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PRODUCTION AND COMPOSITION OF OIL FROM *CANDIDA CURVATA* D

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A strain of the yeast *Candida curvata* D was cultured on deproteinized whey medium. The effect of temperature and acidity on oil biosynthesis yield as well as on lactose utilization and reduction of chemical oxygen demand in the post-culture effluent was studied. The composition of the biomasses and of the oil was compared.

INTRODUCTION

One possibility of alleviating oil shortages is the microbiological biosynthesis of oil, using mainly moulds and yeasts [5, 17]. The biotechnology of microbiological oil ought to satisfy the following requirements [11]:

- the microorganisms used must be predisposed to efficient oil biosynthesis,
- the methods of oil extraction must be easy and cheap,
- the technology must be economically superior to the conventional methods.

The effects of oil biosynthesis also depend on the genetical predispositions of the given microorganisms, the conditions of their culture and on the kind of medium.

According to Hammond et al. [5] the yeast *Candida curvata* D may be used to biosynthesis oil in a whey medium. The abundance of whey in Poland, especially in summer, justifies the study of its possible application in multiplying the biomass of oil-rich yeasts and of its utilization in fodder technology.

The objective of this research was to determine the parameters of *Candida curvata* D biomass cultivation on a whey medium and to give a tentative evaluation of the quality of the oil obtained in the process.

MATERIAL AND METHODS

The experiments were performed with whey from cheese production, deproteinized by thermal-acid coagulation of proteins at 92°C and pH 4.6. The whey was augmented with the following microelements: $(\text{NH}_4)_2\text{SO}_4$ (0.5%), K_2HPO_4 (0.1%), MgSO_4 (0.05%). After having its acidity adjusted to pH 5.4 with a 2 N solution of NaOH, the medium was sterilized at 117°C for 30 min.

The yeasts *Candida curvata* D were cultured on wort-agar slants at 30°C for 48 h. They were then inoculated twice on a whey-agar medium and incubated as before, and then finally transferred to the whey medium.

The yeasts were cultured on a shaker (Elpan type 357) in 100-cm³ Erlenmeyer flasks; the conditions of cultivation were as follows:

- 10% inoculum addition [12],
- flasks filled to 1/5 of their volume,
- time of cultivation — 84 h (determined during preliminary studies [18]);
- temperature: 25, 30 and 35°,
- acidity of the medium: pH 5.0, 5.4 and 5.8.

The active acidity of the medium was maintained on a constant level by adjusting pH with 1 N NaOH solution every 12 h.

The yield of oil biosynthesis was checked at 12-h intervals by determining dry matter yield from 1 dm³ of the medium, lactose content in the medium by Bertrand's method [1], and the chemical oxygen demand (COD) in the effluent after removal of the biomass by centrifugation [6]. At the end of cultivation the oil yield from 1 dm³ of the medium was determined. Five repetitions of the experiment were performed; the Tables contain results which differed from one another by not more than 3%. The biomass characteristic comprised determinations of the following contents: dry matter [1], total nitrogen substances by Kjeldahl's method [1], nitrogen substances precipitated in 12% solution of trichloroacetic acid [1], ash [1] and oil [5].

The determinations in yeast oil included total fatty acid composition [15], separation of oil triglycerides depending on the number of carbon atoms in a molecule [10, 14], fractions of saturated and unsaturated triglycerides [8, 9], fatty acid content in saturated and unsaturated fractions of oil glycerides [8, 9, 15].

RESULTS AND DISCUSSION

The obtained results indicate that the yield of the synthesis of yeast dry matter and of yeast oil as well as the utilization of lactose are little affected by the studied parameters of culture, namely temperature and acidity.

