Zeszyty Problemowe Postępów Nauk Rolniczych
nr 599, 2019, 3–13
DOI 10.22630/ZPPNR.2019.599.18

WASTE FISH OIL AS AN ALTERNATIVE CARBON SOURCE IN MICROBIAL OIL PRODUCTION BY YARROWIA *LIPOLYTICA YEAST*

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Summary. The development of many industries, including food industry, is inherently connected with the rise in the amount of industry waste and the emergence of problems in the areas of their utilization. Due to the rich enzyme apparatus, microorganisms are able to use atypical wastes as a source of carbon and energy with the simultaneous synthesis of valuable metabolites (valorization of waste substrates). A microorganisms characterized by peculiar properties of the use of hydrocarbons and fats is the lipolytic yeast species *Yarrowia lipolytica*. The aim of the study was to evaluate the potential to utilize the fish waste after the process of smoking fish oil as a source of carbon and a lipase inducer in *Y. lipolytica* KKP 379 yeast strain culture as well as to evaluate the potential of microbial oil synthesis with simultaneous valorization of this waste. The obtained results confirmed the ability of the yeast strain to hydrolyze triacylglycerols contained in the oil from the fish smoking process, whose products (free fatty acids) were taken up by the cell and used as a source of carbon and energy. Although the highest efficiency of intracellular lipid biosynthesis by yeast strain *Y. lipolytica* KKP 379 was observed in control mineral medium supplemented with 50 g·dm^{-3} olive oil, storage lipids were also produced in the medium with 5% waste fish oil in amount of 0.187 g per 1 g of dry biomass.

Key words: lipase, microbial oil, waste fish oil, *Yarrowia lipolytica*

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INTRODUCTION

Industry development, including food industry is inseparably linked to the increase in the amount of post-production wastes and the increase in problems related to its management. One of the most troublesome substances in utlization are certainly glycerol and hydrophobic compounds, such as hydrocarbons and waste oils, which are burden to domestic and industrial wastewater treatment plants. Waste oils can accumulate in drain pipes, create hardly permeable layers on the surface of water reservoirs, and, as a result, BOD (Biochemical Oxygen Demand) parameters increase. Moreover wastes may be a source of pathogenic microflora [Boguski, 2008; Yahyaee et al., 2013].

Biotechnological methods use ubiquitous microorganisms whose rich enzymatic apparatus may be helpful in solving the problem of managing industrial wastes. *Yarrowia lipolytica* is a lipolytic yeast species, which definitely can be an example of such microorganisms. In many scientific works carried out on laboratory and industrial scales these yeasts showed the ability to utilize industrial wastes with simultaneous biosynthesis of valuable metabolites (so-called valorization of waste substrates), such as lipolytic enzymes, citric acid, biomass with high protein and fat content (SCP, single cell protein and SCO, single cell oil) [Goncalves et al., 2009; Papanikolaou and Aggelis, 2011]. Due to the unique physiological features of *Y. lipolytica*, which are capable of metabolizing hydrophobic substrates (alkanes, fatty acids and lipids), the ability to accumulate intracellular lipids and development of appropriate genetic tools for transforming *Y. lipolytica* cells caused that this species was selected as a model in studies of microbial oil synthesis in oleaginous yeasts [Krzyczkowska and Fabiszewska, 2015].

Microbial oil is defined as lipids accumulated inside the cell of unicellular organisms. The ease of increasing the scale of production, as well as the short life cycle of microorganisms accumulating intracellular lipids and the independence of their growth from climate conditions and seasons may decide on its widespread use in the near future. In addition, the composition of oil produced by microbiological methods can be modified, including enrichment with *n*-3 and *n*-6 fatty acids, which can be used in the nutrition of infants, adults and animals, ensuring a balanced diet [Martinez et al., 2015].

The aim of the study was to evaluate the possibility of valorization of waste fish oil as a result of its use as a carbon source and inducer of lipolytic enzyme synthesis in the yeast strain *Y. lipolytica* KKP 379. In addition, the ability to synthesize microbial oil during *Y. lipolytica* growth in medium containing the waste substrate was investigated. The biotechnological synthesis of microbial oil and other yeast metabolism products (i.e. lipolytic enzymes) in the batch process using industrial wastes as a carbon source in culture medium is part of a sustainable development strategy. The process can satisfy the growing needs of man for some biotechnological products and on the other hand restore the integrity of the ecosystem without exceeding the long-term limits of its capacity [Stappen, 2006].

