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# COMPARISON OF THE GROWTH OF SOME YEAST STRAINS IN WASTE LUCERNE JUICE *)

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Key words: waste lucerne juice, yeast, increment of biomass, protein content of cells, yield of biomass, specific growth rate, $BOD_5$.

The growth of 14 yeast strains in a lucerne waste juice was compared. The particular yeast cultures differed in efficiency and rate of utilization of sugars from the juice in the attained increments of biomass as well as in the protein content of the cells. The highest increment of biomass from 11.4 to 19.1 g d.m./l. was noted in *C. utilis* Z-2, *C. guilliermondii* K-29 and *C. brumptii* Z-1. The most dynamic growth was observed in *C. utilis* Z-2 and *C. guilliermondii* K-29. As a result of yeast cultivation the $BOD_5$ of the effluent dropped from 30-36.000 to 9-15.000 mg $O_2$/l.

## INTRODUCTION

Recently, there have appeared many papers on the production of leaf protein concentrates from various plants, especially lucerne [2, 3, 10, 11]. The waste product of this process is clear, brown juice.

As it is rich in various organic substances, it may be a good raw material for single-cell protein production [4, 11]. It is obvious that various yeast strains will grow in this medium at varied effectiveness, which can also depend on the lot of juice.

This paper is an attempt at the examination of various yeast strains out of the authors collection in order to select the strains best adapted to lucerne juice, i.e. characterized by a high yield of biomass, a moderately even growth in qualitatively different juice lots and accumulating the highest amount of protein in the cells.

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## MATERIALS AND METHODS

The study was carried out on 14 yeast strains out of the collection of the Department of Technical Microbiology. They had been isolated earlier from various plant habitats as well as from soil. The yeast cultures investigated and their origin given in Table 1.

Table 1. List of investigated yeast species

| Yeast strain | Origin of yeast |
|---|---|
| *Candida brumptii* Z-1<br>*Candida utilis* Z-2<br>*Candida tropicalis* Z-3 | lucerne |
| *Candida guilliermondii* K-29<br>*Candida guilliermondii* K-22 | maize caryopses |
| *Candida scottii* A-311<br>*Candida scottii* A-61<br>*Torulopsis globosa* ADVI | soil |
| *Pichia membranaefaciens* C-4<br>*Candida guilliermondii* C-5<br>*Schizosaccharomyces pombe* C-1<br>*Hansenula anomala* C-2<br>*Kloeckera apiculata* C-3<br>*Candida sp.* R-190 | fruit |

The culture stock was kept on slants of wort agar at a temperature of 4°C. Lucerne juice, extracted on filter press "Wołgar 5", was obtained from the Experimental Station of the Animal Husbandry Institute at Czechnica. The juice was heated up to 85°C and filtrated through a Büchner filter in order to separate the coagulated protein as well as the chloroplast fraction. The clear filtrate was a medium for yeast. The initial pH of the medium was corrected with 10% $H_2SO_4$ up to a value of 4.5 for shaking cultures or up to 4.0 for cultures in the fermenter. All the media were pasteurized for 0.5 hour at 85°C. The media were inoculated with a cell suspension up to about $2\times10^7$ cells/ml. The inoculum had been cultivated earlier in the wort of 7°Blg on a shaker.

Shaking cultures were placed in 750 ml flasks containing 75 ml medium on a rotary shaker operated at 180 r.p.m. at 30°C for 24 hours.

Selected strains were cultivated in a Biostat type fermenter with 1 litre of working volume. They were aerated with 1 litre of air per minute and agitated at 500 r.p.m. The cultivation temperature was 30°C. Cultivation was finished when culture reached the stationary phase of growth.

After shaking cultivation, the whole culture was centrifuged at 4000 r.p.m. Sugars in the supernatant were determined by the Nizovkin method [6], the cells were washed three times and dried up to constant weight at 105°C in order to estimate the cell weight.

From the fermenter, samples were taken at 1-hour intervals in order to determine residual sugars by the Livingstone method cited after Jasiorowski and Zazula [7] and cell concentration. Yeasts were counted in Thom chamber, whereupon the cell production in grams of dry biomass per litre was read out from the plotted dependence curve of cell quantity to biomass weight. In the initial medium and in the post-cultivation liquid of the cultures in the fermenter, the $BOD_5$ was determined in accordance with Polish Standard PN-74/C-04578.

