Journal of Industrial Microbiology & Biotechnology (2000) 25, 298–304

www.nature.com/jim

Citric and gluconic acid production from fig by *Aspergillus niger* using solid-state fermentation

T Roukas

Department of Food Science and Technology, Aristotle University of Thessaloniki, Box 250, 540 06 Thessaloniki, Greece

The production of citric and gluconic acids from fig by *Aspergillus niger* ATCC 10577 in solid-state fermentation was investigated. The maximal citric and gluconic acids concentration (64 and 490 g/kg dry figs, respectively), citric acid yield (8%), and gluconic acid yield (63%) were obtained at a moisture level of 75%, initial pH 7.0, temperature 30°C, and fermentation time in 15 days. However, the highest biomass dry weight (40 g/kg wet substrate) and sugar utilization (90%) were obtained in cultures grown at 35°C. The addition of 6% (w/w) methanol into substrate increased the concentration of citric and gluconic acid from 64 and 490 to 96 and 685 g/kg dry fig, respectively. *Journal of Industrial Microbiology & Biotechnology* (2000) **25,** 298–304.

Keywords: citric acid; gluconic acid; fig; *Aspergillus niger*, solid-state fermentation

Introduction

The fig is the fruit of the fig tree (*Figus carica*), which is chiefly cultivated in the Mediterranean countries and in many areas of America, Africa and Asia. Greece is the primary producer of figs, with an annual harvest of 23,000 and 13,000 tons fresh and dried figs, respectively [1]. The fig contains the following (g/100 g of semidried fig), 23 moisture, 70 total sugars (glucose, fructose, saccharose), 4.3 proteins, 1.3 fat, 1.0 inorganic minerals (calcium, phosphorus iron, sodium, potassium), and 0.9 mg vitamins (vitamin A, niacin, riboflavin, thiamine) [23]. Figs are usually used for production of jams and for human consumption. Because of the high concentration of sugars in the fig, it is important to develop new and attractive uses of these sugars.

Citric and gluconic acids are used in the pharmaceutical, food, and beverage industries as acidifying and flavor-enhancing agents, and are produced from synthetic medium, beet and cane molasses by surface and submerged fermentation using *Aspergillus niger* [13,17,20]. In past years, considerable interest has been shown in using agricultural products such as carob pod, date, maize, citrus and kiwi fruit peel, apple and grape pomace, jerusalem artichoke, and grape must for citric and gluconic acids production by *A. niger* [2,4–6,8,11,15,16,19]. Fig trees have many advantages over traditional crops, such as high carbohydrate yield and good growth in poor soil. The production of citric and gluconic acids from figs by *A. niger* using solid-state fermentation has not been investigated.

The aim of this investigation was to examine the potential of fig as a source of citric and gluconic acids production by *A. niger via* solid-state fermentation, as well as to study the effects of fermentation parameters such as moisture, pH, temperature, and methanol concentration on kinetic parameters of fig fermentation.

Correspondence: Dr T Roukas, Department of Food Science and Technology, Aristotle University of Thessaloniki, Box 250, 540 06 Thessaloniki, Greece
Received 15 April 2000; accepted 11 August 2000

Materials and methods

Microorganism

A. niger ATCC 10577 was used throughout this investigation. It was maintained on potato dextrose agar slants at 4°C and subcultured at intervals from 1 to 2 months.

Inoculum

Cultures were incubated on potato dextrose agar (PDA, Merck) slants at 30°C for 5 days. The spores obtained were suspended in 5 ml of sterile distilled water to prepare the inoculum. Spore numbers were calculated after appropriate dilution of the inoculum, cultivation on PDA and incubation at 30°C for 5 days.

Fermentation medium

Semidried figs (cultivar Kalamon) were obtained from the local market. Figs were chopped into particles 0.3–0.5 cm in diameter. Thirty grams of figs containing 24% moisture and 68% total sugars were placed in 500-ml conical flasks and moistened with the appropriate amount of distilled water to contain 75% moisture. The pH of the substrate was adjusted to 7.0 with 1 N NaOH. The medium was sterilized at 121°C for 30 min and inoculated with 5 ml of inoculum containing 1.0×10^{8} spores/ml. The flasks were incubated at 30°C in a incubator under stationary conditions.

Study of fermentation parameters

Moisture content

A series of conical flasks containing 30 g figs (pH 7.0) were moistened with an appropriate amount of distilled water to contain 60%, 65%, 70%, 75% or 80% moisture. The flasks were inoculated and incubated as above.