MATERIALS AND METHODS

The study was carried out using the *Yarrowia lipolytica* KKP 379, a lipolytic yeast strain from Collection of Industrial Microorganisms of Institute of Agricultural and Food Biotechnology in Warsaw. The yeast strain was stored on agar slants with YPG medium (1% yeast extract, 2% peptone, 2% glucose) with 2% agar under refrigerated conditions at 4°C.

In the experiments, waste oil after the fish smoking process from the fish processing plant located in Grajewo (Podlasie Voivodeship, Poland) was used. The technological process involved mainly fish from the Salmonidae and Scombridae families.

Inoculation culture was carried out in flat-bottom flasks with a volume of 500 cm^3 with 100 cm^3 of sterile YPG medium. The medium was inoculated with yeast biomass using a loop and culture was conducted at 28°C on IKA KS 4000 ic control (IKA-Werke, Germany) rotary shaker at 140 rpm for 24 hours.

Experimental cultures contained 100 cm^3 of YP medium (1% yeast extract – jk sources of vitamins and mineral salts, 2% peptone – organic nitrogen source) with 2% addition of waste fish oil (YPF). Olive oil and glucose were used as a control tests instead of waste fish oil (abbreviated YPO and YPG respectively). Experimental cultures were inoculated with 0.1 cm^3 of inoculation culture. Olive oil was chosen as a control carbon source due to the literature reports indicating the induction of lipase synthesis by fatty acids from triacylglycerol molecules present in vegetable oil [Fickers et al., 2004], while glucose as a non-lipid substrate allowed to determine the activity of extracellular lipases secreted constitutively. Experimental cultures were carried out in flat-bottom flasks at 28°C on a rotary shaker at 140 rpm for 65 hours. Cultures were discontinued after 65 h and then yeast cells were separated from the supernatant by centrifugation in MPW-351R centrifuge (MPW Med. Instruments, Poland) at 8000 rpm for 10 minutes. Supernatant was decanted from the yeast cells and stored for lipolytic activity determination. Yeast biomass was dried in a Radwag MAC 50/NH moisture analyzer (Radwag, Poland) to determine dry mass of yeasts.

The method developed by Kapturowska et al. [2012] was used to determine lipolytic activity. The method involves spectrophotometric measurement of the progress of hydrolysis of *p*-nitrophenyl laurate – a fatty acid ester, where *p*-nitrophenyl laurate is hydrolysed to *p*-nitrophenol. One unit (1 U) of lipase activity was defined as the amount of enzyme able to release 1 μmol of *p*-nitrophenol in 1 minute at 37°C under the conditions of the assay.

The next stage of experiments was performed in bioreactor scale in the BIOFLO 3000 laboratory bioreactor (New Brunswick Scientific, USA) with a working volume of 4 dm^3 at 28°C at 350 rpm and aeration with compressed air, and 0,038% (v/v) yeast inoculation culture was used to inoculate the medium.

The media used in the bioreactor cultures were YPO and YPF media as well as mineral medium according to Bialy et al. [2011] with slightly modification. The mineral medium

consisted of: carbon source 50.0 g·dm^{-3}, Tween 80 1.0 g·dm^{-3}, yeast extract 2.0 g·dm^{-3}, peptone 1.0 g·dm^{-3}, $(NH_4)_2SO_4$ 2.5 g·dm^{-3}, KH_2PO_4 7.0 g·dm^{-3}, Na_2HPO_4 2.5 g·dm^{-3}, $MgSO_4$ 1.5 g·dm^{-3}, $CaCl_2$ 0.15 g·dm^{-3}, $FeSO_4 \cdot H_2O$ 0.16 g·dm^{-3}, $ZnSO_4$ 0.02 g·dm^{-3} and $MnCl_2 \cdot 4H_2O$ 0.08 g·dm^{-3}. Modified mineral media were marked with the symbols MO3 and MO5 (medium containing olive oil as the main carbon source in the amount of 3 and 5%) and MF5 (medium with 5% addition of waste fish oil). Cultures were discontinued when the yeast cells reached stationary growth phase. The growth phases of yeast cells were determined on the basis of the oxygenation of the medium, which was expressed as a relative percentage of dissolved oxygen in the medium in relation to its concentration at the beginning of the culture (dissolved oxygen, dO_2), measured by using of an oxygen electrode in a laboratory bioreactor.

