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Production of Citric Acid from Date Pulp By Solid State Fermentation

M. Mazaheri Assadi[1] and M. Nikkhah[1]

ABSTRACT

A solid state fermentation method was developed for the production of citric acid from date pulp (obtained of date syrup production from the process) by *Aspergillus niger* PTCC 5010. Poor yield was obtained when potassium ferrocyanide treated date pulp were used as substrate, but methanol at a 3-4% concentration markedly increased the formation of citric acid from the waste. The optimum range of pH and moisture for citric acid production were 3.5-4.5, and 70-80% respectively. This method produced about 168g citric acid per kg of date pulp under optimum conditions. On the basis of reducing sugar consumed, the yield was 87% within eight days.

Keywords: *A. niger,* Citric acid, Solid state fermentation.

INTRODUCTION

The date (*Phoenix dactylifera* .L) has been an important crop in the desert region of Middle East Countries. The semidried date called tamer (Mehaia and Cheryan, 1991) contains the following (expressed as g $100g^{-1}$ of date): moisture, 16; total sugars (glucose and fructose), 65; fiber, 6; protein, 2.5; ash, 1.5; and other compounds, 9.

Date production in Iran is considerable; part of this production is exported. part is consumed locally. However, a considerable portion of the crop is spoiled at the time of harvesting as a result of miss-handling and technical problems. Date syrup, a major product from dates, is produced in large quantities in this country, the majority of which is exported and the remainder used as a sugar source in food industries. Date pulp is a by-product resulting from the processing of dates into syrup and represents nearly 50% of the weight of the date syrup. Currently disposal of it poses considerable economic and environmental problems.

Citric acid is a commercially valuable product and has been produced primarily by submerged fungal fermentation of a sucrose or molasses medium (Kappor *et al*., 1982). Recently, considerable interest has been shown in using agricultural products and their waste materials such as citrus and kiwifruit peel (Rodriguez *et al*., 1985; Hang *et al*., 1987); apple and grape pomace (Hang and Woodams, 1984; Hang and Woodams, 1985); pineapple (Tran and Mitchell, 1995) and mandarin orange (Kumagai *et al*., 1981) for citric acid production under solid state fermentation conditions. Solid state fermentation refer to the cultivation of microorganisms on solid materials in the absence of free liquid and has been used for centuries in Asia for the preparation of a variety of fermented food products (Hesseltine, 1972). The major advantages of using solid state fermentation rather than submerged fermentation include: (1) the yields are much higher than those in liquid media, (2) the space taken up by the fermentation vessel required is small relative to yield of product because less water is used and the substrate is concentrated and (3) the operating costs are much lower than those for liquid phase fer-

[1] Biotechnology Center, Iranian Research Organization for Science and Technology, No. 71 Forsat St., Enghelab Ave., Tehran, Islamic Republic of Iran.

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mentation (Hesseltine, 1972).

The objective of this study was to determine the feasibility of citric acid production from date pulp by solid state fermentation.

MATERIALS AND METHODS

Microorganism and Inoculation

The strain of *A. niger* PTCC 5010 was used throughout this investigation. The mutant strain was maintained at 4°C on potato dextrose agar slants and subcultured at intervals from 2-3 months.

Conidia suspension for inoculation was obtained from culture grown on potato dextrose agar at 30°C for six days. The spores were harvested with tween 80 (0.1%) and inoculated in the preculture at 2×10^8 spores/ml substrate.

Substrate

The date pulp used throughout this study was prepared by the method of Mehaia and Cheryan (1991) in a pilot-plant date syrup processing unit. It was dried in a hot air dehydrator at 60°C for 50 hours to a moisture content of about 6% and stored at room temperature (25-30°C) until needed. Prior its use, the dried pulp was ground through the 0.1cm screen of a mill.

