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Original Article

PRODUCTION AND OPTIMIZATION OF CITRIC ACID BY *ASPERGILLUS NIGER* USING MOLASSES AND CORNCOB

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ABSTRACT

Objective: The present study made an attempt to produce commercially valuable citric acid by the fungal strain *Aspergillus niger* from molasses and corncob using submerged fermentation, as the best alternative to the sugar substrate.

Methods: Three types of production media were prepared including control (sucrose) by following standard fermentation conditions. The acid production was indicated by the reduction of pH levels. The citric acid content and residual sugars of the final hydrolysate were estimated by the Marrier and Boulet method and Anthrone Sulphuric acid method respectively.

Results: The control production medium gave yield of 4.6 milligrams per milliter (mg/ml) at pH 3.0 on 10th day. The medium containing molasses and other compositions gave the yield of 10.4 mg/ml, whereas corncob medium and other compositions gave the yield of 5.3 mg/ml at pH 2.5. The medium containing molasses and corncob separately with 5 percent (%) sucrose gave the highest yield of 12.6 mg/ml and 6.7 mg/ml at pH 3.0 respectively. Different factors affecting citric acid production by fermentation were also studied. Sucrose was found superior for maximum citric production at optimum incubation temperature at 30 degree Celsius (^{0}C). The nitrogen supplements, ammonium sulphate and ammonium chloride at a concentration of 0.25 % and 0.5% respectively gave the highest yield, whereas the methanol concentration of 2% was found optimum for obtaining maximum yield of citric acid.

Conclusion: Molasses and corncob when replaced with sucrose in the fermentation medium produced significant amount of citric acid. The results imply the effective use of molasses and corncob as an alternative substrate for the production of commercially valuable, citric acid with a cost effective approach.

Keywords: Citric acid, Pharmaceutical, Molasses, Corncob, *Aspergillus niger,* Submerged fermentation.

INTRODUCTION

Citric acid (2-hydroxy-1, 2, 3-propanetricarboxylic acid) is a nearly universal intermediate and important commercial product of metabolism and its traces are found in virtually all plants and animals tissues. It exists widely in the nature and present as a kind of fruit acid in lemon, orange, pineapple, plum, peas, peach and in animal bones, muscles and blood. It has many applications in food, pharmaceutical and cosmetic industries as an acidulant, flavor, enhancer, preservative, antioxidant and emulsifier and chelation agent [1]. It is accepted worldwide as GRAS (Generally Recognized As Safe), approved by the Joint FAO/WHO Expert Committee on Food Additives [2].

Citric acid derives its name from the Latin word citrus, the citrus tree, the fruit of which resembles a lemon. The acid was first isolated from lemon juice in 1784 by Carl Scheele, a Swedish chemist (1742–1786) [3]. Wehmer (1893) first showed that a "Citromyces" (now *Penicillium*) accumulated citric acid in a culture medium that contained sugars and inorganic salts [4].

In 1917, American food chemist James Currie discovered certain strains of the mold *Aspergillus niger* could be efficient citric acid producers, and the pharmaceutical company Pfizer began the industrial production using this technique [5]. Several other synthetic routes using different starting materials have since been published, but chemical methods have so far proved uncompetitive with fermentation mainly because the starting materials are worth more than the final product [6]. Many other microorganisms have since been found to accumulate citric acid including strains of *A. niger, A. awamori, A. nidulans, A. fonsecaeus, A. luchensis, A. phoenicus, A. wentii, A. saitoi, A. flavus, Absidia sp., Acremonium sp., Botrytis sp., Eupenicillium sp., Mucor piriformis, Penicillium janthinellum, P. restrictum, Talaromyces sp., Trichoderma viride and Ustulina vulgaris* [7, 8]. In addition to molds, several yeast strains are now known to produce large amounts of citric acid from n-alkanes and carbohydrates [9].

Although many microorganisms can be used to produce citric acid, *Aspergillus niger* still remains the main industrial producer exploited for citric acid production [10, 11]. This is because of the fact that the organism has the capacity to utilize varieties of substrates due to its well-developed enzymatic system. *Aspergillus niger* normally a haploid fungus producing white septate hypha which is profusely branched. It produces black conidia, which are found in the chain arising from secondary sterigmata [12].