Initial pH

The substrate consisting of 30 g figs with 75% moisture and at pH 4.0, 5.0, 6.0, 7.0, or 8.0 was inoculated with 5 ml of inoculum and incubated at 30°C for 15 days.

Temperature

The medium (30 g figs, moisture 75%, and pH 7.0) was inoculated with 5 ml of inoculum and incubated at 25, 30, 35 or 40°C for 15 days.

Effect of methanol

Flasks containing medium were supplemented with methanol at concentrations of 3.0, 4.0, 5.0, 6.0, and 7.0 g/100 g wet substrate to investigate the influence of methanol on kinetic parameters of citric acid fermentation. Flasks containing 30 g figs (moisture 75% and pH 7.0) were inoculated with 5 ml of inoculum and incubated at 30°C for 15 days. Methanol was added after sterilization of the medium.

Analytical techniques

At appropriate time intervals, fermentation flasks were removed and the contents analyzed. Mycelium was removed from the flasks by a spatula, washed twice with 100 ml distilled water, and dried at 105°C to constant weight. The fermented mash was mixed with the above 200 ml effluent of the mycelium and the mixture was shaken on a rotary shaker/incubator (Lab-line Orbit-Environ Shaker, Lab-Line Instruments, Melrose Park, IL) at 250 rpm for 30 min at 30°C to extract citric and gluconic acids and residual sugars from the mash. The extract was then centrifuged at 5000×*g* for 15 min and the sediment was treated again as described above with 200 ml distilled water to effect complete extraction of the fermented materials. The supernatants of the two extraction treatments were mixed together and the mixture was used for the determination of total acidity, citric acid, gluconic acid and residual sugars. Total acidity as gluconic acid was assayed by titration of the extract (after appropriate dilution) with 0.01 N NaOH and phenolphthalein as indicator. Citric acid was determined by the method of Saffran and Denstedt [21] and gluconic acid was calculated from the difference between total acidity and citric acid concentration, because no other acids than citric and gluconic acid were detected by HPLC. Separation of the acids was achieved by HPLC using a separon SGX C18 column. Elution was performed with an isocratic solvent of 0.8 ml/min acetonitrile: H_2O (3:7 v/v). Acids were detected at 210 nm. Standard solutions of citric (Sigma, St. Louis, MO) and gluconic (Sigma) acids were prepared and treated as described above by HPLC. The elution times of the peaks were compared to the elution time of standard. Residual sugars were determined as glucose by the method of Dubois *et al* [3]. The pH of the fermented mash

