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The Study of Lactose Effect on Citric Acid Production by *Aspergillus niger* PLA30 in Cheese whey

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Abstract: Citric acid is considered one of the most important organic acid, which is used in various aspects. Fermentation is considered one of the best methods for citric acid production using microorganisms such as *Aspergillus niger*. Cheese whey could be used as a good substrate for the production of citric acid. In this study four levels of lactose 40, 90, 150 and 180 g/l were tested for their effect on the activity of *Aspergillus niger* PLA30 in citric acid production. Results indicated that the optimum lactose concentration to be added to the fermentation medium, which resulted in the production of 25.53ml of acidity and 72.11% real yield. Indicating that modification of raw cheese whey by the addition of lactose can serve as a potential carbon and energy source for the production of citric acid and increase its production.
Key words: Citric acid, *Aspergillus niger*, Cheese whey, Lactose.

Introduction

Citric acid ($CH_2COOH.COH.COOH.CH_2COOH$) is a tricarboxylic acid, soluble in water with a pleasant taste; it is an important acid used in food Industries[1]. It was first commercially-produced in England around 1826 from imported Italian lemons (lemons contain 7- 9% citric acid), lemon juice remained the commercial source of citric acid until 1919, when the first industrial process using *Aspergillus niger* started in Belgium[2].

Many organisms have been found to accumulate citric acid in a medium containing sugar and inorganic salts: *A. niger*, *Aspergillus awamori*, *Aspergillus nidulans*, *Aspergillus fonsecaeus*, *Aspergillus luchensis*, *Aspergillus phoenicus*, *Aspergillus wentii*, *Aspergillus saitoi*, *Aspergillus flavus*, *Absidia* sp., *Acremonium* sp., *Botrytis* sp., *Eupenicillium* sp., *Mucor piriformis*, *Penicillium janthinellum*, *Penicillium restrictum*, *Talaromyces* sp., *Trichoderma viride* and *Ustulinavulgaris*[3].Although many microorganisms can be employed to produce citric acid, *A.niger* is still the main industrial producer[2]. About 99% of world production of citric acid occurs via microbial processes, which can be carried out using surface or submerged cultures[2].

Because of its high solubility, palatability and low toxicity it can be used in food, biochemical, and pharmaceutical industries[1], about 70% of total production of 1.5 million tons per year[4] is used in food and beverage industry as an acidifier or antioxidant to preserve or enhance the flavors and aromas of fruit juices, ice cream, and marmalades.

Citric acid can be produced in surface or submerged cultures[2,5]. The simple surface culture methods used during the first commercial production required intensive labor[2].

According to the substrate employed, surface methods are divided into solids and liquids, the latter being of higher economic importance[2,5].

Surface liquid cultures are usually carried out batchwise, using fermentation broths prepared from wheat bran, potato starch, glucose syrup or molasses in concentrations around 160 g/l. With this technology, *A. niger* strains are less sensitive to oligoelements[2], in the United States. Surface fermentation is still used in industries of small and medium scale because it requires less effort in operation, installation and energy cost. The process is carried out in fermentation chambers where a great number of trays are arranged in shelves[5].

Carbohydrates and wastes that have been considered, experimentally, to produce citric acid by *A. niger* includes inulin[6], date fruit syrup[7], sugar cane molasses[8], soya whey[9], kumara[10], Carob pod[11]and cheese whey[12].

Large amounts of whey are produced worldwide as a by-product of cheese and other dairy products manufacturing. Whey in the Middle Eastern region is generally considered a waste and disposed in the sewage system leaving a small amount for drinking for domestic animals[13].

The aim of this study was to optimize the production of citric acid by *A. niger* from cheese whey fortified with different concentrations of lactose in a liquid surface culture process.

Materials and methods

Culture

A citric acid-producing strain of *Aspergillus niger* PLA30, from (Institute of Food biotechnology in Polonia) was used. The culture was grown on potato dextrose agar at 30°C for 5to 7days and it was sub-cultured three times[14].

Substrate

Cheese whey was supplied by a local cheese manufacturer and stored at 4°C. Protein, lactose, lipids, total solids, pH, acidity, chemical oxygen demand (COD) were measured[15]. The following nutrients and trace elements were added: NH4NO3 (0.5)g/L; KH2PO4 (0.15)g/L; ZnS04 (0.05)g/L; K4[Fe(CN)6] (0.36)g/L.

Four concentration of lactose were tested in this study 40g/L, 90g/L, 150g/L and 180g/L[16].pH was adjusted to 3 using 1M sulfuric acid[17]. Substrate was poured in 500 ml flasks each contains 250ml; they were autoclaved at 121° for 15 minutes.

Fermentation

Surface liquid culture fermentation process was carried out. Each flask was inoculated with the given spore suspension 10^3 spore/ml and incubated at 33°C for three days followed by 29°C for the rest of incubation period, with (95%) humidity[6].

Analytical Procedures

At appropriate time (after 7 days), fermentation samples of total flask contents were taken and analyzed for:

Total acidity:

This test was performed every 24 hours using NaOH 0.01 N for calibration, and total acidity was determined as citric acid. Fermentation ended when no more acidity was produced and the period of time was used to determine the total acids produced during fermentation[18].

- Total acidity formed g / l = NaOH consumed for the calibration of 2 ml X 0.007 X 1000 / sample (ml).
- Total acid yield % = Total productivity of acids (g / l) / the amount of sugars (g / l) × 100.