Citric acid fermentation is one of the rare examples of industrial fermentation technology where academic discoveries have worked in tandem with industrial know how, in spite of an apparent lack of collaboration, to give rise to an efficient fermentation process. Citric acid is one of the most commonly used organic acids in food and pharmaceutical industries having worldwide demand of about 6.08×10^5 ton per year. Citric acid is produced commercially employing various inexpensive and readily available raw materials like molasses, carob pod extract, rape seed oil, apple and grape pomace, kiwi-fruit peel, mandarine orange and brewery wastes have been used as carbon substrates in terrestrial environment [13]. In addition, corncob and other cheap starchy raw material can also be exploited for citric acid production, which will have some cost effective impact on our economy.

The present investigation was therefore, undertaken with a view to determine the feasibility of using raw and cheap materials such as molasses and corncob for citric acid production and optimization under fermentation condition on these substrates.

MATERIALS AND METHODS

Microorganism

Citric acid producing strain (NCIM 1055) was used in the present study. It was obtained from the National Collection of Industrial Microorganism (NCIM), Pune, and Maharashtra. The culture was maintained on Potato Dextrose Agar (PDA) plates at 4 ^{0}C.

Substrate

Substrates used for production of citric acid were (a) cane molasses (b) corncob. Cane molasses was obtained from V. S. S. K. Distillery, Sangli (Maharashtra, India). The other substrate, corncob obtained from the local market was used in the experiments for the comparative study.

A. Pretreatment of molasses

Cane molasses was mixed with an equal quantity of double-glass distilled water. The diluted mixture was kept at 40 ^{0}C for 5 hours (h) followed by centrifugation at 2000 revolutions per minute (rpm) for 5 minutes (min). The clarified liquid was used for the pretreatment methods, given below-

Pretreatment of molasses was done by using Tricalcium phosphate [TCP] method followed Tricalcium Phosphate with Hydrochloric Acid Treatment (TCPH) method [14]. The requisite amount of such pretreated molasses was added to the production media replacing sucrose as a carbon source or in combination with sucrose.

Tricalcium phosphate treatment (TCP)

The pH of molasses was adjusted to 7.0 by addition of 0.1N sodium hydroxide (NaOH) solution and then treated with 2% weight/volume (w/v) tricalcium phosphate followed by autoclaving at 105^{0}C for 5 min. The mixture was cooled and centrifuged at 3000 rpm for 15 min. The insoluble precipitate was discarded and the supernatant was used for further treatment.

Tricalcium phosphate with hydrochloric acid treatment (TCPH)

The pH of TCP-treated liquor was adjusted to 2.0 by the addition of 0.1N hydrochloric acid (HCL) followed by vigorous shaking. The mixture was allowed to stand for 6 h for sedimentation of coarse particles. After centrifugation at 3000 rpm for 20 min, obtained clear supernatant liquid was diluted to 14% sugar level.

B. Pretreatment of corncob

Corncobs were dried in an oven at 40 ^{0}C and pulverized. Dilute acid hydrolysis of corncob powder was done using 0.1N HCL. The hydrolysis reaction was quenched by adding 1.0 M NaOH. Then pH was adjusted to 7 by addition of 0.1N NaOH and hydrolysate was autoclaved 121^{0}C, 15 pounds (lbs) for 20 min. After acid hydrolysis, the reaction mixture was filtered and the filtrate was collected for citric acid production [15].

Fermentation medium and Inoculum preparation

The production (control) medium for citric acid (Composition gram per liter [g/l]: Sucrose-140, Ammonium nitrate-2.23, Dipotassium orthophosphate-1, Magnesium sulphate-0.23) was prepared. Conidial suspensions of *A. niger* obtained from culture grown on PDA slants at 30 ^{0}C for 4-6 days were washed with sterilized 0.8% Tween 80 solution by vigorous shaking. The conidial count was made by Haemocytometer (Sigma Aldrich). One ml of conidial suspension (6.5 × 10^6 conidia/ml) from the slant culture was aseptically transferred to a production medium. The inoculated flask was placed into a rotary shaker at 30^{0}C and 200 rpm for 10 days for proper aeration. The pH was checked in aseptic conditions at every 2 days of interval. Submerged fermentation was carried out in triplicates for each type of medium and conducted in 250 milliliter (ml) Erlenmeyer flasks. At different time intervals, fractions of fermented broth were collected; filtered and thus obtained filtrate was used for the further investigation. Initially the sugars: Sucrose, Glucose and Maltose as sole carbon source were screened for the highest production of citric acid.

