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Citric Acid Production by *Aspergillus niger* Using Molasses and Pumpkin as Substrates

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Abstract: Two starchy substrates like pumpkin and cane molasses were selected for citric acid fermentation by using gamma ray induced mutant strains of 14/20 and 79/20 of *A. niger* under surface culture condition. Citric acid production was also different with various fermentation media by *A. niger* 14/20 and 79/20 strains. It was found to increase with the increase of fermentation period and maximum citric acid was found on day 13. In the presence of Prescott salt citric acid production was found lower than the absence of Prescott salt. Without Prescott salt highest values of citric acid production was found in mixed fermentation medium which were about 14.86 mg/ml and 14.44 mg/ml for *A. niger* 14/20 and 79/20 strains respectively, lowest production of citric acid was found in molasses medium for *A. niger* 14/20 and 79/20 strains were 7.72 and 7.57 mg/ml respectively. Whereas in the presence of Prescott salt, lowest production of citric acid in Pumpkin medium was found 2.86 mg/ml for *A. niger* 14/20 and 2.7 mg/ml for *A. niger* 79/20. Highest amount of citric acid was produced in molasses medium 4.88 mg/ml for *A. niger* 14/20 on day 13 with the presence of Prescott salt whereas 4.75 mg/ml for *A. niger* 79/20 strains respectively. Mixed substrate prepared with molasses and pumpkin media was proved to be the best and potential for citric acid production.

Key words: Citric acid · *Aspergillus niger* · Substrate · Prescott salt

INTRODUCTION

Citric acid is a weak organic acid found in citrus fruits. It is a good, natural preservative and is also used to add an acidic (sour) taste to foods and soft drinks and other food products. Utilization of citric acid includes flavor enhancement, bacterial inhabitant, pH adjustment and as an anti oxidant.

Aspergillus niger is most commonly used for citric acid production [1-3]. This is because of the fact that this organism has capacity to utilize varieties of substrates due to its well-developed enzymatic system [4]. *A niger* is normally a haploid fungus producing white septate hypha which is profusely branched. It produces black mass of conidia, which are found in chain arising from the secondary sterigmata. Citric acid is mainly produced by a fungus *Aspergilus niger* by utilizing starchy and sugar substrates [5,6].

Bangladesh at present has to import cent percent citric acid from foreign countries and as it is a very essential chemical and extensively used in food and pharmaceutical industries. Industrial production of this chemical by fermentation using cheap raw materials will be helpful in economic development of our country. Keeping in view the future requirements and also the availability of cheap raw material, we should develop the process for citric acid fermentation, based on our local discarded starchy substrate such as molasses from sugar mills. In addition, Pumpkin and other cheap starchy raw materials can also be exploited for citric acid production, which will have some cost effective impact on our economy. If citric acid is to be produced in commercial level, a suitable substrate must be looked for with a view to select the substrate, some aspect of citric acid production by microbial fermentation, using a number of indigenous raw materials, have been carried out in present study.

The present investigation was therefore, undertaken with a view to determine the feasibility of using raw and cheap materials such as molasses and pumpkin for citric acid production and optimization under fermentation condition on these substrates.

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MATERIALS AND METHODS

Microorganism Used: Citric acid producing strains of *Aspergillus niger* designated as 14/20 and 79/20 [7] were used in this present study. 14/20 and 79/20 are second step mutants derived from the strain HB3 which is the first step mutant from the wild type strain CA16 ([7-8]. The culture was maintained on agar slants containing 1% malt extract, 1% yeast extract, 1.5% dextrose and 2.5% bacto agar.

Substrates Used: (a) Cane molasses (b) Pumpkin. Cane molasses containing 20% water, 62% sugar, non-sugar contents 10% and 8% inorganic salts (ash contents), making a blackish homogenous liquid with high viscosity. Ash contents include ions such as Mg, Mn, Al, Fe and Zn in variable ratio [9]. The other substrate bright yellow colored pumpkin was used in the experiments for the comparative study.

Fermentation Medium

(A)Preparation of Molasses Medium: Molasses was clarified by appropriate dilution with water and boiled the solution for half an hour. The clarified molasses was then kept overnight for sedimentation of suspended particles. In order to remove the coarse particle in the solution it was filtered through absorbent cotton and sediment was discarded.

