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THE CHEMICAL COMPOSITION OF YEASTS CULTIVATED IN THE PRESENCE OF VARIOUS HYDROCARBONS

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Key words: *Candida scotti*, hydrocarbon, slack wax, hexadecane, naphtha, chemical composition of biomass.

Chemical composition of P-2 yeasts which passed successfully diagnostic tests for the *C. scotti* variety, cultivated in the presence of various hydrocarbon parent substances (slack wax, hexadecane, naphtha) was investigated. No more pronounced effect of the kind of carbon on the chemical composition of biomass was found to exist.

Hydrocarbons, which may constitute the only carbon source in the yeast cultivation process, can be divided — from the point of view of their physical state — into gaseous, liquid and solid.

Till the present time an overwhelming majority of studies devoted to the process of yeasting of petrochemical materials have been based on liquid hydrocarbons due to the much simpler technology compared with that based on gassy and solid substances. This is why the information contained in the literature and concerning the chemical composition of the "hydrocarbon" yeasts refers almost exclusively to yeasts produced just from that group of substrates.

It should also be emphasized that data published in this field usually have a marginal character, as supplementary to the description of bioengineering and technological problems. There are no complex investigations which at the same time would inform about a greater number of components of the yeasts in question (e.g. in addition to the content of proteins also the share of certain aminoacids, fat and ash) and, consequently, would more thoroughly characterise the biomass obtained.

The main purpose of the present study was to determine the content of some yeast components, essential for their nutritive value, the yeasts

Table 1. Percentage content of n-paraffin in slack wax

	Number of carbon atoms in molecule												
	C ₁₁ -C ₁₉	C ₂₀	C ₂₁	C ₂₂	C ₂₃	C ₂₄	C ₂₅	C ₂₆	C ₂₇	C ₂₈	C ₂₉	C ₃₀	>C ₃₀
Percentage share (total amount of n-paraffins = 100%)	2.96	1.69	2.80	5.38	9.75	12.98	14.69	13.37	9.73	8.40	7.73	5.75	4.67

having been obtained in a medium with slack wax as the only source of carbon. Since this material contains n-paraffins (hydrocarbons assimilated by the yeast first) of relatively long chains (see Table 1), then to obtain a comparative material also the chemical composition of yeast produced in the presence of other hydrocarbon substrats i.e. hexadecane ($C_{16}H_{34}$) and naphtha containing n-paraffins of the order of C_9 - C_{13} , was examined.

EXPERIMENTAL

A. MATERIALS

Yeast

In all the cultivation samples whose yield was the subject of this study, the P-2 strains meeting the diagnostic tests for *Candida scottii* [24], were used.

Slack wax

A waste product obtained from the refining of petroleum from Romashkino (Tartar SSR), with the following characteristic:

flowing temperature	43°C
content of n-paraffins	40% (urea meth.)
content of aromatic hydrocarbons	12.9%
content of paraffin-naphtha fraction	86.4%
content of resins	0.68%

The content of normal paraffins in slack wax determined by the earlier given methods [42] is specified in Table 1*).

Naphtha

A commercial product of the following properties:

specific gravity	0.7925 g/cm ³
content of n-paraffins (the urea method)	10.5%
amount of n-paraffins	
< C_9	1.9%
nonane	8.9%
decane	29.5%
dodecane	34.7%
tridecane	19.3%
> C_{13}	5.7%

* The analysis was carried out at the Analytical Research Department of the R. and D. Centre of the Refining Industry at Plock, Poland.

B. METHODS

Cultivation conditions

To obtain comparative material, all the cultures were under practically identical conditions. There were shake cultures in a synthetic medium containing mineral nutrients [42] and a hydrocarbon as the only source of carbon. The concentration of hydrocarbons in terms of n-paraffin did not exceed 1%. The „Difco” yeast extract was used as the source of biotic substances.

Because of the solid state of the slack wax at the temperature of the culture (flowing temperature 43°C), this material — in order to prepare a homogenous emulsion for cultivation samples was dissolved in 2, 6, 10, 14-tetramethylpentadecane not assimilable by the yeasts (samples marked in tables with number "1"), in dewaxed oil (samples marked with number "2"), or were emulsified by means of a surface active agent, cemulsol, a product of condensation of ethylene oxide, with nonylophenol (samples marked with number "3") [43].

