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Utilization of Date Seeds and Cheese Whey in Production of Citric Acid by Candida lipolytica

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ABSTRACT. Date seeds and cheese whey are mainly consisted of organic nitrogen sources, carbohydrates, lipids and minerals. These ingredients were utilized as natural medium for formation of citric acid by *Candida lipolytica*. Yield of citric acid was increased with the increase of fermentation period reaching its maximum at 96 hr. Yeast biomass was closely associated with citric acid formation. The best added carbon source was glucose which pushed the output of citric acid to high titres. The optimum concentration of glucose was 25 mg/ml. $(NH_4)_2SO_4$, NH_4Cl and NH_4NO_3 were used as inorganic nitrogen sources. The experimental organism preferred utilization of inorganic nitrogen sources in the form of ammonia rather than nitrate nitrogen. Addition of different concentrations of date seed ash into the medium increased the output of citric acid which reflected importance of certain elements present in date seed ash such as magnesium, iron, calcium, manganese, zinc and nickel in the biosynthetic formation of citric acid.

Fermentative production of citric acid from raw materials is continuously in progress, especially successful trials in which certain yeasts are utilized in the formation of this important organic acid. As results of many research workers, the main carbon sources are glucose, glycerol, cerelose, and molasses (beet and blackstrap molasses). Finogenova *et al.* (1973) reported that *Candida lipolytica* grown in medium which contained vitamin B_1 (thiamine), glucose, 2.0%, and $(NH_4)_2SO_4$ 0.3% at 28-30°C, a yield up to 26.2% citric acid was achieved. Tachibona (1974) reported that citric acid was accumulated in appreciable amounts when the fermentation medium of *Candida* yeast contained glucose, or glycerol than other carbon sources. Takahoshi and Ikeno

(1974) found that during citric acid formation by *Candida* species in *n*-paraffin medium, addition of lipids at 1-5 gm l^{-1} shortened the yeast growth. *Candida lipolytica* 28 (Fern-P 269) was grown in shaked cultures at 30°C in a medium which contained *n*-paraffins (dodecane 20.0, tridecane 50.0, tetradecane 28.0 and pentadecane 10.0) 4.0% volume, $(NH_4)_2SO_4$ 2.0, corn steep liquor 2.0, vitamin B_1 (thiamine) 100 μg, glycerol 5.0, $CaCO_3$ 10.0 and antifoam agent 0.5 g l^{-1} . The fermentation time was shortened by one day. Masuda *et al.* (1975) grew *Candida lipolytica* in medium which contained purified palm oil 5.0, $(NH_4)_2SO_4$ 0.2, corn steep liquor 0.26, KH_2PO_4 0.0125, $MgSO_4 . 7H_2O$ 0.01, $ZnSO_4$ 0.001, $CaCO_3$ 0.1% and vitamin B_1 (thiamine) 100 μg l^{-1} at 30°C for six days. 0.2% $CaCO_3$ was also added to the fermentation medium when the pH was dropped to less than 4.0. After one to two days of the fermentation process, 0.2% $CaCO_3$ was added to the fermentation medium so that the total addition of $CaCO_3$ was 0.5%. Yield of citric acid was 77.0 gm l^{-1}.

Many other research workers found that it is essential to provide the citric acid producers with carbon, nitrogen and phosphorus sources, vitamins, and micro-nutrients such as iron, copper, zinc, manganese, and lead (Johnson *et al.* 1973, Lewis 1951, Joshi and Ramakrishnan 1959, Kovats 1961, Hanson *et al.* 1961, and Hamissa *et al.* 1982).

The aim of the present research was to utilize local agricultural and industrial by-products, such as date seeds and cheese whey, in a fermentation medium for formation of citric acid by *Candida lipolytica*.

Material and Methods

Cheese whey was obtained from the Danish Saudi Dairy Co. Ltd, Jeddah and date seeds were obtained from Madina, Saudi Arabia.

Analyses

Determination of sugars in date seeds was made by the method of Somogyi (1945). Total protein was determined by the method of Miller and Houghton (1945). Identification and quantitative determination of amino acids present in hydrolysates of date seeds, cheese whey and yeast biomass were by the LKB-4101 fully automatic amino acid analyser (LKB Biochrom Ltd.). The pH value of the fermentation medium was measured by a pH-meter (Corning Scientific Instruments Model 12 research pH - meter). Crude lipids and ash content of date seeds were determined according to the Official Methods of the AOAC (1975). Calcium, iron, aluminium, magnesium, manganese, zinc and nickel present in the ash of date seeds were determined by the atomic absorption spectrophotometer SP-191 Pye Unicam, while citric acid was determined by the method of Marier and Boulet (1958).

