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Citric Acid Production from Waste Substrate by Using Some Fungi

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Authors' contributions

This work was carried out in collaboration among all authors. Author SS designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors SP and AS managed the analyses of the study. Author SD managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Citric acid is one of the most commonly used and easily available multifunctional organic acids. This is widely used in different industrial applications. Citric acid is 2hydroxyl 1,2,3 propanetricarboxylic acid. It is present in many fruits and vegetables. Citric acid is usually found in lemon. The global demand for Citric acid is about $6.08x10^5$per year its uses are increasing day by day. *Aspergillus niger* and *Aspergillus flavus* organisms are used for citric acid production. In this present study citric acid production from the different waste substrates such as Banana peels, Coconut husk, and Rice straw were carried out using *Aspergillus niger* and *Aspergillus flavus* isolated from soil sample. Characterization and identification were done with the help of microscopic examination based on lactophenol cotton blue staining. *Aspergillus niger* and *Aspergillus flavus* are appeared as branched hypae with conidial spore. The production of citric acid was performed by solid state fermentation and estimated on the different fermentation days, different pH and different concentration of substrate. We observed that high level of citric acid production was on 9^{th} day of fermentation as compared to others days of fermentation.

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Keywords: Solid state fermentation; citric acid; banana peels; rice straw and coconut husk; Aspergillus niger and Aspergillus flavus.

1. INTRODUCTION

Citric acid (2- hydroxyl 2, 3- propane tricarboxylic acid) is one of the most common products which have a never-ending demand in the global market and are produced by fermentation. It is a tricarboxylicacid and a universal intermediate product of plant and animalmetabolism. It consists of 3 carboxyl(R-COOH) group (Berovic et al., 2007). Citric acid is a primary agricultural chemical product and consumed throughout the world [1]. Citric acid exists naturally in a variety of fruits and vegetables notably citrus fruits [2, 3]. Citric acid is found most concentrated in lemon and limes, where it can comprise as much as 8% of the dry weight of the fruit.Citric acid was first isolated from lemon juice by Carl Scheele in 1784. Its name citric acid is derived from the Latin word citrus. Wehmer was the first scientist who showed the Penicilliumglaucum *o*n sugar medium assembled citric acid in culture medium presence of sugars and inorganic salt.

Citric acid is a weak organic acid with the formula $C_6H_8O_7$.Citric acid was first produced from the imported Italian lemons. Lemon juice was the first commercial source of citric acid production. Aspergillus niger strain was discovered by Currie in 1917, which are produced citric acid by growing extravagantly in a nutrient medium with ahigh concentration of sugar and mineral salt. Citric acid present in citrus fruits was first crystallized from lemon juice in the form of calcium citrate [4].

Citric acid is produced by the fermentation process employed are solid state fermentation by Aspergillus niger a variety of substrate [5]. Citric acid has been produced by using inexpensive raw material including crude natural products such as hydrolysate of starch, sugar cane bagasse, beet molasses, cassava bagasse, coffee husk, wheat brain, apple pomace, pineapple, waste, potato peels, cassava peels, grape pomace, and citrus waste [1,6,7,8,9]. Citric acid is produced by using a fungus Aspergillus niger from starchy and sugar substrate Kristianses et al 1978, [10].

Citric acid plays an essential role in food and beverage industries to flavour fruit juices, ice cream etc and in the pharmaceutical citric acid is used as a preservative for stored blood,tablets and cosmetic preparation and other industries for applications suchas acidulation, antioxidant, flavor, enhancement, preservation, and plasticization and as a synergistic agent. There have been anincreased in using natural resources such as fruits sugar and agricultural waste for the production of citric acid. Citric acid has application as a function of additive detergent, cosmetics and toiletries (kumar and jain, 2008; Lazar et al., 2011). Citric acidfermentation is one of the primitive fermentations but stillits production is increasing with the passage of time. The over 90% production of citric acid is obtained by fermentation (Khosravi- Daraniet al., 2008). Studies show that 72% of its usage is in food industries and its annual consumption growth rate is about 4-5% and expected to increases more in coming years.

Many micro-organism have been involved in the production of citric acid including bacteria such as *Bacillus licheniformis, B. Subtilis,, Corynebacterium* spp. [11], fungi such as *A. Niger, A. Flavus,A.awamori, A. Foetidus, Penicilliumrestricum* [12,13]. Yeast such as *Candida lipolytica, C. Intermedia and Saccharomyces cervisiae* [14,15, 16]. Among the yeast species,*Yarrowialipolytica*is known as a potential producer of citric acid [17].

One of the most important fungi used in industrialmicrobiology*Aspergillusniger,* has been employed for manyyears for the commercial production of citric acid [18,19,20].However,the worldwide demand for citric acid is increasing fasterthan its production and more economical processes arerequired. *Aspergillusniger*is most commonly used for citric acidproduction. This is because of the fact that this organismhas capacity to utilize varieties of substrates due to its welldeveloped enzymatic system [21]. Although *Aspergillus niger*isthe traditional producer of citric acid, during the last 30 yearsthe use of yeasts for citric acid fermentation processes hasattracted the interest of researchers. Aspergillus niger is normally a haploid fungus producing white septate hypha conidia, which is profusely branched. It produces black mass of conidia, which are found in chain arising from the secondary stigmata.Therefore, our present study is focused on carry out comparative study on production of citric acid from rice straw, banana peels and coconut husk by utilizing the fungus *Aspergillusflavus*and *Aspergillus niger.*

2. MATERIALS AND METHODS

2.1 Isolation of Fungi

Isolation of *Aspergillus* strains was done by using soil samples collected from different sites. *Aspergillus* strains were isolated from soil with the help of serial dilution. Diluted tubes were inoculated on sabouraud dextrose agar media and incubated at 27°C under an incubator for few days. After 3 days of incubation colonies with spore were observed.

