Sci. J. Fac. Sci. Menoufia Univ. Vol. VIII(1994)

UTILIZATION OF SOME CANNERY WASTES IN MICROBIAL PROTEIN PRODUCTION

BY

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ABSTRACT

Carrot leaves, date seeds and guava seeds were acid-hydrolyzed for production of fermentable sugars, on which fodder yeasts were grown. Carrot leaves hydrolyzed with 1% H_2SO_4 at 120°C and solid liquid ratio 1:10 produced 22.54g reducing sugars per 100 g substrate, date seeds gave 33.88 g reducing sugars per 100g substrate when hydrolyzed with 0.2% H_2SO_4 at 185°C, while guava seeds gave 30.72g reducing sugars 100 g substrate wiith 1.2% H2SO4. at 135°C. Both date and guava seeds were hydrolyzed in solid:liquid ratio:1:20.

All the tested strains grew well on the three hydrolyzates media. Candiida: tropicaliis (4) was the best yeast strain, when cultured on date seeds, carrot leaves and guava seeds hydrolyate supplemented wiith malt extract, peptone, yeast extract and some salts. It gave 24.68 22.0 and 17.94 g/L dry yeast with 10.25, 9.18 and 5.81 g/L proteiin, respectiively.

Consiiderable amount of cannery wastes are disposed every year in the food industry factoriies. The peals, pulps or seeds could be utilized as a carbohydrate source for fermentation processes. Some of

these wastes have been used for the production of yeast protein by different authors (Krammer and Itmar, 1968; El-Emery, 1971; Paredes-LLopez et al., 1976; Padhay et al., 1979; Santos, 1980 and Napavarn et al., 1983).

The present work was undertaken to iinvestigate the possibiility of usiing some acid-hydrolysed cannery wastes for yeast protein production.

MATERIAL AND METHODS

Yeast strains:

Cultures off Candida tropicals (4) and (21) Candida I ipolytica (6), and Saccharomyces cerevisiae (29) were obtained from the Miicrobial Chemistry Laboratory, National Research Centre, Cairo. They were grown and maintained on malt agar medium.

Substrate:

Date seeds, guava seeds and carrot leaves used were obtained during the season 1994 from Qaha Factory for Food Industries, Qaha, Egypt.

Acid hydrolysiis of substrate rate materials:

The dry raw materials were ground to 440 mesh size before hydrolysis. Samples were mixed with sulphuriic acid in different concentrations from 0.2 up to 1.2% and different solid:liquid ratios from 1:10 to 1:30. Hydrolysis was carried out at different temperatures (110-135)°C for periods ranging from 15 to 75 min. The filtrate

obtained from each hydrolysis process was neutralised with calcium carbonate, then refiltered and its sugar content was determined.

Yeast propagation:

The neutraliised acid hydrolysates of the above three mentioned cannrery wastes were used as a carbon source for yeast proteiin production. Cultivation was carried out in 250 ml flasks containing 50 ml of the mediium under investigation. The hydrolysates were sterilised separately before adding the inorganic nutrients. THe flasks were inoculated with 2.5 ml of a standard inoculum of the tested yeast strain grown on a basal medium consisting of (g %): $(NH4)_2$ SO4, 0.5; KH_2 PO_4, 0.1 g; $MgSO_4$. $7H_2O_4$, 0.05 g; yeast extract, 0.3 g for 24 hr at 30°C. The inoculated flasks were incubated at 30°C on a rotary shaker (150 rpm). Samples were taken every 24 hr, centrifuged at 4000 rpm for 15 min, then washed thoroughly and dried at 60°C, till constant weight to determine the yeast dry weight and crude protein.

Chemical determinations:

The yeast yield was determined as dry weiight. The nitrogen content was measured by micro-kjeldahl analysis according to A.O.A.C. (1965). Crude protein was calculated as N x 6.25. The total crude protein per litre (TCPL) was calculated by the equation.

TCPL= Biomas (g/L) × Crude protein%)\100

Reducing sugars were determined by the method of Somogyi (1945). The maximum sugar yield wass taken as a criterion for optimum conditions of acid hydroysis.

RESULTS AND DISCUSSION

Hydrolysis of some cannery wastes:

Carrot leaves, date and guava seeds were hydrolysed using sulphuric acid. Different parameters were investigated to obtain optimum hydrolysis. Concentrations of sulphuric acid ranged from 0.2 to 1.2% and temperature from (110-135)°C. The solid: liquid ratio from 1:10 to:30 and the time of hydrolysis from 15 to 75min.