Preparation of Dry Date Seed Hydrolysate

Date seeds were obtained from Madina, Saudi Arabia. Date seeds of the date palm

fruits *(Phoenix dactylifera)* were dried at 90-95°C and then ground to fine powder in a pulverizing mill. The powder was then sieved through a 40-mesh sieve and dried again in an oven until constant weight. A sample of 200 gm of the powdered seeds was placed into a two litre round bottom flask to which one litre of 3N HCl was added. The mixture was refluxed for 7 hr, then cooled to room temperature and filtered through a Buchner funnel. The filtrate was taken and concentrated till dryness. The dry date seed hydrolysate was then kept in a desiccator.

Maintenance of Candida lipolytica *NRRL Y-1552*

The experimental yeast was maintained on the following medium which contained the following ingredients ($g\ l^{-1}$): glucose 10.0, peptone 5.0, yeast extract 5.0, agar 30.0 and distilled water. The ingredients were thoroughly mixed and portioned into test tubes. The test tubes were plugged with cotton wool and sterilized at 110°C for 10 min. The sterilized slants were inoculated with *Candida lipolytica* and incubated at 30°C to obtain luxuriant growth. The slants were kept at 5°C in a refrigerator.

Inoculum Medium

The incolum medium contained the following ingredients ($g\ l^{-1}$): glucose 20.0, yeast extract 5.0, NH_4Cl 2.0, $MgSO_4$. $7H_2O$ 0.5, KH_2PO_4 1.0, $MnSO_4$. $4H_2O$ 0.05, $FeSO_4$. $7H_2O$ 0.005 and distilled water. The ingredients were thoroughly mixed. The initial pH value of medium was adjusted to 5.5 and the medium was dispensed into 250 ml Erlenmeyer flasks in 50 ml portions. The flasks were plugged with cotton wool and sterilized at 120°C for 20 min. When the flasks attained room temperature, they were inoculated with *Candida lipolytica* (growth from one slant per flask containing 50 ml inoculum medium) under aseptic conditions. The flasks were incubated on a rotary shaker at 30°C for 72 hr. At the end of the incubation period the growing cells were ready to be used in the inoculation of the fermentation media.

Fermentation Medium

The fermentation medium contained the following ingredients ($g\ l^{-1}$): dry date seed hydrolysate 30.0, dry cheese whey 15.0, KH_2PO_4 1.0, $MgSO_4$. $7H_2O$ 0.25, $MnSO_4$. $4H_2O$ 0.05, $FeSO_4$. $7H_2O$ 0.005, and distilled water. The initial pH value of the medium was adjusted to 5.5. The fermentation medium was dispensed into 250 ml Erlenmeyer flasks in 50 ml portions. The flasks were plugged with cotton wool and sterilized at 120°C for 20 min. When the flasks attained room temperature they were inoculated with the inoculum medium under aseptic conditions. The percentage of inoculum was 5.0%. The flasks were incubated on a rotary shaker at 30°C for 120 hr. At the end of the fermentation process, final pH of the fermented medium, dry yeast biomasses and citric acid yield were determined.

Influences of different concentrations of dry date seeds hydrolysate and cheese whey on fermentative production of citric acid were also investigated.

Results and Discussion

Chemical Composition of Date Seeds

Date seeds are natural products and they contain a number of ingredients. Some analytical results are shown in Table 1 and Fig. 1. Date seeds contain variable ingredients: minerals, carbohydrates, lipids, and amino acids. As date seeds contain these different ingredients, they may be used as a medium for the growth of *Candida lipolytica* and formation of citric acid. Therefore, date seeds were ground and hydrolysed. The hydrolysate was incorporated into the fermentation medium as miscellaneous ingredients for the fermentative production of citric acid.

Table 1. Chemical constituents present in date seeds

Components	Percentage
A. Elements	g/100 g ash of date seeds
Magnesium	5.900
Iron	4.100
Aluminium	3.250
Calcium	1.400
Manganese	0.150
Zinc	0.088
Nickel	0.032
B. Sugars	g/100 g of date seeds
Soluble sugars	4.00
Insoluble sugar	2.76
C. Total lipids	7.10
D. Ash	2.20
E. Water content	9.50

Whey as an industrial waste product was obtained from the Danish Saudi Dairy Co. Ltd., Jeddah, Saudi Arabia (Ashy *et al.* 1981). Milk whey is a natural product and it contains a number of ingredients. Some analytical results are shown in Table 2. The following amino acids: arginine, lysine, histidine, phenylalanine, tyrosine, leucine, isoleucine, methionine, valine, cystine, alanine, glycine, proline, glutamic acid, serine, threonine and aspartic acid were present in whey as identified by amino acid analyser (Fig 2). As whey contains these miscellaneous ingredients, it may be used besides date seeds hydrolysate as a medium for the fermentative formation of citric acid by *Candida lipolytica*.