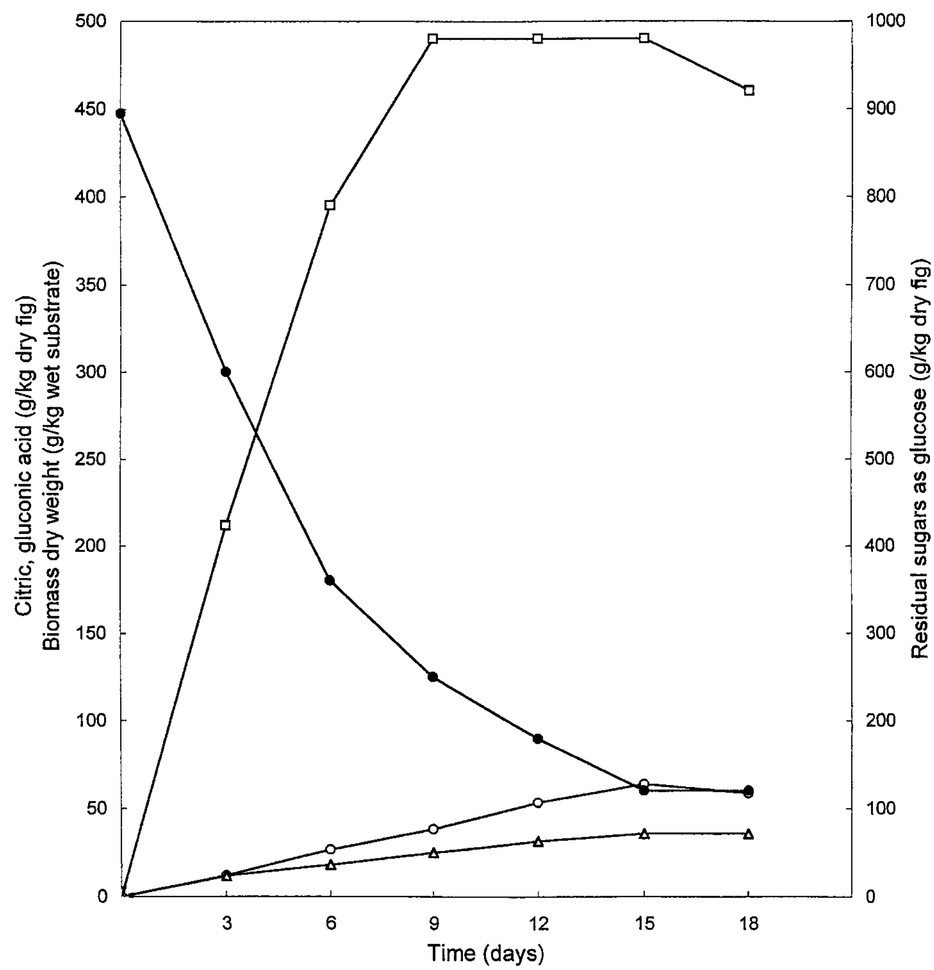

Figure 1 Production of citric and gluconic acid from fig by *A. niger* ATCC 10577 in solid-state fermentation. ○ Citric acid; □ gluconic acid; △ biomass dry weight; ● residual sugars as glucose. Data are by means of duplicate experiments which had a variation that did not exceed the mean value by 3.5% in all cases.

were measured using a knick 646 pH meter equipped with a glass electrode. Citric acid and gluconic acid yields and sugar utilization were expressed as g of acid/100 g sugar utilized and g of sugar utilized/100 g supplied sugar, respectively. Each experiment was repeated twice and the results were reported as averages of two repetitions.

Results and discussion

Citric and gluconic acids production

The production of citric and gluconic acids from figs in solid-state fermentation is shown in Figure 1. The concentration of gluconic acid increased as the fermentation time increased up to 9 days, remained constant between 9 and 15 days, and then decreased. However, the concentration of citric acid was increased with the increase of fermentation time up to 15 days. A longer fermentation time resulted in a decrease of the citric concentration. The decline in concentration of citric acid may have been caused by a decay in the enzyme system responsible for the acid's production [16]. The maximum citric acid (64 g/kg dry fig) and gluconic acid (490 g/kg dry fig) concentrations was obtained after 15 and 9 days, respectively. In relevant work from our laboratory [16], it was found that *A. niger* ATCC 9142 produced 176 g citric acid/kg dry pod when grown in carob pod in solid-state fermentation. Hang *et al* [8] and Hang and Woodams [5,6] reported maximum citric acid concentrations of 100 g/kg dry kiwi fruit peel, 164 g/kg dry apple pomace, and 56 g/kg dry grape pomace for various *A. niger* strains in solid-state fermentation. Moksia *et al* [13] and Buzzini *et al* [2] found that maximal gluconic acid concentrations of 200 and 67.5 g/l were obtained when *A. niger* was grown in synthetic medium and rectified grape must, respectively, in batch culture. Possible reasons for these differences include the strain of organism used, chemical composition of the substrate, fermentation system, and generally, the conditions under which the fermentations took place.

The biomass dry weight followed a pattern similar to citric acid with maximum biomass concentration observed at the same time as the maximum concentration of citric acid was observed (Figure 1). The highest mycelial dry weight (35.5 g/kg wet substrate) was obtained after 15 days of fermentation and then remained constant.