(B)Preparation of Pumpkin Medium: Pumpkin was washed with tap water for several times. Their after pumpkin was sliced thinly and dried in dryer at 50°C. The substrate was powdered by using a grinding machine. Dried powder of pumpkin was hydrolyzed separately in 300 ml solution of 0.05 N HCl and autoclaved at 121°C temperature, 15 Ibs pressure for 20 minutes. The hydrolyzed materials were then filtered through thin cloth.

(C)Preparation of Mixed Substrate Medium: Equal amount of pumpkin and molasses were hydrolyzed in 300 ml solution of 0.05 N HCl and autoclaved at 121°C temperature, 15 Ibs pressure for 20 minutes. The hydrolyzed materials were then filtered through thin cloth. The media was then kept overnight for sedimentation of suspended particles resides in molasses.

Initially 14% sugar, pH 5 and incubation temperature (30ºC) were optimized in case of all used media and the given strains for citric acid fermentation.14% sucrose solution was found better moisture level for citric acid fermentation [10]. A concentration higher than 14 to 18 % however, leads to greater amount of residual sugar making the process uneconomical and when the concentration of the media is more than needed it become inhibitory for citric acid production. And as a result citric acid production also decreases. In the present study, the molasses, pumpkin and mixed substrate media was prepared with calculating the 14% sugar concentration first and thus high yield of citric acid was produced. Begum and Chowdhury [11] investigated that in respect to citric acid production all the strains (318, 79/20, 14/20 and CA16 wild) gave more citric acid at pH 5 than the other pH values (3.5, 4.0, 4.5). In the present study, pH 5 in molasses, pumpkin and mixed substrate media was maintained for optimum production of citric acid. The optimum temperature range of 26-30°C has been proposed for good citric acid yields and rapid rate of accumulation [12]. A temperature of 30ºC was found to be the best for citric acid fermentation (Qp = 0.667 ± 0.02a g/l/h) [13]. The following parameters were selected to find out which one was better for citric acid fermentation: Sugar with Prescott salt and Sugar without Prescott salt (NH_4NO_3, 2.23 g/l; K_2HPO_4, 1.00 g/l and $MgSO_4\,7H_2O$, 0.23 g/l). Fermentation was carried out in 100 ml conical flasks containing 25 ml medium. Titration was done from the 7th day of incubation. Total titrable acid values were determined by freshly prepared 0.1N NaOH. Citric acid and residual sugar were estimated between 3-13 days of culture broth by the Marrier and Boulet method [14] and Anthrone sulphuric acid method [15] respectively.

RESULTS AND DISCUSSION

Increased Weight of Biomass: *Aspergillus niger* strains biomass was increased with the increase period of time. This increase was mainly for the production of mycelial body of the fungus and their sporulation. Without presence of Prescott salt biomass weight was highest for fermented product of *Aspergillus niger* 14/20.

(a) on the Molasses Fermentation Medium: In presence of Prescott salt, wet weight of mycelial body of the fungus *A. niger* 14/20 was found comparatively lower in the molasses fermentation medium than in the absence of Prescott salt on days 3, 5, 7, 9, 11 and 13 (Table 1). In presence of Prescott salt, wet weight of mycelial body of the fungus *A. niger* 79/20 was found comparatively lower in the molasses fermentation medium than in the absence of Prescott salt on the same respective days (Table 2).

Table 1: Weight of biomass at different days of fermentation by *Aspergillus niger* 14/20

		Mycelial weight (mg/ml) after days of fermentation													
		Wet weight							Dry weight						
Substrate	Presence of Prescott salt	0	3	5	7	9	11	13	0	3	5	7	9	11	13
Molasses	With Presence	0	13.85	27.90	38.00	47.90	67.10	82.25	0	3.00	6.18	8.50	11.00	12.50	14.33
Pumpkin	of Prescott salt	0	15.90	23.40	43.20	68.40	92.60	129.76	0	4.61	7.98	12.77	14.00	15.53	16.22
Mixed		0	24.12	36.52	55.00	86.87	116.91	138.08	0	5.84	8.92	13.02	15.62	16.48	17.96
Molasses	Without Presence	0	23.95	32.59	41.20	85.55	115.60	133.00	0	4.92	12.34	18.75	23.40	24.56	29.76
Pumpkin	of Prescott salt	0	27.40	37.89	49.11	104.22	131.60	145.60	0	5.12	13.14	21.00	24.13	26.1	32.25
Mixed		0	31.60	45.40	58.26	121.10	147.80	163.29	0	7.75	16.58	21.85	25.32	29.22	34.94

Table 2: Weight of biomass at different days of fermentation by *Aspergillus niger* 79/20.

