Utilization of Potato-Chips Waste for Production of High Economic Value Products: II- Production of Citric Acid by Solid-State Fermentation

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ABSTRACT

The present study was proposed to evaluate the potential use of potato- chips waste as a substrate for citric acid production by solid-state fermentation. Potato- chips waste could serve as a substrate for citric acid production. Maximum amount of citric acid (23.19g/100g) was produced by *Aspergillus niger* when it was cultivated on the optimized waste medium for 7 days at 30°C. The waste was prepared as follows: it was milled to 1.0-1.5mm, cooked at 121°C for 2hr,adjusted to moisture level of 65% v/w, with pH 3.5 and enriched with 0.0125% w/w nitrogen in form of ammonium nitrate and 4% v/w of methanol was added.

Keywords: potato-chips waste, citric acid, solid-state fermentation

INTRODUCTION

Many tons of potato-chips wastes are discarded yearly by many potato-chips companies leading to serious environmental problems. Thus, there are urgent needs to find suitable applications for disposal of this waste. Since this waste is rich in carbohydrate and other nutrients, it can serve as a cheap substrate for fermentation industries. World- wide demand for citric acid far exceeds its production (Roehr *et al.*, 1983). Developing countries could reduce the burden of citric acid imports on their foreign exchange, they could produce citric acid locally by fermentation of wastes (Tran *et al.*, 1998). Therefore, from economical and environmental viewpoints, the use of potato-chips waste was taken into consideration as an alternative raw material for the production of citric acid. Citric acid is widely used in the food and pharmaceutical industries. In foods, it is used primarily to produce a tart taste and to complement fruit flavours in carbonated beverages, beverage powders, fruit-flavoured drinks, jams, jellies, candy, sherbets, water ices, and wine. It is also used to reduce pH in certain canned foods to make heat treatment more effective, and in conjunction with antioxidants to chelate trace metals and retard enzymatic activity (Yigitoglu, 1992, Haq *et al.*, 2001, Alben & Erkmen, 2004). In the recent years, there has been increasing interest in the use of solid-state fermentation (SSF) process as an alternative to submerged fermentation. This is because SSF has a lower energy requirement, higher product yield with little risk of bacterial contamination, generates less waste water and environmental concerns regarding the disposal of solid waste (Lu *et al.*,1997, Roukas, 1999, Papagianni *et al.*, 1999, Bayraktar & Mehmetoglu,2000). Furthermore, the biosynthesis of citric acid by *Aspergillus niger* is highly sensitive to the concentration of metal ions Fe^{2+}, Mn^{2+}, Zn^{2+}, etc. in submerged or liquid surface fermentation (Kapoor *et al.*,1982, Roehr *et al.*,1983). Inhibition of citric acid production by these ions is ineffective in SSF (Gutierrez-Rozas *et al.*, 1995, Pintado *et al.*, 1998).

The present study was proposed to evaluate the potential use of potato-chips waste as a substrate for citric acid production by SSF, as well as to reduce the citric acid production cost by using cheap substrate in place of costly materials such as molasses, starch, glucose etc., at the same time, eliminate the environmental pollution and hazards.

MATERIALS AND METHODS

Microorganism

A citric acid-producing strain of *Aspergillus niger* ATCC 9142 was used in this study. It was obtained from the Institute of Microbiology and Wine research, Gutenberg-University, Mainz, Germany, maintained on potato dextrose agar slants and stored at 4°C.

Waste resource and preparation

Potato-chips waste was obtained from a factory for potato-chips industry located in Tanta city.

The waste consists of potato peels and tuber pieces. Its chemical composition (dry weight basis) was 61.49% starch, 21.84% crude fiber, 15.22% ash, 0.61% reducing sugar and 0.82% crude protein. The waste was prepared according to the method of Kumar *et al.* (2003) with some modifications as follows: The whole waste was dried at 80°C to a constant weight then milled in kitchen blender to give powder. The milled waste was screened to collect 5 fractions of different particle sizes, less than 0.5 mm, between 0.5 -1.0, 1.0 -1.5, 1.5 and 2.0 -2.5mm.