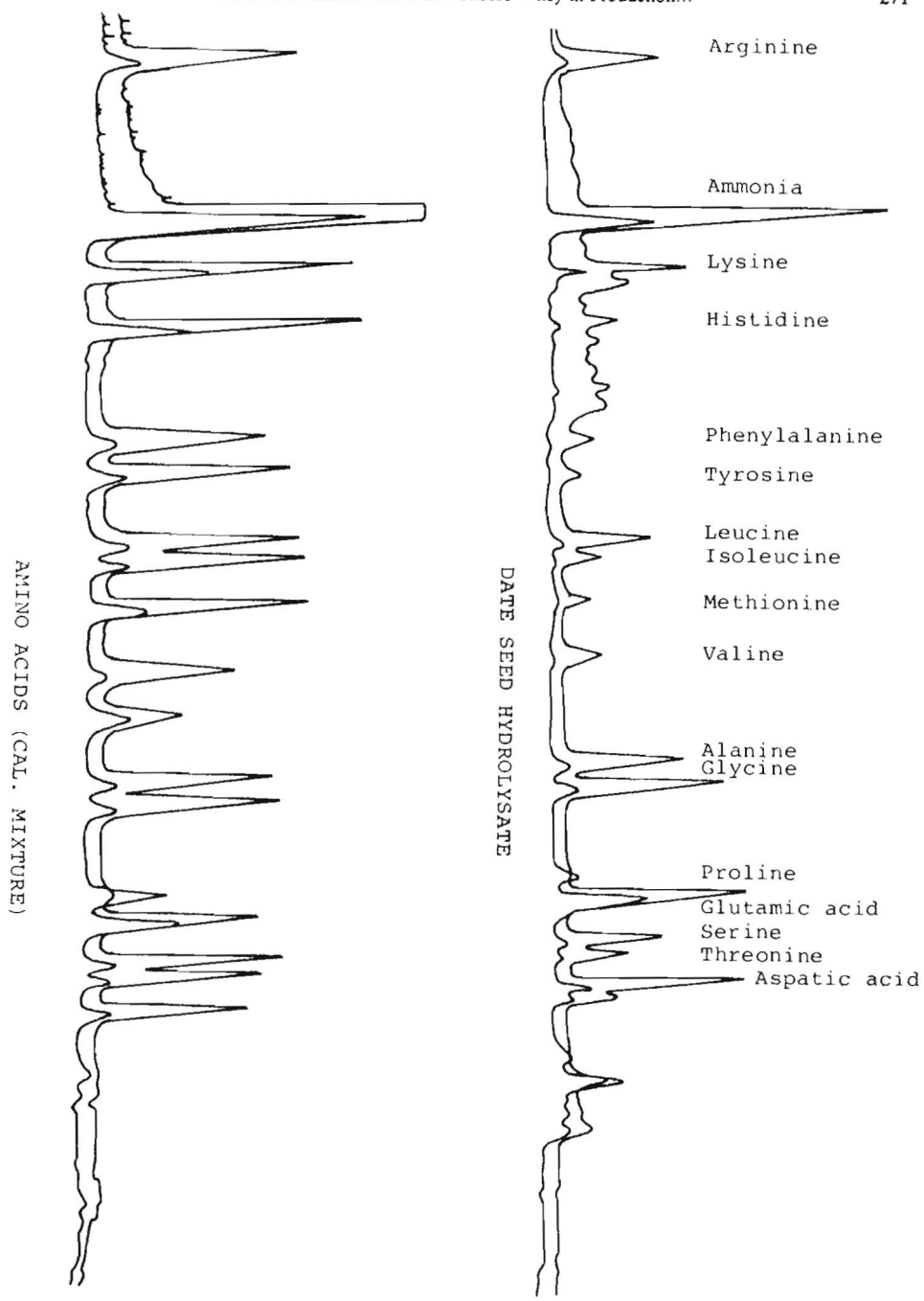

Fig. 1. Amino acids present in the hydrolysate of date seeds as identified by amino acid analyser

