Jurnal Kejuruteraan 34(2) 2022: 317-323
https://doi.org/10.17576/jkukm-2022-34(2)-15

Effective Bioconversion of Locally obtained Apple Waste into Citric Acid using Aspergillus Niger (NRRL 567)

Faheem Akhter[a*], Shaheen Aziz[b] & Fatima Jalal[c]

[a] *Department of Chemical Engineering, Quaid-e-Awam University of Engineering, Science & Technology, Nawabshah, Pakistan*
[b] *Department of Chemical Engineering, Mehran University of Engineering & Technology, Jamshoro, Pakistan*
[c] *Zoology Department, GC University, Faisalabad, Pakistan*

**Corresponding author: faheemakhtar86@quest.edu.pk*

Received 15 July 2021, Received in revised form 17 August 2021
Accepted 19 September 2021, Available online 30 March 2022

ABSTRACT

A viable and sustainable method was used to produce citric acid. Apple waste (pomace) was used as a substrate and displayed a good efficiency for citric acid yield. The fermentation process was carried out using Aspergillus Niger (NRRL 567) strain. This fungal strain showed a competent performance in fermenting apple waste to produce citric acid. Various key parameters were analyzed and optimized such as incubation temperature, amount of substrate, ph, moisture content, nitrogen source, metal ions, methanol inducer and inoculum density. Among such parameters, the highest citric acid yield of 158 g/kg of apple pomace was achieved with the use of methanol inducer. Methanol inducer, moisture content, amount of substrate and nitrogen source were found to have significant impact on CA production. The fungal strain used in the present study is known to possess an impressive biomass fermentation capacity, as also demonstrated by the present work. In Pakistan, this strain has not been analyzed for its efficiency to produce citric acid using pomace of locally cultivated apple, hence the novelty of the present work. All the experimental work, analysis and optimization was accomplished on laboratory scale at Advanced Research Laboratory, Zoology Department, GC University, Faisalabad, Pakistan.

Keywords: SSF; apple pomace; citric acid; substrate; aspergillus niger

INTRODUCTION

Citric acid is a weak organic acid having the chemical formula $C_6H_8O_7$. Its main occurrence is in the citrus fruits like lemon and orange but it can also be produced from agro-waste such as apple pomace by microbial action using solid state fermentation (Angumeenal & Venkappayya 2013). The wide spread presence of citric acid in the animal and plant kingdom is an assurance of its non-toxic nature and it has long been used in the food, beverage, pharmaceutical and cosmetic industries (Ramesh & Kalaiselvam 2011). Citric acid is commercially produced by fungal submerged fermentation of molasses with white rot fungus (*Aspergillus niger*) due to its high productivity at low pH without secretion of toxic by-products (Dhillon et al. 2011a). Solid state fermentation (SSF) offers numerous advantages and has lower energy requirements, produces less waste water and is environment friendly as it resolves the problem of solid waste disposal (Behera 2020). There are many reports on efficient utilization of agro-industrial residues and by-products for citric acid production using less expensive substrates, such as apple and grape pomace, carrot waste, orange and pineapple waste, cassava bagasse, coffee husk, kiwifruit peel, rice and wheat bran (Dhillon et al. 2011a). These residues are very well adapted to SSF due to their cellulosic and starchy nature. The substrate is saturated to about 70% moisture and inoculated with the microorganism. The pH of the process is normally adjusted to 4.5–6.0 and incubated at 28–30°C depending upon the microorganism used (Zafar et al. 2020). Apple pomace is the residue left after juice extraction and constitutes about 25–35% of the weight of fresh fruit. It contains 12.3% fermentable sugar and is rich in carbohydrates, but its protein content is very low (Dhillon et al. 2011b)such as moisture content and inducer (ethanol and methanol. Apple pomace contains 85% carbohydrates (76% natural sugar and 9% uronic acid) and 15% w/v protein (Joshi & Attri, 2006). Water soluble components of apple pomace are composed of monooligosaccharide and water soluble polysaccharide, and water insoluble components including pectic substances, hemicelluloses and cellulose and the total composition ratio is 56:1:17:15:11 in order (Dhillon et al. 2011a). At present, most of the pomace left after juice extraction is dumped on land despite the increasing disposal problem and efforts for by-product utilization (Joshi & Attri 2006). Because of its physical nature, apple pomace is not readily amenable to submerged fermentation and it is necessary to dilute the pomace with water, and yield of citric acid from such a dilute pomace mash is too small to recover economically. In the present study an SSF method is reported for the production of citric acid from apple pomace left after

juice extraction. Samples of apple (*Golden*) pomace were obtained from local juice processing shops.