Basic medium

Basic medium was prepared according to the method of Shankaranand & Lonsane (1994) with some modification as follows: Two grams of prepared milled waste with particle size between 0.5-1.0 mm were introduced into 200ml Erlenmeyer flask and moistened with distilled water adjusted to pH 4.5 with HCl to reach 80 % moisture content and autoclaved at 121°C for 15 min.

Inoculum preparation

The strain was inoculated on potato- dextrose agar slants and incubated at 30°C for 7 days. Five ml of sterile distilled water was added, spores were scraped off, suspended in water. Spores density of 1.0×10^6 ml was checked using a hemocytometer, before inoculation.

Inoculation and fermentation

The basic medium was inoculated by mixing with the spores suspension at a concentration of approximately1.0×10^6 spores g^{-1}, and steady state incubated at 25ºC temperature for 7 days. (Following the method of Shojaosadati & Babaeipour (2002)

Optimization of medium parameters

The strategy adopted was to optimize one particular parameter at a time and then include it at its optimum value in the next optimization step.

Extraction of citric acid

Citric acid was extracted according to the method of Kumar *et al.* (2003) with some modifications as follows: A 10 ml of distilled water was added per g of fermented material. The mixture was agitated for 2hr on a rotary shaker then, filtered through filter paper Whatman No 1. The residual solids were washed with water three times and the filtrate and washings were made up to 200ml with distilled water and used for estimation of citric acid.

Determination of citric acid

Citric acid was determined by the acetic anhydride and pyridine according to the method of Marier & Boulet (1958). All determinations were carried out in triplicate and the recorded results are their average values.

RESULTS AND DISCUSSION

Factors affecting citric acid production by *Aspergillus niger* ATCC 9142:

Effect of sterilization period on waste

The basal medium was heat treated at 121ºC for different periods of time (from 15 min to 4hrs) for cooking and sterilization at the same time. The results presented in Fig (1) show that, the minimum citric acid accumulation (8.76g/100g) was detected when the medium treated with heat for 15 min, (the normal sterilization time).The maximum amount of citric acid (12.1g/100g) was obtained when the medium was heated for 2hrs. The increasing of heat treatment time more than 2hrs did not correspond to increase of citric acid production. The enhance of citric acid production may be attributed to that, the heat treatment makes the nutrients in the waste more available for the microbial consumption, where as denaturation of the protein content makes the cell wall of the waste tissue permeable. The obtained results are in agreement with those of Kumar *et al.* (2003). They reported that, the proper heat treatment of the fermentation substrate increased its susceptibility to microbial attack.

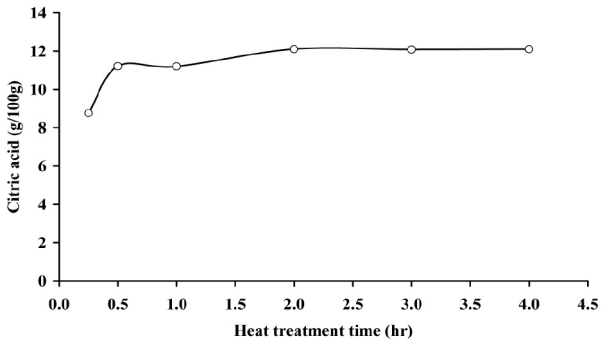

Fig. 1: Effect of heat treatment period of the waste on citric acid production

Fermentation conditions: Temperature 25ºC, pH 4.5, particle size 0.5-1.0 mm, 80% moisture level and 7 days cultivation period.