The results presented in Tables 1-4 indicated that each waste needed a specific mode of hydrolysis for optimum sugar production. Optimization of the acid hydrolysis process showed that date seeds could produce 33.8 g reducing sugars per 100 g substrate when hydrolysed with 0.2% sulphuriic acid at 135°C, solid:liquid ratio 1:20, for 60 min. However, guava seeds gave 30.7 g/100 g substrate, when hydrolyzed with 1.2% acid at solid liquid ratio 1:20 at 135°Cfor 60min. Carrot leaves produced 22.5 g/100g substrut, when hydrolyzed with 1.0% acid at 120°C, solid liquid ratio 1:10, for 60 min.

Table 1. Effect of acid concentration on hydrolysis* of some cannery wastes.

H_2SO_4 %	Reducing sugare g/L.		
	Carrot Leaves	Date seeds	Guava seeds
Control	0.89	0.88	0.45
0.2	2.95	7.53	2.69
0.4	4.46	5.37	3.71
0.6	5.02	4.92	3.82
0.8	6.13	4.89	4.25
1.0	8.15	4.85	5.71
1.2	7.90	3.65	6.13

* At 125°C, for 30 min, and solid:liquid ratio 1:10.

Table 2. Effect of solid:liquid ratio on the amount of reducing sugars in the hydrolysis* of some cannery wastes.

Solid:liquid ratio	Reducing sugare g/L.		
	Carrot Leaves	Date seeds	Guava seeds
1:10	8.75	7.53	6.13
1:20	4.38	6.87	5.84
1:30	2.89	4.02	3.14

* Hydrolysis was carried 125°C for 30 min. using H_2SO_4 at (1.0, 0.2 and 1.2%) for carrot leaves, date seeds and guave seeds respectively.

Table 3. Effect of temperature on acid hydrolysis of the tested wastes.

Temperature °C	Reducing sugare g/L.		
	Carrot Leaves	Date seeds	Guava seeds
100	6.32	4.10	1.76
120	8.75	5.39	3.31
125	8.15	6.87	5.84
130	7.56	7.03	6.67
135	6.23	9.87	9.74

* For 30 min, solid:liquid ratio (1:10) and H_2SO_4 at (1.0, 0.2 and 1.2%) for carrot leaves, date seeds and guava seeds respectively.

In this connection, it may be mentioned that acid hydrolysis at 120°C applied to sunflower seeds husks and green plantain skin was recommended by Eklund et al., (1976) and Pujol and Bahar (1983), for obtaining substrates giving high yields of some yeasts.

Table 4. Effect of time of hydrolysis on amount of reducin sugars and % hydrolysis considering the optimum conditionsn of hydrolysis for each waste.

Time of hydrolysis (in min:)	Carrot leaves*		Date seeds**		Guave seeds***	
	Reducng sugars g/L.	% hydrolysis	Reducng sugars g/L.	% hydrolysis	Reducng sugars g/L.	% hydrolysis
15	3.95	18.23	6.45	28.12	7.34	34.44
30	8.75	39.21	9.87	32.16	9.74	36.36
45	15.03	48.14	16.13	37.18	15.18	36.66
60	22.50	53.80	16.94	38.96	15.36	36.88
75	22.40	56.32	13.23	39.74	14.73	38.95

* Carrot leaves : H_2SO_4 . 1 %, 120°C, Solid : liquid 1 : 10.
** Date seeds : H_2SO_4 .0.2 %, 135°C, Solid : liquid 1 : 20.
*** Guava seeds : H_2SO_4 .1.2 %, 132°C, Solid : liquid 1 : 30.

Protein synthesis by the tested yeasts

Comparative studies on some fodder yeasts using acid hydrolysates for each tested waste were performed. Data in Table 5 showed that Candida tropicalis (4) was the most efficient yeast for protein production. It gave best yields after 3 days' incubation period on all waste hydrolysates. It produced 28.9%, 43.1% and 29.1% crude protein on carrot leaces, date seeds and guava seeds hyydrolysates

respectively. Similar results were obtained by veksler (1970); Anna (1972) and Jarosz et al. (1974) using hydrolysed potato pulp. Nemat et al. (1986), also found that C. Tropicalis was the most efficient yeast for protein production on acid hydolysed maize stems.

For increasing the growth and protein yield of the chosen yeast, ammonium sulphate in the basal medium was replaced by ammonium hydroxide or urea or peptone as nitrogen sources in media with acid hydrolysates of the tested wastes. The amount of reducing sugars in all media was 2%.

The results recorded in Table 6 indicated that ammonium sulphate (M_1) was not efficient for yeast production, while urea (M_2) increased relatively both the biomass and protein yields. Okada et al. (1980) found that urea with citrus waste seeds hydrolysate increased the protein formation by Candida Sp. On the other hand, when ammonium hydroxide was used as nitrogen source (M_3), it increased the protein content of the yeast, but did not affect greatly the yeast yield. The protein content became 47.3%, 45.8% and 34.5% on using date seeds, carrot leaves and guava seeds hydrolysates respectively. This was in agreement with the results obntained by Rale (1984). He obtained maximum biomass of C. utilis when grown on pine-apple cannery wastes supplemented with 0.3% ammonia.