		Mycelial weight (mg/ml) after days of fermentation													
		Wet weight							Dry weight						
Substrate	Presence of Prescott salt	0	3	5	7	9	11	13	0	3	5	7	9	11	13
Molasses	With Presence	0	13.00	27.05	36.66	47.11	64.89	79.25	0	2.20	5.18	7.40	9.91	10.68	12.33
Pumpkin	of Prescott	0	14.11	21.35	39.22	62.71	88.57	123.33	0	3.26	6.48	9.40	10.00	11.21	12.22
Mixed		0	21.42	33.72	46.00	80.47	111.21	129.28	0	4.00	6.72	10.15	11.12	11.88	12.36
Molasses	Without Presence	0	22.88	31.15	38.40	85.55	116.33	128.26		4.00	10.64	16.75	21.40	23.00	27.72
Pumpkin	of Prescott	0	25.14	34.25	43.74	98.62	124.20	137.95	0	4.31	11.20	18.06	21.19	22.27	27.89
Mixed		0	27.75	40.11	52.20	119.10	139.23	155.29	0	5.20	13.29	17.75	21.82	22.54	29.74

Wet-weight for both strains of fungus *A. niger* 14/20 and 79/20 were statistically significant. On the other hand, significantly higher amount of dry weight of mycelial body of the fungus *A. niger* 14/20 was found 29.76 mg/ml on day 13 with the absence of Prescott salt. In presence of Prescott salt dry weight was significantly lower for each day measurement. Without the presence of Prescott salt biomass weight was significantly high for fermented product of *A. niger* 14/20 in molasses medium. Without the presence of Prescott salt biomass weight of *A. niger* 79/20 fermented product was respectively lower than *A. niger* 14/20 strain.

(b) on the Pumpkin Fermentation Medium: In presence of Prescott salt, wet weight of mycelial body of the fungus *A. niger* 14/20 was found comparatively lower in the pumpkin fermentation medium than in the absence of Prescott salt on days 3, 5, 7, 9, 11 and 13. In presence of Prescott salt, wet weight of mycelial body of the fungus *A. niger* 79/20 was found comparatively lower in the pumpkin fermentation medium than in the absence of Prescott salt on the same respective days. Wet-weight for both strains of fungus *A. niger* 14/20 and 79/20 were statistically significant. On the other hand, significantly higher amount of dry weight of mycelial body of the fungus *A. niger* 79/20 was found 27.89 mg/ml on day 13 with the absence of Prescott salt. In presence of Prescott salt dry weight was significantly lower for each day measurement. Without the presence of Prescott salt biomass weight was significantly higher for fermented product of *A. niger* 14/20 in pumpkin medium than *A. niger* 79/20.

(C) on the Mixed Fermentation Medium: In presence of Prescott salt, wet weight of mycelial body of the fungus *Aspergillus niger* 14/20 was found 24.12, 36.52, 55.00, 86.87, 116.91 and 138.08 mg/ml respectively in the mixed fermentation media on day 3, 5, 7, 9, 11 and 13. In the absence of Prescott salt, wet weight was found 31.60, 45.40, 58.26, 121.10, 147.80 and 163.29 mg/ml on the respective day. On the other hand, significantly higher amount of wet weight of mycelial body of the fungus *A. niger* 79/20 was found 155.29 mg/ml on day 13 with the absence of Prescott salt. In presence of Prescott salt dry weight was significantly lower for each day measurement.

In presence of Prescott salt, dry weight of mycelial body of the fungus *A. niger* 14/20 was found comparatively lower in the mixed fermentation medium than in the absence of Prescott salt on days 3, 5, 7, 9, 11 and 13. In presence of Prescott salt, dry weight of mycelial body of the fungus *A. niger* 79/20 was found comparatively lower in the mixed fermentation medium than in the absence of Prescott salt on the same respective days. Dry-weight for both strains of fungus *A. niger* 14/20 and 79/20 were statistically significant. Without the presence of Prescott salt biomass weight was significantly higher for fermented product of *A. niger* 14/20 than that of *A. niger* 79/20 on the same mixed medium. Through this result we observe that the biomass production was not same in all fermentation medium. Without presence of Prescott salt biomass weight was significantly higher for fermented product of *A. niger* 14/20 than *A. niger* 79/20 when compared.