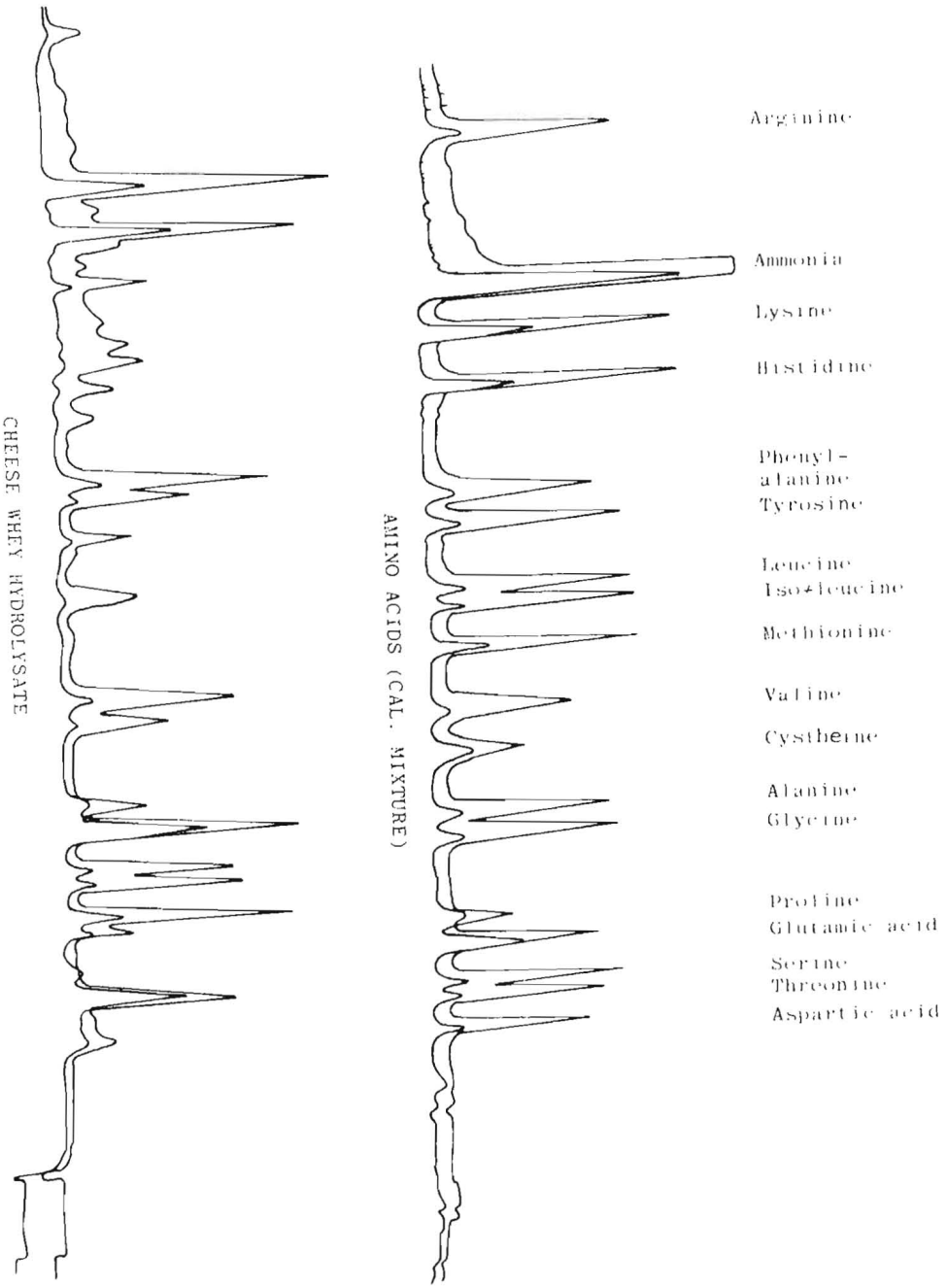

Fig. 2: Amino acids present in the hydrolysate of cheese whey as identified by amino acid analyser.

Table 2. Chemical constituents present in cheese whey

Components	Percentage
	g/100 g
Ash	1.0
Lactose	5.0
	mg/100 g of ash
Calcium	21.0
Magnesium	1.3
Manganese	Undetected
Iron	Undetected

Fermentation Period

The results obtained (Table 3) show that the fermentation period influenced biosynthesis of citric acid by *Candida lipolytica*. Citric acid yield was increased with the increase of incubation period reaching its optimum at 72 hr, above which a decline in the yield of citric acid was recorded. The initial pH value of the medium was adjusted to 5.5, and when the fermentation process began, the final pH value of the fermented medium was shifted towards acidic side (2.34-3.3), and this shift was due to formation of organic acids, especially citric acid. Cellular biomasses of *Candida lipolytica* increased with the increase of fermentation period reaching its maximum at 72 hr, above which a decrease in cellular biomasses was recorded. Formation of citric acid increased with the increase of cellular biomasses, therefore the output of citric

Table 3. Fermentation period and biochemical aspects of citric acid biosynthesis by *Candida lipolytica*.

Incubation Period	Final pH * Value	Dry biomass (mg ml^{-1})	Citric acid (mg ml^{-1})
24	3.9	5.2	10
48	2.8	10.5	12
72	2.6	12.8	17
96	2.7	12.7	17
120	2.5	10.6	16
144	2.4	9.4	15

* The initial pH value of the fermentation medium was adjusted to 5.5.

acid yield was associated with the anabolism of cellular constituents of the experimental organism.