Preculture Medium

Date syrup was diluted with distilled water in order to obtain 10% (w/v) total sugar concentration. About 0.1g/l potassium ferrocyanide was added to the medium. The liquid was centrifuged at 5000 g for 20 minutes and the pH of supernatant adjusted to 5.5 with NaOH, 1 mol/l (Roukas and Kotzekidou, 1997). The centrifuged supernatant was used for production of *A. niger* mycelium pellets. The preculture medium was performed in a 500-ml conical flask containing 50 ml treated date syrup. The medium was sterilized at 121°C for 15 min and inoculated with 1ml of the inoculum to give a final concentration of 2×10^8 spores/ml. The flask was incubated on a rotary shaker (220 rpm and 4cm eccentric throw) at 30°C for 29 hours.

Solid State Fermentation

Portions of 100g dried pulp were rehydrated with distilled water in 250-ml erlenmeyer flasks to give different moisture ranges (60, 70, 80 and 90%). The solid state medium was treated with potassium ferrocyanide (0, 0.25, 0.5, 0.75 and 1 g/kg) before sterilization. Each flask at different initial pH values (3.5, 4.5, 5.5 and 6.5) was inoculated with 8% of mycelium pellets, and incubated at 30°C for eight days. Methanol, 0-4%, was added to the flasks before fermentation. At the end of the fermentation period, the fermented material was extracted according to the method of Hang and Woodams, 1987 in which the citric acid was extracted with distilled water. The extracts were analyzed for residual sugar and citric acid.

Analytical Methods

Moisture, protein (N×6.25), fats and ash were determined by AOAC (1960) methods. Reducing sugars were estimated for the 3,5 dinitrosalicylic acid reaction with glucose as standard (Miller, 1954). pH was measured using a Metrohm 620-pH meter equipped with a glass electrode. Citric acid of the fermented pulp was determined by the colorimetric method of Marrier and Boulet (1958) using a Unicam 8620 spectrophotometer. Each experiment was repeated three times. The indicated results are the mean values of these results, which have been treated using the ANOVA analysis (Lazer and Lellouch, 1983).

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Table 1. Effect of moisture content on citric acid production.

Moisture (%)	Reducing sugar (g/kg)	Sugar consumed (%)	Citric acid Yield (g/kg)	Citric acid Yield (%)[a]
60	168.7	45.6	115.1	68.2
70	178.0	48.1	129.7	72.9
80	189.3	51.1	134.0	70.8
90	189.9	51.3	118.9	62.6

Incubation period 8 days.
Production medium: date pulp 20g; moisture 80%.
[a] based on sugar consumed

RESULTS

The date pulp used in this study was found to have the following composition: moisture, 6.2%; crude protein (N×6.25), 8.2%; crude fat, 0.5%; crude ash, 3.1%, reducing sugar as glucose, 26.6% and sucrose, 10.4%.

Effect of Moisture

The effect of moisture on the solid state fermentation of citric acid production with date pulp is shown in Table1. From this table, it is clear that a moisture level of 70-80% is optimum for the production of citric acid. At higher moisture levels, the percentage of citric acid production decreased whereas sugar consumption increased. The citric acid yields were much lower when the moisture was increased to 90%.

Effect of Potassium Ferrocyanide

The production of citric acid from pretreated date pulp using potassium ferrocyanide to precipitate the heavy metals, is shown in Table 2. It was observed that potassium ferrocyanide at different concentrations had no effect or a slightly increased productivity and yield of citric acid. However the highest values of citric acid concentration (139.1 g/kg) and sugar utilization (50.2%) were obtained in culture grown in 0.75 g/kg potassium ferrocyanide treated medium.

Effect of Initial pH

An important factor that affects the performance of date pulp fermentation is the initial pH of the substrate. The purpose of this experiment was to determine the optimum initial pH of date pulp that would re-

Table 2. Effect of potassium ferrocyanide on citric acid production**.**

$K_4[Fe\ cn_6)]$ (g/kg)	Reducing sugar (g/kg)	Sugar Consumed (%)	Citric acid Yield (g/kg)	Citric acid Yield (%)[a]
0	179.8	48.6	131.6	73.2
0.25	180.3	48.7	134.6	74.6
0.5	183.2	49.5	137.7	75.2
0.75	185.6	50.2	139.1	74.9
0.1	179.3	48.5	132.0	73.6

Incubation period 8 days
Production medium: date pulp, 20g; moisture 80%.
[a] based on sugar consumed.