Citric acid is one of the most important acids due to its diverse uses and applications as shown in Table 1 (Vandenberghe et al. 2016). As per one of the estimates, the global demand of citric acid has reached to 1.7 million tons per year and this rate is increasing by 5% each year (Ciriminna et al. 2017). Just like other countries, citric acid demand is growing every year in the developing country like Pakistan. Unfortunately, Pakistan has failed to produce citric acid using its local resources. Instead, citric acid is imported from other countries, mainly China. In 2008 alone, Pakistan imported 12,341 tons of citric acid from china and therefore spent a huge amount of 12.2 million dollars on import (Nadeem et al. 2014). This demand has been increasing by almost 15% each year since 2006 as shown in the Table 2. Therefore, the present study aimed to unveil the fermenting potential of *Aspegillus Niger* (NRRL 567) strain to produce citric acid using locally cultivated apple pomace and to encourage the local production. Moreover, it resolves the solid waste disposal issue by following an environment friendly method, hence eco-friendly. All the experimental work of the present study was conducted at and with the help of Advanced Research Lab at the Zoology Department, GC University, Faisalabad. They provided all the necessary materials and gave access to all the required equipment to accomplish this work.

TABLE 1. Applications of citric acid

Industry	Applications
Beverages	Provides tartness and complements fruits and berries flavors. Increases the effectiveness of antimicrobial preservatives. Used in pH adjustment to provide uniform acidity.
Jellies, Jams and Preserves	Provides tartness. pH adjustment.
Candy	Provides tartness. Minimizes sucrose inversion. Produces dark color in hard candies. Acts as acidulant.
Frozen Fruits	Lowers pH to inactivate oxidative enzymes. Protects ascorbic acid inactivating trace metals.
Dairy Products	As emulsifier in ice creams and processed cheese. Acidifying agent in many cheese products and as an antioxidant.
Fats and Oils	Synergist for other antioxidants as sequestrant.
Pharmaceuticals	As effervescent in powders and tablets in combination with bicarbonates. Provides rapid dissolution of active ingredients. Acidulant in mild astringent formulation
Cosmetics and toiletries	pH adjustment, antioxidant as a metallic ion chelator, buffering agent.
Industrial Applications	Sequestrant of metal ions, neutralizant, buffer agent
Metal cleaning	Removes metal oxides from surface of ferrous and nonferrous metals, for preparational and operational cleaning of copper and iron oxides.
Others	In electroplating, copper plating, metal cleaning, leather tanning, printing inks, bottle washing compounds, floor cement, textiles, photographic reagents, concrete, plaster, refractories and moulds, adhesives, paper, polymers, tobacco, waste treatment, etc.

Souce: Angumeenal & Venkappayya (2013); Dhillon et al. (2011a); Vandenberghe et al. (2016)

TABLE 2. Citric acid demand and import in Pakistan

Year	Value Total Imports (US$ M)	Quantity of Imports from China (US$ M)	Value of Imports from China (US$ M)	Quantity of Imports from China (Kg) (approx.)	Unit-value US$ per Kg
2005	6.6	8,130	4.7	6,391,832	0.80
2006	6.4	8,171 (5%)	5.5	7,470,395	0.77
2007	9.5	10,795 (32%)	8.0	9,842,410	0.85
2008	12.2	12,341 (14%)	11.2	11,447,169	1.00

Souce: Nadeem et al. (2014)

MATERIALS & METHODOLOGY

MICROORGANISM

Aspergillus Niger (NRRL567) was provided by Advanced Research Lab, Zoology Department, GC University Faisalabad. The choice of this strain was due to its hyper production capacity (Lotfy et al. 2007). The obtained pure culture in Eppendorf was stored and preserved in a freezer until further use.

SUBSTRATE

To prepare the substrate, apples of "Golden" breed were bought from local market, their juice was extracted and the pomace (solid residue) was sun dried for 7 days. Pomace was further dried in oven at 100 °C for 4 hours followed by grinding in a Ball Mill to obtain powdered form. To initiate fermentation, all the flasks were filled with 50 mL broth/15 g dry pomace, covered airtight, autoclaved and incubated (Dhillon et al. 2011b).

