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Full Length Research Paper

Citric acid production from whey with sugars and additives by *Aspergillus niger*

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Citric acid (CA) production by *Aspergillus niger* ATCC9642 from whey with different concentrations of sucrose, glucose, fructose, galactose riboflavin, tricalcium phosphate and methanol in surface culture process was studied. It was found that whey with 15% (w/v) sucrose with or without 1% methanol was the most favourable medium producing the highest amount (106.5 g/l) of citric acid. Lower CA was produced from whey with other concentrations of sugars and other additives used. Highest biomass of *A. niger* was produced with the addition of riboflavins. In general, extension of the fermentation (up to 20 days) resulted in an increase in CA and biomass, and decrease in both residual sucrose and pH values.

Key words: Citric acid, *Aspergillus niger*, whey fermentation, surface culture.

INTRODUCTION

Citric acid (CA), a carboxylic organic acid, soluble in water with a pleasant taste, is the most important acid used in the food industries. Until about 1920, all commercial CA was produced from lemon and lime juices (King and Cheetham, 1987). Rohr et al (1983) reported that CA can be produced by fermentation process using species of microorganisms namely *Aspergillus niger*, a fungus which was used commercially for the first time in 1923. They also indicated that factors affecting the production of CA by fermentation include the nutritional composition of the media, environmental conditions, deficiency of manganese and other metals, pH, and dissolved oxygen tension. The influence of types and concentrations of sugars (Hossain et al., 1984; Xu et al., 1989), chelating effect on metal ions (Roukas and Kotzekiodou, 1997), ammonium nitrate and aeration (Bayraktar and Mehmetoglu, 2000) on CA production by *A. niger* have also been studied. At present time CA is produced commercially using mutant strains of *A. niger*, and with a significant amount by *Saccharomycopsis lipolytica* (Good et al., 1985), *Pencillium simplicissimum* (Franz et al., 1993) and *A. foeitidus* (Tran et al., 1998). Other carbohydrates and wastes that have been considered, experimentally, to produce CA by *A. niger* includes inulin (Drysdale and McKay, 1995), date fruit syrup (Roukas and Kotsekiodou, 1997), sugar cane molasses (Gupta, 1994), soya whey (Khare, 1994), kumara (Lu, 1995), Carob pod (Roukas, 1998) and cheese whey (El-Samragy, 1996; Hossain et al., 1984).

Large amounts of whey are produced world wide 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. The aim of this study was to produce citric acid by *A. niger* from cheese whey fortified with different concentrations of sucrose, glucose, fructose, galactose, tricalcium phosphates, methanol and riboflavin in a liquid surface culture process.

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Table 1. Citric acid production from whey with different sugar concentrations by *A. niger* in a surface culture process.

Media	Citric Acid (g/L)* during different incubation time (days)						
	4	6	8	10	12	14	16
Whey	0.6	1.27	2.43	2.28	0.13	0.0	0.0
Whey + 5% sucrose	5.51	13.38	14.22	0.90	0.00	0.00	0.00
Whey + 10% sucrose	4.80	9.16	15.50	27.30	37.59	35.29	19.90
Whey + 15% sucrose	4.10	9.10	18.25	30.48	47.46	79.09	106.50
Whey + 5% glucose	2.56	7.94	3.46	0.00	0.00	0.00	0.00
Whey + 10% glucose	1.99	4.74	21.13	26.64	3.84	0.00	0.00
Whey + 15% glucose	2.05	4.35	14.47	31.38	54.18	46.11	29.90
Whey + 5% fructose	2.05	5.38	9.22	0.77	0.00	0.00	0.00
Whey + 10% fructose	2.05	3.84	7.22	14.47	32.28	31.13	17.02
Whey + 15% fructose	1.79	2.82	9.22	23.95	45.85	48.24	59.66
Whey + 5% galactose	0.51	3.27	5.89	1.92	0.00	0.00	0.00
Whey +10% galactose	0.19	1.73	7.49	16.84	27.35	34.13	28.16
Whey +15% galactose	0.13	0.38	3.71	12.23	22.26	40.99	57.76

*Values are the average of duplicate samples.

MATERIALS AND METHOD

A. niger

A. niger ATCC 9642 stock culture was reactivated and cultivated by streaking a loopfull of the culture on Petri dishes containing solidified acidified(with 10% tartaric acid) potato dextrose agar (PDA) and incubated at 25^{0}C for 5 days. Spores formed were washed out twice with 10 ml distilled sterilized water each time. Spore suspensions containing about log 8/ml were prepared and used as inoculums for the fermentation process.

Fermentation Media

Whey from the dairy plant of the University of Jordan was used as the basal fermentation media. Its proximate composition was determined. Sucrose, glucose, fructose and galactose sugar solutions of 5, 10, and 15 % (w/v) each were added to the whey in the fermentation process. Different concentrations of tricalcium phosphate (TCP), methanol (1, 2, 3, 4 and 5%), and riboflavin (10, 20, 30, 40 and 50 mg/L) were also used to fortify the fermentation media. Surface liquid culture fermentation process was carried out in a 500 ml Erlemyer flask containing 100 ml media. Each flask was inoculated with the given spore suspension and incubated at 30^{0}C for up to 20 days.