Utilization of Sugar Increases at Various Days of Fermentation: Sugar utilization rate was different in various media during citric acid production by *Aspergillus niger* 14/20 and 79/20. Prescott salt was also found to have statistically significant effect on sugar utilization for citric acid production except on days 0 and 3. In presence of Prescott salt, sugar utilization was significantly lower than absence of Prescott salt in the molasses, pumpkin and mixed fermentation media during production of citric acid. With the increase of fermentation period sugar concentration in the medium was reduced and maximum reduction was found on day 13.

(a) on the Molasses Fermentation Medium: Highest amount of sugar was utilized by *A. niger* 14/20 in the absence of Prescott salt on the day 13 on molasses fermentation medium and the value of sugar utilization is statistically significant on the respective days except 0 and 3 days in presence or absence of salt (Table 3).

For the *A. niger* 79/20 in the presence of Prescott salt, residual sugar was found 145, 142.8, 134, 127.8, 115.4, 106.4 and 99.06 mg/ml respectively in the fermentation medium on day 0, 3, 5, 7, 9, 11 and 13. Lowest residual sugar was 65.21 mg/ml on 13 day without the presence of Prescott salt means highest sugar utilization (Table 4). Maximum amount of sugar was utilized by *A. niger* 14/20 in the absence of Prescott salt on the day 13 on molasses fermentation medium than *A. niger* 79. The values of sugar utilization on days 0 and 3 are not statistically significant but the following respective days sugar utilization in presence of Prescott salt and without salt both are significant for the strains *A. niger* 14/20 and 79/20.

(B)on the Pumpkin Fermentation Medium: In presence of Prescott salt, utilization of residual sugar of the fungus *Aspergillus niger* 14/20 was found 144.23, 141.75, 139.39, 130.1, 111.36, 105.35 and 100.28 mg/ml respectively in the pumpkin fermentation medium on day 0, 3, 5, 7, 9, 11 and 13. On the other hand, the residual sugar 143, 137.9, 129.69, 116.37, 100.25, 75.21 and 63.51 mg/ml was found respectively without the presence of Prescott salt on the same respective days. Highest amount of sugar was utilized by *A. niger* 14/20 in the absence of Prescott salt on the day 13 on the pumpkin fermentation medium. In each respective day sugar utilization is significant except on day 0 (Table 4). Significant higher amount of sugar was utilized by *A. niger* 14/20 in the absence of Prescott salt on the day 13 on the pumpkin fermentation medium than *A. niger* 79/20.

Table 3: Sugar utilization at different days of fermentation during citric acid production by *Aspergillus niger* 14/20.

		Estimated sugar concentration (mg/ml) at different period (days) of fermentation						
Substrate	Presence of Prescott salt	0	3	5	7	9	11	13
Molasses	With Presence of Prescott salt	145.30	142.5	132.3	125.4	112.8	103.60	95.83
Pumpkin		144.23	141.75	139.39	130.10	111.36	105.35	100.28
Mixed		145.68	140.13	137.13	131.10	116.50	107.79	101.49
Molasses	Without Presence of Prescott salt	145.50	142.62	128.17	113.84	97.81	73.29	63.24
Pumpkin		143.00	137.90	129.69	116.37	100.25	75.21	63.51
Mixed		144.80	138.90	137.20	119.30	102.10	75.15	62.39

Table 4: Sugar utilization at different days of fermentation during citric acid production by *Aspergillus niger* 79/20

		Estimated sugar concentration (mg/ml) at different period (days) of fermentation						
Substrate	Presence of Prescott salt	0	3	5	7	9	11	13
Molasses	With Presence of Prescott salt	145.0	142.8	134.0	127.8	115.4	106.4	99.06
Pumpkin		144.7	142.5	141.1	132.1	114.2	107.1	101.8
Mixed		145.5	141.9	139.3	132.8	119.0	110.3	104.1
Molasses	Without Presence of Prescott salt	145.4	142.3	130.1	116.2	99.91	76.05	65.21
Pumpkin		143.5	139.5	131.0	118.8	104.4	79.05	67.07
Mixed		145.50	140.8	139.3	122.8	106.19	80.19	68.29