Date Seed Hydrolysate and Whey

The results obtained (Table 4) show that the different concentrations of date seed hydrolysate affected the biosynthetic formation of citric acid. Citric acid yield was increased with the increase of date seed hydrolysate reaching its maximum at 55 mg ml^{-1}. A drop in the final pH value of the fermentation medium was recorded, and the shift in pH value to acidic conditions was mainly due to formation of organic acids, especially citric acid. Yeast cell biomass was also increased with the increase of hydrolysate concentration.

Total sugars present in date seed hydrolysate were insufficient to give good yield of citric acid. Therefore, the fermentation medium lacked incorporation of enough carbon source to perform good yield of citric acid.

Table 4. Influences of date seed hydrolysate and whey concentration on citric acid formation by *Candida lipolytica*.

Ingredients (mg ml^{-1})	Final pH * Value	Dry biomass (mg ml^{-1})	Citric acid (mg ml^{-1})
Date seed hydrolysate			
30 *	2.7	11	16
35	2.4	11	17
40	2.3	12	19
45	2.3	13	20
50	2.4	14	22
55	2.6	14	23
60	2.4	15	22
Whey			
15**	2.6	12	17
20	2.4	13	19
25	2.3	14	20
30	2.3	16	27
35	2.4	17	27
40	2.4	18	24

* The initial pH value of the fermentation medium was adjusted to 5.5.

** The basal fermentation medium contained date seed hydrolysate 30.0 mg and whey 15.0 mg/ml.

Addition of different concentrations of whey into the fermentation medium partially increased citric acid yield. The citric acid output increased with the increase of whey concentration reaching its maximum at 30 mg ml^{-1}. The final pH value of the fermented medium was shifted towards acidic side, and yeast cell biomass increased with the increase of whey concentration.

Date seed hydrolysate and whey were good nutrient sources for the growth of *Candida lipolytica,* especially amino acids and other naturally occurring constituents, but they lacked sufficient carbon sources to be elaborated by the organism to citric acid. Therefore, the fermentation medium contained date seed hydrolysate and whey was supplemented with different concentrations of glucose, maltose and sucrose to increase its potentiality for citric acid formation.

Role of Different Concentrations of Carbon Sources on Citric Acid Production

Different carbon sources such as maltose, sucrose and glucose were added separately into the ingredients of fermentation medium. The amount of each experimental carbon source was 5.0 to 30.0 mg ml^{-1}. The media contained the different concentrations of carbon sources with the other ingredients of fermentation medium were thoroughly mixed and adjusted to pH 5.5. The media were dispensed into 250 ml Erlenmeyer flasks in 50 ml portions. The flasks were plugged with cotton wool and sterilized at 120°C for 20 min. When the flasks attained room temperature, they were inoculated with the inoculum medium under aseptic conditions. The percentage of inoculum was 5.0%. The flasks were incubated on a rotary shaker at 30°C for 120 hr. At the end of the fermentation process, the required determinations were carried out.

The results obtained (Table 5) show that addition of different concentrations of glucose, maltose and sucrose separately into the fermentation medium markedly influenced the biosynthetic formation of citric acid by *Candida lipolytica*. The most efficient carbon source was glucose. Citric acid yield increased with the increase of glucose concentration reaching its maximum at 25 mg ml^{-1}. The final pH value of the fermented medium was shifted to acid side, and yeast cell biomass also increased with the increase of glucose concentration. Supplement of the fermentation medium with different concentrations of maltose and sucrose was also inductive to the biosynthetic formation of citric acid by *Candida lipolytica,* but their potentialities were lesser than glucose. The suitabilities of carbon sources according to their potentialities for citric acid formation were descendignly arranged as follows: glucose > maltose > sucrose.

Influences of Inorganic Nitrogen Sources on Citric Acid Production

Different concentrations of inorganic nitrogen sources such as $(NH_4)_2SO_4$, NH_4Cl, NH_4NO_3, $NaNO_3$ were added separately to the ingredients of fermentation medium. The concentrations of inorganic nitrogen sources were in the range of 1.0 to 5.0 mg ml^{-1}. The ingredients were thoroughly mixed and adjusted to pH 5.5. The media were dispensed into 250 ml Erlenmeyer flasks in 50 ml portions. The flasks were

Table 5. Effects of different carbon sources on biosynthetic formation of citric acid by *Candida lipolytica*.