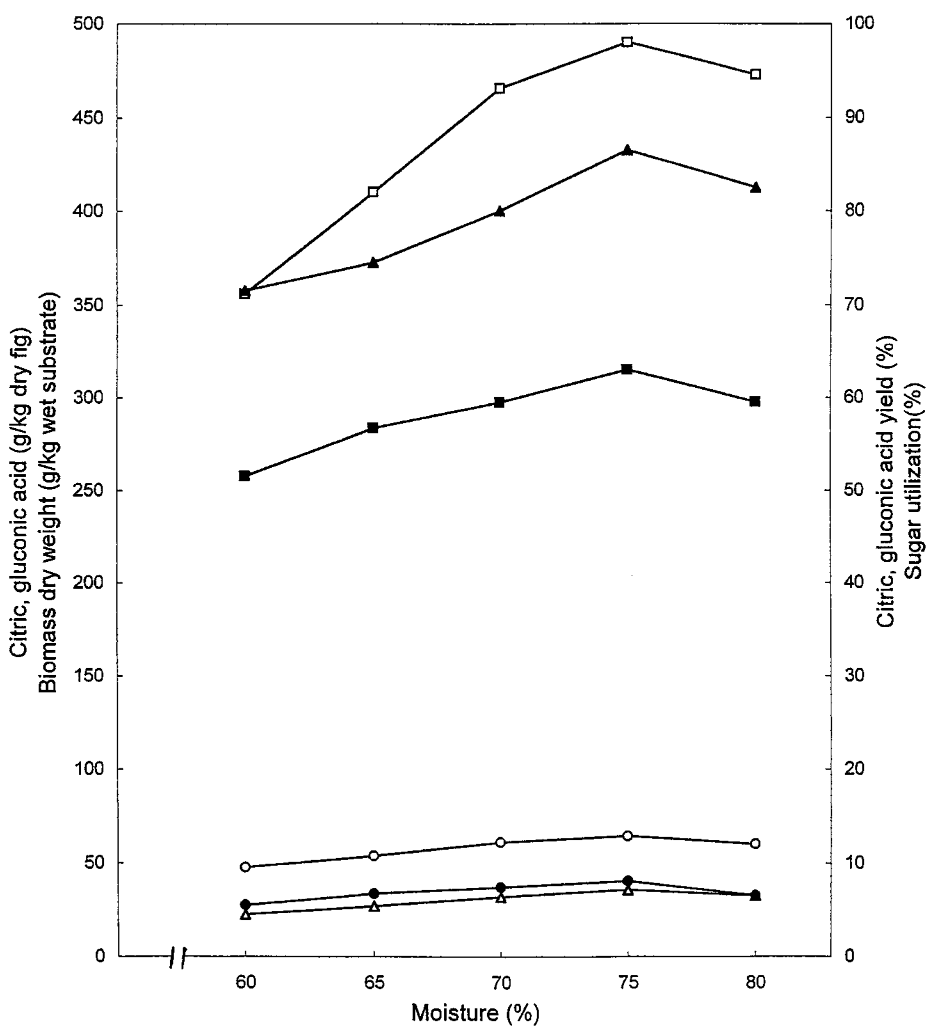

Figure 2 Kinetic parameters of fig fermentation by *A. niger* ATCC 10577 at different moisture levels. ○ Citric acid; □ gluconic acid; △ biomass dry weight; ● citric acid yield; ■, gluconic acid yield; ▲, sugar utilization. Data are by means of duplicate experiments, which had a variation that did not exceed the mean value by 4.3% in all cases.

As expected, the concentration of residual sugars decreased during the fermentation, coinciding with an increased in biomass, citric acid and gluconic acid production (Figure 1). The concentration of residual sugars fell rapidly during the first 6 days of fermentation, after which it decreased slowly. This was due to a rapid increase of gluconic acid observed at the same time. When the maximal concentration of citric acid was achieved, 8% and 63% of sugars consumed was converted to citric and gluconic acids, respectively, whereas the total amount of utilized sugars was 86.5%.

Effect of moisture content

One important factor that affects the performance of a solid-state fermentation is the moisture content of solids. The purpose of this experiment was to determine the optimum moisture level of figs that would result in the highest citric and gluconic acids concentration. All fermentation parameters were increased with the increase in moisture content from 60% to 75% and then decreased (Figure 2). The highest values of biomass dry weight (35.5 g/kg wet substrate), citric acid (64 g/kg dry fig), gluconic acid (490 g/kg dry fig), citric acid yield (8%), gluconic acid yield (63%), and sugar utilization (86.5%) were achieved at a moisture level of 75%. Decreasing the moisture level from 75% to 60% resulted in a decrease in the kinetic parameters. The decrease in moisture level is advantageous because the chance of contamination in the fermentation medium is reduced; however, there is a lower limit of moisture content below which *A. niger* may not produce citric acid. This may be due to the higher osmotic pressure levels at lower moisture contents [10]. Ngadi and Correia [14] reported that low substrate moisture contents in solid-state fermentation resulted in suboptimal product formation due to reduced mass transfer processes such as diffusion of solutes and gas to the cell during fermentation.