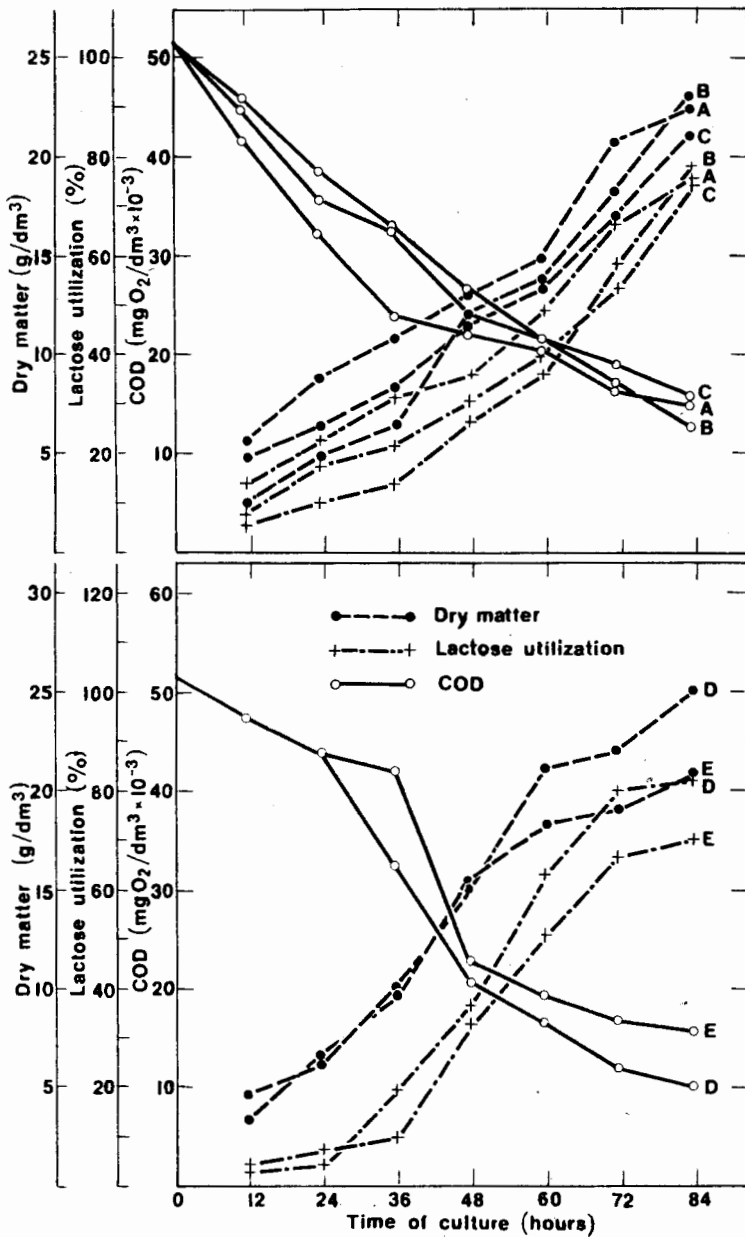


Fig. Dry matter yield, lactose utilization and COD of the effluent during *Candida curvata* D culture

The yeasts *Candida curvata* D actively utilized lactose when active acidity of the medium ranged from pH 5.0 to 5.8. Yeasts multiplied at 25°C and active acidity pH 5.0 fermented lactose with particular intensity starting from the 48th h of culture. Lactose utilization by the yeasts was about 75% (Fig.). Yeast cultured on the medium with pH 5.4 utilized lactose at a slower rate in the initial period, a rapid intensification of this process occurred after 60 h of culture. The lactose utilization in these conditions was better (76.8%). Similar utilization of lactose in the medium, 73.38%, was observed in yeast culture on medium with active acidity pH 5.8 (Fig.).

The yield of yeast dry synthesis ranged from 20.96 to 22.87 g/dm³ of the medium, with the best results obtained when the active acidity of the medium was pH 5.4.

The evaluation of oil biosynthesis yield confirmed these results. 6.70 and 6.10 g oil/dm³ of the medium were obtained when active acidity of the media was pH 5.4 and 5.8, respectively (Table 1). By far the worst results were obtained with the yeast culture on media with pH 5.0.

Table 1. Yield of oil biosynthesis and chemical composition of *Candida curvata* D biomass depending on the conditions of yeast culture

Analysis Culture	Yield of oil biosynthesis		Dry matter	Oil	Total nitrogen substances N _i × 6.25	Nitrogen substances precipitated in 12% TCA N _{pTCA} × 6.25	Ash	Other components
	g/l dm ³	g/100 g of lactose	g/l dm ³	% of dry matter	% of dry matter		% of dry matter	% of dry matter
A-25°C, pH 5.0	5.70	15.07	22.40	25.45	26.26	19.12	9.90	38.39
B-25°C, pH 5.4	6.70	17.36	22.87	29.98	24.24	21.20	8.70	37.08
C-25°C, pH 5.8	6.10	16.60	20.96	29.10	21.12	16.01	8.43	41.35
D-30°C, pH 5.4	7.40	17.70	25.23	29.39	20.36	15.06	8.17	42.14
E-35°C, pH 5.4	6.02	16.98	20.96	28.73	26.32	18.54	9.80	35.15

The culture of the yeasts *Candida curvata* D led to a considerable reduction of COD in the effluent and thereby. The COD was reduced from 52 000, the value in whey, to 12 000 (culture at pH 5.4), 14 000 (culture at pH 5.0) and 15 000 (pH 5.9) (Fig.). The reduction was thus by 71.16-76.93%. The high COD values, unwelcome from the point of view of natural environment protection, were due to partial fermentation of lactose and to the presence in the medium of mineral substances and low-molecular nitrogen substances. The oil content in dry matter of yeast

biomass ranged from 25.45 to 29.98% (Table 1). The highest oil content in dry matter was in the biomass obtained at 25°C and pH 5.4. It was observed that lower oil content in biomass dry matter markedly facilitated the separation of biomass by centrifugation.