CULTURE SPREADING

The preserved culture of pure *Aspergillus Niger* (NRRL 567) was extended by cultivating on the PDA media Petri plates via streaking method (Behera 2020) and incubated at 30 ^{0}C for 96 hours. The cultures were renewed every 4 weeks.

INOCULUM PREPARATION

Culture inoculum is a method of growing microorganism, *Aspegillus Niger* (NRRL 567) in this case, in a liquid media. The inoculum was prepared by separating the broth of the boiled potatoes and mixing dextrose as 2% v/v. Dextrose broth was autoclaved, poured in the flasks and inserted with *Aspergillus Niger* culture already grown on petri plates using loop, ethanol and burner. Flasks were then placed in the Shaking Incubator at 30 ^{0}C for 24 hours (Adeoye et al. 2015).

SOLID STATE FERMENTATION (SSF)

In order to initiate the SSF, substrate broth and inoculum were mixed in flasks and shaken vigorously to ensure proper mixing. The flasks containing the SSF media (Substrate Broth + Inoculum) were wrapped properly with aluminum foil and incubated (Joshi & Attri 2006).

HPLC

High Performance Liquid Chromatography is a technique to make quantitative and ultimate analysis of different liquids present in a mixture (Zafar et al. 2020). It works on the principle that each component present in a mixture has a different flow rate thus determining the component on the basis of flow rate. In the present study, the citric acid was analyzed using HPLC (Perkin Elmer Series 00, USA). Sulfuric acid (0.001 N) in HPLC grade water at 6 ml per min was used as a mobile phase. The components were detected by refractive index detector and quantified using Tubochrom 4 software provided by the suppliers. All the chemicals were of the HPLC grade. The fermented media was shaken vigorously and filtered through filter paper. The liquid filtered extract was subjected to centrifuge (Model Biofuge 13, Heraeus, SEPATECH, Germany) at 8000 rpm for 5 minutes. 50 µml of the clear solution was taken as a sample and injected in to HPLC. The response was recorded and calculated by the integrating recorder which determined the amount of citric acid by measuring the peak area with reference to the peak area of standard injection.

OPTIMIZATION OF PARAMETERS

Different parameters affecting the citric acid production were examined and evaluated for their optimum values. Total of 7 parameters were analyzed for their optimum values; Incubation Temperature, Amount of Substrate, pH, Moisture Content, Nitrogen Source & Metal Ions, Methanol Inducer and Inoculum Density. Each of the parameters were explored to reach optimum values for the maximum citric acid production. The method employed for altering the parameters to make optimizations is known as "one factor at a time", in which one dependent variable is changed whereas the remaining ones are kept constant. The multifactor designing consumes a lot of time and efforts to reach a one-handed optimization due to interactions among the factors. During the fermentation process, the overall effect of variables is affected. To optimize each of the parameters, a total of 35 flasks were prepared each containing fermented media (Inoculum+Substrate). The amount of substrate in each flask was 15 grams of the dried apple pomace whereas 50 ml of the inoculum was mixed. Each flask was ready made with the method mentioned earlier in this paper. For each of the samples, the citric acid yield was calculated using HPLC (Lotfy et al. 2007).

RESULTS & DISCUSSION

OPTIMIZATION OF PARAMETERS

Fermentation process is affected by various parameters. The optimum values of such parameters ensure the highest yield of citric acid. Hence, the key affecting parameters were analyzed to obtain their optimum values.

INCUBATION TEMPERATURE

Incubation temperature has a significant impact on the citric acid production (Ambati & Ayyanna 2001). To achieve the results, 5 prepared flasks with fermented media were incubated at different temperatures of 20, 25, 30, 35 and 40 ^{0}C for 72 hours keeping all other parameters constant. After incubation, each of the flask was mixed with 200 ml of distilled water and filtered using whatman 41 filter paper. The filtrate of each flask was subjected to HPLC to analyze citric acid yield. Figure 1 shows the results where the maximum citric acid production was achieved at 30 ^{0}C with 108 g/Kg of dry substrate. Lower or higher temperature resulted in reduced citric acid production, hence 30 °C being the optimum temperature.