Effect of initial pH

The effect of initial pH on kinetics of fig fermentation is shown in Figure 3. All fermentation parameters except citric acid yield increased with the increase of initial pH from 4 to 7, and decreased somewhat at pH 8. The citric acid yield remained practically constant over the pH range 4–8. The highest values of biomass dry weight (35.5 g/kg wet substrate), citric acid (64.0

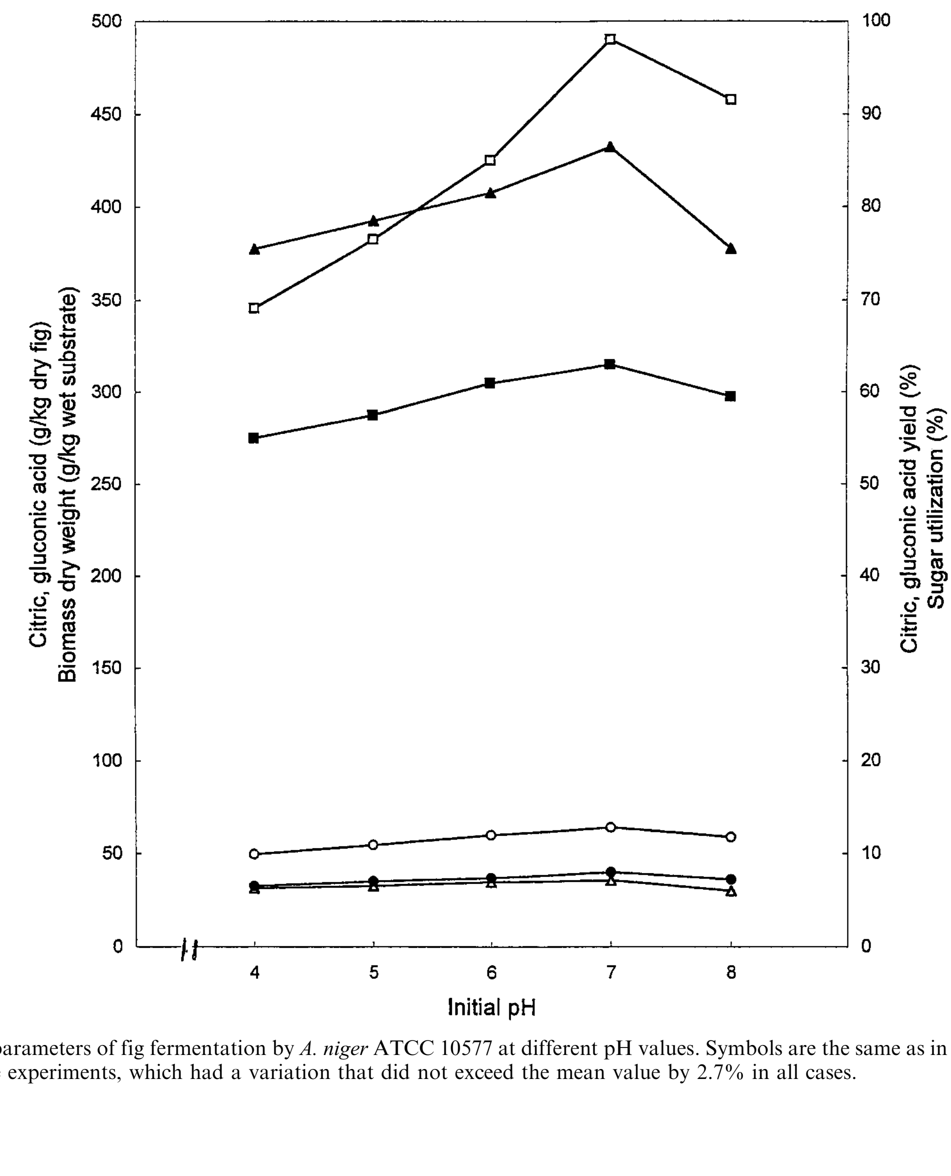

Figure 3 Kinetic parameters of fig fermentation by *A. niger* ATCC 10577 at different pH values. Symbols are the same as in Figure 2. Data are by means of duplicate experiments, which had a variation that did not exceed the mean value by 2.7% in all cases.

g/kg dry figs), gluconic acid concentration (490.0 g/kg dry figs), citric acid yield (8%), gluconic acid yield (63%), and sugar utilization (86.5%) were obtained at an initial pH of 7.0.