Table 5: Total titratable acidity, citric acid, residual sugar concentration and mycelial dry weight of *Aspergillus niger* strain 14/20 on 13[th] day in sucrose medium at pH 5 and temperature 30ºC under stationary condition in different media

Medium	Presence of Prescott salt	Sugar supplied (mg/ml)	TTA (ml 0.1N NaOH/ml medium)	Citric acid produced (mg/ml)	Mycelial dry weight (mg/ml)	Residual sugar (mg/ml)	Sugar utilized (mg/ml)	Sugar utilized (%)	Citric acid in relation to sugar supplied (% w/w)
Molasses	With Prescott salt	145.3	2.61	4.88	14.33	95.83	49.47	34.47	3.36
Pumpkin		144.23	1.77	2.87	16.22	100.28	43.95	30.47	1.98
Mixed substrate		145.68	2.41	4.76	17.96	101.49	44.19	30.33	3.26
Molasses	Without Prescott salt	145.50	4.00	7.72	29.76	63.24	82.26	56.54	5.31
Pumpkin		143.0	5.80	10.35	32.25	63.51	79.49	55.58	7.24
Mixed substrate		144.8	6.94	14.86	34.94	62.39	82.41	56.91	10.26

Table 6: Total titratable acidity, citric acid, residual sugar concentration and mycelial dry weight of *Aspergillus niger* strain 79/20 on 13[th] day in sucrose medium at pH 5 and temperature 30ºC under stationary condition in different media

Medium	Presence of Prescott salt	Sugar supplied (mg/ml)	TTA (ml 0.1N NaOH/ml medium)	Citric acid produced (mg/ml)	Mycelial dry weight (mg/ml)	Residual sugar (mg/ml)	Sugar utilized (mg/ml)	Sugar utilized (%)	Citric acid in relation to sugar supplied (% w/w)
Molasses	With Prescott salt	145	2.15	4.75	12.33	99.06	45.94	31.68	3.28
Pumpkin		144.7	1.17	2.7	12.22	101.8	42.90	29.65	1.87
Mixed substrate		145.5	2.05	4.48	12.36	104.1	41.40	28.45	3.08
Molasses	Without Prescott salt	145.4	3.55	7.57	27.72	65.21	84.47	58.09	5.21
Pumpkin		143.5	4.9	10.21	27.89	67.07	76.43	53.26	7.11
Mixed substrate		145.5	6.15	14.44	29.74	68.25	77.26	53.31	9.92

(c) on the Mixed Fermentation Medium: In presence of Prescott salt, residual sugar was found 145.68, 140.13, 137.13, 131.10, 116.50, 107.79 and 101.49 mg/ml respectively in the fermentation medium by *Aspergillus niger* 14/20 on day 0, 3, 5, 7, 9, 11 and 13. On the other hand, the residual sugar without the presence of Prescott salt in mixed fermentation medium was 144.80, 138.9, 137.2, 119.3, 102.1, 75.15 and 62.39 mg/ml respectively on the same respective days. Utilization of residual sugar without the presence of Prescott salt in mixed fermentation medium was significantly higher than with the presence of Prescott salt except 0, 3 and 5 days (Table 4).

In presence or absence of salt sugar utilization were also significant statistically (Fig. 6). Utilization of residual sugar without the presence of Prescott salt in mixed fermentation medium was significantly higher than with the presence of Prescott salt by *A. niger* 14/20 and 79/20. But *A. niger* 14/20 utilized significantly more sugar than by *A. niger* 79/20 utilized from the same fermentation medium.

TTA Values at Different Period of Fermentation During Citric Acid Production: Total titratable acidity (TTA) value of citric acid against 0.1 N NaOH was determined at different fermentation period during citric acid production by *Aspergillus niger* 14/20 and 79/20. TTA value was found just proportional to the utilization of sugar in the fermentation medium. In the presence of Prescott salt TTA value was found significantly lower than the absence of Prescott salt.

(a) on the Molasses Fermentation Medium: TTA value for citric acid production by *A. niger* 14/20 was significantly lower in presence of Prescott salt, than found for in absence of Prescott salt. Significantly highest TTA value was found in absence of Prescott salt on 13 day and TTA value was 4 (Table 5). Significantly higher amount of TTA value for citric acid was found in 14/20 strain of *A. niger* than *A. niger* 79/20 in the absence of Prescott salt on the day 13 on the molasses fermentation medium (Table 6). TTA values for citric acid in either presence or absence of Prescott salt are also statistically significant when compared except 3 and 5 days.