Citric Acid Determination

Citric acid (CA) was determined titrimetrically (AOAC, 1995) by using 0.1 N NaOH and phenolphthalin as indicator and calculated as % according to the following formula:

$$\%CA = \frac{\text{Normality X volume of NaOH X Equiv. wt. of CA}}{\text{Weight of sample (g) X 10}}$$

Biomass, residual sugars and pH determination

Biomass, residual sugars and pH values were determined according to AOAC, (1995). To determine biomass, the whole fungal culture growth was filtered with Whatman filter paper #4, washed with distilled water (250 ml) and dried at 105^{0}C to constant weight. Results was expressed as g/l. Residual sugars were determined using SL50 UV-Visible Spectrophotometer and expressed as g/l glucose. Culture pH was measured by Analoge WPA pH meter. The initial pH of the fermentation culture was adjusted to 3 using 1 N of HCl and/or NaOH.

RESULTS AND DESCUSSION

The proximate composition of cheese whey used as the basic fermentation media in this study was found to be 4.9% lactose, 1.0% crude protein, 0.5% ash, 0.2% fat, 6.4% total soluble solid (TSS) and 93.3% water. Table 1. shows citric acid production by *A. niger* from whey as a basic fermentation media, and with different concentrations of sucrose, glucose, fructose and galactose. Low amount of CA (2.43 g/L) was produced from whey alone. Adding different sugars to whey enhanced CA production with a maximum value of 106.5 g/L with 15% sucrose. Significantly lower values were obtained using same concentration of other sugars. The poor CA production from whey alone is believed to be at least partly due to the presence of galactose moiety of lactose in the whey (Hossain et al., 1984). It was believed that *A. niger* can readily utilize galactose its presence or that of its metabolic products causes inhibition of citric acid production and also reduce the rate of glucose utilization. These authors found that galactose interferes with the glucose repression of the key enzyme, 2-oxoglutarate dehydrogenase. There is a strong relationship between citric acid production and the

Table 2. Citric acid production and biomass from whey + 15% sucrose with different concentrations of riboflavin, tricalcium phosphate and methanol by *A. niger* ATCC 9642 in a surface culture media.

Media	Citric Acid (g/L)* during different incubation time (days)							Biomass (g/l) after 16 days
	4	6	8	10	12	14	16	
Whey alone	0.60	1.27	2.43	2.28	0.13	0.00	0.00	14.3
Whey + 15% sucrose	3.71	7.30	19.21	28.05	38.94	69.16	92.86	33.9
Whey + 10% sucrose + 10 mg/L ribpflavin	1.67	3.97	20.87	45.47	62.37	51.10	18.44	42.2
Whey + 10% sucrose + 20 mg/L ribpflavin	1.28	3.07	7.94	22.54	43.93	46.62	30.61	43.1
Whey + 10% sucrose + 30 mg/L ribpflavin	0.51	2.94	6.53	19.60	48.54	56.48	43.29	46.0
Whey + 10% sucrose + 40 mg/L ribpflavin	0.64	2.95	9.67	26.96	43.87	49.89	33.49	42.9
Whey + 10% sucrose + 50 mg/L ribpflavin	0.13	1.54	6.28	22.41	36.70	46.49	31.00	42.0
Whey + 15% sucrose + 1% tricalcium PO_4	5.19	11.40	17.55	24.49	34.20	37.14	14.73	37.3
Whey + 15% sucrose + 2% tricalcium PO_4	4.48	8.58	16.39	18.19	27.92	32.40	30.23	34.4
Whey + 15% sucrose + 3% tricalcium PO_4	6.40	11.53	13.58	21.77	28.69	33.17	31.25	34.2
Whey + 15% sucrose + 4% tricalcium PO_4	4.35	11.53	16.14	23.05	30.74	40.60	45.60	38.9
Whey + 15% sucrose + 5% tricalcium PO_4	3.97	7.68	11.01	15.50	23.28	41.24	5.51	31.1
Whey + 15% sucrose + 1% methanol	0.64	1.15	3.59	10.31	34.45	68.14	92.40	37.4
Whey + 15% sucrose + 2% methanol	0.32	0.38	0.90	3.97	15.75	40.86	76.46	37.2
Whey + 15% sucrose + 3% methanol	0.98	3.97	4.10	4.10	4.74	5.76	12.04	12.3
Whey + 15% sucrose + 4% methanol	0.64	2.63	3.07	3.14	3.27	4.67	11.27	8.1
Whey + 15% sucrose + 5% methanol	0.45	2.18	2.69	2.69	3.71	4.48	5.44	6.9

*Values are the average of duplicate samples.