Effect of temperature

As shown in Figure 4, the citric acid concentration, citric acid yield, gluconic acid concentration, and gluconic acid yield increased with the increase in fermentation temperature from 25 to 30°C and decreased above 30°C. This was due to denaturation of the enzyme system at high temperatures [10]. Biomass dry weight and sugar utilization increased slightly as the fermentation temperature increased from 25 to 40°C and then decreased. Szewczyk and Myszka [22] found that the temperature did not strongly affect the growth rate in the range of 28–34°C for *A. niger* in solid-state fermentation. The maximum citric acid concentration, citric acid yield, gluconic acid concentration and gluconic acid yield were obtained at 30°C whereas biomass dry weight and sugar utilization were maximum at 40°C. These results agree with those of Hang and Woodams [7] who studied the effect of temperature on citric acid production from grape pomace by solid-state fermentation.

Effect of methanol

The effects of methanol are shown in Figure 5. All the fermentation parameters except biomass dry weight increased with the increase in methanol concentration from 3% to 6% (w/w) and decreased as the methanol concentration was increased beyond 6%. However, the biomass dry weight remained almost constant with the increase in methanol concentration from 3% to 7%. The highest values of citric acid concentration (96.0 g/kg dry figs), citric acid yield (11%), gluconic acid concentration (685.0 g/kg dry figs), and sugar utilization (97.7%) were obtained in the presence of methanol at a concentration of 6% (w/w). Hang *et al* [9] and Roukas and Kotzekidou [18] reported that addition of 1–4% (v/v) methanol resulted in a marked increased in the amount of citric acid formed by *A. niger* on spent grain liquor and brewery wastes, respectively. The observed increases in citric acid concentration show that methanol has a profound effect on the metabolism of sugars by *A. niger*. The mechanism by which methanol stimulates citric acid production from sugars is not clear. Maddox *et al* [12] reported that the effect of methanol is at the cell permeability level, allowing metabolites to be excreted from the cell.