Crude protein in the yeast biomass was determined by the Kjeldahl method.

## RESULTS

Tables 2 to 5 give the results of shaking cultures of particular yeast strains in waste lucerne juice.

Table 2. Growth of *C. brumptii* Z-1 in various lots of waste lucerne juice (24-hour shaking cultures)

| Date of lucerne harvesting | Initial sugar conc. (%) | Utilization of sugar % | Increment of biomass g. d.m./l | Cell yield on consumed sugar ($Y_S$) | Protein % of dry biomass |
|---|---|---|---|---|---|
| 6.06.1978 | 2.98 | 72.5 | 16.1 | 0.74 | 46.2 |
| 14.06.1978 | 2.15 | 94.4 | 13.0 | 0.64 | 43.7 |
| 20.06.1978 | 2.41 | 87.5 | 11.9 | 0.56 | 45.3 |
| 27.06.1978 | 2.07 | 95.7 | 11.4 | 0.57 | 45.9 |
| 4.07.1978 | 2.78 | 74.4 | 13.8 | 0.66 | 43.6 |
| 11.07.1978 | 2.26 | 94.1 | 12.3 | 0.59 | 46.9 |

Table 3. Growth of *C. utilis* Z-2 in various lots of waste lucerne juice (24-hour shaking cultures)

| Date of lucerne harvesting | Initial sugar conc. (%) | Utilization of sugar % | Increment of biomass g d.m./l | Cell yield on consumed sugar ($Y_S$) | Protein % of dry biomass |
|---|---|---|---|---|---|
| 6.06.1978 | 2.98 | 92.9 | 17.2 | 0.62 | 41.0 |
| 14.06.1978 | 2.15 | 93.0 | 12.0 | 0.60 | 41.3 |
| 20.06.1978 | 2.41 | 87.5 | 14.4 | 0.68 | 40.2 |
| 27.06.1978 | 2.07 | 92.7 | 12.0 | 0.62 | 40.6 |
| 4.07.1978 | 2.78 | 94.2 | 15.5 | 0.59 | 41.1 |
| 11.07.1978 | 2.26 | 96.9 | 12.9 | 0.61 | 41.2 |

Table 4. Growth of *C. guilliermondii* K-29 in various lots of waste lucerne juice (24-hour shaking cultures)

| Date of lucerne harvesting | Initial sugar conc. (%) | Utilization of sugar % | Increment of biomass g d.m./l | Cell yield on consumed sugar ($Y_S$) | Protein % of dry biomass |
|---|---|---|---|---|---|
| 6.06.1978 | 2.98 | 92.9 | 19.1 | 0.68 | 37.3 |
| 14.06.1978 | 2.15 | 93.0 | 13.1 | 0.65 | 40.5 |
| 20.06.1978 | 2.41 | 87.5 | 14.6 | 0.69 | 37.6 |
| 27.06.1978 | 2.07 | 90.3 | 11.9 | 0.63 | 38.1 |
| 4.07.1978 | 2.78 | 94.4 | 15.9 | 0.59 | 41.0 |
| 11.07.1978 | 2.26 | 96.9 | 13.5 | 0.62 | 39.8 |

Table 5. The results of some shaking cultures of the remaining yeast strains in waste lucerne juice