Citric acid production using molasses and corncob

Three experiments were carried out to find out the possibilities of using molasses and corncob as an alternate substrate for citric acid production. The pretreated molasses and corncob were analyzed for their sugar content (carbohydrate estimation) [16, 17] and then added separately at requisite volume in production medium. Control production medium was maintained in the first experiment, in the second experiment, sucrose was replaced by molasses [14% volume/volume (v/v)] and corncob (14 % w/v) respectively, and in the third experiment, 5 % (7.5 gram) sucrose was added to the molasses and corncob in the production medium separately for observing the enhanced activity molasses and corncob respectively. The fermentation conditions were maintained carefully throughout the process.

Recovery process

After the completion of the fermentation process, the incubated broth was filtered for the separation of pellet form of fungal culture and the fermentation broth, which acts as the source of citric acid. Lime ($Ca(OH)_2$) was added to the fermentation broth to allow precipitation of citric acid in the form of calcium citrate. Again, the precipitate was treated with dil. sulphuric acid (H_2SO_4) to precipitate insoluble calcium sulphate, and then filtered. The precipitated solution containing citric acid was purified by passing through a column of carbon granules. The obtained solution was evaporated for getting purified citric acid in crystal form [21].

Optimization of citric acid productivity

In order to optimize the citric acid production, different chemical and physical parameters such as sugar concentration, nitrogen source, concentration of methanol and incubation temperature were screened in case of control production medium as well as from an alternate substrate medium. Effect of nitrogen supplements was studied by adding ammonium sulphate and ammonium chloride (0.25-1.0 %) to the basal medium. Methanol (0-5 %) was also added to the flasks before fermentation to check its effect on citric acid production. To analyze the effect of incubation temperature on citric acid production the flasks containing production media after inoculation were incubated at different temperatures (25 ^{0}C-35 ^{0}C).

Qualitative analysis for citric acid

Qualitative detection of citric acid in the filtrate obtained after fermentation was done by thin layer chromatography (TLC) for confirming the production of citric acid by fermentation process.

Analytical method

The citric acid content and residual sugars of the final hydrolysate were estimated by the Marrier and Boulet method [18] and Anthrone Sulphuric acid method respectively [19]. Biomass and pH values were determined according to Association of Official Analytical Chemists, (AOAC) 1995 [20].

Statistical analysis

All the experiments were repeated 3 times till the data obtained were statistically valid as an analysis of variance was carried out for all data using Graph Pad software (GraphPad InStat version 3.00, GraphPad Software, San Diego, CA, USA).

RESULTS AND DISCUSSION

Screening of molasses and corncob

Cane molasses contained 20% water, 62% sugar; non-sugar contents 10% and 8% inorganic salts (ash contents), making a blackish homogenous liquid with high viscosity. Ash contents include ions such as magnesium (Mg), manganese (Mn), aluminum (Al), iron (Fe) and zinc (Zn) in a variable ratio. The corncob powder used throughout this study was found to contain the following: crude protein 3.0%; crude fat 0.57%; crude fiber 34.0%; carbon content 44.0%; sugars content 38.0%; ash content 1.5% and moisture 8.0%. The screening indicated that molasses and corncob are a suitable alternative for biosynthesis of citric acid by *A. niger* due to its nutritional content [22].

Fermentation process was carried out carefully under maintained fermentation parameters up to the end of the process for production of citric acid. Citric production was obtained using molasses and corncob using *A. niger* with optimum production at the 10th day. Earlier studies report that the factors affecting citric acid production by fermentation includes the nutrient composition of the media, environmental conditions, deficiency of manganese [23], types and more/less (±) concentration of sugars, chelation effect on metal ions [24], ammonium nitrate and aeration [25]. Thus, effect of different

factors on the fermentation process and yield of citric acid was also studied. After recovery process, a purified citric acid was obtained.

Effect of carbon source and sugar concentration

The citric acid production was analyzed by using carbon sources such as maltose, sucrose and glucose shown in table 1. Maximum citric acid (4.6 mg/ml) production was recorded in sucrose incorporated medium and lowest yield (3.8 mg/ml) was in the case of maltose supplemented medium.

Sucrose solution (14%) was found better moisture level for citric acid fermentation [26]. A concentration higher than 14-18% however, leads to a greater amount of residual sugar making the process uneconomical and when the concentration of the media is more than needed it becomes inhibitory for citric acid production, resulting in decreased citric acid production. In the present study, molasses, corncob and mixed media were prepared with the 14% sugar concentration first and thus the high yield of citric acid was produced.