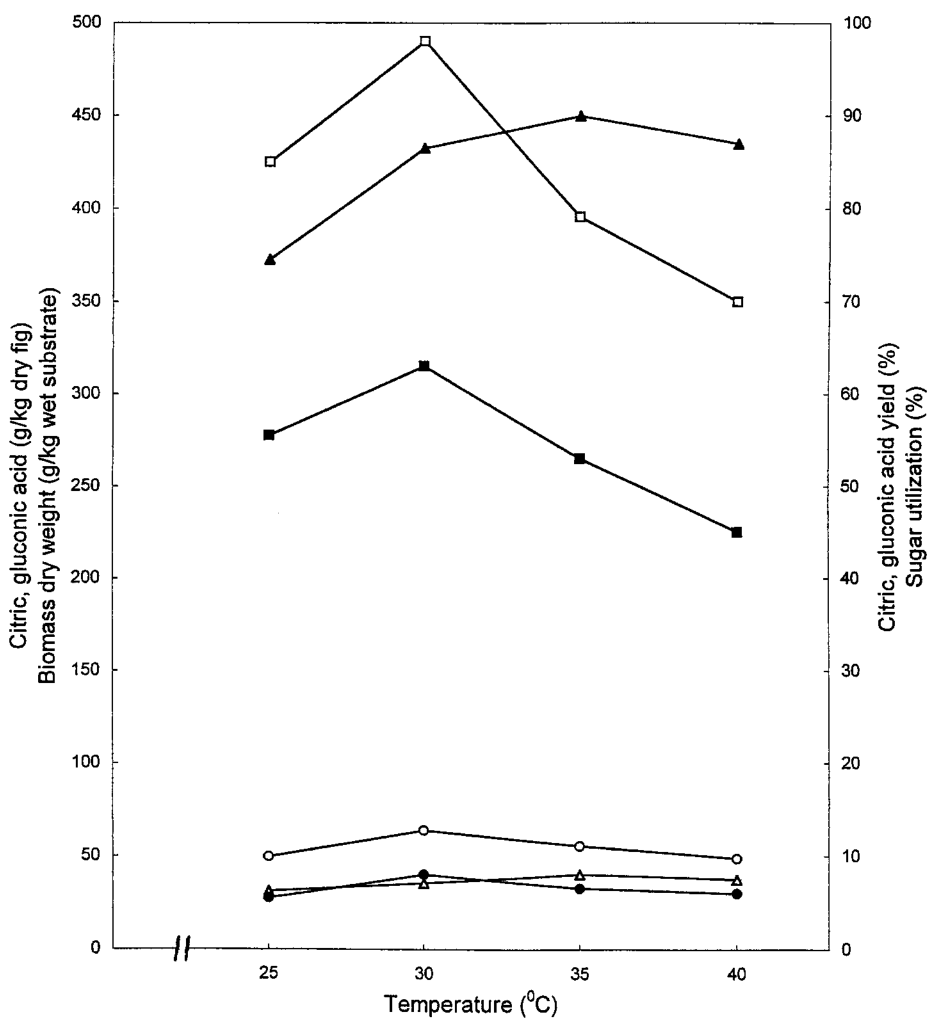

Figure 4 Kinetic parameters of fig fermentation by *A. niger* ATCC 10577 at different temperatures. Symbols are the same as in Figure 2. Data are by means of duplicate experiments, which had a variation that did not exceed the mean value by 4.0% in all cases.

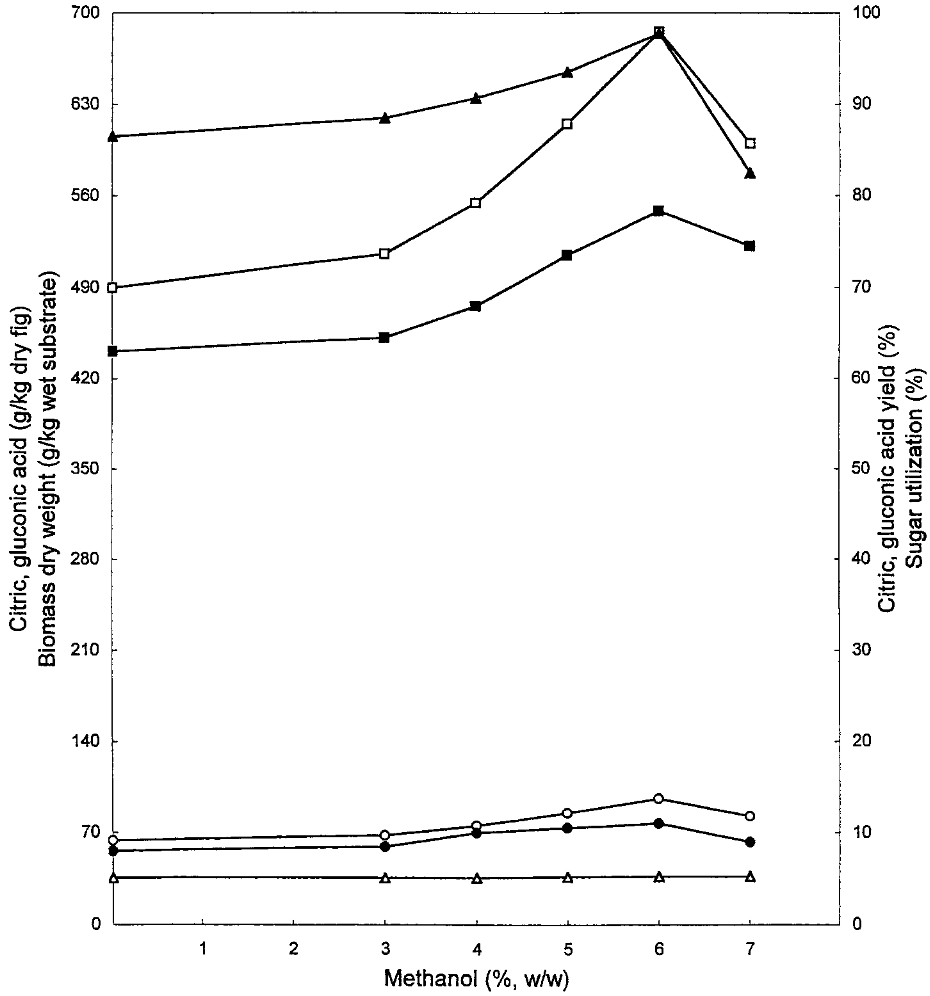

Figure 5 Effect of methanol addition on kinetic parameters of fig fermentation by *A. niger* ATCC 10577 in solid-state fermentation. Symbols are the same as in Figure 2. Data are by means of duplicate experiments, which had a variation that did not exceed the mean value by 4.5% in all cases.

In conclusion, our results showed some important aspects of citric and gluconic acids production from figs by *A. niger* in solid-state fermentation. Optimum conditions are moisture 75%, initial pH 7.0, and temperature 30°C. Addition of methanol at concentrations up to 6% (w/w) resulted in a marked increase in citric and gluconic acids concentration. The fig was an attractive medium for the production of citric and gluconic acid by *A. niger*.