2.2 Identification of Fungal Isolates

Aspergillus strainswere identified by using the cotton blue staining method. Colonies are identified as *Aspergillus flavus*and *Aspergillus niger*. Inoculum was maintained on sabouarud dextrose agar slants, for citric acid production.

2.3 Samples Collection

Sample such as Banana peels; Rice straw and Coconut husk were collected from the local market.

2.4 Pre-treatment of Samples

The collected samples were oven-dried at 60°C for 2hour and used for the production of citric acid. Substrates were cut into small pieces and grind into the grinder, used for fermentation to produce citric acid [22].

2.5 Preparation of Fermentation Media

Fermentation: Solid substrate fermentation is carried out to produce citric acid

The basal medium was prepared by introducing different dry substrates in different concentrations (5gram, 10gram and 15gram) into a separate 100ml Erlenmeyer flask. The medium was supplemented with nitrogen supplement and by adding ofammonium phosphate, potassium hydrogen phosphate and peptone to the basal medium. The flask was cotton plugged and autoclaved at 151lbs for 15minutes. After cooling at room temperature each medium was inoculated with *Aspergillus flavus*and *Aspergillus niger*dilution suspension and incubated in arotary shaker at 30^0C for different days (6^{th}, 7th, 8^{th} and 9^{th} days).

2.6 Effect of Variables on Citric Acid Production

The effect of pH was investigated on citric acid production. The range of pH investigated was 4, 6, 8, 10 .and the temperature was 30°C. The citric acid production was studied at different fermentation periods such as days 6, 7, 8, and 9. The total titratable acidity was also determined by 0.1N NaOH. The effect of different concentrations 5gm, 10gm and 15gm of the substrate was carried out and inoculums size $6.0X10^6$of the substrate was also studied. All experiments were incubated in a rotary shaking incubator.

2.7 Filtration

The medium was diluted with sterile distilled water and then filtered through sterile paper to get filtrate.

2.8 Citric Acid Determination

Citric acid was determined by the pyridine acetic anhydride method. Now day's acetic anhydride is not easily available in the market so that I havebeenused acetyl chloride instead of acetic anhydride [23,24,25].

2.9 Estimation of Reducing Sugar

Reducing sugar was estimated by phenol sulphuric acid method [26]

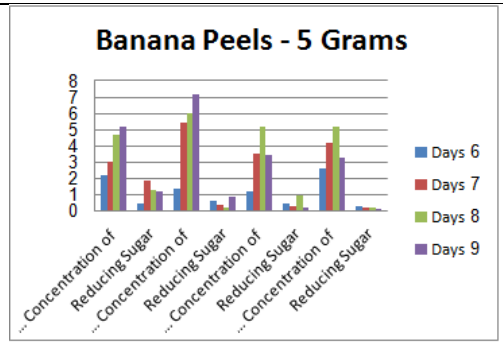
3. RESULTS AND DISCUSSION

3.1 Effect of Different Range of Ph for Citric Acid Production

The Aspergillus niger and *Aspergillus flavus* were produced higher citric acid at pH 4. But *Aspergillus niger* was the best citric acid produced as compared to *Aspergillus flavus.* Iralapati .V. and Kummari .S. [27] reported that higher citric acid were produced from fresh banana peels by Aspergillus niger Mostly the fungal strains are seemed to be grown well under acidic conditions ranging from 3 to 6 [28]. The highest production of citric acid by using the rice husks at temperature 30°C and primary pH 4 [29].

***1.Aspergillus flavus* – Banana Peels – 5 Gram**

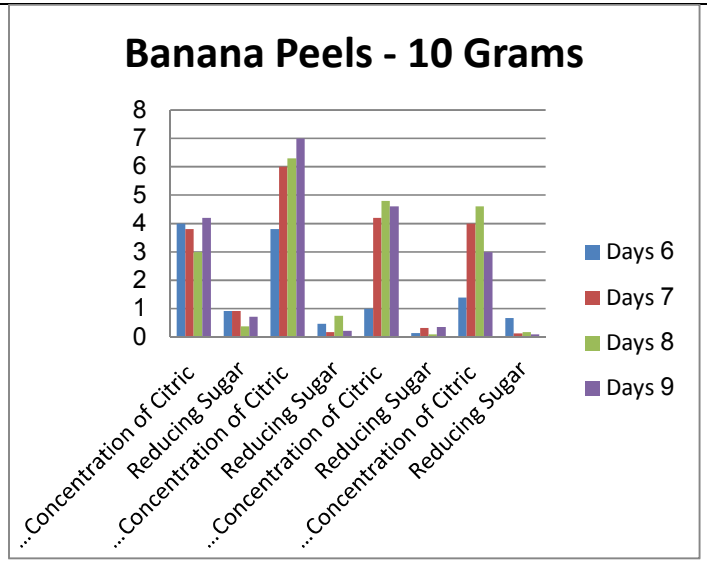
pH Value	Fermentation Days	Concentration of Citric Acid	Reducing Sugar
pH4	6 Days	2.2	0.424
	7 Days	3	1.845
	8 Days	4.7	1.297
	9Days	5.2	1.225
pH6	6 Days	1.4	0.612
	7 Days	5.4	0.363
	8 Days	6	0.176
	9Days	7.2	0.836
pH8	6 Days	1.2	0.487
	7 Days	3.5	0.254
	8 Days	5.2	0.913
	9Days	3.4	0.204
pH10	6 Days	2.6	0.298
	7 Days	4.2	0.171
	8 Days	5.2	0.163
	9Days	3.3	0.007



In this table showing that *Aspergilusflavus*is produced high amount of citric acid in 6 pH from 5gm of banana peels in 9th days of fermentation