Effect of initial moisture content of medium

The experiment was carried out at different moisture levels (50, 60, 65 and 70%). The results presented in Fig (2) show that, the production of citric acid using solid-state fermentation was strongly affected by the medium moisture content. The maximum production of citric acid (12.43g/ 100g) was detected at 65 % v/w of moisture level. On the other hand, the lowest amount of citric acid was excreated at 50% moisture content. This may be due to that, the low moisture level reduced the substrate availability to the fungus and mass transfer processes such as diffusion of the solutes and gas to the cell during the fermentation (Nagadi & Correia, 1992, Kumar *et al.*, 2003). From the same results, it can be noted that, the increase of moisture content beyond the optimal level was corresponded with the decrease in citric acid excretion. This possibly attributed to that, the higher moisture content reduced the porosity of the medium consequently poor heat, mass transfer and poor oxygen availability (Kargi *et al.*, 1985, Kumar *et al.*, 2003).

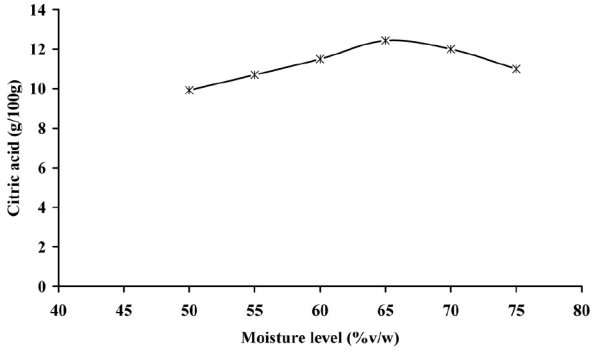

Fig. 2: Effect of initial moisture content of medium on citric acid production

Fermentation conditions: Temperature 25°C, pH 4.5, particle size 0.5-1.0 mm, the medium was heat treated for 2hrs at 121°C before inoculation and 7 days cultivation period.

Effect of particle size of waste:

Five different particle sizes of milled waste (ranged from < 0.5 mm to 2.5 mm) were used for preparing fermentation medium. The results given in Table (1) show that, the lowest amounts of citric acid (11.5 g/100g and 11.98/100g) were obtained in the media containing either the smallest particle size (<0.5 mm) or the largest ones (between 2.0 - 2.5 mm) respectively. The decrease in citric acid production in case of small particle size may be attributed to the improper in air diffusion and distribution, poor mass and low heat transfer, whereas large particle size provides smaller surface area hence reduces the substrate availability to the fungus (Shojaosadati & Babaeipour, 2002, Kumar *et al.*, 2003). The medium with particle size ranged between 1.0 -1.5 mm stimulated maximum release of citric acid (12.53 g/100g), since it exhibited high porosity and resulted in better heat and mass transfer which increased citric acid production (Lu & Maddox, 1998).

Table 1: Effect of particle size of potato- waste on citric acid production

Particle size (mm)	<0.5	0.5-1.0	1.0 -1.5	1.5 -2.0	2.0 -2.5
Citric acid (g/100g)	11.50	12.33	13.31	12.53	11.98

Fermentation conditions: Temperature 25°C, pH 4.5, particle size 1.0-1.5 mm, moisture level 65 %, the medium was heat treated for 2hrs at 121°C before inoculation and 7 days cultivation period.

Effect of addition of ground rice straw:

In previous experiments, it was noted that the milled waste media were agglomerated after sterilization with different degrees according to their moisture content and particle sizes. In this experiment, a try was carried out to prevent the agglomerations by mixing ground rice straw with the milled waste. The results presented in Table (2) indicate that, the addition of ground rice straw up to 1 % enhanced citric acid production. The highest citric acid yield (13.73 g/100g) was observed when 1% (w/w) of rice straw was mixed with the waste medium. The addition of ground rice straw with more than 1% (w/w) did not cause any increase in citric acid production. Enhancement of citric acid production by addition of milled rice straw can perhaps be attributed to that, it may prevent the agglomerations which affects uniform mixing of the substrate and therefore affects growth, heat (metabolic heat) transfer, mass (O_2 intake and CO_2 evolution) and citric acid formation (Kumar *et al.*, 2003).