The most favourable content of nitrogen substances in dry matter was found in biomass obtained at 25°C and active acidity pH 5.0 (26.26%) and at 35°C and pH 5.4 (26.32%) (Table 1). Yeast biomasses with higher oil content had less protein (24.24% at pH 5.4, and 21.12% at pH 5.8).

About 80% of total nitrogen substances was accounted for by protein, i.e. by nitrogen substances precipitated with a 12% solution of trichloroacetic acid. The ash content in yeast biomass ranged from 8.17 to 9.90% and was highest in the biomass obtained at pH 5.0 (Table 1).

The effect of temperature of yeast culture on oil biosynthesis yield was determined next. In cultures maintained at 30°C the degree of lactose utilization rose to 83.26% (Fig.), and the post-culture effluent contained only 0.84% lactose. The increase of temperature to 30°C enables either a more thorough fermentation of lactose or the reduction of culture duration. The degree of lactose utilization in the medium was reflected in the yield of yeast dry matter synthesis (25.23 g/dm³ of the medium) and the yield of oil synthesis (7.40 g/dm³ of the medium). The high transformation of medium components into biomass components led to a reduction of the effluent's COD to 10 000, which means that the COD re-

Table 2. The fatty acid composition of *Candida curvata* D oil

Fatty acid	Fatty acid content (%)	
	upper layer	bottom layer
C _{12:0}	0.27	—
C _{14:0}	0.78	1.26
C _{15:0}	trace	0.20
C _{16:0}	27.19	28.74
C _{16:1}	0.73	0.78
C _{17:0}	0.25	0.23
C _{18:0}	14.04	13.97
C _{18:1}	49.10	47.78
C _{18:2}	6.22	6.36
C _{18:3}	0.75	0.41
C _{20:0}	0.50	—
C _{20:1}	0.17	0.27
C _{20:2}	—	—
C _{22:0}	—	—
C _{22:1}	—	—
Saturated	43.03	44.40
Monosaturated	50.00	48.83
Polyunsaturated	6.97	6.77

duction in this experiment was by about 81%. A further increase of culture temperature improved neither the yield of yeast dry matter synthesis (20.96 g/dm³ of the medium) nor the final lactose content in the effluent (1.48%). The oil synthesis yield was lower (6.02 g/dm³ of the medium) and the COD of the affluent after culture was 15 500 (Table 1).

The biomass of yeasts obtained at 30°C had the lowest dry matter content. It is noteworthy, however, that the oil content in this dry matter was high, namely 29.33% (Table 1). A high content of oil in the biomass goes together with reduced total nitrogen substances and protein content. A further increase of culture temperature led to an increase of dry matter content in the biomass, a reduction of oil content, and to higher content of nitrogen substances (Table 1).

The oil extracted from the yeasts *Candida curvata* D divided into two layers, the upper being light-coloured and clear, and the bottom being dark and viscous. Subsequent analyses of the oil were performed for the

Table 3. The content of saturated and unsaturated tryglicerides of *Candida curvata* D oil

Triglyceride	Triglycerides content % of raw oil	
	upper layer	bottom layer
Saturated	6.71	3.93
Unsaturated	62.69	26.50

Table 4. The content of fatty acids in saturated and unsaturated *Candida curvata* D oil

Fatty acid	Fatty acids content (%)			
	upper layer		bottom layer	
	triglycerides			
	saturated	unsaturated	saturated	unsaturated
C _{12:0}	1.37	—	—	—
C _{14:0}	3.58	0.73	16.17	3.04
C _{15:0}	0.56	—	2.42	1.93
C _{16:0}	47.55	29.34	48.41	25.57
C _{16:1}	0.53	0.53	2.65	4.34
C _{17:0}	0.86	—	1.41	2.73
C _{18:0}	26.73	13.17	12.15	16.78
C _{18:1}	11.87	52.27	16.79	35.05
C _{18:2}	1.71	3.60	—	8.63
C _{18:3}	0.41	0.15	—	0.95
C _{20:0}	4.81	—	—	0.98
C _{20:1}	—	0.21	—	0.98
C _{20:2}	—	—	—	—
C _{22:0}	—	—	—	—
C _{22:1}	—	—	—	—