Cultivation was carried out at pH = 5.0 in two variants differing by temperature, which in variant I was $30^{\circ} \pm 1^{\circ}\text{C}$. These two different temperatures were used due to the formerly noticed small increase in yeast yield in case of temperature elevated by several degrees. Temperature may also effect the chemical composition of yeast, e.g. the content of carbohydrates in them [33].

The yeast yield obtained was filtered using the Schott funnel washing it first with distilled water of 55-60°C and, later with a mixture of dehydrated alcohol and petrol ether (1:4).

The yeasts under investigation represented average samples obtained after mixing the yield from a number of cultures produced under identical conditions.

Analytical methods

The content of dry substance, protein, phosphorus and ash was determined according to the obligatory standards for the chemical composition of fodder yeast [25]. The content of aminoacids was established by the gas chromatography method in a Pye Unicam Apparatus*). Yeast hydrolysates (hydrolysis of 6N HCl at 110°C for 24 h) were purified on the IR-120H Amberlit cationite. Then the aminoacids were transformed into N-triacetyl-n-butyl esters and, in the form of these derivatives, injected into the chromatograph. The content of methionine and

*) The analysis was carried out at the Department of Instrumental Analysis of the Institute of Fermentation Industry, Warsaw.

tryptophan was determined by the colorimetric methods (colour compounds: methionine with sodium nitroprusside complex, a tryptophan oxidation product in acid waterless medium) in separate samples [18, 19].

The fractionation of carbohydrates was carried out by the Weinfurter method [41] depending on the thermal treatment of yeast in an alkaline and acid medium. The content of fractions was determined according to Rapport and Raderecht [29].

Vitamins from group B were determined by A.O.A.C. methods [28] after Hrdy and Urbanova [13] and Myszkowska [23].

The yeast was subjected — to liberate vitamins from them — to the acid hydrolysis (H_2SO_4) and enzymatic hydrolysis (taka-diastrase, papaine).

Vitamins fractioning was carried out in a column filled with SD-5 cationite or "florisil" adsorbent [12, 22]. The eluate were examined spectrophotometrically in a "Specol" apparatus by the measurement of fluorescence (thiamine, riboflavine) or extinction (pyridoxine, niacine)*.

Fat was determined by extraction with ethyl ether [16].

Yeast-nucleic acids were determined spectrophotometrically, by Spi-ryn's method [32].

C. RESULTS AND DISCUSSION

The results of investigation of the chemical composition of yeast obtained from sample cultures are given in Tables 2-5.

Table 2. Chemical composition of P-2 yeasts produced in the presence of various hydrocarbon substrates

Sample No	Carbon source	Content in D.S. in %					
		protein (N×6.25)	carbo- hydrates	fat	phosphorus (P ₂ O ₅)	ash	nucleic acids
1	slack wax	49.5	16.9	3.25	3.21	6.3	5.4
1a		49.2	18.6	3.18	3.08	6.1	5.4
2		48.7	17.4	2.87	3.16	5.8	5.8
2a		49.0	17.7	2.69	3.37	6.4	5.1
3		48.9	18.2	2.94	3.10	7.0	5.5
3a	48.0	17.8	3.28	3.40	6.6	5.0	
4	hexadecane	46.1	19.8	4.10	3.09	5.2	6.9
4a		46.4	20.1	3.22	3.36	6.5	6.5
5	naphtha	47.0	19.0	2.91	2.45	5.8	6.9
5a		45.2	19.3	3.42	3.41	6.2	7.1

*) I wish to express my deep gratitude to Dr W. Krakowiak from the Institute of Biochemistry of the Łódź Technical University for carrying out the analyses.