At the end of the culture, the yeast biomass was separated from the supernatant in Sigma 4-15 (Sigma Laborzentrifugen GmbH, Germany) high speed centrifuge (8000 rpm, 7 minutes, 10°C). Yeast biomass was dried in a Napco Vacuum Oven Model 5831 dryer (Napco Inc., USA) at 80°C until it became dry. Dried yeasts were ground in mortar with sand and placed in a filter paper thimbles. Lipids from yeast biomass were extracted by continuous extraction using Soxhlet apparatus with *n*-hexane (150 cm^3) as a extraction solvent. The process was ended up after 18 solvent transfers through the Soxhlet apparatus, which took about 2,5 hours. When the extraction was complete, the solvent was evaporated in a Buchi Rotavapor R-200 (Buchi AG, Switzerland).

Statistical analysis was carried out using Statistica 12.0 software (TIBCO Software Inc., USA). The results were analyzed using one-way analysis of variance and Tukey's post-hoc test. The significance level was $\alpha = 0{,}05$.

RESULTS AND DISCUSSION

It was found that waste oil originating from the smoking process of fish was used as a carbon source and stimulated the synthesis of extracellular lipases during growth of *Y. lipolytica* yeast cells (Table 1). An average dry biomass yield of 11.43 g d.m.·dm^{-3} was obtained in medium with olive oil (YPO) and a biomass yield of 10.69 g s.s.·dm^{-3} in waste fish oil medium (YPF) (Table 1). Almost twice lower biomass yield was obtained in culture in YPG control medium (5.95 g s.s.·dm^{-3}) containing glucose as a carbon substrate. Low biomass yield in the control medium in comparison to media containing lipid carbon sources indicate the preference of *Y. lipolytica* yeast for the assimilation of hydrophobic compounds. Moreover the enzymatic apparatus of the studied species is directed into hydrolysis of lipids present in culture medium.

In the olive oil medium 2-fold higher lipolytic activity was observed compared to the results for the medium with waste fish oil (0.373 and 0.198 U·cm^{-3}, respectively, Table 1). The difference in lipolytic activity may result from the content of oleic acid in both lipid substrates. According to Fabiszewska et al. (2017) olive oil contains average 75%, and in fish waste oil there is only 16% of oleic acid in total fatty acids content. The lipolytic activity observed in yeast cultured in glucose-containing medium (0.063 U·cm^{-3}) differed significantly compared to media with a lipid carbon source, which are inducers of lipolytic enzymes synthesis [Fickers et al., 2011; Mazurczak et al., 2017]. The obtained results

Table 1. Effect of carbon source on extracellular lipolytic activity and yield of dry biomass of *Y. lipolytica* KKP 379 yeast after 65 h of shaken culture

Tabela 1.Wpływ źródła węgla na zewnątrzkomórkową aktywność lipolityczną oraz plon biomasy szczepu drożdży *Y. lipolytica* KKP 379 po 65 h hodowli wytrząsanej

	Culture medium – Podłoże hodowlane		
	YPF	YPO	YPG
Extracellular lipolytic activity Zewnątrzkomórkowa aktywność lipolityczna [U·cm^{-3}]	0.198 ±0.011^{b}	0.373 ±0.013^{c}	0.063 ±0.001^{a}
Dry biomass yield Plon biomasy [g d.m.·dm^{-3}]	10.69 ±0.54^{b}	11.43 ±0.69^{b}	5.95 ±1.03^{a}

Diferent letters (a-c) mean significant difference ($p < 0.05$) /Różne litery (a-c) oznaczają istotne różnice ($p < 0,05$).

Source/Źródło: own study based/opracowanie własne.

confirmed the ability of the yeast strain to hydrolyze the triacylglycerols contained in the fish waste oil, whose products (free fatty acids) were used as energy source and building blocks for cell growth.

In the second stage of the experiments yeast fed-batch cultures were carried out in the laboratory bioreactor in 5 media: YPR (rich medium with 2% waste fish oil), YPO (rich medium with 2% olive oil), MO3 and MO5 (mineral media with 3% and 5% olive oil) and MR5 (mineral medium with 5% waste fish oil). The highest content of cellular lipids, and thus the microbial oil yield was found for MO5 medium when olive oil was used as a source of carbon and nitrogen source was limited (Fig. 1). The cellular lipids content reached 0.356 g g·d.m.$^{-1}$. In the MF5 medium yeast cells contained almost twice less oil than in the MO5 medium and amounted to 0.187 g g·d.m.$^{-1}$. In the case of YP series media, the microbial oil content in cells was (0.004 g g·d.m.$^{-1}$ and 0.025 g g·d.m.$^{-1}$, for YPF and YPO respectively).