| Yeast strain | Initial sugar concentr. (%) | Utitization of sugar % | Icnrement of biomass g d.m./l | Cell yield on consumed sugar ($Y_S$) | Protein % of dry biomass |
|---|---|---|---|---|---|
| *C. guilliermondi* C-5 | 2.15 | 92.6 | 13.8 | 0.69 | 35.1 |
| | 2.41 | 95.0 | 11.0 | 0.48 | 36.5 |
| *C. guilliermondii* K-22 | 2.15 | 91.6 | 14.0 | 0.7 | 35.6 |
| | 2.41 | 95.8 | 10.9 | 0.47 | 37.1 |
| *P. membranaefaciens* C-4 | 2.15 | 94.2 | 10.7 | 0.53 | 39.9 |
| | 2.07 | 48.8 | 5.2 | 0.51 | 49.7 |
| | 2.26 | 90.7 | 9.8 | 0.48 | 40.9 |
| *C. scottii* A-61 | 2.98 | 82.2 | 11.6 | 0.47 | 38.9 |
| | 2.15 | 90.7 | 8.7 | 0.45 | 40.3 |
| | 2.41 | 84.2 | 9.1 | 0.45 | 39.1 |
| *T. globosa* ADVI | 2.15 | 94.4 | 11.3 | 0.55 | 40.9 |
| | 2.41 | 83.8 | 11.1 | 0.53 | 40.7 |
| | 2.26 | 92.0 | 9.8 | 0.47 | 41.8 |
| *C. tropicalis* Z-3 | 2.15 | 91.6 | 3.9 | 0.2 | 43.7 |
| *Schiz. pombe* C-1 | 2.15 | 94.8 | 7.7 | 0.37 | 39.6 |
| *H. anomala* C-2 | 2.15 | 94.3 | 7.2 | 0.35 | 35.7 |
| *K. apiculata* C-3 | 2.15 | 91.8 | 7.6 | 0.38 | 39.9 |
| *C. scottii* A-311 | 2.15 | 96.7 | 9.2 | 0.44 | 39.2 |
| *Candida sp.* R-190 | 2.15 | 92.1 | 8.2 | 0.41 | 40.5 |

*C. brumptii* Z-1 in the examined lots of juice containing from 2.07 to 2.98% sugars gave increments of biomass at a level of 11.4-16.1 g d.m./l (Table 2). It is significant that in the presence of higher sugar concentrations (above 2.5%) a substantial, not utilized amount of this substrate remained in the medium. Apart from sugars, yeast metabolized also other compounds from the medium, which testifies to a high yield of biomass

compared to the sugar utilized, i.e., from 0.56 to 0.74. In the biomass an average 45% of protein was found.

In cultures *C. utilis* Z-2 higher increments of cells, from 12 to 17.2 g dry matter/l were observed in corresponding juice lots (Table 3).

Sugars were better utilized than in the cultures of the previous strain, and the yield of biomass in the particular media was more even. In the biomass of *C. utilis* Z-2 less protein (about 41%) was found, and, similarly to *C. brumptii*, its level does not substantially depend on the juice lot.

Equally good results were obtained in cultures of *C. guilliermondii* K-29 (Table 4). The biomass increments were higher, in the range of 11.9 to 19.1 g dry matter/l, there were also high yields of biomass from 0.59 to 0.69. On the other hand, there were higher fluctuactions of protein content in cells. According to the type of juice they ranged from 37.3 to 41.0%.

Table 5 lists some results of cultivation of the remaining 11 yeast strains. Two successive strains of *C. guilliermondii* C-5 and K-22 grew in lucerne juices in a very similary way. A practically analogous sugar utilization, similar increments of biomass as well as an approximate protein content of dry biomass were found. In the particular media the yield of biomass compared to the suggar utilized was very differentiated (from 0.47 to 0.7), whereas the protein content of cells was low and moderately even. Characteristic of the cultures of *P.membranaefaciens* C-4 was an almost constant yield of biomass in qualitatively different lots of juice; it was close to a theoretical value of 0.5. Thus the strain did not utilize from the medium any other compounds as a source of carbon and energy and grew poorly in some lots of juice.

Two yeast strains, isolated from soil: *C. scotti* A-61 and *T. globosa* ADVI grew in lucerne juice less effectively then previous cultures originating from plant habitats. Characteristic of all remaining cultures was a low yield of biomass on utilized sugar. The lowest yield factor, 0.2, was observed in *C. tropicalis* Z-3, which clearly testifies to the fermentative metabolism of this yeast.

On the basis of the results of shaking cultures three yeast strains best growing in lucerne juice were selected. The results of cultivation of these strains in juice of 1 litre volume in the fermenter are presented in Table 6. In the selected lots of juice, containing 2.07 to 2.41% sugars, the strains under examination attained biomass increments from 12.0 to 14.8 g d.m./l. The cell yield from sugar ($Y_s$) was high and equalized for all the strains, approximating 0.6. Two strains, *C. utilis* Z-2 and *C. guilliermondii* K-29, grew very dynamically in the juice examined, at a specific growth rate ($\mu$) equal to 0.68 and 0.66 $h^{-1}$ respectively. The strain *C. brumptii* Z-1 grew much slower. The latter culture was at the same time characterized by the highest protein content of cells, amounting to 53.1%.