Carbon sources (mg ml^{-1})	Final pH* Value	Dry biomass (mg ml^{-1})	Citric acid (mg ml^{-1})
Control**	2.7	14.5	25
Glucose			
5	2.6	15.0	27
10	2.4	15.5	30
15	2.7	25.0	32
20	2.9	16.7	35
25	3.1	17.6	40
30	3.0	17.4	40
Maltose			
5	2.9	14.7	26
10	2.8	15.2	29
15	3.2	14.8	32
20	3.1	14.0	33
25	2.7	15.5	34
30	3.4	17.4	36
Sucrose			
5	3.0	15.0	27
10	2.9	15.4	28
15	2.7	14.3	30
20	3.2	15.7	32
25	3.3	16.0	33
30	3.5	17.6	32

* The final pH value of the fermentation medium was adjusted to 5.5.

** No addition of the experimental carbon sources.

plugged with cotton wool and sterilized at 120°C for 20 min. When the flasks attained room temperature, they were inoculated with the inoculum medium under aseptic conditions. The percentage of inoculum was 5.0%. The flasks were incubated on a rotary shaker at 30°C for 120 hr. At the end of the fermentation process, the required determinations of final pH value, dry yeast biomasses and citric acid yields were conducted.

The results obtained (Table 6) show that different concentrations of inorganic nitrogen sources affected partially biosynthetic formation of citric acid. The fermentation medium was supplemented with different inorganic nitrogen sources, namely

Table 6. Influences of inorganic nitrogen sources on citric acid formation by *Candida lipolytica*.

Inorganic nitrogen sources (mg ml^{-1})	Final pH* Value	Dry biomass (mg ml^{-1})	Citric acid (mg ml^{-1})
Control**	2.9	16.5	39
$(NH_4)_2SO_4$			
1	2.7	16.7	42
2	2.8	16.9	45
3	2.6	17.0	44
4	2.4	16.7	43
5	2.3	17.0	42
NH_4Cl			
1	2.8	15.8	40
2	2.7	16.1	42
3	2.8	15.7	40
4	2.6	16.2	38
5	2.9	17.0	37
NH_4NO_3			
1	2.7	16.4	40
2	2.9	15.8	41
3	3.0	14.7	39
4	3.1	15.2	39
5	3.0	14.3	37
$NaNO_3$			
1	4.9	14.5	37
2	5.0	13.4	35
3	5.2	14.5	34
4	5.4	14.0	34
5	4.7	12.0	33

* The initial pH value of the fermentation medium was adjusted to 5.5.
** No addition of the experimental inorganic nitrogen sources to the fermentation medium.

$(NH_4)_2SO_4$, NH_4Cl and $NaNO_3$. The best inorganic nitrogen source was $(NH_4)_2SO_4$. Citric acid yield slowly increased with the increase of $(NH_4)_2SO_4$ concentration reaching its maximum at 2 mg ml^{-1}, and a slight increase in yeast biomas was obtained with the increase of $(NH_4)_2SO_4$. The final pH value of the fermented medium was shifted to acid conditions. $(NH_4)_2SO_4$ and NH_4Cl were more efficient inorganic nitrogen sources than $NaNO_3$. The experimental organism preferred utilization of inorganic nitrogen sources in the form of ammonia nitrogen rather than nitrate nitrogen.

The fermentation medium already contained date seed hydrolysate and whey which contained miscellaneous ingredients, especially organic nitrogen sources, therefore the medium was rich in organic nitrogen sources. Addition of inorganic sources in the form of $(NH_4)_2SO_4$ slightly increased yield of citric acid.

Role of Different Concentrations of Date Seed Ash on Citric Acid Formation

Different concentrations of date seed ash were incorporated into the fermentation medium. The ash concentrations ranged from 0.1 to 1.0 mg ml^{-1}. The ingredients of the medium were thoroughly mixed and adjusted to pH 5.5. The media were dispensed into 250 ml Erlenmeyer flasks in 50 ml portions. The flasks were plugged with cotton wool and sterilized at 120°C for 20 min. When the flasks attained room temperature, they were inoculated with the inoculum medium under aseptic conditions. The percentage of inoculum was 5.0%. The flasks were incubated on a rotary shaker at 30°C for 120 hr. At the end of fermentation process, the required determinations of final pH value, dry yeast biomasses and citric acid yields were carried out.

The results obtained (Table 7) show that different concentrations of date seed ash influenced partially the biosynthetic formation of citric acid. Date seed ash contained magnesium, iron, aluminium, calcium, manganese, zinc and nickel. The presence of these elements affected citric acid formation. Citric acid yield increased with the increase of ash, reaching its maximum at 0.2 mg ml^{-1} above which a slight decrease in citric acid yield was obtained.

Table 7. Role of date seed ash on the fermentative formation of citric acid by *Candida lipolytica*.

