *Lactobacillus spp.* Incubation was done at different time periods (12, 24, 48 and 72 hours) and was then checked at time intervals for the production of lactic acid.

Determination of Lactic Acid Yield

The spectrophotometric determination of lactic acid was done by adding test solution of iron chloride (III) taken at the concentration of 0.3% measuring the optical density of the resulting solution at the wavelength of 380-405nm and quantifying the solution of lactate in the initial solution by using a calibration chart.

Determination of Moisture Content

The determination of the moisture content was done by the Ahamed, *et al*., (2004) method. Before determination, samples were reduced to fine form out of which 4g was weighted out into crucible. The sample was spread evenly across the dish and weighed as rapidly as possible to minimize loss of moisture. The sample was then dried for 4 hours at 125^{0}C in forced draft air oven and cooled to a room temperature in a dessicator and then weighed accurately. The moisture content was reported in percentage. The formula use was as follows:

Where:

A = weight of sample (g)

B = weight of dish + sample prior to drying (g)

C = weight of dish + sample after dying (g)

B – C = loss in weight of sample after drying (g)

Determination of crude protein content

The Macrokjeldahl digestion method as described by FAO (1986) was adopted for the determination of total nitrogen content. The potatoes and pineapple samples were weighed and oxidized by concentrated sulphuric acid in the presence of a catalyst and the nitrogen converted to ammonium sulphate. This was made alkaline by addition of sodium hydroxide and the liberated ammonia was distilled and estimated. The percentage nitrogen was calculated as follows:

Where:

v = millilitre of 0.1N acid added minus millilitre of 0.1N NaOH used to neutralize the ammonia nitrogen.

W = weight of sample (g)

The crude protein content was found by multiplying the percentage nitrogen as obtained by the Kjeldah method by 6.25.

Determination of ash content

This was done according to the method of Dacera, *et al*., (2009). A total of 5g of the sample was weighed and put into a weighed porcelain dish. This was then dried at 100^0 for 3-4 hours in an oven. After which the porcelain was removed and heated gently over a bunsen flame until the content turned black. The dish and the content was transferred to a muffle furnace and ignited at 500 – 600^0C until free from carbon for 8 hours. After which it was removed from muffle furnace and moistened with a few drops of water. This was re- dried in oven at 100^0C for 3-4 hours, and re-ashed at 500 – 600^0C for another hour. It was removed from muffle furnace, allowed to cool for a moment, placed in a dessicator until cool, and then weighed. The result was calculated and expressed as percentage ash using the formula below:

Where: A = weight of sample (g)
B = weight of dish and contents after drying (g) C = weight of empty dish (g)

Determination of fat content

The method of Dacera, *et al*., (2009) was adapted. Some 4g of sample in a fine form was accurately weighted into a thimble lined with a circle of filter paper. The thimble with contents was placed in a 50ml beaker and dried in a mechanical convertion oven for 6 hours at 100^0C. The thimble and contents was then transferred to soxhlet extraction apparatus. The beaker was rinsed several times with ethyl ether, adding rising to the apparatus. The sample contained in the thimble was extracted with ethyl ether in a soxhlet extraction apparatus for 8 hours at a condensation rate of 3-6 drops per second. At the completion of the extraction, the fat extract was transferred from the extraction flask into a pre-weighed evaporating dish with several rinsings of ethyl ether. The evaporating dish was placed in a fumehood with the fan on. The ethyl ether was allowed to evaporate off until no odour of it was detectable. The dish with contents was dried in a mechanical convection oven for 30 minutes at 100^0C. It was removed from the oven, cooled in a dessicator and the dish plus contents was weighed. The calculation was done using the formula below:

Where: w1 = weight of empty evaporating dish (g)
w2 = weight of evaporating dish + contents after drying (g) S = weight of sample (g)

Determination of carbohydrate

The phenol – sulfuric acid method using colorimeter was used to determine the total concentration of carbohydrate present in the sample. A clear aqueous solution of the carbohydrates to be analysed was placed in a test tube, then phenol and sulfuric acid were added. The solution turns yellow- orange color as a result of the interaction between the carbohydrate and the phenol. The absorbance at 420nm was proportional to the carbohydrate concentration initially in the sample .The sulfuric acid causes all non- reducing sugar to be converted to reducing sugar, so as to determine the total sugar present . The method was done by preparing calibration curve using series of standards of known carbohydrate concentration.