two layers separately. The oil was found to contain a large share of oleic acid, about 48% (Table 2), with palmitic acid being in second place (about 28%). Two more fatty acids, the stearic and the linolic, occurred in considerable quantities, the others being present in relatively small amounts. Worth stressing is that the studied oil contained about 44% of unsaturated acids. Compared with vegetable oil, the yeast oil contains twice as much oleic, stearic and palmitic acids, and considerably less linolic acid [7, 13]. These results are similar to those quoted by other authors [11].

An attempt was made to divide the yeast oil glycerides into saturated and unsaturated triglycerides with the method of column chromatography on Florisil. It turned out that 30-70% of the applied raw oil could be recovered in the form of triglycerides (Table 3). This means that this method of triglycerides separation is useless without a prior purification of the oil. The complete inefficiency of the method is indicated by the fatty acid composition in the separated fractions (Table 4). As we can see, in the case of the so called "saturated" fractions there were large quantities of oleic and linolic acids (except in the oil fraction from the bottom layer). Thus, the characteristic of triglycerides of the saturated and unsaturated fractions must be preceded by methodological studies.

The yeast oil triglycerides were also separated depending on the number of carbon atoms in the molecule, which ranged from 48 to 54 (Table 5). The triglycerides are not much differentiated as regards the length of the carbon chain. Molecules with 52 carbon atoms dominate and this makes the obtained yeast oil similar to most vegetable oils.

Table 5. Separation of triglycerides in *Candida curvata* D oil according to the number of carbon atoms in the molecule

Number of carbon atoms in triglyceride molecule	Fractions (%)	
	upper layer	bottom layer
46	—	—
48	3.17	2.77
50	16.06	25.44
52	49.56	58.20
54	31.21	13.59

The satisfactory yeast biomass yield and the very high oil content in the biomass justify further studies, notably concerning biosynthesis intensification leading to an, acceleration of biomass multiplication. The fatty acid composition in the oil from *Candida curvata*, similar to that in other vegetable oils, confirms the assumption that it is applicable in fodder technology.

CONCLUSIONS

1. 7.40 g oil/1 dm³ of the medium and a roughly 81% reduction of chemical oxygen demand in the post-culture effluent may be obtained in the culture of the yeasts *Candida curvata* D on a whey medium at 30°C and active acidity of the medium amounting to pH 5.4.

2. The microbiological oil contains about 44% of saturated acids. Dominant among fatty acids are oleic, palmitic, stearic and linolic.

3. The triglycerides of *Candida curvata* D are poorly differentiated as regards carbon chain length. Molecules with 52 carbon atoms predominate.

4. The laboratory-scale results justify a continuation of studies on a micro-technological scale, with particular attention devoted to aeration conditions and the N : C ratio in the medium.

The authors are aware of the fact that toxicological studies of the oil obtained from biomass of *Candida curvata* will be necessary taking into account the toxicogenic properties of these yeasts.

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WARUNKI BIOSYNTETY I CHARAKTERYSTYKA TŁUSZCZU Z DROŹDZY *CANDIDA CURVATA* D

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Streszczenie

Drożdże *Candida curvata* D namnażano na podłożu z serwatki odbiałczonej. Badano wpływ temperatury i kwasowości czynnej środowiska na wydajność biosyntezy tłuszczu oraz dynamikę wykorzystania laktozy i stopień redukcji ChZT odcieku pohodowlanego. Porównano skład chemiczny namnożonych biomas oraz przeprowadzono charakterystykę jakościową uzyskanego tłuszczu.

Najlepsze rezultaty uzyskano w temperaturze 30°C przy kwasowości czynnej podłoża pH 5,4 (25,53 g s.s. drożdży/1 dm³ i 7,40 g tłuszczu/1 dm³). Tłuszcz z drożdży zawierał ok. 44% kwasów nasyconych. Spośród kwasów tłuszczowych dominowały kwasy: oleinowy (ok. 48%), palmitynowy (ok. 28%), stearynowy i linolowy. Próba rozdziału trójglicerydów tłuszczu drożdży wykazała, że liczba atomów węgla w cząsteczce mieściła się w przedziale od 48 do 54. Obraz trójglicerydów był mało urozmaicony pod względem długości łańcucha z przewagą trójglicerydów zawierających 52 atomy węgla w cząsteczce.