Ash (mg ml^{-1})	Final pH* Value	Dry biomass (mg ml^{-1})	Citric acid (mg ml^{-1})
Control**	2.7	16.4	45
0.1	2.9	15.7	50
0.2	2.8	16.2	54
0.3	2.6	15.6	50
0.4	2.5	15.5	47
0.5	2.9	16.2	45
0.6	2.6	16.0	46
0.7	2.8	15.8	47
0.8	3.1	15.0	40
0.9	3.2	14.7	40
1.0	3.0	14.5	40

* The final pH value of the fermentation medium was adjusted to 5.5.
** No addition of ash.

Amino Acids Present in Yeast Biomass

The results obtained (Table 8) show that the following amino acids: alanine, valine, glycine, isoleucine, leucine, proline, threonine, serine, methionine, phenylalanine, aspartic acid, glutamic acid, lysine, tyrosine, arginine, cysteine and other undetectable amino acids were present in yeast biomass. Total proteins present in the yeast biomass was 40%. Yeast proteins contained eight essential amino acids: Valine, leucine, isoleucine, threonine, methionine, phenylalanine, lysine and arginine. Therefore, cell biomass of *Candida lipolytica* could contribute good source of proteins.

Table 8. Amino Acids present in cell biomass of *Candida lipolytica*.

Amino acids	% in the dry biomass	% in protein
Alanine	2.48	6.20
Valine	2.28	5.70
Glycine	2.02	5.06
isoleucine	2.18	5.46
Leucine	2.74	6.86
Proline	1.96	4.90
Threonine	2.10	5.26
Serine	3.12	7.80
Methionine	0.54	1.36
Phenylalanine	1.38	3.46
Aspartic acid	4.70	11.76
Glutamic acid	6.84	17.10
Lysine	3.20	8.00
Tyrosine	1.42	3.56
Arginine	1.38	3.46
Cysteine	0.66	1.66
Undetermined amino acids	1.00	2.40
Total	40.00	100.00

Conclusion

Citric acid is one of the organic acids known for a long time as the principal acid of citrus fruits. Its widespread presence in nature indicates that it has a great physiological significance which, nowadays, can be confirmed by great varieties of applications. Citric acid is the principal food acid used in preparation of soft drinks and syrups, desserts, jams, jellies, wines, candy, preserved fruits, frozen fruits and vegetable

juices. Citric acid is also used in gelatin food products, and artificial flavours compounded materials such as soft drink tablets and powders. In pharmacy, citrates are used in blood transfusion, and the free acid is used in effervescent products. Citric acid is widely used in cosmetics, electroplating, metal cleaning, leather tanning, printing, reactivation of oil wells, inks, floor cements, silvering compounds, algicide formulation, dyeing of fabrics and removal of contamination of radioactive isotopes.

The discovery of citric acid formation by fermentation is still very interesting point of research, as many workers devoted their efforts to produce citric acid by fungi, namely *Aspergillus niger*. Recently, yeasts are successfully used in the fermentative production of citric acid.

In the present work, *Candida lipolytica* was used as citric acid producer. The basal fermentation medium contained natural products, namely date seed hydrolysate and whey as miscellaneous ingredients for the biosynthetic formation of citric acid. Date seed hydrolysate and whey contained good nutrients, especially organic nitrogen sources for the growth of *Candida lipolytica* and the biosynthetic formation of citric acid. The deficiency of carbon source was overcome by the addition of glucose into the fermentation medium which increased the output of citric acid. Date seeds and whey are cheap natural products which could be utilized in formation of citric acid. They contained also other valuable ingredients, especially lipids and minerals, which were beneficial to the fermentative formation of citric acid. Lipids are active-surface agents and could be utilized as carbon sources in absence of carbohydrates, and also the presence of certain elements such as calcium, iron, manganese, zinc and nickel induced the experimental organism to give good yield of citric acid. Biochemical aspects of the fermentative formation of citric acid by *Candida lipolytica* were characterized with a drop in the final pH value of the fermentation medium, formation of yeast cell within 72 hr of the fermentation period, and formation of citric acid was closely associated with increase of yeast biomass. From the economic view-point, utilization of cheap raw materials such as date seeds and whey in formation of citric is more better than to use expensive ingredients in the form of carbohydrates and other organic nitrogen sources.

Saudi Arabia occupies an advanced position among the date producing countries in the World. The percentage of seeds in the total weight of date is about 12.5. The date seeds are considered as agricultural waste product and represent a big source as a raw material for the production of several products such as citric acid.

References

AOAC (1975) *Official Methods of Analysis of Association of Official Analytical Chemists*. Washington, DC.

Ashy, M.A., Khalil, A.M., Abou-Zeid, A.A. and **Abou-Alnaga, K.O.** (1981) Utilization of cheese whey in production of Milk-clotting enzymes, *Agric. Wastes* **3:** 277-284.