Residual sugars at the end of fermentation:

This test was conducted after the completion of the fermentation process for indicating the activity of fungal strain, the remaining ratio should be less than 1%, and the used method was fast Fehling[19].

The mycelia dry weight:

It was determined by filtering through Buchner, washing three times with distilled water and drying to constant weight at 105°C.

Detection of the presence of sulfuric acid:

Which is considered harmful in citric acid production, and it was done by adding several drops of CaCl2 (10%) to the fermentation solution then heated till boiling, cooled, filtered and a white precipitate of calcium sulfate CaSO4 resulting from the presence of sulfuric acid in the fermentation solution was noticed[19].

Estimation of the formed citric acid:

This method is based on the purification and separation of citric acid, followed by the measurement of its spectral absorbance at 294 nm wavelength using spectrophotometer. One ml of fermentation solution was filtered then diluted to 100 ml with distilled water, 5 ml of potassium bromide were added acidified with sulfuric acid then saturated potassium permanganate was added until the appearance of crimson color, 10 ml of saturated iron sulfate were added so the color changed into light yellow, then 20 ml of chloroform were added to with shacking for 3 minutes, the final liquid was measured for its spectral absorbance at 294 nm wavelength, citric acid concentration was measured using standard curve[20].

Statistical analysis

Means and standard deviations were calculated from 3 independent replicate trials and subjected to analysis of variances using SPSS version 20. Differences between means were evaluated by comparing according to L.S.D using SPSS version 20.

Results and Discussion

Chemical composition of the cheese whey used in this study:

As shown in Table (1).

Table (1) The chemical composition of serum gene used in fermentation processes

COD mg/l	Acidity%	pH	Total Solids%	Lipids%	Lactose%	Protein%
65000	0.15	6.5	5.95	0.40	4	0.9

The effect of different levels of lactose on citric acid production by the studied fungal strain *Aspergillus niger*PLA30:

Four levels of lactose were tested for their effect on the activity of *Aspergillus niger*PLA30 in citric acid production, statistical analysis showed that there were significant differences in acidity values when different levels of lactose were tested, as shown in table (2) acidity was the highest (8.52%) when 40g/l lactose were tested after 7 days of fermentation, while when 90g/l lactose was used the highest acidity (17.36%) was after 11 days of fermentation, and it was observed that the increase of lactose concentration could increase the acidity to reach its highest level (25.53%) when 150g/l lactose were added to the fermentation medium after 14 days of fermentation followed by a tiny drop in acidity (24.53%) when 180g/l lactose were added at the 16th day of fermentation, this decrease in acidity during the use of higher levels of lactose more than 150g/l could be because of the increase of osmosis pressure in the fermentation medium, these results are close to those mentioned by[13].

Table (2) Total acidity produced when different levels of lactose were used during bio-production of citric acid

Total Acidity during Fermentation Period								Lactose Levels g/l
Highest Acidity	**16**	**15**	**14**	**11**	**11**	**9**	**7**	
8.25[d]	-	-	-	-	6.41	7.12	8.52	40
17.36[c]	-	-	-	16.47	17.36	16.86	14.53	90
25.53[a]	23.51	25.11	25.53	24.19	18.48	14.79	12.11	150
24.53[b]	24.53	23.99	22.77	20.14	17.99	13.45	9.51	180

Different character within the column indicate the presence of significant differences at the level of $p < 0.05$

As shown in table (3) the highest total yield was 74.55% when 150% lactose was added, and it was significantly decreased when different levels of lactose were used. The highest real yield (72.11%) also obtained when 150% lactose was added, and it was also significantly decreased during the addition of other levels of lactose, these results agree with those mentioned by[21]. None sulfuric acid was detected during fermentation. Mycelia weight was significantly affected by the change in lactose levels, that the increase of lactose level led to the increase of it, when lactose concentration was 40g/l biomass yield was 12.22 g/l which is close to the biomass yield obtained by[22] which was 12.5 g/l at an initial lactose concentration of 43 g/L, while the highest biomass yield was 22.57 g/l when lactose concentration was 180g/l, these results agree with[23]. The data also indicated that the increase of lactose concentration led to a significantly increase in residual sugar resulting from the fermentation process and this could be directly utilized as animal feeds, fertilizers or soil conditioners[24].

Table (3) fermentation indicators using different levels of lactose

Biomass g/l	Residual Sugars %	Real Yield %	Total Yield %	Total Acids g/l	Lactose Levels g/l
12.22[a]	0.43[d]	42.51[d]	47.69[d]	29.82[d]	40
16.88[c]	0.59[c]	56.21[c]	59.56[c]	60.76[c]	90
19.78[b]	0.85[b]	72.11[a]	74.55[a]	89.35[a]	150
22.57[a]	1.61[a]	64.34[b]	67.51[b]	85.85[b]	180

Different character within the column indicate the presence of significant differences at the level of $p < 0.05$

These results indicate that the optimum lactose concentration to be added to the fermentation medium during fermentation by *Aspergillus niger* PLA30 is 150g/l which resulted in the production of 25.53ml of acidity and 72.11% real yield,these results agree with those obtained by[17], indicating that modification of raw cheese whey by the addition of lactose can serve as a potential carbon and energy source for the production of citric acid and increase its production.

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