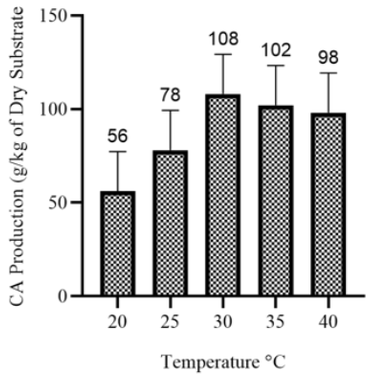

FIGURE 1. Effect of Incubation Temperature

AMOUNT OF SUBSTRATE

Effect of substrate was analyzed by preparing 6 flasks containing the fixed quantity of 50 ml of inoculum and varying quantities of substrate as 10, 12, 15, 18, 20 and 25 grams (Amato et al. 2020). All the samples were incubated at constant temperature of 30 ^{0}C for 72 hours. After incubation, each flask was mixed with distilled water, filtered and subjected to HPLC for citric acid concentration. As shown in the Figure 2, the highest citric acid concentration was achieved when 15 grams of substrate was used for SSF while keeping the inoculum quantity constant with 50 ml.

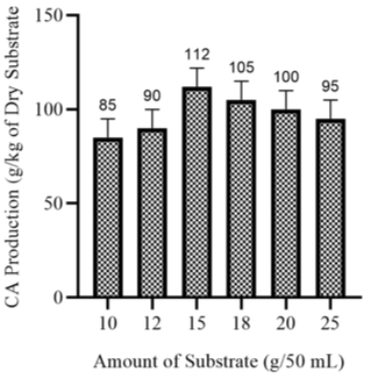

FIGURE 2. Effect of Amount of Substrate

pH

Microbial growth need a suitable environment for the fermentation which depends on pH (Yu et al. 2018). To analyze the optimum pH, 5 samples with different pH of 3.0, 4.0, 5.0, 6.0 and 6.5 were prepared and processed for fermentation. Substrate, inoculum and temperature fixed as 50 ml, 15g and 30 °C respectively. pH was changed using 0.1N NaOH and 0.1N HCL. As the Figure 3 shows, the highest production of CA was achieved at pH 5.0 with 110 g/Kg of dry substrate. Lower or higher pH values reduced the CA yield.

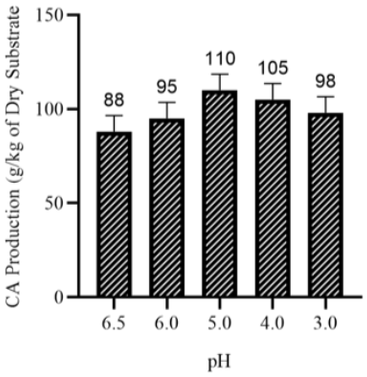

FIGURE 3. Effect of pH

MOISTURE CONTENT

Moisture content is essential for fermentation. Optimum moisture content for microorganism like *Aspergillus Niger* is very crucial as this fungal strain is shown to grow and function efficiently in an optimized humid environment (Yu et al. 2018). 5 prepared samples were varied in their moisture content as 60%, 70%, 75%, 80% and 85% while the remaining parameters kept constant. The results shown in the Figure 4 reveal the highest CA production achieved at 75% moisture content. The lowest production was observed at 60% moisture content. Above or below 75% moisture content, the yield was reduced.

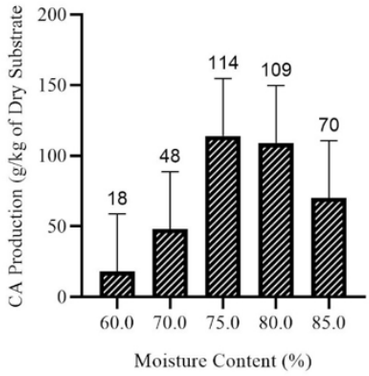

FIGURE 4. Effect of Moisture Content

NITROGEN SOURCE & METAL IONS

Nitrogen Source such as ammonium salts and metal ions such as magnesium and copper are observed to have a great impact on CA production (Francisco et al. 2020). Present study used ammonium nitrate, magnesium sulphate, dihydrogen phosphate and copper sulphate as nitrogen and metal ion sources. Concentration used for each of the compound is given in the table 3. Total of 6 flasks were prepared with 15g of substrate and 50 ml of inoculum. 3 flasks contained nitrogen and metal ion compounds whereas the remaining 3 were without the compounds. Results shown in figure 5 indicate a higher yield of citric acid in the flasks containing nitrogen and metal ions compared to flasks missing the compounds. As shown in table 4, the mean increase yield of 11.86% was observed in the flasks containing nitrogen source and metal ions vs flasks without the source and ions.