Table 2: Effect of addition of ground rice straw to the milled potato waste on citric acid production

Ground rice straw % (w/w)	0.5	1	1.5	2
Citric acid (g/100g)	13.53	13.73	13.61	12.6

Fermentation conditions: Temperature 25°C, pH 4.5, particle size 1.0-1.5 mm, moisture level 65 %, the medium was heat treated for 2hrs at 121°C before inoculation and 7 days cultivation period.

Effect of initial pH of the medium:

For studying the effect of initial pH, the fermentation media were adjusted to initial pH values ranged between 1.5 and 5.0 using HCl. The results given in Fig (3) reveal that, in general, the fungus was able to produce citric acid at all tested pH values. The accumulation of citric acid increased modulatory with the increase of the pH values to reach its maximum at pH 3.5. Above this value, the production of citric acid slightly decreased. These results are in accordance with those of Tran *et al.*, (1998) who reported that, optimal production of citric acid occurred at pH 3.5. On the other hand, these results are in disagreement with that of Kubicer & Roehr, (1986) who recommended that, the pH should be kept below 2.0 because at higher pH values, *Aspergillus niger* accumulates gluconic acid, especially when the pH is around 4.0. On the contrary, Alben & Erkmen, (2004) reported that, the highest amount of citric acid was produced in medium with an initial pH 6.5.

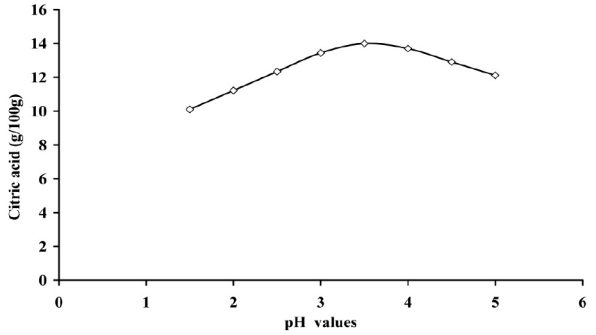

Fig. 3: Effect of initial pH of medium on citric acid production

Fermentation conditions: Temperature 25°C, particle size 1.0-1.5 mm, moisture level 65 %, the medium was heat treated for 2hrs at 121°C before inoculation and 7 days cultivation period.

Effect of cultivation temperature:

Fermentation was conducted at the temperatures of 26, 28, 30 and 32°C. As shown in Table (3), maximum citric acid yield (13.98g/100g) was detected at 30°C. This result is quite similar with the result stated by Hang & Woodams, (1998), who reported that, fermentation temperature has a profound influence on fungal production of citric acid from corn cobs and the highest production was found at 30°C. At higher temperatures, the production was retarded. The decrease in citric acid production at temperatures beyond 30°C may be attributed to a problem in solid-state fermentation, since overheating is a common problem and could potentially decrease citric acid production (Tran *et al.*, 1998).

Table 3: Effect of cultivation temperature on citric acid production

Temperature °C	26	28	30	32	34
Citric acid (g/100g)	13.65	13.63	13.98	12.81	11.11

Fermentation conditions: pH 3.5, particle size 1.0-1.5 mm, moisture level 65 %, the medium was heat treated for 2hrs at 121°C before inoculation and 7 days cultivation period.

Effect of enrichment with nitrogen sources

The waste media were supplemented with different nitrogen compounds at concentrations of 0.0062, 0.0125, 0.025 and 0.05 nitrogen/100 dry waste. The results given in Table (4) reveal that, nitrogen content of the media have a great effect on citric acid production by *Aspergillus niger* ATCC9142, since all tested nitrogen sources enhanced citric acid production at concentration of 0.0125 % (w/w) or below. Beyond this limit, a strong suppression on citric acid accumulation was noted. The highest amount of citric acid was produced in medium enriched with nitrogen at concentration of 0.0125 % (w/w) in the form of ammonium nitrate. Alben & Erkmen, (2004) reported that, nitrogen content of the fermentation medium is a limiting factor in citric acid production. In contrast with these results, Xu *et al.*, (1989) reported that, the optimal concentrations of ammonium sulfate were 5.0 and 2.5 g/l (= 0.106 % and 0.053 % g nitrogen) for the submerged and filter paper cultivation system. On the other hand, Demirel *et al.* (2005), reported that, the maximum citric acid production was obtained at 0.05 g nitrogen/l of medium, whereas it significantly decreased at 0.3g/l. Similar trend was