Table 3. Contents of amino acids in P-2 yeasts in g/16 g N

Sample No	Carbon source	Ala-nine	Valine	Iso-leucine	Threo-nine	Leu-cine	Gly-cine	Serine	Proli-ne	Hy-droxy-pro-line	Aspar-tic acid	Phe-nylala-nine	Gluta-mic acid	Tyro-sine	Ly-sine	Met-hio-nine	Tryp-to-phan	Sum of amino acids	
																		all	exo-genic
1	slack wax	7.8	5.6	4.3	2.6	6.4	5.2	1.5	3.5	0.6	7.6	3.2	8.6	1.8	6.8	1.3	1.1	67.9	31.3
1a		6.6	4.6	3.7	2.3	5.5	4.7	1.5	3.1	2.5	7.9	3.1	10.2	1.9	7.3	1.1	1.1	67.2	28.7
2		7.0	4.7	4.4	2.6	6.7	5.7	1.5	3.5	0.6	7.7	3.2	8.7	1.5	6.7	1.3	1.2	67.3	30.8
2a		7.3	5.3	4.6	2.5	6.5	5.0	1.6	3.5	0.5	7.7	3.3	8.6	1.8	6.6	1.2	1.1	67.3	31.1
3		6.9	5.2	4.6	2.4	6.7	5.2	1.6	3.4	0.5	7.5	3.2	8.4	1.8	6.6	1.3	1.0	66.3	31.0
3a		6.7	4.5	2.5	2.8	6.3	5.5	1.7	3.3	0.6	8.3	3.6	8.9	1.9	7.5	1.1	1.3	66.6	29.6
4	hexa-decane	6.7	4.8	4.3	2.2	6.0	4.8	1.3	3.0	—	7.0	2.8	10.0	1.8	7.2	1.0	0.8	63.7	29.1
4a		7.1	4.9	4.4	2.4	5.8	4.9	1.3	2.6	—	7.6	3.0	10.5	0.2	6.9	1.2	0.8	63.6	28.3
5	na-phtha	6.1	4.2	3.9	—	7.7	5.6	—	3.2	0.4	8.0	3.6	10.3	0.9	7.3	0.8	0.9	62.8	28.4
5a		5.7	4.0	3.5	2.2	5.5	4.5	1.5	3.0	1.8	7.2	2.8	9.8	1.9	6.6	1.0	0.8	61.8	26.4
FAO standard			4.2	4.2	2.8	4.8						2.8			4.2	2.2	1.4		22.6

Table 4. Content of carbohydrate fractions in P-2 yeasts in terms of glucose in mg/g D.S.

Sample No	Carbon source	Glycogen			Glucane	Mannane	Total of fractions
		acid	alkaline	total			
1	slack wax	23.5(14)	86.4(51)	109.9(65)	48.9(29)	10.2(6)	169.0
2		29.4(17)	92.3(53)	121.7(70)	39.9(23)	12.7(7)	174.2
3		32.3(18)	89.1(49)	121.4(67)	51.8(28)	8.8(5)	182.0
4	hexadecane	39.9(20)	95.9(48)	135.8(68)	42.5(22)	19.7(10)	198.0
5	naphtha	35.8(17)	96.2(47)	132.0(64)	41.1(23)	20.1(13)	193.2

Values quoted in brackets determine the percentage share in the sum of fractions

Table 5. Content of group B vitamins in P-2 yeasts in mg/g D.S.

Sample No	Carbon source	Thiamine	Riboflavine	Pyridoxine	Niacine	Pantothenic acid
1	slack wax	7.9	61	6.0	219	84
1a		7.9	71	6.8	209	78
2		8.7	70	6.1	214	96
2a		7.1	70	9.3	232	111
3		6.8	63	6.5	225	95
3a		8.2	88	6.5	245	71
4	hexadecane	10.5	69	7.9	215	53
4a		10.4	83	7.1	219	39
5	naphtha	10.0	83	6.7	249	40
5a		10.8	90	10.5	261	51

Table 2 specifies the content of components most frequently discussed in the literature. Generally it should be said that most results obtained are contained within the limits quoted in the literature.

Temperature difference (by 5°C) in both sample variants (with the "a" index and without it) did not affect in a visible way the chemical composition of the biomass obtained. This remark is also true for the results given in the remaining tables.