Table 1: Citric acid production using different carbon source by *A. niger* on 10th day

S. No.	Carbon source	Citric acid (mg/ml)
1	Maltose	3.8±0.06
2	Glucose	4.0±0.02
3	Sucrose	4.6±0.01

Note: Here the sole source of carbon in production (control) medium was the respective sugar (14%), rest of the components were kept unchanged. Values are mean±S. E. M (n = 3)

The fungus *A. niger* utilized the sugar compound and produced 4.6 mg/ml whereas in the second experiment molasses and corncob used as a sugar substrate in the production medium yielded 10.4 mg/ml and 5.3 mg/ml as showed in table 2. The combination of two sugars in the medium improves the yield of citric acid [27, 28]. In the third experiment, when the molasses and corncob were added with 5% of sucrose to production media separately produced 12.6 mg/ml & 6.7 mg/ml respectively as evident by the pH reduction from 6.5 to 3.0. Sucrose combines with molasses and corncob, enhances the utilization of the sugar substrate from molasses and corncob and induces the production of citric acid. A maximal citric acid production rate is obtained at 14% of the sugar in the control medium whereas the combination of the two sugars such as sugar from molasses and corncob and 5% sucrose improve the yields of citric acid up to 17% and 20% respectively than in case of individual molasses and corncob production medium (fig. 1).

Further fractions collected on 12th day (after 288 hours) revealed that the amount of citric acid obtained remained constant as that of 10th day. This concluded that, it was the maximum amount of citric acid that can be obtained from the fermentation medium, after which the mycelial mat began to dry.

Sucrose has relatively low molecular weight and it is readily transported into microbial cells for hydrolysis by intracellular enzymes [29]. This is the main reason for using sucrose-based substrate in citric acid production. The chemical nature of the sugar source has a marked effect on citric acid production by *A. niger* [30]. Sucrose is traditional commercial substrate for citric acid production although glucose, fructose and maltose have also been used as substrates for citric acid production. The increase in citric acid production and biomass values was accompanied with a steady decrease in sugar along the incubation time [31].

Table 2: Effect of different sugar concentration on citric acid production from molasses and corncob by *A. niger*

S. No.	Production media	Citric acid (mg/ml) at different time intervals (days)					
		0 (0 hrs)	2 (48 hrs)	4 (96 hrs)	6 (144 hrs)	8 (192 hrs)	10 (240 hrs)
1	Control	0	2.6±0.05	3.0±0.05	3.6±0.01	4.1±0.07	4.6±0.01
2	Molasses	0	5.8±0.02	6.9±0.01	8.2±0.02	9.6±0.05	10.4±0.12
2. a	Molasses+5% sucrose	0	6.5±0.04	7.7±0.04	9.0±0.04	11.5±0.2	12.6±0.05
3	Corncob	0	2.8±0.04	3.5±0.01	4.1±0.07	4.9±0.08	5.3±0.05
3. a	Corncob+5% sucrose	0	3.1±0.03	4.0±0.03	4.8±0.06	5.9±0.05	6.7±0.08

Note: Control production medium was maintained in the first experiment (1), in the second experiment, sucrose replaced by molasses (14% v/v) and corncob (14% w/v) respectively (2&3), and, 5% (7.0g) sucrose was added to the molasses and corncob in the production medium separately for observing the enhanced activity (2. a & 3. a) respectively in the third experiment. Values are mean±S. E. M (n = 3)

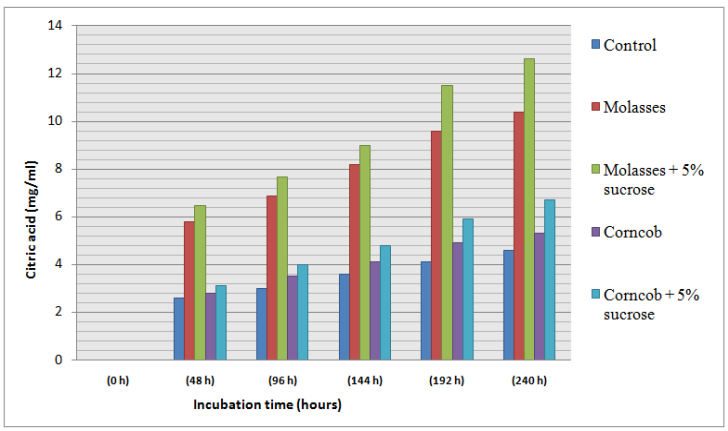

Fig. 1: Citric acid production in different media compositions at different time interval. Values are mean±SEM (n = 3)