Determination of crude fibre

Crude fibre was determined by the method of Ahamed *et al*., (2004), 0.5g processed sample each was boiled in 150lm of 1.25% H_2SO_4 solution for 30min under reflux. The boiled sample was washed in several portion of hot water using a twofold cloth to trap the particles. It was returned to the flask and boiled again in 150ml of 1.25% NaOH for another 30min under same condition. After washing in several portion of hot water the sample was allowed to drain dry before being transferred quantitatively to a weighed crucible where it was dried in the oven at 150◦C to a constant weight. It was thereafter taken to a muffle furnace where it was burnt only ash was left of it. The weight of the fibre was determined by difference and calculated as a percentage of the weight of sample, analyzed thus:

$$\text{Crude fibre(\%)} = \frac{W2 - W1}{\text{Weight of sample}}$$

Results

Table 1: list of *lactobacillus species* isolated from nono from different locations

Sample location	Number of samples collected (n=40)	*Lactobacillus spp* Isolated
GWA	**10**	*L. plantarum* *L. delbrueckii* *L. bulgaricus*
YEL	**10**	*L. fermentus*
WUN	**10**	*L. rhamnosus*
MUD	**10**	*L. bulgaricus* *L. lactis* *L. acidophilus* *L. delbrueckii*

Bacteria isolates	Frequency of isolates (n=10)	Percentage frequency (%)
L. plantarum	1	10
L. delbrueckii	2	20
L. bulgaricus	2	20
L. fermentus	1	10
L. salivarius	1	10
L. rhamnosus	1	10
L. lactis	1	10
L. acidophilus	1	10

© GSJ

Keys- *L= Lactobacillus,* N = number of isolates, % = percentage

Table 3: COMPARATIVE LACTIC ACID YIELD ACCORDING TO SPECIES AND SUBSTRATES USED

Isolate	Temperature ◦C	pH	Substrate conc (g)	Time (hour)	Mineral source (g)	Nitrogen source (g)	Carbon source (g)	Lactic acid yield (g/L) SWP	Lactic acid yield (g/L) PP
L.plantarum	40	5	10	60	0.02	0.5	0.5	15.5	11.4
L.rhomnosus	40	5	10	60	0.02	0.5	0.5	10.3	10.0
L.delbrueckii	40	5	10	60	0.02	0.5	0.5	31.0	30.2
L. bulgaricus	40	5	10	60	0.02	0.5	0.5	33.6	31.6
L. lactis	40	5	10	60	0.02	0.5	0.5	13.0	14.4

Keys = conc –concentration g- gram, SWP- sweet potato pp- pineapple, g/l – gram per litre

Table 4: COMPARATIVE OPTIMIZED CONDITION FOR LACTIC ACID PRODUCTION BASED ON SELECTED ISOLATES AND SUBSTRATE

Isolate	Temperature ◦C	pH	Time (hour)	Substrate conc	Carbon source (g)	Mineral source (g)	Nitrogen source (g)	Lactic acid yield(g/L) SWP	Lactic acid yield(g/L) PP
L. delbrueckii	45	5	48	10	0.5	0.02	0.5	39.5	36.3
L. bulgaricus	40	4	48	10	0.5	0.02	0.5	41.5	39.3

Keys = concentration g- gram, SWP- sweet potato pp- pineapple, g/l – gram per litre

© GSJ

Table 5: COMPARATIVE PROXIMATE COMPOSITION OF SUBSTRATES

Parameters Analyzed	SUBSTRATE AND PROXIMATE CONTENT (%) Before Fermentation		SUBSTRATE AND PROXIMATE CONTENT (%) After Fermentation	
	SWP	PP	SWP	PP
Moisture	8.24	6.78	11.21	10.46
Carbohydrate	72.60	75.83	53.43	45.32
Protein	4.64	3.69	6.67	8.89
Ash	4.56	2.61	5.77	4.79
Crude fibre	3.79	10.80	2.16	2.49
Fat	2.02	5.31	4.57	7.62