***2. Aspergillusflavus* – Banana Peels – 10 Gram**

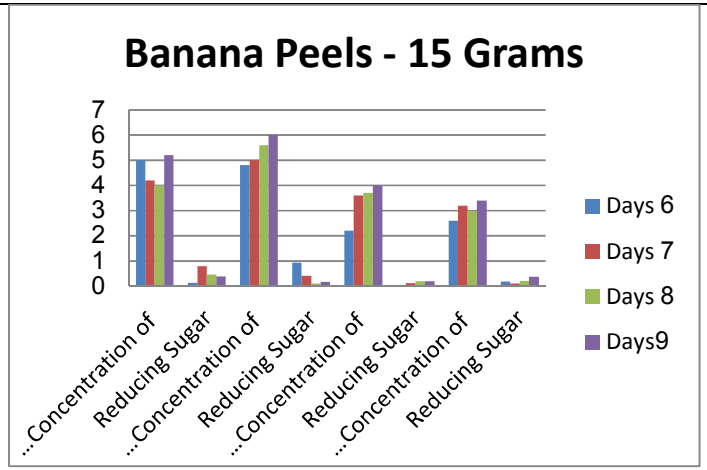
pH Value	Fermentation Days	Concentration of Citric Acid	Reducing Sugar
pH4	6 Days	4	0.915
	7 Days	3.8	0.913
	8 Days	3	0.378
	9Days	4.2	0.718
pH6	6 Days	3.8	0.467
	7 Days	6	0.177
	8 Days	6.3	0.745
	9Days	7	0.215
pH8	6 Days	1	0.141
	7 Days	4.2	0.323
	8 Days	4.8	0.096
	9Days	4.6	0.354
pH10	6 Days	1.4	0.674
	7 Days	4	0.133
	8 Days	4.6	0.176
	9Days	3	0.096



In this table showing that *Aspergilusflavus*is produced high amount of citric acid in 6 pH from10gm of banana peels in 9th days of fermentation

***3. Aspergillus flavus* – Banana Peels – 15 Gram**

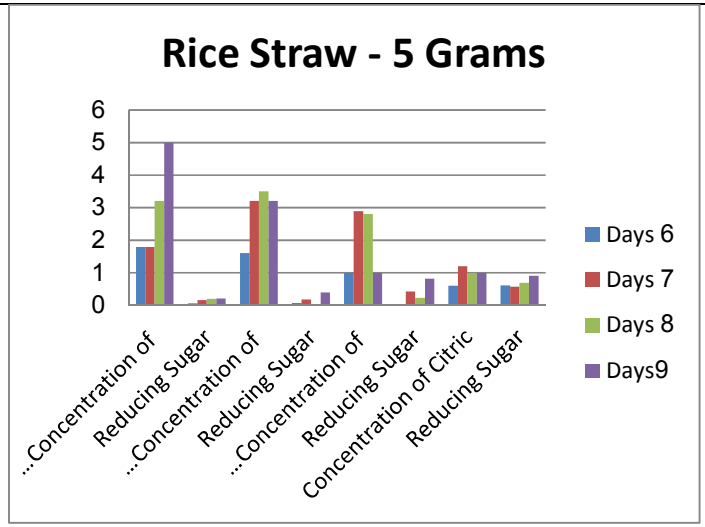
pH Value	Fermentation Days	Concentration of Citric Acid	Reducing Sugar
pH4	6 Days	5	0.13
	7 Days	4.2	0.787
	8 Days	4	0.456
	9Days	5.2	0.387
pH6	6 Days	4.8	0.928
	7 Days	5	0.403
	8 Days	5.6	0.098
	9Days	6	0.172
pH8	6 Days	2.2	0.034
	7 Days	3.6	0.121
	8 Days	3.7	0.19
	9Days	4	0.19
pH10	6 Days	2.6	0.178
	7 Days	3.2	0.109
	8 Days	3	0.205
	9Days	3.4	0.37



In this table showing that *Aspergilusflavus*is produced high amount of citric acid in 4 pH from15gm of banana peels in 9th days of fermentation

***4. Aspergillus flavus*– Rice Straw – 5 Gram**

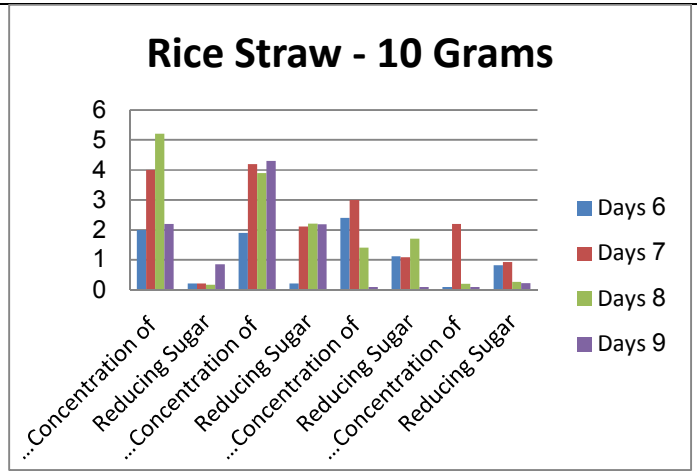
pH Value	Fermentation Days	Concentration of Citric Acid	Reducing Sugar
pH4	6 Days	1.8	0.061
	7 Days	1.8	0.16
	8 Days	3.2	0.202
	9Days	5	0.209
pH6	6 Days	1.6	0.071
	7 Days	3.2	0.176
	8 Days	3.5	0.008
	9Days	3.2	0.397
pH8	6 Days	1	0.029
	7 Days	2.9	0.43
	8 Days	2.8	0.229
	9Days	1	0.81
pH10	6 Days	0.6	0.606
	7 Days	1.2	0.575
	8 Days	1	0.692
	9Days	1	0.907



In this table showing that *Aspergilusflavus*is produced high amount of citric acid in 4 pH from5gm of rice straw in 9th days of fermentation