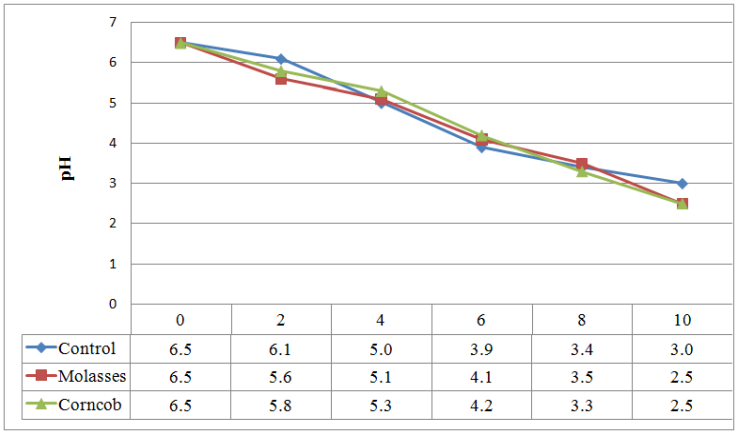

Fig. 2: Production of citric acid indicated by pH reduction. Values are mean±SEM (n = 3)

pH profile

There was a gradual reduction of pH (Fig.2) noticed in all the experiments. When the molasses and corncob were added with the production medium, the pH gets slowly reduced at regular intervals, from the initial pH of 6.5 reduced to 2.5 on the 10th day. This change of pH indicates the production of citric acid in the production medium. Actually, the acid production starts with the initial stage idiophase (between 80h-120h) of fungal growth. In this stage, the pH is generally stated at five. The yield is gradually increased and it reaches to maximum rate at the late idiophase (180–220 h) [32, 33].

In this stage, the pH must be around three in order to suppress the oxalic acid and gluconic acid formation. In control production medium, the initial pH 6.5 is gradually reduced to 3.0 during fermentation whereas 2.5 in the case of molasses, corncob and combined media. The pH value maintained at the beginning of fermentation was important for a specific biomass formation.

Effect of nitrogen supplements

Effect of nitrogen sources on citric acid productivity by *A. niger* is shown in table 3. Supplementation of the control, molasses and corncob production medium with ammonium sulphate (0.25% w/v) gave an increase in citric acid production from 4.6 mg/ml to 8.8 mg/ml, 10.4 mg/ml to 15.2 mg/ml and 5.3 mg/ml to 8.0 mg/ml respectively. In the case of ammonium sulphate, decrease in concentration (<0.25%) does not show any significant increase citric acid production and the amount of citric acid obtained remained constant. Further lowering of ammonium sulphate concentration leads to decrease in production of citric acid. Ammonium chloride (0.5% w/v) supplement also increased the yield of citric acid from 4.6 mg/ml to 8.0 mg/ml, from 10.4 mg/ml to 14.7 mg/ml and from 5.3 mg/ml to 7.1 mg/ml in control, molasses and corncob production medium respectively. Any increase or decrease other than ammonium sulphate (0.25% w/v) and ammonium chloride (0.5% w/v) concentration, resulted in the disturbance of fungal growth and subsequently citric acid production.

Table 3: Effect of nitrogen supplements on citric acid production

Concentration (% w/v)	Citric acid (mg/ml)		
	Control	Molasses	Corncob
Ammonium sulphate			
0.25	8.8±0.05	15.2±0.11	8.0±0.06
0.5	6.1±0.08	13.4±0.11	5.6±0.12
0.75	3.7±0.03	10.0±0.12	4.8±0.08
1.0	3.0±0.05	9.4±0.06	4.0±0.05
Ammonium chloride			
0.25	7.1±0.05	13.4±0.12	6.7±0.08
0.5	8.0±0.08	14.7±0.17	7.1±0.11
0.75	4.0±0.06	9.6±0.14	5.0±0.08
1.0	2.7±0.03	7.3±0.17	3.8±0.04

Values are mean±SEM (n = 3)

Nitrogen had been reported to be an important factor in fermentation processes due to an increase in C/N ratio [34]. Nitrogen constituent has a profound effect on citric acid production because nitrogen is not only important for metabolic rates in the cells but it is also basic part of cell proteins. Fermentation media for citric acid biosynthesis should consist of substrates necessary for the growth of microorganism primarily the carbon, nitrogen and phosphorus sources. Citric acid production is directly influenced by the concentration and nature of the nitrogen source, which is completely metabolized during fermentation periods. Physiologically, ammonium salts are preferred, such as urea, ammonium nitrate and sulphate, peptone, malt extract, etc. [35]. Citric acid starts to appear when the nitrogen concentration falls below a low limiting value. Acid ammonium compounds are preferred because their consumption leads to pH decrease, which is essential for the citric fermentation. However, it is necessary to maintain pH values in the first days of fermentation prior to a certain quantity biomass production. High nitrogen concentrations increase fungal growth and sugar consumption but decrease the amount of citric acid produced [36].