Protein content in the yeast under investigation was relatively low, within 45-49% of dry substance. According to data quoted by various authors, the amount of protein in petrochemical yeast shows great variations, from 42 to 68% of D.S. [1, 2, 3, 7, 9, 11, 15, 20, 21, 19], and the values contained within this range concern yeasts from various systematic groups (genus, species) produced in the presence of various substrates (natural petroleum fractions of various characteristics, pure n-paraffins of various chain length) and under different cultivation

conditions. Thus it would be difficult to give a comparative analysis of these values. Particularly interesting are i.a. the results obtained by Miller [20] who confronts the amount of nitrogen in yeast (mixture *C. intermedia* and *C. lypolytica*) produced in the presence of n-paraffins of different chain lengths. Raw protein content obtained from a calculation (N.6.25) amounts in the case of liquid n-paraffins (C_{16} - C_{18}) from 42 to 48.5%, whereas in yeasts from solid paraffins, of longer chains (C_{20} - C_{36}) these values are clearly higher — from 51 to 55%.

The results of the author's own investigations made it possible to obtain a similar, although somewhat smaller differentiation of protein content: more of it in yeast obtained on a medium containing slack wax (n-paraffins of longer chain) than in the case of the remaining shorter n-paraffin chain substrates.

One of the very few information concerning the chemical composition of yeast produced in the presence of the slack wax, available in the literature, may be found in another publication by Miller [21], according to which their content of protein varied from 52 to 55%. Simek and Szechenyi [36] noticed similar variations in protein content in yeast also produced from slack wax, and they suggested that these variations might be connected with the kind of the nitrogen nutrients in the medium. In the case of ammonium sulphate the yeast contained less protein than when urea was used as the source of nitrogen.

The content of carbohydrates in yeasts under investigation indicates that — like in the case of protein — certain small but pronounced differences in the amounts of carbohydrates in yeasts produced in the presence of various hydrocarbons, can be noticed. The concentration of carbohydrates in yeasts coming from slack wax is some 10% lower than in yeasts produced in the presence of other substrates.

From the very few data about the amount of carbohydrates in "petrochemical" yeasts, published so far [2, 7, 8, 9, 10, 15] it results that they may vary within a wide range, from 12 to 26% of D.S. The relatively lowest content has been reported by Czepigo [11], i.e. from 12 to 20%, the highest by Anin [2] in the *C. lypolytica* yeasts (31%) and *C. pelliculosa* yeasts (36%) obtained in the presence of petroleum fraction containing 58% of n-paraffins.

Fat content varies within narrow limits, from approx. 2.7 to 4.2%, some differences were noticed depending on the kind of substrate used.

The appropriate data quoted, on the other hand, in the literature, are highly different [1, 3, 7, 8, 9, 11, 15, 21] and they oscillate within a wide range from 1.3% (1) to 23% [15, 35].

It seems that the reason for such great differences should be sought in the methods of yeast isolation and cleaning of the remaining of the non-metabolised hydrocarbons.

Depending on the kind of solvent and the method of washing (ratio, time, temperature), various amounts of compounds which may affect the results of fat determination by the classic extraction method, are removed.

The content of phosphorus and ash in yeasts being tested is maintained — independent of the substrate within similar limits not deviating from typical values encountered in fodder yeasts of carbohydrate origin.

Amin [2] and Champagnat's [8, 9] data concerning the content of phosphorus in petrochemical yeasts were found. The first of them reports very low amounts of P_2O_5 for yeasts obtained in the presence of liquid petroleum fraction (58% n-paraffin): for *C. pelliculosa* 0.46% D.S., for *C. tropicalis* 0.68% and for *C. lipolytica* 1.57%. According to Champagnat the content of P_2O_5 in the "BP concentrate" amounts 2.8%. The ash content in the "hydrocarbon" yeasts, as quoted in the literature varies within the wide range, from 1.9 to 10% of D.S. [1, 2, 3, 7, 8, 9, 11, 15, 39]; Amin [2] quotes an exceptionally low content of 1.9% D.S. for *C. pelliculosa* yeasts.