Note worthy is the fact that the protein content in the biomass of

Table 6. Growth of selected yeast strains in waste lucerne juice

| Microorganism | Initial sugar concentr. % | Increment of biomass g d.m./l | Cell yield on consumed sugar $Y_S$ | Protein % of dry biomass | Specific growth rate ($\mu$) $h^{-1}$ | $BOD_5$ $10^3$ $mgO_2/l$ | |
|---|---|---|---|---|---|---|---|
| | | | | | | before | after cultivation |
| *C. utilis* Z-2 | 2.15 | 12.4 | 0.59 | 50.3 | 0.68 | 30 | 11 |
| *C. brumptii* Z-2 | 2.41 | 14.8 | 0.63 | 53.1 | 0.35 | 32.5 | 9 |
| *C. guilliermondii* K-29 | 2.07 | 12.0 | 0.60 | 46.1 | 0.66 | 36 | 15 |

Culture conditions: 1 liter of juice, pH 4,5; aeration 1 l/min; agitation 500 r.p.m.; 30°C

yeast grown in the fermenter was definitely higher than in than obtained in shaking cultures. An important result of yeast cultivation in lucerne juice was an outstanding lowering of pollution of this waste, expressed in a decrease in $BOD_5$ from 30-36.000 down to 9-15.000 mg $O_2$/l.

## DISCUSSION

Summing up the results presented above, one can say that the yeast strains investigated differed in efficacity and utilization rate of sugars from juice, in the attained increments of biomass as well as in the protein content of cells. In the case of some yeast cultures these basic parameters of growth were substantially dependent on the juice lot, while for others they were not. Generally speaking the major part of cultures, during 24-hour shaking cultivations, utilized an average of over 90% sugars. Sugars were metabolized more slowly by strains isolated from soil. The highest oscillations of this index between 48.8% to 94.2%, according to the sort of juice, were found in cultures *P. membranaefaciens* C-4. Most cultures isolated from fruit juices as well as *C. tropicalis* Z-3, of which the parent medium was lucerne, metabolized sugars but attained low increments of biomass, which may testify to a marked fermentative metabolism of these strains.

Highest increments of biomass, between 11.4 to 19.1 g dry matter, were noted for *C. guilliermondii* K-29, *C. utilis* Z-2 and *C. brumptii* Z-1. The latter two cultures have been isolated from lucerne juice. Apart from sugars, all three species of yeast had the capability of utilization from the medium, other compounds as a source of carbon and energy. They showed a high yield of biomass substantially exceeding the theoretical value 0.5. At the same time *C. utilis* Z-2 and *C. guilliermondii* K-29 were characterized by a more even yield od biomass from sugar, which for *C. brumptii* Z-1 oscillated between 0.56 to 0.74 in various lots of lucerne juice. In yeasts grown in lucerne juices unriched in any nitrogen, from 35.1 to 53.1% of protein in dry mass was found. The protein content of yeast seems to be a feature more depending on a yeast strain and culture conditions than on the type of juice. Yeasts grown on a shaker contained much less protein than those grown in the fermenter. In the first case the protein content of cells did not exceed 47% in yeast *C. brumptii* Z-1, the richest in this component, which means that it was much lower than that reported in literature for fodder yeasts obtained from various carbohydrate substrates [1, 8]. On the other hand, it is known that the nitrogen content of waste lucerne juice is high, often amounting to 3 g/l [2]; therefore it should not be a limiting factor in this process provided that growing yeasts are able to utilize the forms of nitrogen occurring

there. Besides, when we cultured three yeast strains, selected out of the 14 strains tested in lucerne juice in the fermenter, we found much more proteins in their biomass. Similar effect was noted by Janshekar [5] who showed that the protein content of yeast grown on molasses depends, among other things, on temperature, pH as well as oxygen supply. Thus the problem remains still open. In our studies we will take into account the nitrogen balance in yeast cultures we plan to optimize culture conditions in order to obtain a biomass with the highest possible protein content and we shall further isolate the appropriate yeast strains.