Keys -SWP=sweet potato peel, PP=pineapple peel, %= percentage

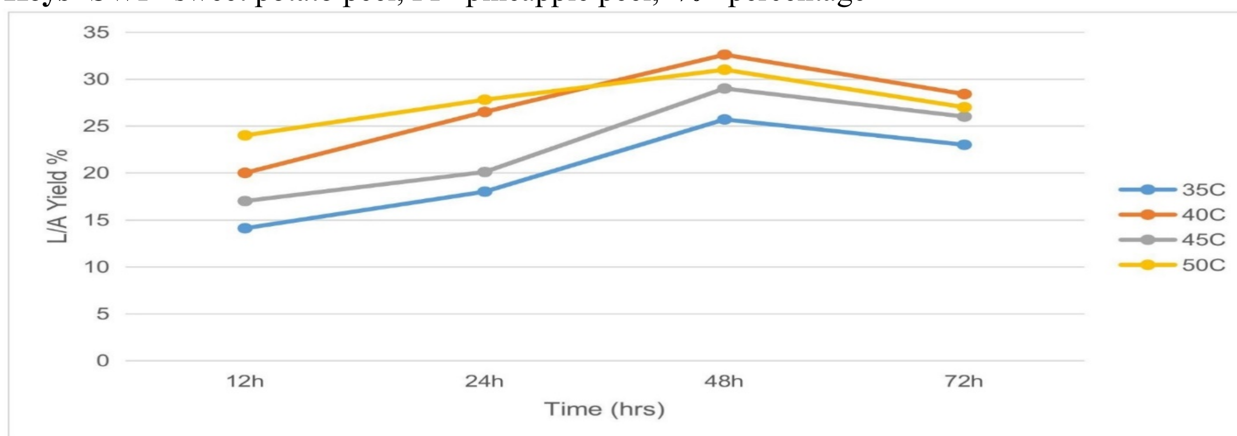

Figure 1: ***Effect of temperature on lactic acid yield with time using Pineapple peels (L. delbruecKii)***

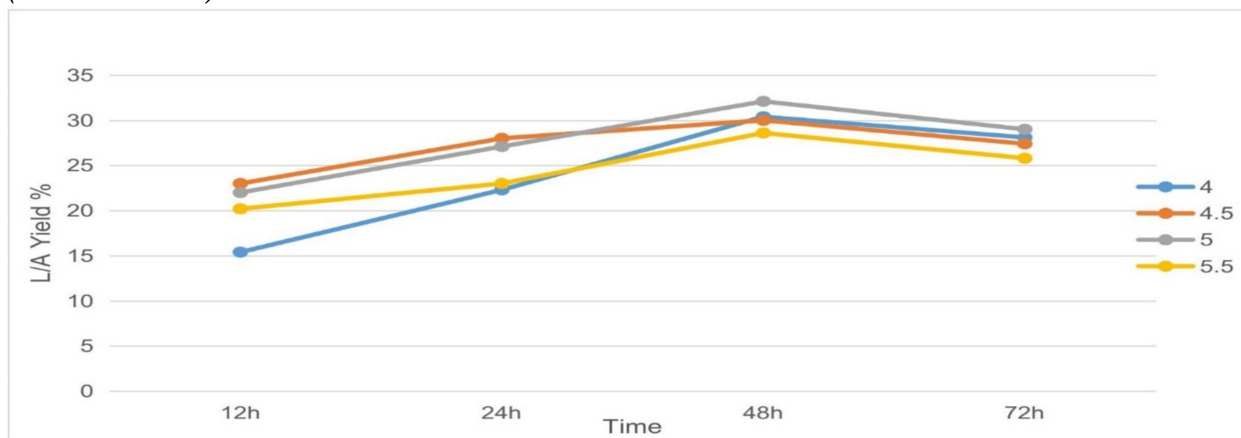

Fig 2: ***Effect of pH on lactic acid yield with time using Pineapple peels (L. delbrueckii)***