(b) on the Pumpkin Fermentation Medium: Significantly higher amount of TTA value for citric acid was found in the absence of Prescott salt on the day 13 in the pumpkin fermentation medium by *A. niger* 14/20 than *A. niger* 79/20.

(c) on the Mixed Fermentation Medium: Significantly highest amount of TTA value for citric acid was found by *A. niger* 14/20 in the absence of Prescott salt in the day 13 on the mixed fermentation medium. And *A. niger* 79/20 produces significantly lower amount of citric acid than *A. niger* 14/20.

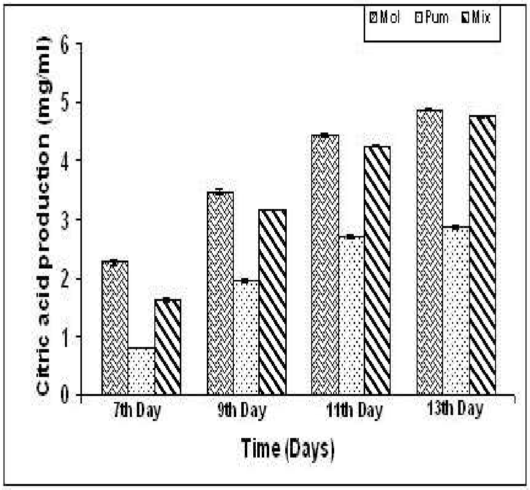

Fig. 1: Citric acid production at different days of fermentation in various substrates (with Prescott salt) by *Aspergillus niger* 14/20.

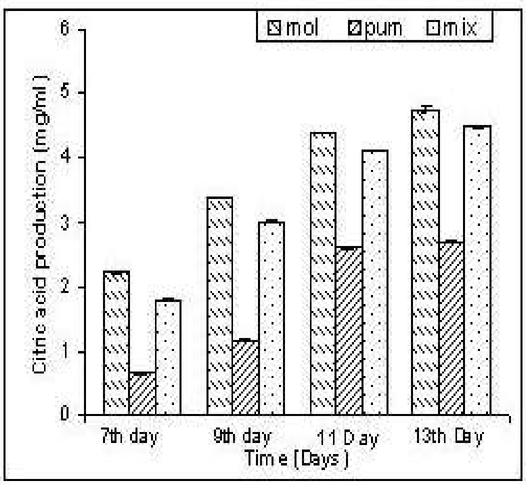

Fig. 2: Citric acid production at different days of fermentation in various substrates (with Prescott salt) by *A. niger* 79/20.

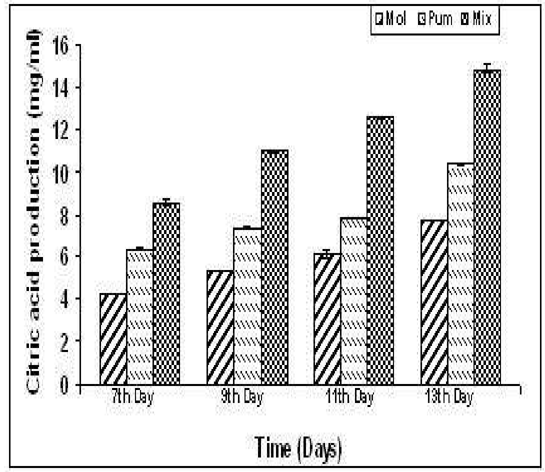

Fig. 3: Citric acid production at different days of fermentation in various substrates (without Prescott salt) by *A. niger* 14/20.

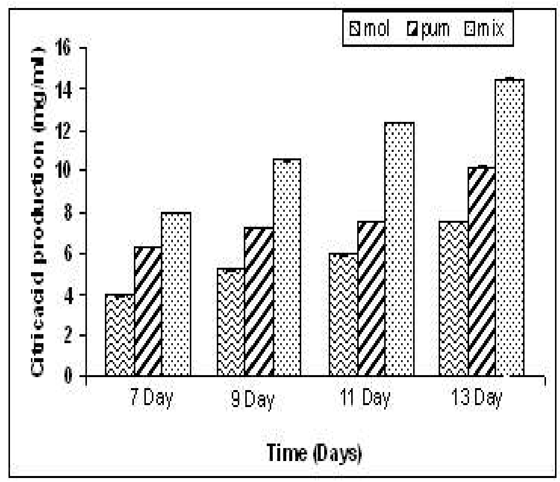

Fig. 4: Citric acid production at different days of fermentation in various substrates (without Prescott salt) by *A. niger* 79/20.