References

1 Agricultural Statistics of Greece. 1990. National Statistical Service of Greece, Athens.
2 Buzzini P, M Gobbetti, J Rossi and M Ribaldi. 1993. Utilization of grape must and concentrated rectified grape must to produce gluconic acid by *Aspergillus niger* in batch fermentations. *Biotechnol Lett* 15: 151–156.
3 Dubois M, KA Gilles, JK Hamilton, PA Rebers and F Smith. 1956. Colorimetric method for determination of sugars and related substances. *Anal Chem* 28: 350–356.
4 Esuoso KO, RA Oderinde and JI Okogun. 1991. Citric acid production from imumu *Cyperus esculentus* and maize *Zea mays*. *J Ferment Bioeng* 71: 200–202.
5 Hang YD and EE Woodams. 1984. Apple pomace: a potential substrate for citric acid production by *Aspergillus niger*. *Biotechnol Lett* 6: 763–764.
6 Hang YD and EE Woodams. 1985. Grape pomace: a novel substrate for microbial production of citric acid. *Biotechnol Lett* 7: 253–254.
7 Hang YD and EE Woodams. 1986. Utilization of grape pomace for citric acid production by solid-state fermentation. *Am J Enol Vitic* 37: 141–142.
8 Hang YD, BS Luh and EE Woodams. 1987. Microbial production of citric acid by solid state fermentation of kiwifruit peel. *J Food Sci* 52: 226–227.
9 Hang YD, DE Splittstoesser, EE Woodams and RM Sherman. 1977. Citric acid fermentation of brewery waste. *J Food Sci* 42: 383–384.
10 Kargi F, JA Curme and JJ Sheehan. 1985. Solid-state fermentation of sweet sorghum to ethanol. *Biotechnol Bioeng* 27: 34–40.
11 Kim DM and HS Kim. 1992. Continuous production of gluconic acid and sorbitol from Jerusalem artichoke and glucose using an oxidoreductase of *Zymomonas mobilis* and inulinase. *Biotechnol Bioeng* 39: 336–342.
12 Maddox IS, M Hossain and JD Brooks. 1986. The effect of methanol on citric acid production from galactose by *Aspergillus niger*. *Appl Microbiol Biotechnol* 23: 203–205.
13 Moksia J, C Larroche and JB Gros. 1996. Gluconate production by spores of *Aspergillus niger*. *Biotechnol Lett* 18: 1025–1030.
14 Ngadi MO and LR Correia. 1992. Solid-state ethanol fermentation of apple pomace as affected by moisture and bioreactor mixing speed. *J Food Sci* 57: 667–670.
15 Rodriguez JA, J Echevarria, FJ Rodriguez, AD Sierra and O Martinez. 1985. Solid state fermentation of dried citrus peel by *Apergillus niger*. *Biotechnol Lett* 7: 577–580.

16 Roukas T. 1999. Citric acid production from carob pod by solid-state fermentation. *Enzyme Microb Technol* 24: 54–59.
17 Roukas T and E Alichanidis. 1991. Citric acid production from beet molasses by cell recycle of *Aspergillus niger*. *J Ind Microbiol* 7: 71–74.
18 Roukas T and P Kotzekidou. 1987. Influence of some trace metals and stimulants on citric acid production from brewery wastes by *Aspergillus niger*. *Enzyme Microb Technol* 9: 291–294.
19 Roukas T and P Kotzekidou. 1997. Pretreatment of date syrup to increase citric acid production. *Enzyme Microb Technol* 21: 273–276.
20 Rugsaseel S, S Morikawa, K Krimura and S Usami. 1995. Stimulation of citric acid production in *Aspergillus niger* by addition of viscous substances in shake flask. *Appl Microbiol Biotechnol* 42: 839–843.
21 Saffran M and OF Denstedt. 1948. A rapid method for the determination of citric acid. *J Biol Chem* 175: 849–855.
22 Szewczyk KW and L Myszka. 1994. The effect of temperature on the growth of *A. niger* in solid state fermentation. *Bioprocess Eng* 10: 123–126.
23 Watt BK and AL Merrill. 1963. Composition of foods. Agriculture Handbook No 8, Consumer and Food Economics Research Division, Agriculture Research Service, United States Department of Agriculture.