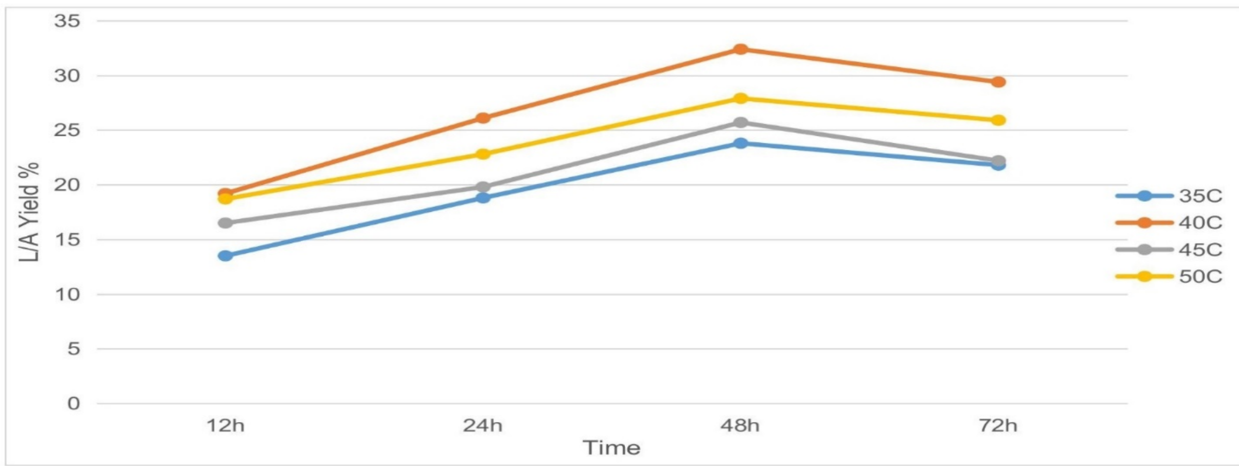

Figure 3*: Effect of temperature on lactic acid yield with time using Pineapple peels (L. bulgaricus)*

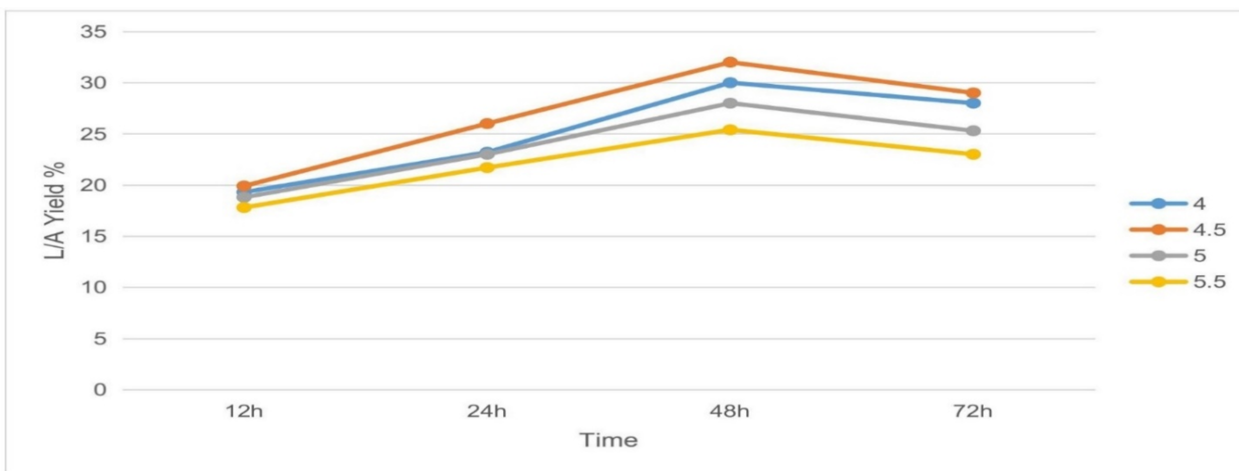

Figure 4: *Effect of pH on lactic acid yield with time using Pineapple peels (L. bulgaricus)*