Table 4: Effect of enrichment with nitrogen sources on citric acid production by *Aspergillus niger* ATCC9142

Nitrogen source / Conc. % w/w	Urea	Ammonium phosphate	Ammonium sulfate	Ammonium nitrate
	Citric acid (g/100g)			
0.0062	14.83	15.00	15.34	16.0
0.0125	15.88	16.31	16.19	17.0
0.025	15.88	13.48	12.83	11.71
0.05	11.3	12.1	10.95	9.32

Fermentation conditions: Temperature 30°C, pH 3.5, particle size 1.0-1.5 mm, moisture level 65 %, the medium was heat treated for 2hrs at 121 oC before inoculation and 7 days cultivation period.

previously described by Shankaranand & Lonsane, (1994), who observed that, at high nitrogen concentrations (0.0318 and 0.042 %) in form of ammonium sulfate, a decrease of citric acid production by 20 and 31% occurred, respectively. Kristiansen & Sinclair (1979) explained the influence of nitrogen on the production of citric acid, as the cytoplasm in hyphae flows toward the tip where the new cells are formed. Meanwhile, aged cells suffer from nitrogen limitation, become carbon stores and would produce citric acid. The number of cells produced would increase with the nitrogen concentration and a similar increase will be observed in the flow of cytoplasm toward new cells if the nitrogen concentration was elevated, the rate of the formation of storage cells would increase, resulting higher yields of citric acid. It is known that, citric acid is produced in the mitochondria. If the flow is significant, streaming of cytoplasm is transported to no producing tip hyphae, which is not suffering nitrogen limitation and citric acid production, this means that, citric acid would decrease at both lower and higher nitrogen levels, for this reason, the optimum nitrogen concentration must be used.

Effect of addition of alcohols to the fermentation medium:

To identify the effect of alcohols on citric acid production, methanol and ethanol were filter- sterilized and added to the media after autoclaving at concentrations ranged from 1% to 6% v/w. The results shown in Fig (4) indicate that, the addition of both methanol and ethanol to the fermentation media stimulated the production of citric acid; however methanol was more efficient than ethanol in this respect. Maximum citric acid, i.e. 19.33 and 18.81 g/100g was obtained by addition of 4 and 3 %(v/w) methanol and ethanol, respectively. These results are in agreement with those reported by Tran *et al.* (1998), Hang & Woodams, (1998), Kumar *et al.* (2003), Alben & Erkmen, (2004) and Demirel *et al.*, (2005). The improvement of citric acid yield by alcohols is a general phenomenon with *Aspergillus niger* strain in despite of alcohols are not assimilated by the fungus (Tran *et al.*, 1998), the exact mechanism of alcohols effects on stimulating citric acid accumulation is still not clear, however it is likely that, alcohols increased the permeability level of the cell membrane allowing metabolites such as citric acid to be excreted from the cells (Rouskas, 2000, Haq *et al.*, 2003). It is also observed that, addition of excess alcohols more than the optimum level, led to a strong suppression of citric acid production. This observation was previously reported by Tran *et al.* (1998).