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Table 3. Effect of initial pH on citric acid production.

Initial pH	Reducing sugar (g/kg)	Sugar Consumed (%)	Yield of (g/kg)	Citric acid (%)[a]
3.5	175.1	47.3	133.9	76.4
4.5	190.1	51.4	149.9	78.8
5.5	180.9	48.9	140.2	77.5
6.5	176.5	47.7	134.1	75.9

Incubation period 8 days.
Production medium: date pulp 20g; moisture 80%.
[a] based on sugar consumed

result in the highest citric acid concentration. As shown in Table 3, the citric acid concentration, citric acid yield, and sugar utilization increased with an increase of up to 4.5 and decreased beyond this value. The citric acid yield and sugar utilization were about 78.8% and 51.4% respectively when the initial pH was adjusted to 4.5. The highest value of citric acid concentration (149.9 g/kg) was achieved at an initial pH of 4.5.

Effect of Methanol

Methanol has an enhancing effect on fungal production of citric acid from date pulp. The addition of methanol at a concentration of 3-4% (v/w) resulted in a marked increase in the amount of citric acid formed. All the solid state fermentation parameters increased at methanol concentrations up to 4% (v/w) and decreased as the methanol concentration was increased beyond 4%. The highest values of citric acid concentration (156.4 g/kg), citric acid yield (81.5%) and sugar utilization (52%) were obtained in the presence of methanol at a concentration of 4% (v/w) (Table 4).

Table 4. Effect of methanol concentrations on citric acid production.

Methanol (%)	Reduucing sugar (g/kg)	Sugar Consumed (%)	Citric acid Yield (g/kg)	Citric acid Yield (%)[a]
0	178.1	48.1	145.9	81.9
1	180.2	48.7	148.4	82.3
2	184.4	49.8	152.9	82.9
3	191.0	51.6	150.3	83.9
4	192.5	52.0	168.0	87.2
5	179.4	48.4	129.9	72.4

Incubation period 8 days.
Production medium: date pulp 20g moisture 80%,
pH=4.5.

Figure 1 demonstrates the time course of citric acid production by *A.niger* PTCC 5010 grown on date pulp in the presence of 4% methanol. The production of citric acid approximately parallels the consumption of sugars; citric acid production increased rapidly at between 3-4 days of fermentation and continued up to tenth day. The pH of the fermentation dropped from an initial value of 4.5 to 2.3 after six days, then relatively constant sugar was consumed throughout the fermentation.

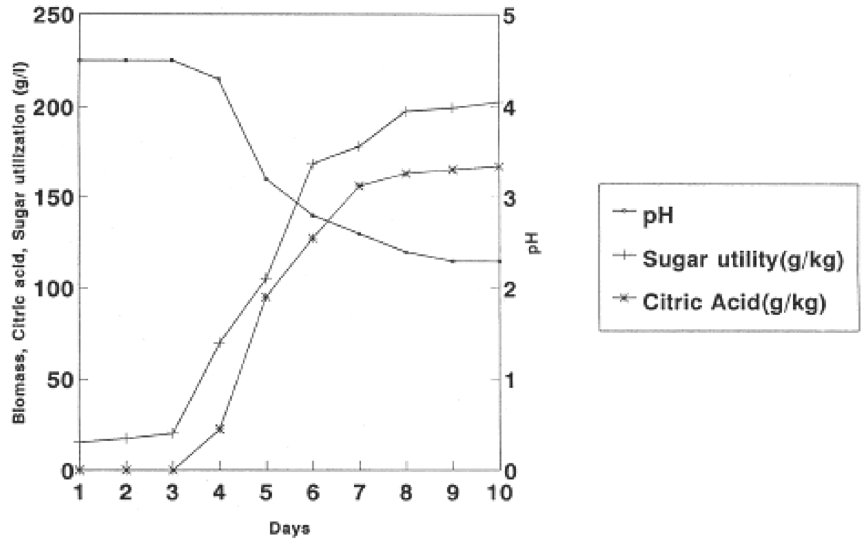

Figure 1. Time course of citric acid production by *A. niger* PTCC 5010 on date pulp.