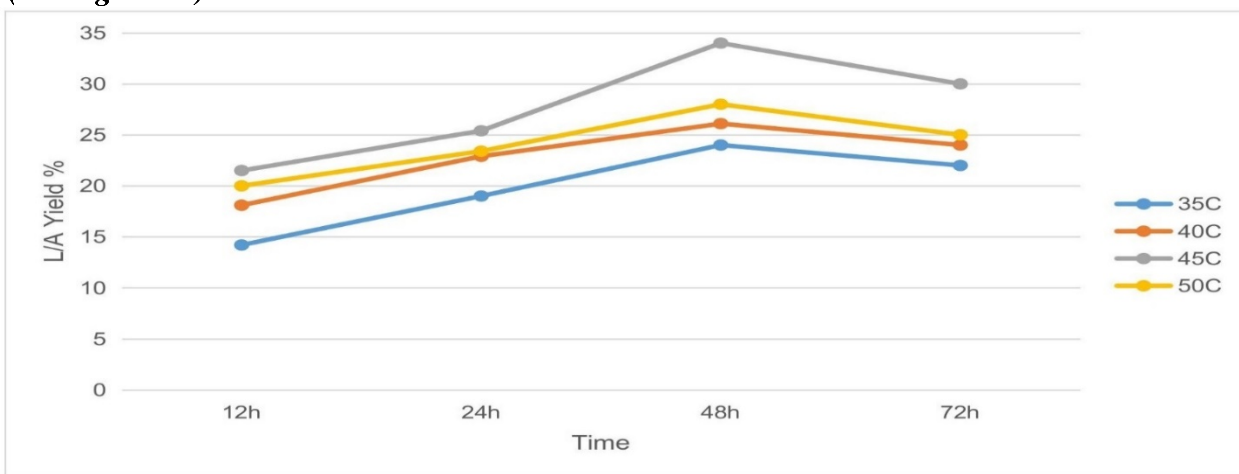

Figure 5: *Effect of temperature on lactic acid yield with time using sweet Potatoe peels (L. delbrueckii)*

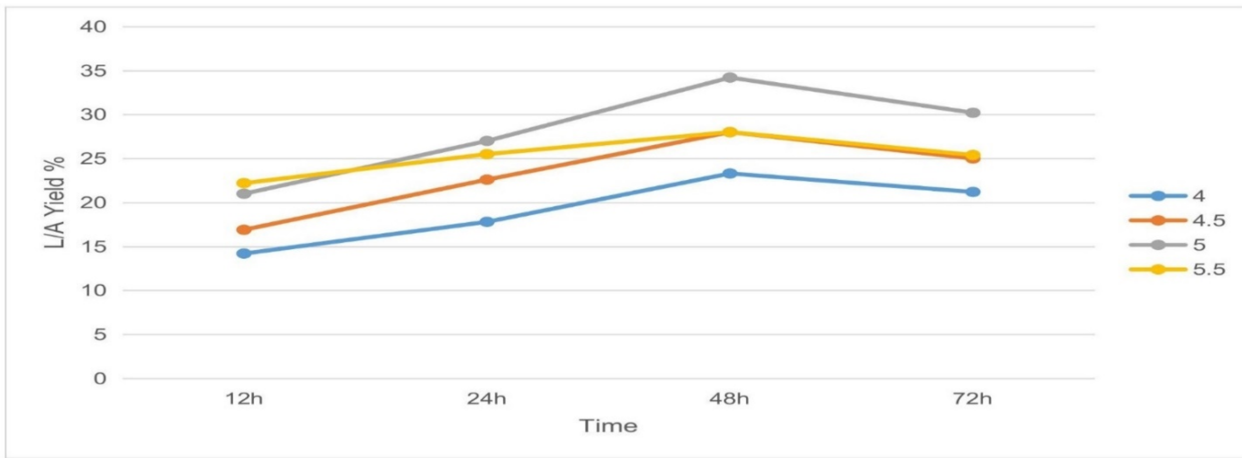

Fig 6: ***Effect of pH on lactic acid yield with time using sweet Potatoe peels (L. delbrueckii)***