Effect of methanol

Maximum citric acid production was obtained at 2% methanol concentration. The citric acid production at 2% methanol concentration in control, molasses and corncob was 8.6 mg/ml, 16.3 mg/ml and 12.1 mg/ml respectively as shown in table 4. The presence of methanol in fermentation media increases citric acid production by *A. niger*. Is the inductive effect of methanol for citric acid production may be due to reduction of the inhibitory effects of metal ions. The use of low molecular weight alcohols i.e. methanol,

isopropanol as adjuncts to the culture medium greatly increased citric acid production in both surface and submerged cultures. Such uses have made it possible to ferment directly crude carbohydrate substrates [37, 38]. Methanol, ethanol, n-propanol, isopropanol neutralize the negative effect of the metals in citric acid production. Even so, optimal amount of methanol and ethanol depends upon the strain and the composition of the medium. Alcohols have been shown to act principally on membrane permeability in microorganisms by affecting phospholipids composition. Other studies showed that alcohols stimulate citric acid production by affecting growth and sporulation on space organization of the membrane or changes in lipid composition of the cell wall [39].

Table 4: Effect of methanol on citric acid production

Methanol concentration(% v/v)	Citric acid (mg/ml)		
	Control	Molasses	Corncob
1	5.1±0.12	11.1±0.08	6.0±0.12
2	8.6±0.16	16.3±0.14	12.1±0.07
3	6.3±0.23	13.1±0.12	10.1±0.17
4	3.7±0.08	8.8±0.06	5.0±0.16
5	1.7±0.14	5.0±0.11	2.8±0.12

Values are mean±SEM (n = 3)

Effect of incubation temperature

Effect of temperature on the production of citric acid is shown in table 5. A temperature of 30^{0}C was found to be the optimum for citric acid production in the present study. The mold produced only a small amount of citric acid at 25 ^{0}C in ten days. Sporulation however, was more marked at 35 ^{0}C than at lower temperatures. Temperature of a fermentation medium is one of the critical factors that have a profound effect on the production of citric acid by fermentation of agricultural wastes [40]. At low temperature, the low citric acid production was attributed to low enzyme activity. Further increase in the temperature (above 30 ^{0}C) results in decreased biosynthesis of citric acid. It may be due to high temperature that can cause denaturation of enzyme citrate synthase and accumulation of other by-product acids such as oxalic acid and enzyme catabolite repression and it also inhibits the culture development [41].

Table 5: Effect of temperature on citric acid production

Temperature (^{0}C)	Citric acid (mg/ml)		
	Control	Molasses	Corncob
25	2.2±0.11	6.4±0.12	2.4±0.15
30	4.6±0.20	10.4±0.14	5.3±0.21
35	3.9±0.08	9.0±0.17	4.1±0.13

Values are mean±SEM (n = 3)

The citric acid, residual sugar, TTA Values and biomass

Citric acid production and residual sugar profiles during fermentation by *A. niger* are given in table 6. Citric acid values steadily increased with fermentation time with a maximum of 4.6 mg/ml, 10.4 mg/ml, and 5.3 mg/ml in control, molasses and corncob production medium respectively at 10th day of fermentation. In the present study, a parallel relationship between citric acid production and the consumption of sugar was also observed. Production of citric acid approximately paralleled the consumption of sugar. At the end of the fermentation process, a significant reduction in residual sugar from 140 mg/ml to 77.12 mg/ml, 140 mg/ml to 60.24 mg/ml and 140 mg/ml to 63.24 mg/ml was observed in control, molasses and corncob production medium respectively. Further increase in the incubation period does not enhance the citric acid production. It may be due to the age of the fungus used [42]. Biomass is a fundamental parameter in the characterization of microbial growth. A steady increase in biomass throughout the fermentation period was observed with a maximum of 13.27 mg/ml, 21.8 mg/ml and 18.5 mg/ml for control, molasses and corncob respectively at the 10th day. Total titratable acidity (TTA) value of citric acid against 0.1 N NaOH was determined at different fermentation period during citric acid production by *A. niger*. TTA value was found just proportional to the utilization of sugar in the fermentation medium.