Citric Acid Production Increases at Different Periods of Fermentation in Various Substrates: Citric acid production was also different with various fermentation media by *Aspergillus niger* 14/20 and 79/20 strains. It was found to significantly increase with the increase of fermentation period and maximum citric acid production was found on day 13. In the presence of Prescott salt citric acid production was found significantly lower than the absence of Prescott salt.

On the Molasses Fermentation Medium: In the presence of Prescott salt, production of citric acid by *A. niger* 14/20 was found statistically significant for each days sample (Fig. 1). Such as for 13 days citric acid production was 4.88 mg/ml. Again, citric acid production on day 3, 5, 7, 9, 11 and 13 without the presence of Prescott salt was 1.21, 2.51, 4.21, 5.35, 6.12 and 7.72 mg/ml respectively (Fig. 3). In absence of Prescott salt the values were also statistically significant. And in the presence of Prescott salt production of citric acid by *A. niger* 79/20 was highly significant (Fig. 2) Citric acid production on day 3, 5, 7, 9, 11 and 13 without the presence of Prescott salt was 1.06, 2.08, 3.93, 5.25, 5.94 and 7.57 mg/ml respectively (Fig. 4). Significantly highest amount of citric acid was produced by *A. niger* 14/20 in the absence of Prescott salt on the day 13 on the molasses fermentation medium.

On the pumpkin fermentation medium: In presence of Prescott salt, production of citric acid of the fungus *A. niger* 14/20 was significantly lower than in absence of salt found in the pumpkin fermentation media on day 3, 5, 7, 9, 11, 13. On the other hand, the highest citric acid production on day 13 without the presence of Prescott salt was 10.35 mg/ml. In presence of Prescott salt, production of citric acid of the fungus *A. niger* 79/20 found in the pumpkin fermentation media on day 3, 5, 7, 9, 11 and 13 was 0.18, 0.32, 0.66, 1.86, 2.59 and 2.7 mg/ml respectively. On the other hand, the citric acid production on day 3, 5, 7, 9, 11 and 13 without the presence of Prescott salt was 1.71, 3.33, 6.25, 7.19, 7.57 and 10.21 mg/ml respectively. Highly significant amount of citric acid was produced by *A. niger* 14/20 than *A. niger* 79/20 in the absence of Prescott salt on the day 13 in the pumpkin fermentation medium.

On the Mixed Fermentation Medium: In presence of Prescott salt, citric acid production in mixed fermentation medium by *Aspergillus niger* 14/20 on day 3, 5, 7, 9, 11 and 13 was found 0.49, 0.93, 1.64, 3.16, 4.27 and 4.76 mg/ml respectively. On the other hand, citric acid production on day 3, 5, 7, 9, 11 and 13 without the presence of Prescott salt in mixed fermentation medium was 2.37, 4.55, 8.55, 10.95, 12.55 and 14.86 mg/ml respectively. Citric acid production was highly significant in each day. In presence of Prescott salt citric acid production in the fermentation medium by *A. niger* 79/20 was lower than without the presence of Prescott salt. Citric acid production on 13 day was 14.44 mg/ml in mixed fermentation medium. Production of citric acid without the presence of Prescott salt in mixed fermentation media is significantly higher than with the presence of Prescott salt.

Through this result it was observed that the production of citric acid was not same in all fermentation media. Without the presence of Prescott salt significantly highest citric acid production was found in mixed fermentation medium throughout the fermentation period and lowest production of citric acid was found in molasses media, which was also statistically significant. Whereas with the presence of Prescott salt significantly lowest production of citric acid was found in pumpkin medium and highest amount of citric acid was produced in molasses medium throughout the fermentation period. And in both cases *A. niger* 79/20 produces significantly lower amount of citric acid than *A. niger* 14/20.

From the experiments it appears that the two substrates molasses and pumpkin could be used for the production of citric acid but in case of mixed substrate the citric acid production is much economically feasible. Mixed substrate media was proved more potential substrate for citric acid production. It is apparent from the results that the maximum yield of citric acid was not dependent only upon the total titrable acid value but also on sugar level and biomass.

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