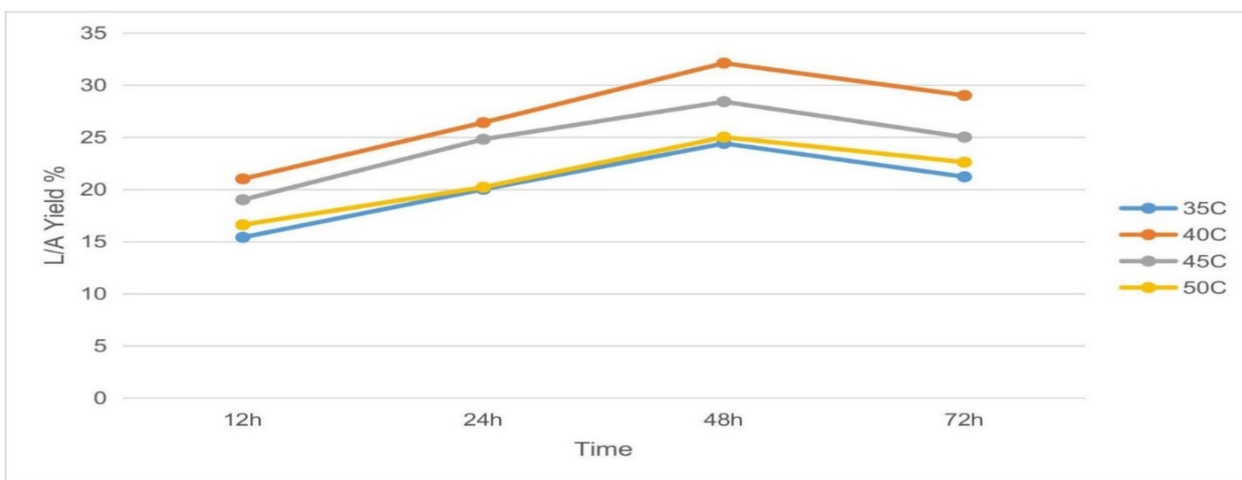

Figure 7: ***Effect of Temp on lactic acid yield with time using sweet Potatoe peels (L. bulgaricus)***

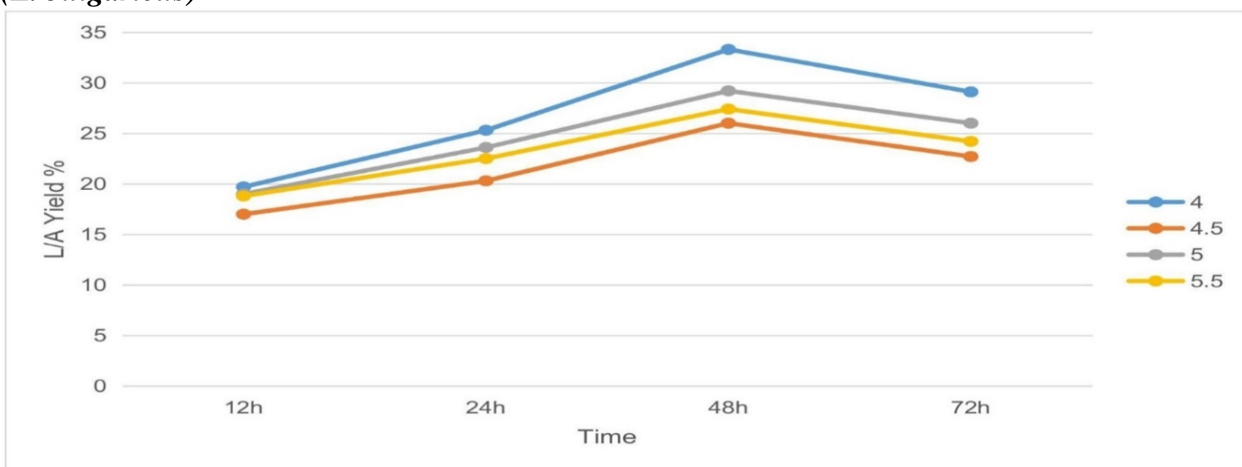

Figure 8*:* ***Effect of pH on lactic acid yield with time using sweet Potatoe peels (L. bulgaricus)***