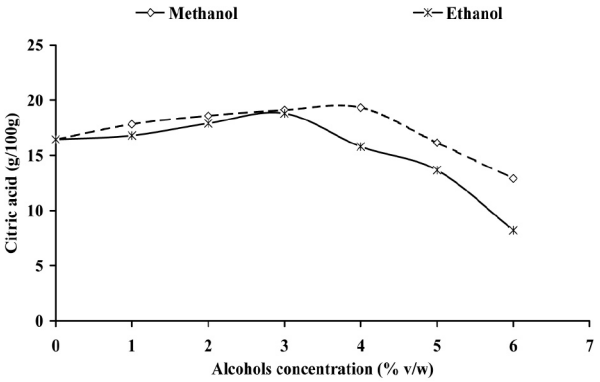

Fig. 4: Effect of addition of alcohols to the fermentation media on citric acid production

Fermentation conditions: Temperature 30°C, pH 3.5, particle size 1.0-1.5 mm, moisture level 65 %, the medium was heat treated for 2hrs at 121°C before inoculation 0.0125% w/w ammonium nitrate and 7 days cultivation period.

Effect of fermentation time:

The fungus was cultivated for different periods (i.e. 1 to 9 days) under previously optimized nutritional and environmental factors to study the effect of fermentation time on citric acid production. As shown in Fig (5) citric acid started to appear on the first day and increased moderately up to 3rd day then, followed by an additional increase of the production up to 7th day of the fermentation at which the maximum production (23.19g/100g) was occurred. Extending the fermentation time more than 7 days, impaired the citric acid excretion.

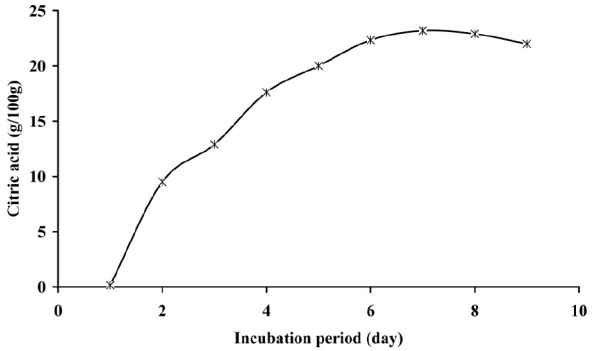

Fig. 5: Effect of incubation time

Fermentation conditions: Temperature 30°C, pH 3.5, particle size 1.0-1.5 mm, moisture level 65 %, the medium was cooked for 2hrs at 121°C before inoculation 0.0125% w/w ammonium nitrate and 4 % v/w methanol.

ACKNOWLEDGEMENT

We are grateful to Prof. Dr. Samir Metwalli for his help during writing this manuscript.

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الاستفادة من مخلفات صناعة شرائح البطاطس في إنتاج منتجات ذات قيمة إقتصادية عالية ٢ ـ إنتاج حامض الستريك

سمير السناط و محمد قريش

قسم الصناعات الغذائية -كلية الزراعة - جامعة كفر الشيخ - كفر الشيخ- مصر

هدفت هذه الدراسة إلى الإستفادة من مخلفات صناعة شرائح البطاطس في إنتاج حامض الستريك بغرض خفض تكلفة الإنتاج وفي نفس الوقت تجنب الأضرار البيئية التي قد تنجم عن التخلص من تلك المخلفات في البيئة. أوضحت النتائج أن مخلفات شرائح البطاطس تصلح لإنتاج حامض الستريك وكانت أقصي كمية منتجة هي ٢٣,١٩ جم/١٠٠ جم بيئة جافة و ذلك عندما تم زراعة فطر الأسبرجلس علي بيئة مثلي من المخلف لمدة سبعة أيام علي درجة حرارة ٣٠ درجة مئوية و ذلك باستخدام البئية المثلي التي تم ضبطها كالتالي: تم طحن المخلفات لكي تكون حجم الجزيئات من ١,٠ – ١,٥ مم. وتم معاملتها حراريا لمدة ساعتين علي درجة حرارة ١٢١ درجة مئوية. ثم أضيف مطحون قش الأرز إليها بنسبة ١٪ وزن / حجم لمنع تكتل المخلف. وتم تدعيم المخلف بالنيتروجين بتركيز ٠,٠١٢٥ ٪ وزن / وزن في صورة نترات أمونيوم ثم يضاف الميثانول إلي البيئة بتركيز ٤٪ بعد التعقيم.