***5. Aspergillus flavus* – Rice Straw – 10 Gram**

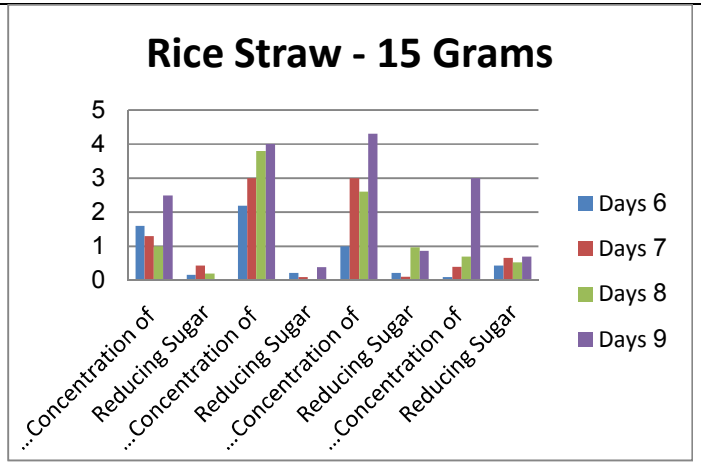
pH Value	Fermentation Days	Concentration of Citric Acid	Reducing Sugar
pH4	6 Days	2	0.204
	7 Days	4	0.214
	8 Days	5.2	0.171
	9Days	2.2	0.854
pH6	6 Days	1.9	0.203
	7 Days	4.2	2.109
	8 Days	3.9	2.209
	9Days	4.3	2.187
pH8	6 Days	2.4	1.122
	7 Days	3	1.092
	8 Days	1.4	1.708
	9Days	0.1	0.096
pH10	6 Days	0.1	0.818
	7 Days	2.2	0.934
	8 Days	0.2	0.263
	9Days	0.1	0.229



In this table showing that *Aspergilusflavus*is produced high amount of citric acid in 4 pH from10gm of banana rice straw in 8th days of fermentation

***6. Aspergillus flavus*– Rice Straw – 15 Gram**

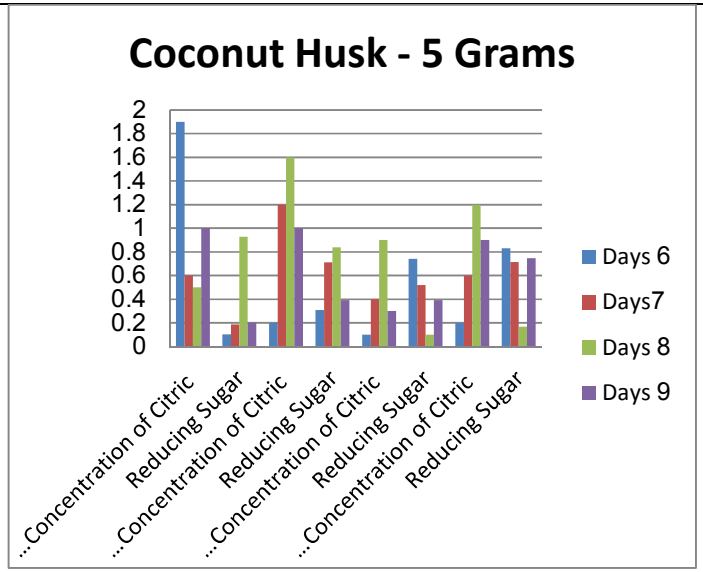
pH Value	Fermentation Days	Concentration of Citric Acid	Reducing Sugar
pH4	6 Days	1.6	0.159
	7 Days	1.3	0.439
	8 Days	1	0.199
	9Days	2.5	0.014
pH6	6 Days	2.2	0.212
	7 Days	3	0.098
	8 Days	3.8	0
	9Days	4	0.391
pH8	6 Days	1	0.215
	7 Days	3	0.107
	8 Days	2.6	0.979
	9Days	4.3	0.874
pH10	6 Days	0.1	0.432
	7 Days	0.4	0.653
	8 Days	0.7	0.532
	9Days	3	0.701



In this table showing that *Aspergilusflavus*is produced high amount of citric acid in 8 pH from15gm of rice straw in 9th days of fermentation

***7. Aspergillus flavus* – Coconut Husk – 5 Gram**

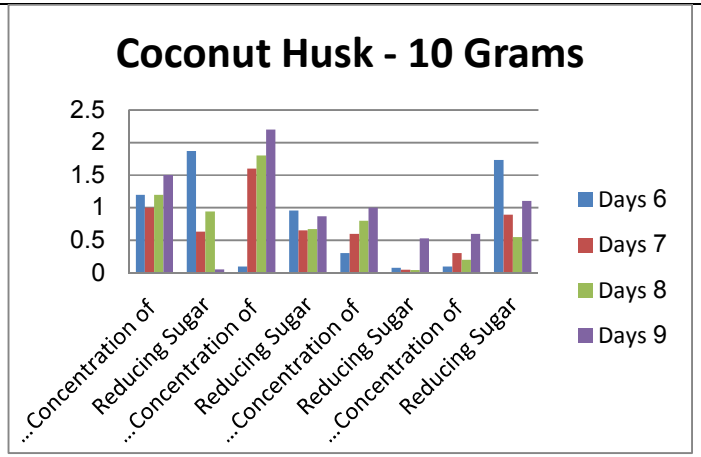
pH Value	Fermentation Days	Concentration of Citric Acid	Reducing Sugar
pH4	6 Days	1.9	0.103
	7 Days	0.6	0.188
	8 Days	0.5	0.93
	9Days	1	0.203
pH6	6 Days	0.2	0.307
	7 Days	1.2	0.712
	8 Days	1.6	0.84
	9Days	1	0.392
pH8	6 Days	0.1	0.74
	7 Days	0.4	0.52
	8 Days	0.9	0.1
	9Days	0.3	0.391
pH10	6 Days	0.2	0.829
	7 Days	0.6	0.716
	8 Days	1.2	0.167
	9Days	0.9	0.745