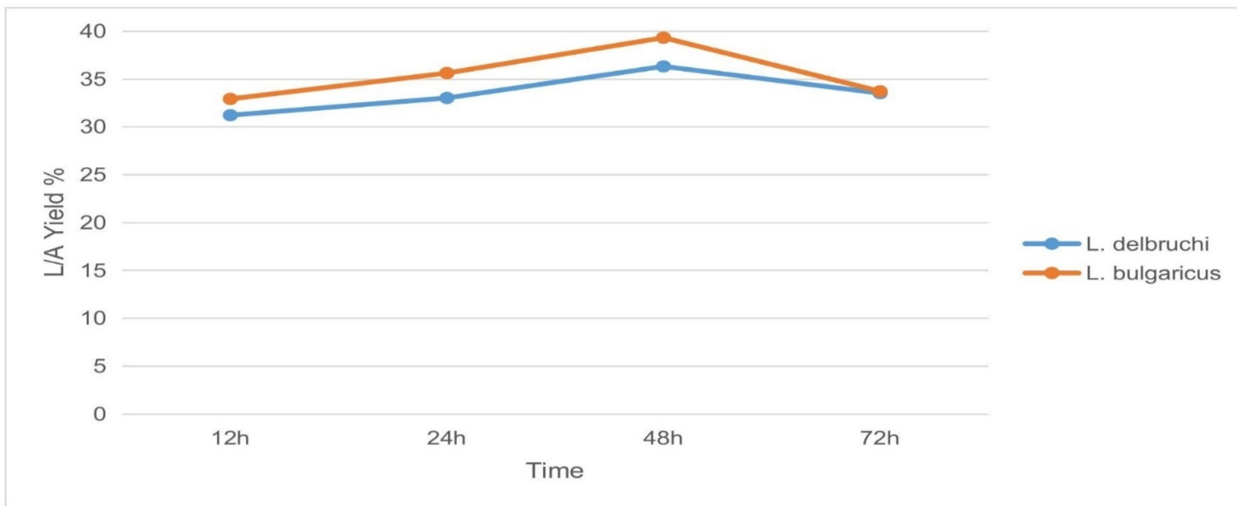

Fig 9: ***Production of lactic acid yield at optimal condition using Pineapple peels***

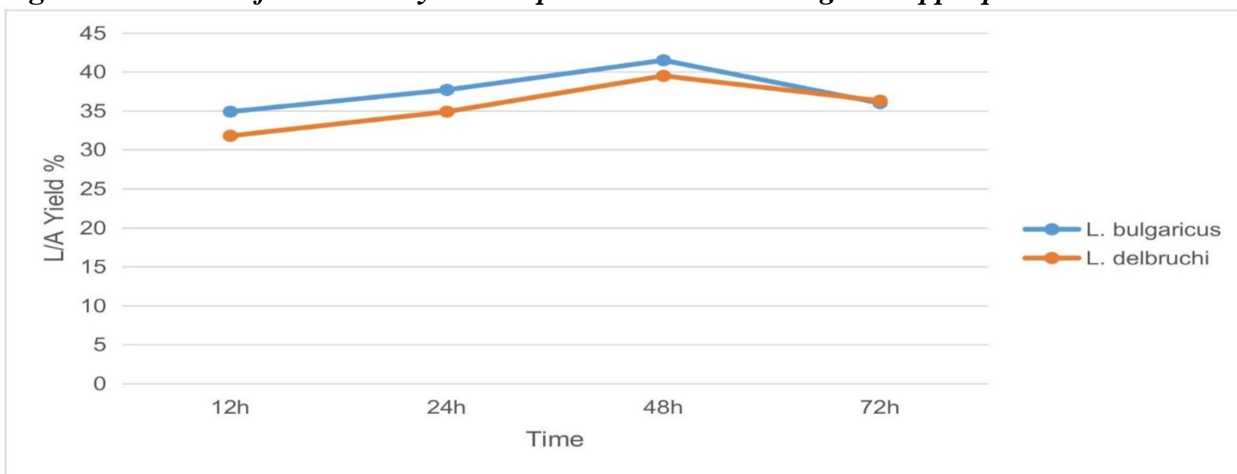

Figure10: ***Production of lactic acid yield at optimal condition using sweet potatoe peels***

Summary

The results revealed lactic acid production of 41.5g/l, 39.5g/land 39.3/l, 36.3g/l at 35°C 40°C,45°C and 50°C for both sweet potato peel and pineapple peel medium with *Lactobacillus specie, lactobacillus bulgaricus and lactobacillus delbrueckii* respectively. It was observed that 40°C and 45°C were the best temperatures for the maximum (41.5g/land 39.3g/l) production of lactic acid for both sweet potato peel and pineapple peel medium respectively. The proximate analysis of both sweet potato and pineapple peels shows that the fermented peels has high nutritional content of protein, moisture, ash and fat while carbohydrate and crude fiber content reduced after fermentation for both substrate this shows that fermentation has a great influence and can bring a desirable changes into product.

Conclusion

The comparative production of lactic acid from pineapple and sweet potato peels using *lactobacillus species* was carried out, five of the isolates were able to produce lactic acid Two of the five isolates namely *L. bulgaricus* and *L. debrueckii* gave maximum yield of lactic acid using potatote and pineapple peels. The process of lactic acid production using solid state fermentation offers several advantages as a result of low substrate cost, low energy consumption and time. The production of a notably, effective lactic acid was achieved at 40ºC, pH of 4.0 and incubation time of 48 hours, the result obtained shows that the effect of optimization parameters has a significant difference on lactic acid yield at $P \geq 0.05$ on the production process.