TABLE 3. Concentration of nitrogen source & metal ions

Compounds	Concentration
Ammonium Nitrate	0.45%
$MgSO_4$	0.025%
H_2PO_4	0.1%
Copper Sulphate	0.004%

TABLE 4. Effect of nitrogen source and metal ions on citric acid production after 72 hours of incubation at 30 degree centigrade

S. No	NH_4NO_3 Concentration (g/50mL)	$MgSO_4$ Concentration (g/50mL)	H_2PO_4 Concentration (g/50mL)	$CuSO_4$ Concentration (g/50mL)	Citric Acid Production (g/Kg of dry substrate)	Mean Increase in Yield %
1	Nil	Nil	Nil	Nil	98	11.86
2	Nil	Nil	Nil	Nil	100	
3	Nil	Nil	Nil	Nil	97	
4	0.06	0.006	0.025	0.001	109	
5	0.06	0.006	0.025	0.001	113	
6	0.06	0.006	0.025	0.001	108	

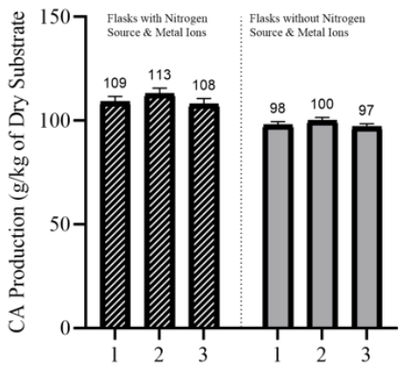

FIGURE 5. Effect of nitrogen source and metal ions

METHANOL INDUCER

Inducers are seen to have a great influence on citric acid production (Francisco et al. 2020). Present study used methanol as an inducer to observe its impact on CA production. A total of 10 flasks were prepared, 5 containing inducer and 5 without the inducer. Amount of inducer in 5 flasks was 4% (v/w). All the flasks were incubated at 30 ^{0}C for 72 hours, mixed with distilled water, filtered and subjected to HPLC for CA yield. As shown in figure 6, methanol inducer is found to have a significant impact over CA production. Furthermore, methanol inducer produced the highest citric acid yield so far in this study with 158 g/Kg of dry substrate, as shown in the figure 7 showing the HPLC graph of the sample with highest yield.

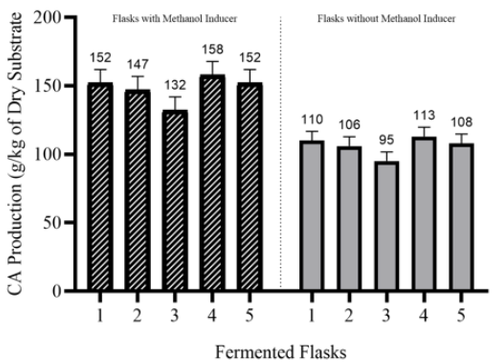

FIGURE 6. Effect of Methanol Inducer

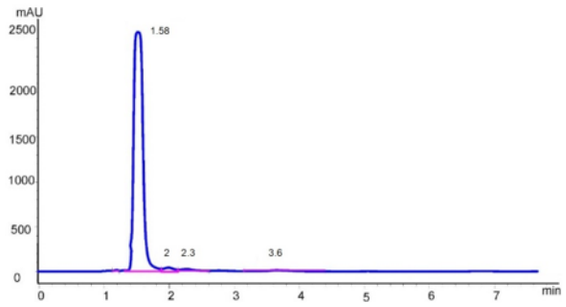

FIGURE 7. HPLC Graph

INOCULUM DENSITY

To analyze the effect of inoculum density over CA production, 5 flasks were prepared, each containing the different densities as $1x10^3$, $1x10^4$, $1x10^5$, $1x10^6$ and $1x10^7$ and fermented (Dhillon et al. 2011b). Inoculum Densities were measured using Haemocytometer (Bright Line Z359629-1EA). As indicated by the results (Figure 8), the highest CA concentration of 109 g/kg was observed in the flask containing $1x10^5$ spores/mL.