In this table showing that *Aspergilusflavus*is produced high amount of citric acid in 4 pH from5gm of banana peels in 6th days of fermentation

***8. Aspergillus flavus* – Coconut Husk – 10 Gram**

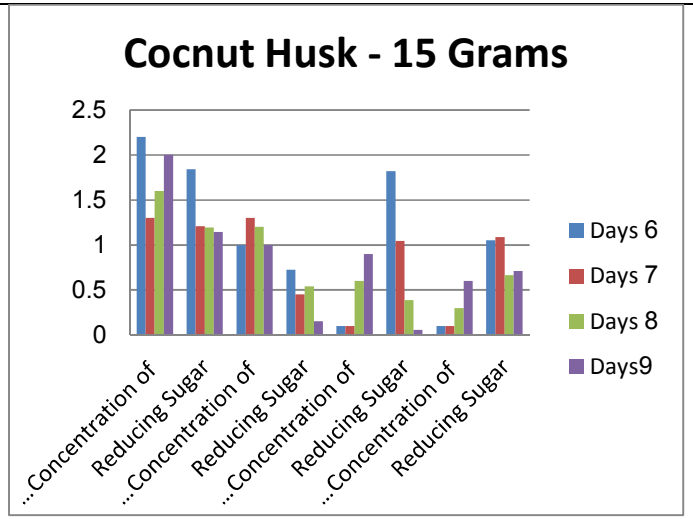
pH Value	Fermentation Days	Concentration of Citric Acid	Reducing Sugar
pH4	6 Days	1.2	1.871
	7 Days	1	0.635
	8 Days	1.2	0.942
	9Days	1.5	0.053
pH6	6 Days	0.1	0.96
	7 Days	1.6	0.652
	8 Days	1.8	0.674
	9Days	2.2	0.865
pH8	6 Days	0.3	0.075
	7 Days	0.6	0.046
	8 Days	0.8	0.045
	9Days	1	0.531
pH10	6 Days	0.1	1.731
	7 Days	0.3	0.894
	8 Days	0.2	0.554
	9Days	0.6	1.106



In this table showing that *Aspergilusflavus*is produced high amount of citric acid in 6 pH from10gm of coconut husk in 9th days of fermentation

***9. Aspergillus flavus* – Coconut Husk – 15 Gram**

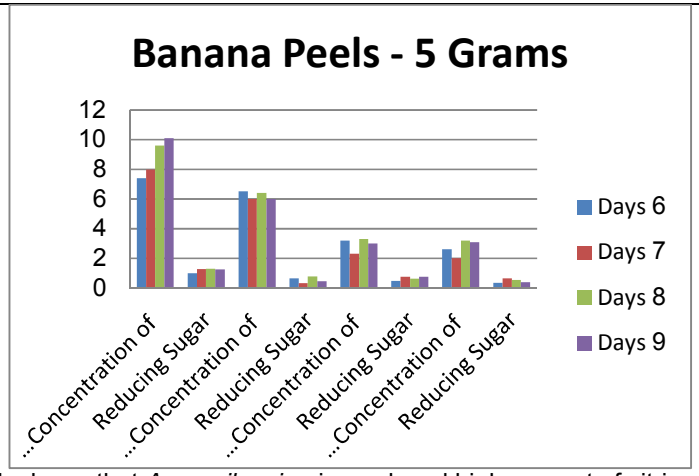
pH Value	Fermentation Days	Concentration of Citric Acid	Reducing Sugar
pH4	6 Days	2.2	1.843
	7 Days	1.3	1.209
	8 Days	1.6	1.193
	9Days	2	1.143
pH6	6 Days	1	0.725
	7 Days	1.3	0.451
	8 Days	1.2	0.54
	9Days	1	0.153
pH8	6 Days	0.1	1.82
	7 Days	0.1	1.047
	8 Days	0.6	0.387
	9Days	0.9	0.06
pH10	6 Days	0.1	1.054
	7 Days	0.1	1.091
	8 Days	0.3	0.665
	9Days	0.6	0.712



In this table showing that *Aspergilusflavus*is produced high amount of citric acid in 4 pH from15gm of coconut husk in 6th days of fermentation

***10. Aspergillus niger* - Banana Peels – 5 Gram**

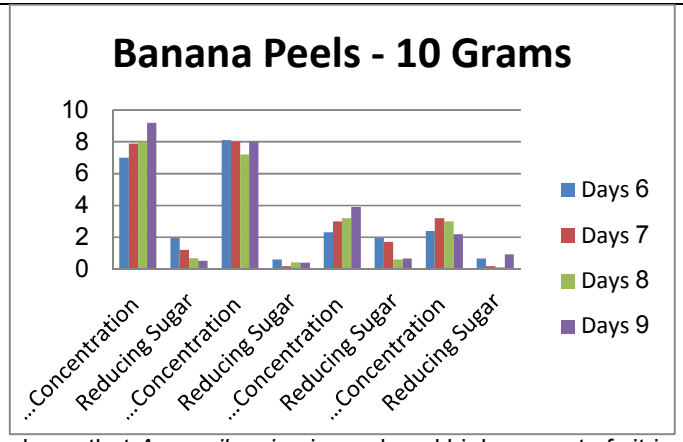
pH Value	Fermentation Days	Concentration of Citric Acid	Reducing Sugar
pH4	6 Days	7.4	0.984
	7 Days	8	1.265
	8 Days	9.6	1.287
	9Days	10.1	1.245
pH6	6 Days	6.5	0.657
	7 Days	6	0.342
	8 Days	6.4	0.785
	9Days	6	0.456
pH8	6 Days	3.2	0.469
	7 Days	2.3	0.765
	8 Days	3.3	0.621
	9Days	3	0.765
pH10	6 Days	2.6	0.346
	7 Days	2	0.654
	8 Days	3.2	0.538
	9Days	3.1	0.389



This graph shows that *Aspergilusniger*is produced high amount of citric acid in 4 pH from5gm of banana peels in 9th days of fermentation.