Finogenova, T.V., Illarionova, V.L. and **Lozinov, A.B.** (1973) Production of citric acid and iso-citric acid by *Candida lipolytica* growing on *n*-alkanes, *Mikrobiologiya* **42:** 790-794.

Hamissa, F.A., Abou-Zeid, A.A. and **Radwan, A.A.** (1982) Induction and selection of improved yeast mutants for citric acid production, *Agric. Wastes* **4:** 17-23.

Hanson, R.S., Srinivoson, V.R. and **Orin, H.** (1961) Citric acid formation in *Bacillus* strains, *Biochem. biophys. Res. Commun.* **5:** 457-460.

Johnson, E.M., Knight, E.C. and **Walker, T.K.** (1973) The mechanism of the formation of organic acids by mould fungi, *Biochem. J.* **31:** 903.

Joshi, A.P. and **Ramakrishnan, G.V.** (1959) Mechanism of formation and accumulation of citric acid in *Aspergillus niger, Enzymologia* **12:** 43-61.

Kovats, J. (1961) Studies in submerged citric acid fermentation. *Acta microbiol. pol.* **4:** 275-287.

Lewis, K.F. (1951) Studies on the mechanism of citric acid production, *J. Am. chem. Soc.* **73:** 2500-2503.

Marier, J.R. and **Boulet, M.** (1958) Estimation of citric acid, *Dairy Sci.* **41:** 1983.

Masuda, M., Oshima, T. and **Ikeno. Y.** (1975) Fermentation production of citric acid, *Japan Kokai* **75,** 158,486 (Cl.C 120) 12 Dec. 1975. Appl, **74,** 60,706, 31 May 1974, 6 p.

Miller, L. and **Houghton, J.A.** (1945) Modification of Kjeldahl, *J. biol. Chem.* **159:** 373.

Somogyi, M. (1945) A new reagent for the determination of sugars. *J. biol. Chem.* **160:** 61.

Takahoshi, N. and **Ikeno, Y.** (1974) Citric acid production by *Candida*. (Hitachi Chemical Co. Ltd.) *Japan Kokai* **74,** 30, 589, 19 March, 1974. Appl. 14 July, 1972, 4 p.

Tachibona, S. (1974) Citric acid production by *Candida* (Iwata Kogaku Co. Ltd.) *Japan Kokai* **74,** 100,486, 21 September, 1974. Appl. 1 February, 1973, 3 p.

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الاستفادة من نوى البلح وشرش اللبن في إنتاج حمض الستريك بواسطة الخميرة كانديدا ليپوليتكا

أبو زيد علي أبو زيد، أحمد عمر بغلف، جلال الدين أعظم خان، صالح سعيد مخاشن

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دلت التحاليل الكيميائية على أن نوى البلح يتكون من مواد نيتروجينية، عضوية، كربوهيدراتية، ليبيدات، وبعض العناصر. ولقد أمكن استخدام نوى البلح وشرش اللبن في تكوين وسط طبيعي لاستزراع فطرة الخميرة كانديدا ليپوليتكا لتكوين حمض الستريك تخمرياً. وبينت النتائج أن من أهم الخصائص الحيوية التي تصطحب تكوين حمض الستريك: انخفاض درجة الأس الهيدروجيني لوسط التخمر، كما أن كمية حمض الستريك المفرزة في الوسط الغذائي تزداد بزيادة فترة التخمر إلى أن تصل إلى ٩٦ ساعة. ولقد أظهرت النتائج أن زيادة كمية خلايا الخميرة تكون مصحوبة بزيادة في التكوين الحيوي لحمض الستريك. ولقد ازدادت كفاءة الوسط الغذائي بإضافة بعض السكريات مثل الجلوكوز، المالتوز، والسكروز، وكان سكر الجلوكوز بتركيز ٢٥ جرام / لتر أفضلها ملاءمة لزيادة كمية حمض الستريك. وعند إضافة بعض الأملاح غير العضوية مثل كبريتات الأمونيوم، وكلوريد الأمونيوم، ونترات الأمونيوم إلى وسط التخمر، كان لكبريتات الأمونيوم أثر قليل في زيادة كمية حمض الستريك

الناتجة، وذلك لاحتواء نوى البلح وشرش اللبن على كميات من المواد النيتروجينية الملائمة لنمو فطرة الخميرة وإنتاج حمض الستريك. وقد أدت إضافة رماد نوى البلح إلى وسط التخمير إلى زيادة في كمية حمض الستريك المتكونة تخمرياً. ويدل هذا على أهمية بعض العناصر الموجودة في نوى البلح مثل المغنسيوم، والحديد، والكالسيوم، والزنك، والنيكل في التفاعلات الإنزيمية المتعلقة بالتكوين الحيوي لحمض الستريك.