Recommendation

i. It is recommended that both sweet potato and pineapple peels may be used as low-cost substrates for lactic acid production.
ii. A well fermented sweet potato and pineapple peels could be used to solve the feed needs of animals.
iii. The optimization of the production process enables the fermentation process economically viable and sustainable.
iv. The production of lactic acid is still limited by the final production cost which is associated with the downstream process that requires many step,it is therefore necessary to develop more efficient and viable technologies.

REFERENCES

Ahmed, O. H., Husni, M. H. A., Anuar, A. R. & Hanafi, M. M. (2004). Towards sustainable use of potassium in pineapple waste. *The Scientific World Journal*. 4:1007-1013.

Cheesebrough, M. (2002). *District Laboratory Practice in Tropical Countries* Part Two (2) Cambridge University Press, UK.

Dacera, D. D. M., Babel, S. &Parkpian, P. (2009). Potential for land application of contaminated sewage sludge treated with fermented liquid from pineapple wastes. *Journal of Hazardous Materials*.167(1): 866-872.

Ezejiofor, T. I. N. Enebaku, U. E. & Ogueke, C. (2014). Waste to wealth-value recovery from agro-food processing wastes using biotechnology: A review. *Br Biotechnol J*. 4(4):418-481.

Galina, N., Olga, M. & Victoria, S. (2017). Waste Degradation and Utilization by Lactic AcidBacteria: Use of Lactic Acid Bacteria in Production of Food Additives.*Bioenergy and Biogas*. 2(1):1-42.

Gunders, D. (2012). Wasted: how America is losing up to 40 percent of its food from farm to fork to landfill, Vol. iP:12-06-B, Natural Resources Defense Council accessed 25 October 2016.

Gustavsson, J. Cederberg, C. Sonesson, U. Otterdijk, R. V. & Meybeck, A. (2011). Global Food Losses and Food Waste. Extent, Causes and Prevention. Food and Agriculture Organization of the United Nations. USA.

HPA. (2010). Health Protection Agency: Motility Test. National Standard Method BSOP TP 21 Issue 2.

John, R. P. Nampoothiri, K. M. &Pandey, A. (2007). Fermentative production of lactic acid from biomass: an overview on process developments and future perspectives. *Applied Microbiology and Biotechnology*. 74(3):524-534.

Nelson, M. L. (2010). Utilization and application of wet potato processing Co-products for finishing Cattle. *Journal of Animal Science*.88: 133-142.

Parimala, M. & Muthusamy, P. (2017). Screening and Optimization of Lactic Acid

Production from Lactobacillus Strains by Using Agro Waste Residues. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*.8(4) 1054-1064.

Pundir, R., Rana. S., Kashyap, N. & Kaur, A. (2013). Probiotic Potential of Lactic acid Bacteria Isolated from food Samples – an in Vitro study. *Journal of Applied Pharmacological Science*. 3(3):085-093.

Reddy, G., Altaf, M., Naveena, B., Venkateshwar, M., & Kumar, E. V. (2013). Amylolytic bacterial lactic acid fermentation—a review. *Biotechnology advances*. 26(1): 22-34.

Rodríguez-Couto S. (2008). Exploitation of biological wastes for the production of value- added products under solid-state fermentation conditions. *Biotechnol Journal*. 3:859-870.

Somnath, D. Pramanik, A. Das, A. K. R. Paul, S. Jana, S. &Pramanik, P. (2017). Isolation and characterization of *Lactobacillus spp*. from curd and its pharmacological application in probiotic chocolate. *Journal of Phytopharmacology*.6(6):335-339.

Sonomoto, K. & Yokota, A. (2011). In: *Lactic acid bacteria and Bifidobacteria*. Caister Academic Press Norfolk UK: 211- 218.

Tobias, I. N. E., Uchechi, E. E. &Chika, O. (2012). Waste to Wealth- Value Recovery from Agro-Food 2 Processing Wastes Using Biotechnology: A Review. SDI Paper Template Version 1.6.

USDA, (2010). United States Department of Agriculture. "Manure and Nutrient Management"2261http.//www.csrees.usda.gov/manurenutrientmanagement.

Wee, Y. J. Kim, J. N. & Ryu, H. W. (2006). Biotechnological production of lactic acid and its recent applications. *Food Technology and Biotechnology*. 44(2):163-172.