***11. Aspergillus niger* - Banana Peels – 10 Gram**

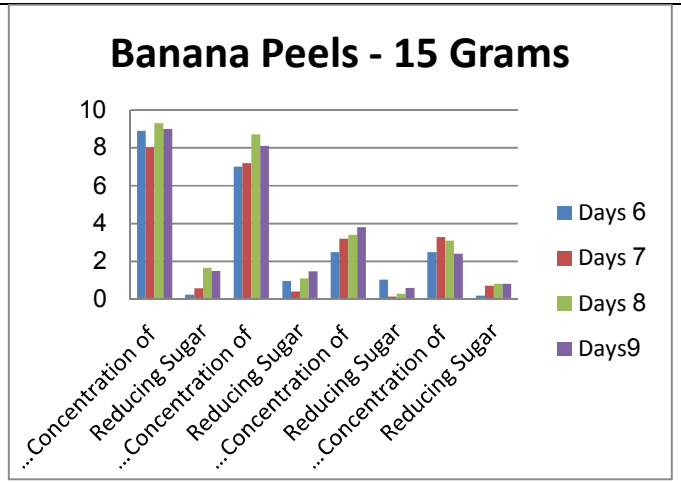
pH Value	Fermentation Days	Concentration of Citric Acid	Reducing Sugar
pH4	6 Days	7	1.945
	7 Days	7.9	1.213
	8 Days	8	0.678
	9Days	9.2	0.518
pH6	6 Days	8.1	0.598
	7 Days	8	0.192
	8 Days	7.2	0.431
	9Days	8	0.387
pH8	6 Days	2.3	1.941
	7 Days	3	1.723
	8 Days	3.2	0.596
	9Days	3.9	0.654
pH10	6 Days	2.4	0.654
	7 Days	3.2	0.193
	8 Days	3	0.138
	9Days	2.2	0.921



This graph shows that *Aspergilusniger*is produced high amount of citric acid in 4 pH from 10gm of banana peels in 9th days of fermentation.

***12. Aspergillus niger* - Banana Peels – 15 Gram**

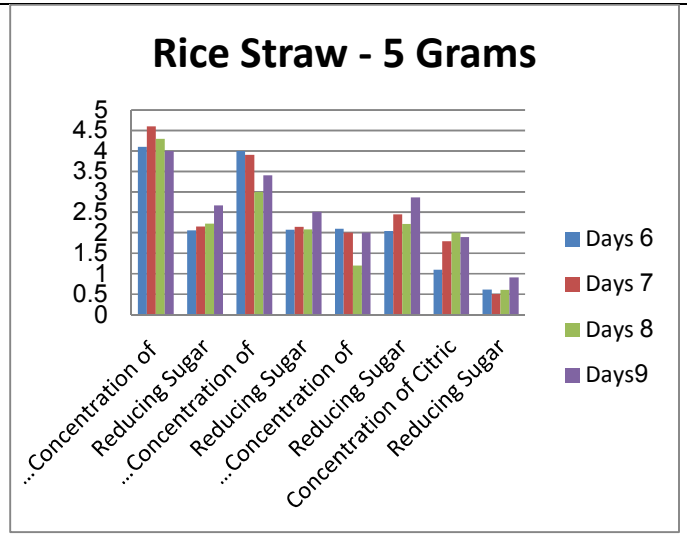
pH Value	Fermentation Days	Concentration of Citric Acid	Reducing Sugar
pH4	6 Days	8.9	0.23
	7 Days	8	0.587
	8 Days	9.3	1.656
	9Days	9	1.487
pH6	6 Days	7	0.968
	7 Days	7.2	0.413
	8 Days	8.7	1.098
	9Days	8.1	1.472
pH8	6 Days	2.5	1.037
	7 Days	3.2	0.134
	8 Days	3.4	0.286
	9Days	3.8	0.61
pH10	6 Days	2.5	0.185
	7 Days	3.3	0.715
	8 Days	3.1	0.813
	9Days	2.4	0.815



This graph shows that *Aspergilusniger*is produced high amount of citric acid in 4 pH from 15gm of banana peels in 8th days of fermentation.

***13. Aspergillus niger* - Rice Straw – 5 Gram**

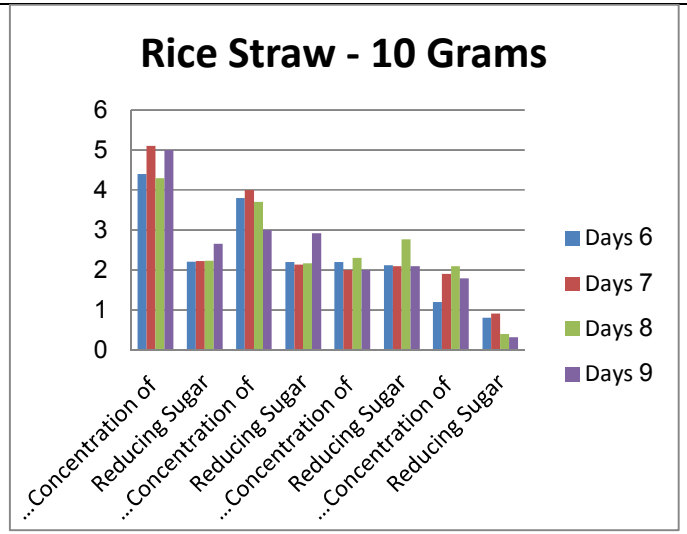
pH Value	Fermentation Days	Concentration of Citric Acid	Reducing Sugar
pH4	6 Days	4.1	2.063
	7 Days	4.6	2.161
	8 Days	4.3	2.221
	9Days	4	2.674
pH6	6 Days	4	2.076
	7 Days	3.9	2.146
	8 Days	3	2.084
	9Days	3.4	2.51
pH8	6 Days	2.1	2.045
	7 Days	2	2.452
	8 Days	1.2	2.217
	9Days	2	2.864
pH10	6 Days	1.1	0.616
	7 Days	1.8	0.505
	8 Days	2	0.602
	9Days	1.9	0.917



This graph shows that *Aspergilus niger*is produced high amount of citric acid in 4 pH from 5gm of rice straw in 7th days of fermentation.