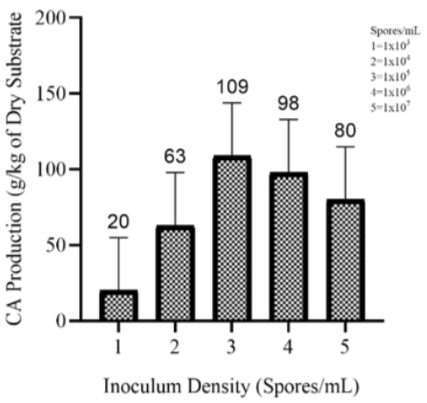

FIGURE 8. Effect of Inoculum Density

DISCUSSION

In the present study, a considerable amount of citric acid is produced via *Aspegillus Niger* (NRRL 567) strain using apple pomace as a substrate. When the apple pomace is added in the production medium, the pH is found to reduce slowly from 6.5 to 3.5 on the tenth day, indicating citric acid production.

It is reported in the present study that the factors affecting CA production include incubation temperature, amount of substrate, pH, moisture content, nitrogen source and metal ions, methanol inducer and inoculum density, which resonates with the results observed by other studies (Joshi & Attri, 2006; Lotfy et al. 2007; Yu et al. 2018; Zafar et al. 2020). The optimum incubation temperature for maximum CA yield was found to be 30 degree centigrade. Lower or higher temperature showed reduced yield. It may be due to high temperature causing denaturation of enzyme-citrate synthase and accumulation of other by-product acids such as oxalic acid and enzyme catabolite repression (Ambati & Ayyanna 2001; *Method for Increasing Citric Acid Production by Aspergillus Niger Fermentation* 2018). Furthermore, concentration of substrate affects the CA yield. The type of carbohydrates utilized *by Aspergillus* Niger to produce citric acid is sucrose, which is present in apple pomace. 15 g of substrate produced the highest yield when 50 mL of inoculum was used for fermentation. This may be due to the fungal inoculum density used, hence able to consume all the sucrose (Zafar et al. 2020). Inoculum prepared with higher fungal density may give a varying optimum value for substrate concentration. Similarly, pH affected the CA production, optimum pH being 5. It may apply specifically for the fungal strain used in the present study, while the pH values may differ depending on the other strains used, usually giving high yields between pH 5-6 (Behera, 2020). Moisture content was reported to affect CA production, optimum being 75% *for Aspregillus* Niger (NRRL 567) strain. Compared to 60 % moisture content, 6 times higher citric acid yield was observed at 75% moisture. Lower or higher moisture content produced lower CA yield, which may be because of metabolic activities coupled with consequent product synthesis having adverse effects in fungal SSF (Dhillon et al. 2011b). Nitrogen source was supplied through ammonium salt whereas metal ion compounds of magnesium, phosphate and copper were used as metal sources. A total of 11% enhanced citric acid yield was observed when using nitrogen and metal ion sources. Fungus uses nitrogen source and metal ions as nutrients for growth and biosynthesis of citric acid, hence crucial during fermentation (Francisco et al. 2020). Methanol inducer was found to have a significant impact on CA production. The highest citric acid yield of 158 g/kg of dry substrate was achieved using methanol inducer. Methanol inducer showed 29% enhanced citric acid production. As shown in the previous studies, alcohol induced fermentation leads to enhanced citric acid production (Francisco et al. 2020). Furthermore, exploring inoculum density, highest CA yield was indicated to be 109 g/kg of dry substrate when $1x10^5$ spores/mL were used.

CONCLUSION

In the present study, apple pomace was used as a substrate to produce citric acid via solid state fermentation. *Aspergillus Niger* (NRRL 567) used to carry out fermentation is shown to possess a significant potential for citric acid production. Various parameters were shown to affect the citric acid production such as incubation temperature, amount of substrate, pH, moisture content, nitrogen source, metal ions, methanol inducer and inoculum density. Each of these parameters produced varying amounts of citric acid, hence optimized. The highest yield of 158 g of citric acid per kg of apple pomace was observed when methanol inducer was used and all other parameters kept at constant optimum values. Furthermore, the apple waste is usually disposed, hence the present study can be used to produce citric acid on a commercial scale by utilizing the waste of locally cultivated apple. This can not only reduce the biomass but also reutilize it to produce a value-added product like citric acid. Authors believe that the commercial scale production of citric acid via the present work could also be cost friendly. Moreover, the commercial scale bio-reactors could be established besides apple processing industries to process the apples waste for citric acid production.

ACKNOWLEDGEMENT

Authors are thankful to Advanced Research Lab, Zoology Department, GC University, Faisalabad for their assistance, support and provision of lab access for the present study.