Moreover, the use of pepton (M_4) with the tested hydrolysates increased the yeast biomass. Nevertheless, the addition of malt extract to peptone medium (M_3) was greatly favourable for yeast growth. It helped to obtain 24.6 g/L biomass, when the yeast grown on date seeds hydrolysartes, while it gave 22.0 and 17.9 g/L dry weight on carrot leaves and guava seeds hydrolysates respectively.

It may be concluded that certain acid- hydrolysed cannery wastes- under certain conditions- could serve as a low cost substrate for the productionn of microbial protein for example, the date seeds hydrolysate with peptone and malt extract medium (M_5), was preferabel for the growth of C. tropicalis (4) as it could produce 24.6 g/L biomass with 10.2 g/L protein.

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Table 5. Biomass and crude protein content of different yeast strains grown on a basel medium with wastes hydrolysates

Waste	Incubation period (h)	C. tropicatis (4)		C.lipolytica (6)		C. tropicalis (21)		S. cerevisise (29)	
		Dry wt. g/L.	Crude protein %	Dry wt. g/L.	Crude protein %	Dry wt. g/L.	Crude protein %	Dry wt. g/L.	Crude protein %
Carrot leaves	24	6.12	24.30	4.45	19.14	4.56	18.60	5.91	22.05
	48	6.84	26.40	5.25	21.16	6.20	19.44	6.39	22.60
	72	9.48	28.93	6.00	27.50	8.56	21.17	6.70	23.27
	96	9.06	25.76	6.91	22.87	8.36	18.84	5.90	21.88
Date seeds	24	4.16	31.20	6.80	25.25	3.24	37.11	4.10	21.85
	48	6.28	38.16	6.88	36.16	3.86	40.52	5.10	22.14
	72	6.91	43.10	6.55	42.16	4.10	43.14	5.29	35.95
	96	6.01	39.12	5.18	41.28	5.01	41.28	6.00	32.69
guava seeds	24	6.28	24.30	7.14	22.59	3.14	21.56	3.96	21.61
	48	10.12	28.11	9.28	26.59	3.35	21.67	5.86	22.70
	72	11.75	29.10	10.76	29.88	5.27	23.93	5.79	31.11
	96	9.76	26.14	9.88	21.19	5.11	19.72	6.50	26.05

Basal medium : $(NH_4)_2 SO_4$, o.5 g, $KH_3 PO_4$, 0.1 g, $MgSO_4 .7H_2O$, 0.05 g; yeast extract, 0.3 g and 100 ml of tested hydrolysate.

الاستفاده من بعض مخلفات الاغذيه فى انتاج البروتين

د./ محمود هزاع

قسم النبات- كليه العلوم بنها- جامعه الزقازيق

تم تحليل أوراق الجزر ونوى البلح وبذور الجوافه بواسطه حمض الكبريتيك لانتاج السكريات اللازمه لنمو خميره العلف وقد اعطيت أوراق الجزر ٢٢,٥ جرام مختزله لكل ١٠٠ جرام من الماده الصلبه وذلك عند معاملتها بحامض الكبريتك يتركز ١٪ عند درجه حراره ١٢٠م سكريات مختزله من نوى البلح لكل ١٠٠ جرام ماده صلبه وذلك ٠,٢٪ حامض الكبريتيك عند درجه حراره ١٣٥م ونسبه المواد الصلبه الى السائله ١:٢٠ بينما أعطت بذور الجوافه ٣٠,٧ جرام سكريات مختزله لكل ١٠٠ جرام بذور وذلك باستخدام ١,٢٪ حامض الكبريتيك ودرجه حراره ١٣٥م نسبه المواد الصلبه الى السائله ١:٢٠ ولقد نمت جميع سلالات الخميره المختبره نموا ملحوظا على المحاليل السكريه الناتجه من تحليل المخلفات الثلاثه السابقه. وقد كانت خميره كانديدا تروبيكالس أفضل السلالات فقد أعطت ٢٤,٦ ، ٢٢، ١٧,٩ جرام بروتين لكل لتر من الخميره الجافه وذلك عند زراعتها فى المحلول السكرى مضافا اليه ٢، ١٠، ٩,٢ ، ٥,٨ جرام بروتين لكل لتر بيتون وخلاصه الشعير وخلاصه الخميره مع بعض الاملاح الذى نتج نوى تحليل البلح وأوراق الجزر وبذور الجوافه على التوالى.