***14. Aspergillus niger*- Rice Straw – 10 Gram**

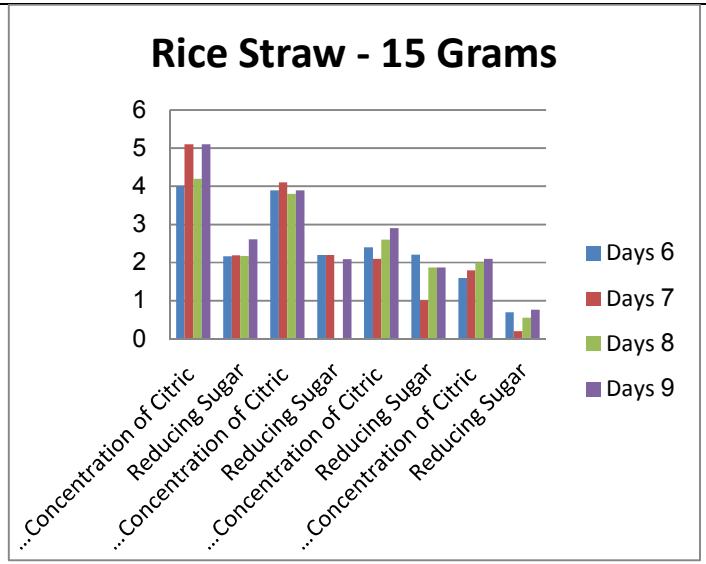
pH Value	Fermentation Days	Concentration of Citric Acid	Reducing Sugar
pH4	6 Days	4.4	2.214
	7 Days	5.1	2.224
	8 Days	4.3	2.231
	9Days	5	2.654
pH6	6 Days	3.8	2.204
	7 Days	4	2.132
	8 Days	3.7	2.165
	9Days	3	2.916
pH8	6 Days	2.2	2.121
	7 Days	2	2.098
	8 Days	2.3	2.768
	9Days	2	2.098
pH10	6 Days	1.2	0.808
	7 Days	1.9	0.914
	8 Days	2.1	0.403
	9Days	1.8	0.319



This graph shows that *Aspergilusniger*is produced high amount of citric acid in 4 pH from 10gm of rice straw in 9th days of fermentation.

***15. Aspergillus niger* - Rice Straw – 15 Gram**

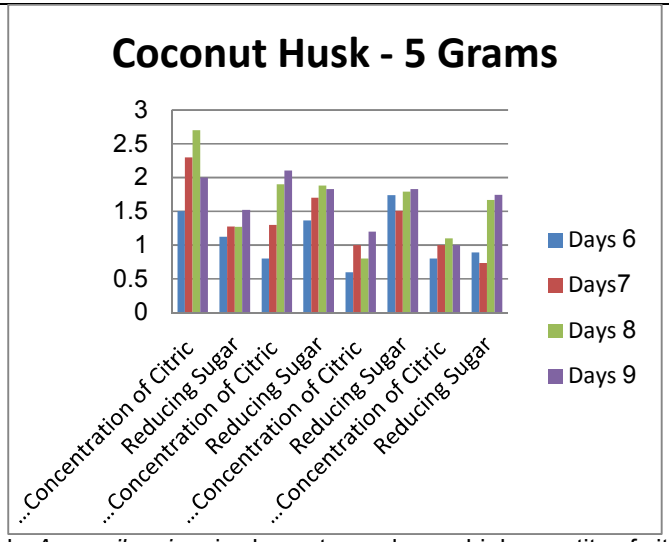
pH Value	Fermentation Days	Concentration of Citric Acid	Reducing Sugar
pH4	6 Days	4	2.169
	7 Days	5.1	2.189
	8 Days	4.2	2.179
	9Days	5.1	2.614
pH6	6 Days	3.9	2.202
	7 Days	4.1	2.198
	8 Days	3.8	2. 196
	9Days	3.9	2.091
pH8	6 Days	2.4	2.205
	7 Days	2.1	1.007
	8 Days	2.6	1.879
	9Days	2.9	1.873
pH10	6 Days	1.6	0.702
	7 Days	1.8	0.201
	8 Days	2	0.561
	9Days	2.1	0.761



This graph shows that *Aspergilusniger* produces a high quantity of citric acid at 4 pH after 9 days of fermentation from 15 gm of rice straw.