## CONCLUSIONS

1. Waste lucerne juice is a good medium for yeasts isolated mainly from it.

2. The cell yield of yeast strains, *C. utilis* Z-1, *C. guilliermondii* K-29 and *C. brumptii* Z-1, best growing in lucerne juice, was high, and amounted to an average of 0.6 on utilized sugars, which shows that in addition to sugars, yeasts metabolize also non-sugar compounds as a carbon and energy source.

3. The protein content of yeasts grown in lucerne juice was the characteristic feature of a strain and first of all depended on culture conditions, less on the type of juice. The highest amount of protein, 43.6 to 53.1%, was found in the biomass of *C. brumptii* Z-1.

4. Yeast cultivation resulted in an about 3-fold reduction of $BOD_5$ of the waste lucerne juice.

## LITERATURE

1. Bujak S.: Acta Microbiol. Polon., 1952, **1** (1), 65.
2. Glapś J., Korniewicz A., Przysiecka M., Ryś R.: Roczn. Nauk Zootechn., 1975, 2, 201.
3. Höllo J., Koch L., Koch B.: Nowe kierunki pozyskiwania i wykorzystania białka spożywczego, Warszawa 25-26 XI 1971.
4. Irgens R. L., Clarke J. D.: Europ. J. Appl. Microb., 1976, **2** (4), 231.
5. Janshekar H.: A dissertation submit. to the Swiss Federal Institute of Technology for the degree of Doctor of Technical Sciences, Zurich 1979.
6. Jarosz K., Muszkat T., Skibniewski C., Suchodolski J., Urbański M.: Kontrola produkcji w przemyśle spirytusowym WPLiS, Warszawa 1955.
7. Jasiorowski H., Zazula M.: Roczn. Nauk Roln., 1962, 79 (B), 741.
8. Łabędziński S.: Postęp naukowo-techniczny a intensyfikacja produkcji białka. Katowice, March 1978.
9. Polska Norma PN-74/C-04578, O7.
10. Oelshlegel F. J., Schroeder J. R., Stahmann M. A.: J. Agric. Food Chem., 1969, **17** (4), 791.
11. Upit A. A., Krauze I. J., Beker M. E., Pinte A. M.: Tezisy dokładow Sympozjuma: Biotechnołogija i Bioinżinierija, Izd. Zinatne, Riga 1978.

12. Woldergiorgis G.: Dissertation Abstracts International 1977, B 37 [6], 2776.

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## PORÓWNANIE WZROSTU WYBRANYCH SZCZEPÓW DROŻDŻY W ODPADOWYM SOKU Z LUCERNY

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Streszczenie

Porównano wzrost 14 szczepów drożdży wyodrębnionych z różnych środowisk naturalnych w odpadowym soku w produkcji koncentratów białkowych z lucerny. Badania te prowadzono w celu wytypowania szczepów drożdży najlepiej przystosowanych do wzrostu w tym środowisku. Szczepy te mogą znaleźć zastosowanie w produkcji drożdży paszowych na odpadowych sokach roślinnych.

Poszczególne szczepy różniły się efektywnością i szybkością utylizacji cukrów zawartych w soku, wydajnością biomasy oraz poziomem białka w komórkach. Najlepsze przyrosty biomasy od 11,4 do 19,1 g ss/l dawały *C. utilis* Z-2, *C. guilliermondii* K-29 i *C. brumptii* Z-1 (tab. 2, 3). Cechowała je wysoka wydajność wzrostu w przeliczeniu na zużyte cukry, wyższa od teoretycznej, co świadczyło o tym, że szczepy te wykorzystywały ze środowiska również inne związki organiczne w charakterze źródła węgla i energii. Najwięcej białka 53,1% stwierdzono w biomasie *C. brumptii* Z-1 (tab. 6). Najdynamiczniej rosły w soku *C. utilis* Z-2 i *C. guilliermondii* K-29, ze specyficzną szybkością wzrostu bliską $\mu = 0{,}68\ h^{-1}$. Efektem hodowli drożdży była ok. 3-krotna redukcja $BZT_5$ soku (tab. 6).