The yeast-nucleic acids content in yeasts under investigation varies only slightly, depending on the substrate. A relatively smaller content of nucleic acids — by some 10-20% — was found in the yeast produced in the presence of slack wax, compared with other materials; this may be connected with the nature of carbon source (length of n-paraffin chains). Information about nucleic acids in yeasts are scant and for being uniform. According to Birolaud [6] their content varies from 5 to 6%, according to Ilnicka-Olejniczak — from 3 to 7% of D.S. According to the results of original investigations the content of nucleic acids in fodder yeast of carbohydrate origin (Haszewo) was 5.7% [27].

Information about the content of nucleic acids in yeasts produced in the presence of liquid hydrocarbon is reported by: Czepigo [11], (6-12%, natural fraction of petroleum), Achremowicz [1], 7.3%, liquid n-paraffins), and Birckensteadt [7], (4-5% n-paraffins). No data concerning their content in yeasts from solid hydrocarbon materials are available.

Table 2 shows the content of 16 amino-acids determined in yeasts under investigation. From its analysis it results that, like in the case of raw protein, there is a certain difference between the total amount (the sum of 16 amino acids) and the content of essential amino acids in yeasts obtained in the presence of various hydrocarbon substrats. 10% more on the average is contained in the biomass coming from the slack wax. It is not possible to compare exactly the set of all the determined amino acids with the results reported by other authors, because various sets not identical with those presented in Table 2 are quoted in the publications. On the other hand, the analysis of content of individual amino acids indicates that the concentrations of the majority of them in yeasts

produced from slack wax are contained within the limits quoted by other authors [1, 3, 4, 7, 8, 11, 15]. These yeasts contain obviously less treonic (all the data found in the literature exceed 4-5%) and glutamic acid (average literature values are over 11%).

The total amount of exogenic aminoacids from the point of view of human needs [29] is relatively low and it amounts in the slack-wax yeasts to some 31%, and in yeasts from the remaining two liquid substrats — to about 26 and 29%. The relatively values quoted in literature are higher; for example 37.6% (liquid n-paraffins) and 39.8% (gaseous oil) according to Champagnat (10%, 36.4% (liquid n-paraffins) according to Kaemmerer [15]; 35 and 32% (gaseous oil) according to Amin [4] and 32.1% (liquid n-paraffins) according to Achremowicz [1]. Only Birckensteadt [7] quotes a lower, 25.8% content (liquid n-paraffins).

Table 3 shows the contents of the four fractions of polysaccharides in yeasts, i.e. acid and alkaline glycogen, glucane and mannane. The concentration of the individual fractions is similar in yeasts produced from various substrats. The content of glycogen is about 65-70% of the sum of all fractions; alkaline glycogen content is approximately three times higher than that of acid glycogen. Relatively highest differences can be seen in the content of mannane — yeasts obtained in the presence of slack wax contain approximately two times less of it.

Saubenowa [34] collected many data concerning the contents of carbohydrates in yeasts, but these concern almost exclusively the carbohydrate nourishing substances. Saubenowa applies a classification of the yeast saccharides to the "reserve" (glycogen, trehalose) and "build-up" (glucane and mannane) ones; she says, however, that without any objections only the glucane may be included into the latter group. On other hand, the role of mannane in the living functions of the cell are not sufficiently explained. The fact, that it constitutes a part of invertase, that the biochemical and physico-chemical properties of the cell surface depend, to some extent, on it and, finally, that it is characterised by high instability indicate how difficult it is to restrict the role of this polysaccharide only to the build-up base. According to Saubenowa under difficult growth conditions (to which certainly the cultures produced in the presence of the slack wax can be included) the yeast may contain no mannane at all. This can be explained not by the lack of the cell ability to its synthesis, but by the defectiveness of yeasts produced on the poorly balanced substrats. This results in the weakening of the chemical bonds between mannane and other cell wall ingredients, and leads to its mechanical wash-out. The quantitative relationship between the "reserve" carbohydrates and the "build-up" ones in the yeasts vary within wide limits. According to Saubenowa [34] the first ones may account for from 32 (*S. paradoxus*) to 76% (*Schizosaccharom. pombe*), whereas the build-up variety — from 20 to 61% of the total of all carbohydrates.