DECLARATION OF COMPETING INTEREST

None

REFERENCES

Adeoye, A. O., Lateef, A. & Gueguim-Kana, E. B. 2015. Optimization of citric acid production using a mutant strain of Aspergillus niger on cassava peel substrate. *Biocatalysis and Agricultural Biotechnology 4*(4): 568–574.

Amato, A., Becci, A., & Beolchini, F. 2020. Citric acid bioproduction: the technological innovation change. In *Critical Reviews in Biotechnology* (Vol. 40, Issue 2, pp. 199–212). Taylor and Francis Ltd.

Ambati, P., & Ayyanna, C. 2001. Optimizing medium constituents and fermentation conditions for citric acid production from palmyra jaggery using response surface method. *World Journal of Microbiology and Biotechnology 17*(4): 331–335.

Angumeenal, A. R., & Venkappayya, D. 2013. An overview of citric acid production. *LWT - Food Science and Technology 50*(2): 367–370.

Behera, B. C. 2020. Citric acid from Aspergillus niger: a comprehensive overview. *Critical Reviews in Microbiology 46*(6): 727–749.

Ciriminna, R., Meneguzzo, F., Delisi, R., & Pagliaro, M. 2017. Citric acid: Emerging applications of key biotechnology industrial product. In *Chemistry Central Journal* (Vol. 11, Issue 1). BioMed Central Ltd.

Dhillon, G. S., Brar, S. K., Verma, M., & Tyagi, R. D. 2011a. Utilization of different agro-industrial wastes for sustainable bioproduction of citric acid by Aspergillus niger. *Biochemical Engineering Journal 54*(2): 83–92.

Dhillon, G. S., Brar, S. K., Verma, M., & Tyagi, R. D. 2011b. Enhanced solid-state citric acid bio-production using apple pomace waste through surface response methodology. *Journal of Applied Microbiology 110*(4): 1045–1055.

Francisco, J. C. E., Rivera, W. L., & Vital, P. G. 2020. Influences of carbohydrate, nitrogen, and phosphorus sources on the citric acid production by fungal endophyte Aspergillus fumigatus P3I6. *Preparative Biochemistry and Biotechnology 50*(3): 292–301.

Joshi, V., & Attri, D. 2006. Solid state fermentation of apple pomace for the production of value added products. *Indian Journal of Natural Products and Resources (IJNPR) 5*(4): 289–296. https://books.google.com.pk/books?hl=en&lr=&id=-Z5voRIHZ54C&oi=fnd&pg=PA180&dq=12.%09Joshi,+V.+K.,+and+Devender+Attri.+%22Solid+state+fermentation+of+apple+pomace+for+the+production+of+value+added+products.%22+Pollution+in+Urban+Industrial+Environment+:+1

Lotfy, W. A., Ghanem, K. M., & El-Helow, E. R. 2007. Citric acid production by a novel Aspergillus niger isolate: II. Optimization of process parameters through statistical experimental designs. *Bioresource Technology 98*(18): 3470–3477.

Method for increasing citric acid production by Aspergillus niger fermentation. 2018.

Nadeem, S., Ahmed, I., Abdul Muttalib, I., Tufail, M., & Khan, M. S. 2014. Citric acid future prospects for Pakistan, a short review. *Applied Mechanics and Materials* 625: 61–64.

Ramesh, T., & Kalaiselvam, M. 2011. An Experimental Study on Citric Acid Production by Aspergillus niger Using Gelidiella acerosa as a Substrate. *Indian Journal of Microbiology 51*(3): 289–293.

Vandenberghe, L. P. S., Rodrigues, C., de Carvalho, J. C., Medeiros, A. B. P., & Soccol, C. R. 2016. Production and Application of Citric Acid. *Current Developments in Biotechnology and Bioengineering: Production, Isolation and Purification of Industrial Products* 557–575.

Yu, B., Zhang, X., Sun, W., Xi, X., Zhao, N., Huang, Z., Ying, Z., Liu, L., Liu, D., Niu, H., Wu, J., Zhuang, W., Zhu, C., Chen, Y., & Ying, H. 2018. Continuous citric acid production in repeated-fed batch fermentation by Aspergillus niger immobilized on a new porous foam. *Journal of Biotechnology* 276–277: 1–9.

Zafar, M., Arshad, F., Faizi, S., Anwar, Z., Imran, M., & Mehmood, R. T. 2020. HPLC based characterization of citric acid produced from indigenous fungal strain through single and Co-Culture fermentation. *Biocatalysis and Agricultural Biotechnology* 29.