Table 6: Total titratable acidity, citric acid, residual sugar concentration and mycelial dry weight (biomass) on 10th day under submerged condition in different media

Medium	Sugar supplied (mg/ml)	TTA (ml 0.1N NaOH/ml medium)	Citric acid produced (mg/ml)	Mycelial dry weight (mg/ml)	Residual sugar (mg/ml)	Sugar utilized (mg/ml)	Sugar utilized (%)	Citric acid in relation to sugar supplied (% w/w)
Control	140.0	2.7±0.05	4.6±0.01	13.27±0.04	77.12±0.08	62.88±0.04	55.08	3.28
Molasses	140.0	5.6±0.08	10.4±0.12	21.8±0.17	60.24±0.07	79.76±0.08	43.02	7.42
Corncobs	140.0	2.7±0.05	5.3±0.05	18.5±0.17	63.24±0.05	76.76±0.06	45.17	3.78

Values are mean±SEM (n = 3)

CONCLUSION

Citric acid is commercially produced from various sources by *A. niger*. To the economic point of view, the media containing molasses and corncob with 5% sucrose gives more benefit at lower cost when compared to a control media. Molasses and corncob have been proved to have the ability to produce citric acid and it can be used as alternate and cost effective source of sugar substrate for citric acid production at industrial scale worldwide in the near future. This study propose the use of molasses and corncob for fungal production of citric acid representing an efficient perspective of minimizing molasses and corncob waste disposal problems, indirectly reducing the population health hazards faced due to indiscriminate dumping of the waste and concomitantly producing organic acids of valuable importance for food and pharmaceutical industries. Increased demand for citric acid has led to searching for high yielding fermentable strains of microorganisms and cheaper fermentation substrate in many countries. Further study of genetic amelioration of producer strain for significant optimization of all citric acid production process from alternative substrates, will prove a powerful tool in the citric acid market.

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CONFLICT OF INTERESTS

Declare None

REFERENCES

1. Rohr MA. Century of citric acid fermentation and research. Food Technol Biotechnol 1998;36:163-71.
2. Pandey A, Soccol RC, Rodriguez-Leon JA, Nigam P. Production of organic acids by solid-state fermentation. In: Solid-State Fermentation in Biotechnology-Fundamentals and Applications. Asiatech Pub Inc 2001;7:113-26.