These differences may depend on the location of the yeasts in the given systematic group, on the culture parameters, the kind and concentration of substrate, the content of toxic components (e.g. furfural), etc. Essential changes in the total content of carbohydrates and their individual fractions result from the yeast drying and storage processes [5].

Table 4 shows the content of group B vitamins in yeasts. From this Table it results that there are no particular differences in this field in biomasses coming from different substrates. Although some 20% less thiamine and some 40-50% more pantothenic acid were found in yeasts obtained in the presence of slack wax, but compared with the range of variations reported by others authors these differences are relatively small. According to the literature the contents of vitamins B group in "petrochemical" yeasts oscillate within wide limits, i.e.:

— thiamine from 3 to 20 $\mu\text{g/g}$. D-S. riboflavine — from 17 to 180 $\mu\text{g/g}$ D.S, pyridoxine from 9 to 57 $\mu\text{g/g}$ D.S, niacine from 125 to 1000 $\mu\text{g/g}$ D.S and pantothenic acid from 10 to 640 $\mu\text{g/g}$ D.S.

Against the background of the majority of information quoted, the yeasts under investigation have the vitamin content close to the lower limit of those quoted by other authors. From a comparison of the amount of vitamins detectable in "petrochemical" and "carbohydrate" yeasts it results that they are of the same order and that they coincide in some cases. According to certain comparative studies the yeasts produced in the presence of hydrocarbons as the only source of carbon may contain more vitamins than those coming from carbohydrate substrats [17, 26].

D. SUMMARY

In the summary of the results obtained it should be said that the P-2 yeasts investigated and meeting the requirements of diagnostic tests for *C. scotti*, have independent of the kind of the hydrocarbon raw material used in the investigations, the chemical composition similar to that of "petrochemical" yeasts described in other authors' studies. A comparison of the amount of individual components with the majority of results quoted in the literature indicates that the content of proteins, amino acids and vitamins group B is close to the lower limit of values quoted in the literature.

The small differences in certain components of yeasts produced in the presence of various hydrocarbon substrates (slack wax, hexadecane, naphtha) do not permit one to determine unambiguously the relationship between the kind of carbon source and the chemical composition of the biomass obtained.

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CHARAKTERYSTYKA SKŁADU CHEMICZNEGO DROŻDZY WYHODOWANYCH W OBECNOŚCI RÓŻNYCH SUBSTRATÓW WĘGLOWODOROWYCH

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Streszczenie

Wykonano badania składu chemicznego drożdży P-2 spełniających testy diagnostyczne gatunku *Candida scotti* wyhodowanych w obecności różnych substratów węglowodorowych (gacz parafinowy, heksadekan, nafta), jako jedyne źródła węgla. W celu uzyskania materiału porównawczego próby hodowlane prowadzono w praktycznie identycznych warunkach. Badania obejmowały oznaczenia zawartości białka, węglowodanów (z uwzględnieniem 4 frakcji: kwaśnego i alkalicznego glikogenu, glukanu i mannanu), tłuszczu, fosforu, popiołu, kwasów nukleinowych, aminokwasów, witamin grupy B (tiaminy, ryboflawiny, pirydoksyny, niacyny, kwasu pantotenowego). Porównanie wyników badań własnych ze spotykanymi sporadycznie w literaturze wykazuje, że zawartości białka (tabela 2, wahania od 45 do 49% s.m.), aminokwasów egzogennych (tabela 3, wahania od 26 do 31% s.m.) oraz witamin grupy B (tabela 5) zbliżone są do dolnej granicy wartości podawanych przez innych autorów. Zaobserwowano niewielkie zróżnicowanie ilości niektórych komponentów drożdży (białko, węglowodany, mannan) w zależności od rodzaju substratu, wobec którego zostały wyhodowane. Drożdże pochodzące z gaczu parafinowego posiadają od 5-10% więcej białka, ok. 10-15% mniej węglowodanów (tab. 2) i ok. dwukrotnie mniej mannanu (tabela 4) w stosunku do biomasy uzyskanej z pozostałych rodzajów substratów (heksadekan, nafta). Stwierdzone niewielkie różnice nie pozwalają jednakże w jednoznaczny sposób określić związku między rodzajem źródła węgla a składem chemicznym uzyskanej biomasy.