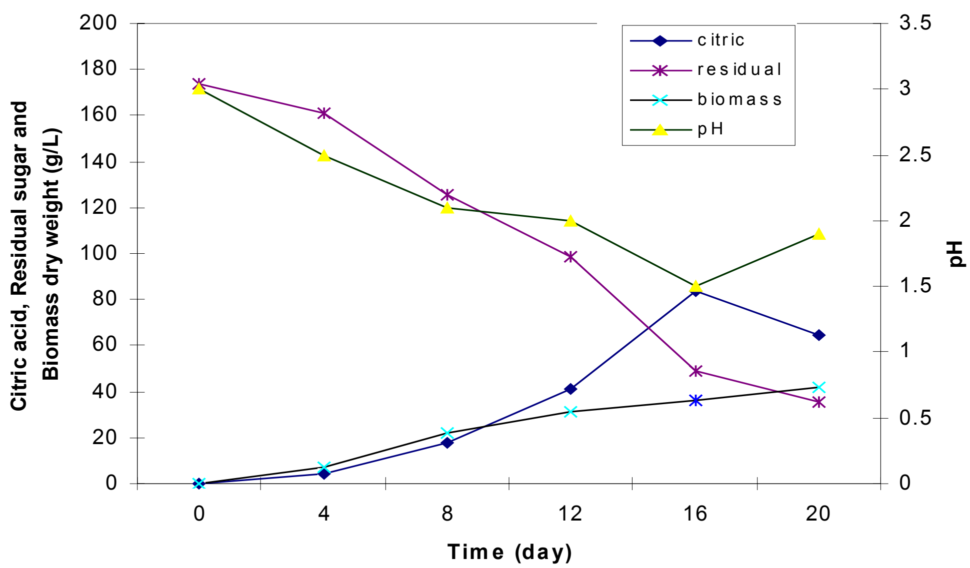

Figure 1. Citric acid, Residual sugar, Biomass and pH values from whey with 15% sucrose by *A. niger* ATCC 9642 in a surface culture process during 20 days fermentation period.

activities of this enzyme and pyrovate dehydrogenase in cell free extracts (Moddax et al., 1986). Hossain et al. (1985) explained that the nature of sugar source has a marked effect on citric acid production by *A. niger*. Sucrose is the traditional commercial substrate for CA production. Glucose, fructose and maltose have also been used as substrates for CA production (Xu et al., 1989). Sucrose is of relatively low molecular weight and readily transported into microbial cells for hydrolysis by intracellular enzymes (Drysdale and McKay, 1995)

The result of the influence of different concentrations of each of riboflavin, TCP and methanol added to whey media containing 15% sucrose on citric acid and biomass production are presented in Table 2. The highest citric acid values of 92.46-92.86 g/L were produced in the whey media containing 15% sucrose with or without 1% methanol, respectively. Much lower CA values were obtained with the addition of riboflavin and TCP throughout 16 days fermentation period. Higher methanol concentration (up to 5%) caused drastic decrease in CA production reaching its minimum (5.4 g/L) with the addition of 5%. CA values steadily increased with incubation time. Relatively higher biomass values (42-46 g/l) were found in the cultures containing riboflavin after 16 days. Lower values (31.1-38.9 g/l) were recorded in the cultures with the TCP. Biomasss in the cultures containing methanol decreased from 37.4g/l with 1% methanol to 6.9g/l with 5% methanol.

Previous reports (Moddax et al., 1986; Hossain et al., 1985) stated that the presence of methanol in the fermentation media may increase CA production by *A. niger.* The inductive effect of methanol for citric acid production may be due to reduction of the inhibitory effect of metal ions (Kiel et al., 1981). In the absence of methanol little or no citric acid was produced from galactose. The addition of TCP to date extract induced CA production which probably chelates high levels of inhibitory metal ions like Mn, Fe, and Zn present in the date extract (Roukos and Kotzekidou, 1997). Adding TCP, riboflavin and methanol to whey may cause similar adverse effects by chelating certain metal ions like Cu^{+2} which is reported to be necessary component in the structure of the productive fungal pellets (Benuzzi and Segoria, 1997).

Citric acid, residual sugar, biomass and pH values from whey (with 15% sucrose) fermentation media by *A. niger* during 20 days are presented in Figure 1. A gradual increase was obtained in both citric acid values with fermentation time, reaching its maximum of 83.7 mg/L after 16 days, followed by a decrease to 42 g/L after 20 days. Biomass increase continues to the 20th day. These increases in CA and biomass values were accompanied with steady decrease in residual sugar from 173.8 g/L initially to a minimum of 35.6 g/L after 20 days, as well as in pH values from 3.0 initially to 1.5 after 16 days. In conclusion, we found in this study that using whey alone as a natural fermentation medium for citric acid production was inferior to whey fortified with sugars. Maximum citric acid was produced from whey with 15% sucrose.

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