DISCUSSION

In this study our main objective was to produce citric acid from date pulp, a process about which not much information is available. The moisture content of the date pulp had a profound influence on the production of citric acid by *A.niger* PTCC 5010 in a solid state fermentation system. The yield of citric acid based on the sugar consumed varied according to the initial moisture of the date pulp. As shown in Table 1, the best citric acid yield was obtained when initial moisture was adjusted to between 70-80%.

The sporulation of *A.niger*, however, was more noticeable at a higher rather than a lower moisture level. There is general agreement to admit a double effect of hexacyanoferrate on citric acid production: a positive, one causing the formation of complexes with metals that precipitate or are biologically inactive, and a negative one by inhibiting growth. It is not clear, however, if the inhibition is due to a direct effect or to a diminution in the content of metals to levels restrictive of growth. Some authors suggest the existence of an excess of free hexacyanoferrate (Cejkove *et al*., 1966; Horitsu and Clark, 1966). In general, the optimum concentration that appears in the literature shows a great deal of variability even with the same type of substrate and must be estabilished empirically. Roukas and Kotzekidou (1997) reported that some treatments to improve the yield by deionization of date syrup with potassium ferrocyanide were not very successful. In our observation, the only effect of hexacyanoferrate treatment, was a light increase in the yield of citric acid (Table 2).

The data, in turn, support the hypothesis (Crueger, 1990) that the solid state fermentation system has less sensibility to heavy metals. Among the most important conditions governing a successful citric acid fermentation, consideration of pH is rather complicated. Because citric acid is being produced all the time during the acid producing phase, the pH will drift down- wards during a batch fermentation whatever the starting value unless this is very low. The initial pH used will depend on the substrate. (Milsom,1986). In our investigation, as shown in Table 3, a pH of 4.5 is optimum for the production and there is a lower risk chance of infection by adventitious organisms.

Methanol markedly enhanced fungal production of citric acid from date pulp (Table

4). The high stimulation methanol is not assimilated by *A. niger* and its exact role in stimulating the production of citric acid is still not known. Maddox *et al*., (1986) and Kappoor *et al*., (1982) reported that methanol affects the permeability properties of the mold and a greater excretion of citric acid. The high stimulation effect of methanol can be attributed to the inhibition of spore formation and an increase of the microorganism's tolerance to high levels of minerals contained in date pulp. The results obtained in this study agree with those of Roukas and Kotzekidou (1997) who studied the effect of methanol on citric acid production from date syrup by surface fermentation. They reported that the addition of methanol at a concentration of 4% (v/w) resulted in a remarkable increase in the amount of citric acid produced.

CONCLUSIONS

The results showed some important aspects of citric acid production from date pulp by *A. niger* On the basis of the present results, it can be concluded that the pretreatment of date pulp medium with hexacyanoferrate is not required but the addition of methanol to the medium significantly improved the production of citric acid. The addition of methanol at concentrations up to 4% (w/v) resulted in a marked increase in the citric acid concentration. The optimum pH and moisture for citric acid production were 4.5 and 70-80%.

The date pulp was an attractive medium for the production of citric acid by *A. niger*. This natural medium is economically very suitable, especially for a major date-producing country such as Iran.

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تولید اسید سیتریک از تفاله خرما با استفاده از فرومانتاسیون بستر جامد

م. مظاهری اسدی و م. نیکخواه

چکیده

در این تحقیق امکان تولید اسیدسیتریک از تفاله خشک شده خرما (۳۷٪ قند) با استفاده از *A. niger* PTCC 5010 بر روی فاز جامد مورد بررسی قرار گرفت. هنگامی که از فروسیانید پتاسیم در تیمار تفاله خرما استفده شده میزان تولید بسیار کاهش یافت در حالیکه غلظت ۴-۳ درصد متانول تولید اسیدسیتریک را افزایش داد. میزان آپتیم pH و رطوبت در تولید اسیدسیتریک به ترتیب ۴/۵- ۳/۵ و ۸۰-۷۰ درصد بود. در این روش ۱۶۸ گرم اسید سیتریک در هر کیلو تولید شده و براساس قند مصرفی تولید ۸۷ درصد در مدت ۸ روز بود.