***16. Aspergillus niger* - Coconut Husk – 5 Gram**

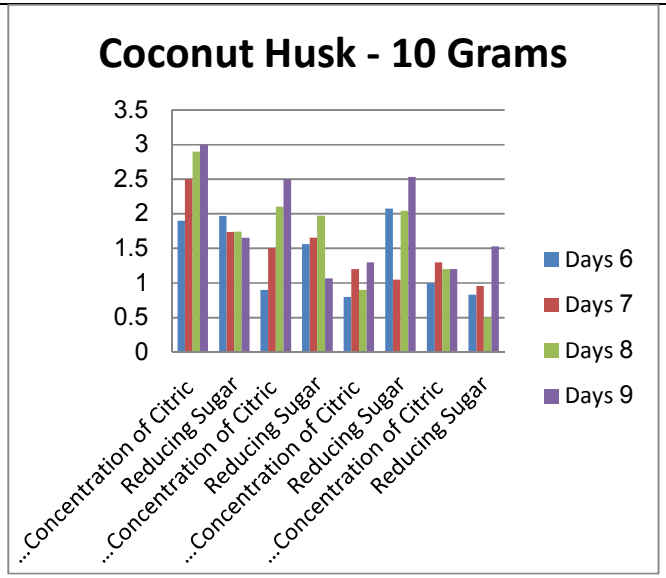
pH Value	Fermentation Days	Concentration of Citric Acid	Reducing Sugar
pH4	6 Days	1.5	1.123
	7 Days	2.3	1.278
	8 Days	2.7	1.27
	9Days	2	1.523
pH6	6 Days	0.8	1.367
	7 Days	1.3	1.702
	8 Days	1.9	1.88
	9Days	2.1	1.832
pH8	6 Days	0.6	1.74
	7 Days	1	1.51
	8 Days	0.8	1.79
	9Days	1.2	1.831
pH10	6 Days	0.8	0.891
	7 Days	1	0.731
	8 Days	1.1	1.671
	9Days	1	1.745



In this graph, *Aspergilusniger* is shown to produce a high quantity of citric acid at 4 pH from 5gm of coconut husk on the 8 day of fermentation.

***17. Aspergillus niger*-Coconut Husk – 10 Gram**

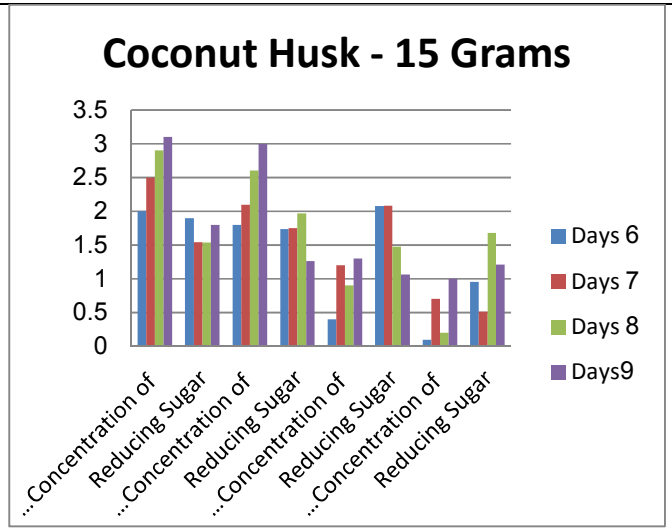
pH Value	Fermentation Days	Concentration of Citric Acid	Reducing Sugar
pH4	6 Days	1.9	1.971
	7 Days	2.5	1.735
	8 Days	2.9	1.742
	9Days	3	1.653
pH6	6 Days	0.9	1.56
	7 Days	1.5	1.652
	8 Days	2.1	1.974
	9Days	2.5	1.065
pH8	6 Days	0.8	2.075
	7 Days	1.2	1.046
	8 Days	0.9	2.045
	9Days	1.3	2.531
pH10	6 Days	1	0.831
	7 Days	1.3	0.954
	8 Days	1.2	0.503
	9Days	1.2	1.529



In this graph, *Aspergilusniger*shows that a high quantity of citric acid is produced at 4 pH from 10gm of coconut husk on the 9th day of fermentation.

***18. Aspergillus niger* -Coconut Husk – 15 Gram**

pH Value	Fermentation Days	Concentration of Citric Acid	Reducing Sugar
pH4	6 Days	2	1.895
	7 Days	2.5	1.543
	8 Days	2.9	1.535
	9Days	3.1	1.798
pH6	6 Days	1.8	1.735
	7 Days	2.1	1.751
	8 Days	2.6	1.97
	9Days	3	1.263
pH8	6 Days	0.4	2.075
	7 Days	1.2	2.085
	8 Days	0.9	1.479
	9Days	1.3	1.062
pH10	6 Days	0.1	0.954
	7 Days	0.7	0.513
	8 Days	0.2	1.678
	9Days	1	1.208



This graph shows that *Aspergilusniger* develops high amounts of citric acid at 4 pH after 9 days of fermentation from 5 gm of coconut husk.

3.2 Effect of Different Substrate Concentration on Citric Acid Production

The Aspergillus niger and Aspergillus flavus were produced higher citric acid at the temperature 30^{o}c. The higher amount of citric acid production has been seen on 15gram substrate concentration. But *Aspergillus niger* is the best citric acid produces as compare to *Aspergillus flavus.* Perhaps this is due to the fact that the substrate can contain sugars that at higher concentrations inhibit the production of citric acid. Hossain et al. and Orthafer et al. stated that citric acid production is inhibited by certain sugars, such as galactose and arabinose. This means that the concentration of 2% of the substrate could encourage the production of citric acid, but an increase of 2% would result in higher levels of certain sugars present in the pulp that are inhibitory to the production of citric acid.

3.3 Effect of Time Course of Citric Acid Production

The *Aspergillus niger* has incubated for several hours. It was produced the maximum amount of citric acid on 216 hrs, followed by 144 hrs of incubation.Similarly [30].

Reported the maximum amount of citric production occurred at 192hrs of incubation.

4. CONCLUSION

In conclusion, a solid state fermentation method has been developed for the production of citric acid from banana peels, coconut husk and rice straw by *Aspergillus niger* and *Aspergillus flavus.* A maximum citric acid production from 15gm of banana peels was obtained under optimum condition as compare to coconut husk and rice straw. This study indicates that the use of banana peels, coconut husk and rice straw for fungal production of citric acid might represent an efficient method of minimizing waste disposal problems and concomitantly producing organic acid of valuable importance for food and pharmaceutical industries [31, 32, 33].

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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