3. Scheele C. Sammtliche physische and chemische werke, Hermbstadt, Berlin. Crells Ann 1784;2:1.
4. Wehmer C. Note sur la fermentation Citrique. Bull Soc Chem France 1893;9:728.
5. Currie JN. The citric acid fermentation of *A. niger*. J Biol Chem 1917;31:5.
6. Grimoux E, Adams P. Synthese de lácide citrique. CR Hebd Seances Acad Sci 1880;90:1252.
7. Yokoya F. Citric acid production. In: Industrial Fermentation Series, Campinas, SP. Brazil 1992;4:1-82.
8. Papagianni M. Advances in citric acid fermentation by *Aspergillus niger*: Biochemical aspects, membrane transport and modeling. Biotechnol Adv 2007;25:244-63.
9. Mattey M. Biochemistry of citric acid production by yeasts. In: Kristiansen B, Mattey M, Linden J. Citric acid biotechnology. London: Taylor and Francis 1999;7:33-54.
10. Usami S. Production of citric acid by submerged culture. Mem School Sci Eng Waseda Uni 1978;42:17-26.
11. Steinbock FA, Held I, Choojun S, Harmsen HM, Kubicek-pranz EM, Kubicek CP. Regulatory aspect of carbohydrate metabolism in relation to citric acid accumulation by *Aspergillus niger*. Acta Biotechnol 1991;11:571-81.
12. Alexopolous CJ. Introductory mycology. 4th ed. John Wiley, Sons Inc; 1962.
13. Roukas T. Fermentation of citric acid with cellulase hydrolite by *Aspergillus niger*. Appl Biochem Biotechnol 1998;74:43-53.
14. Kundu S, Panda T, Majumdar SK, Guha B, Bandyopadhyay KK. Pretreatment of Indian cane molasses for increased production of citric acid. Biotechnol and Bioeng, John Wiley & Sons, Inc; 1984;26:1114-21.
15. Amenaghawon NA, Nwaru KI, Aisien FA, Ogbeide SE, Okieimen CO. Application of Box-behnken design for the optimization of citric acid production from corn starch using *Aspergillus niger*. Br Biotechnol J 2013;3:236-45.
16. Sadashivam S, Manickam A. Biochemical methods. 3rd ed. New Age International; 1966.
17. Dubois M, Gills KA, Hamilton JK, Rober PA, Smith F. Colorimetric method determination sugars and related substances. Anal Chem 1956;28:350.
18. Marier IR, Boulet M. Direct determination of citric acid in milk with an improved pyridine-acetic anhydride method. J Dairy Sci 1958;4:1683-92.
19. Morse EE. Anthrone in estimating low concentration of sucrose. Anal Chem 1947;19:1012-3.
20. AOAC, Official methods of analysis. 16thed. Association of official analytical chemists, Washington; 1995.
21. Dubey RC. Primary metabolites: organic acids. In: A textbook of biotechnology. 4th ed. S Chand, Company Ltd, New Delhi; 2003.
22. Prescott S, Dunn A. Industrial Microbiology. CBS Publishers and Distributors, New Delhi, India; 1987;4:710-5.
23. Rohr M, Kubicek CP, Kominek J. Citric acid. In: Biotechnology, VCH Publishers: Weinheim 1983;3(1):331-73.
24. Roukas T, Kotzekiodou P. Pretreatment of date syrup to increase citric acid production. Enzyme Microb Technol 1997;21:273-6.
25. Bayraktar E, Mehmetoglu U. Production of citric acid using immobilized conidia of *Aspergillus niger*. Appl Biochem Biotechnol 2000;87:117-25.
26. Mahin AA, Hasan SM, Khan MH, Begum R. Citric acid production by *A. niger* 79/20 *Aspergillus niger* through solid-state fermentation in sugarcane bagasse. Bangladesh J Microbiol 2007;4(1):9-13.
27. Begum AA, Choudhury N, Islam MA. Citric acid fermentation by gamma ray induced mutants of *Aspergillus niger* in different carbohydrate media. J Ferment Eng 1990;70(4):286.
28. Shu P, Johnson MJ. Effect of the composition of the sporulation medium on citric acid production by *Aspergillus niger* in submerged culture. J Bacteriol 1947;54:161-7.
29. Elholi MA, Al-Delaimy KS. Citric acid production from whey with sugars and additives by *Aspergillus niger*. Afr J Biotechnol 2003;2(10):356-9.
30. Hossain M, Brooks JD, Maddox IS. The effect of sugar source on citric acid production by *Aspergillus niger*. Appl Microbiol Biotechnol 1984;19:393-7.
31. Xu DB, Madrid CP, Rohr M, Kubicek CP. The influence of type and concentration of the carbon source on production of citric acid by *Aspergillus niger*. Appl Microbiol Biotechnol 1989;30:553-8.
32. Gupta JK, Heding LG, Jorgensen OB. Effect of sugars, hydrogen ion concentration and ammonium nitrate on the formation of citric acid by *Aspergillus niger*. Acta Microbiol Acad Sci Hung 1976;23:63-70.
33. Guebel DV, Darias NVT. Optimization of the citric acid production by *Aspergillus niger* through a metabolic flux balance model. Electron J Biotechnol 2001;4(1):1-17.
34. Pandey A. Solid-state fermentation. Biochem Eng J 2003;13:81-4.
35. Grewal HS, Kalra KL. Fungal production of citric acid. Biotechnol Adv 1995;13:209-34.
36. Kiel H, Gurin R, Henis Y. Citric acid fermentation by *Aspergillus niger* on low sugar concentration and cotton waste. Appl Envt Microbiol 1981;42:1-4.
37. Ingram LO, Buttke TM. Effects of alcohols on microorganism's. Adv Microb Physiol 1984;25:253.
38. Vandenberghe LPS, Soccol CR, Pandey A, Lebeault JM. Review: Microbial production of citric acid. Braz Arch Biol Technol 1999;42:263-76.
39. Moyer AJ. Effect of alcohols on the mycological production of citric acid in surface and submerged culture. Appl Microbiol 1953;1:1-7.
40. Hang YD, Woodams EE. Utilization of grape pomace for citric acid production by Solid state fermentation. Am J Enol Vitic 1986;37:141-2.
41. Panda T, Kundu S, Majumdar SK. Studies on citric acid production by *Aspergillus niger* using treated Indian cane molasses. J Microbiol 1984;52(2):61-6.
42. Arzumanov TE, Shishkanova NV, Finogenova TV. Biosynthesis of citric acid by *Yarrowia lipolytica* repeat-batch culture on ethanol. Appl Microbiol Biotechnol 2000;53:525-9.