YP medium was a rich source of organic nitrogen in the form of peptone and yeast extract, as well as a rich source of vitamins and minerals. The medium is well selected for high yeast biomass yield, but it was not useful in the production of SCO, because a higher concentration of microbial oil in *Y. lipolytica* cells was achieved in mineral medium (Fig. 1). It is also worth noting that the increase in the content of carbon source (olive oil) in the mineral medium positively influenced the examined feature. A threefold increase in the content of lipids extracted from yeast cells cultured in MO5 medium was observed (0.356 g g·dm^{-3}) compared to cells grown in MO3 medium (0.119 g g·dm^{-3}). According to literature data, in media where carbohydrates or glycerol are used as a carbon source, lipids are stored by yeast cells in de novo route. At the same time, a lack of nitrogen in the medium promotes lipid storage because it indirectly contributes to the inhibition of the tricarboxylic acid cycle (Krebs cycle). Excess of carbon may then be directed into lipid synthesis [Ageitos et al., 2011; Bialy et al., 2011; Papanikolaou and Aggelis, 2011]. In the studies described in this paper, lipid substrates were used in medium, so the ex novo pathway, which is independent of nitrogen limitation, was expected to take part in microbial oil synthesis. Meanwhile, the use of a high ratio of carbon to nitrogen source content in the culture medium turned out to be equally beneficial and reasonable as it was claimed for de novo route, what was also hypothesized by Fabiszewska et al. (2019).

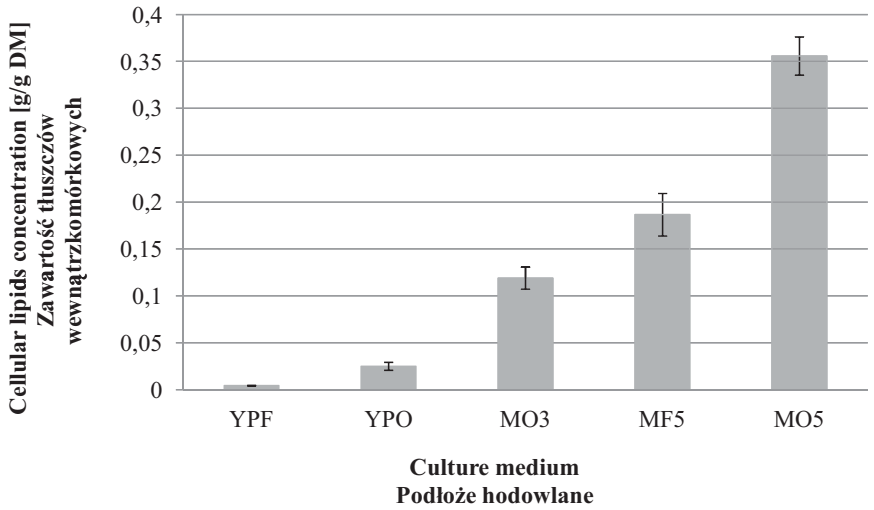

Fig. 1. Content of microbial oil in *Y. lipolytica* yeast cells depending on the culture medium and carbon substrate contained (YPF – rich medium with 2% waste fish oil, YPO – rich medium with 2% olive oil, MO3 – nitrogen limited mineral medium with 3% olive oil, MO5 – nitrogen limited mineral medium with 3% olive oil, MF5 – nitrogen limited mineral medium with 5% waste). Vertical bars represent confidence interval 0.95

Rys. 1. Zawartość oleju mikrobiologicznego w komórkach drożdży *Y. lipolytica* w zależności od zastosowanego podłoża hodowlanego i zawartości źródła węgla (YPF – bogate podłoże z 2% odpadowym olejem rybim, YPO – bogate podłoże z 2% oliwy z oliwek, MO3 – podłoże mineralne z limitowaną zawartością źródła azotu i 3% oliwy z oliwek, MO5 – podłoże mineralne z limitowaną zawartością źródła azotu i 5% oliwy z oliwek, MF5– podłoże mineralne z limitowaną zawartością źródła azotu i 5% odpadowego oleju rybiego). Pionowe słupki przedstawiają 95% przedziały ufności

Source/Źródło: own study based/opracowanie własne.

Yeast cells growth was limited in mineral medium with 5% waste fish oil (MF5), what can be seen by analyzing Figure 2. The cultivation time was extended in MF5 medium in comparison to MO5 medium by about 20 hours (Fig. 2, 3). The authors made an attempt to explain the observations. The ex novo biosynthesis of microbiological oil depends to a large extent on the activity of enzymes that catalyze the hydrolysis of hydrophobic compounds. Lip2p is a main enzyme specific for triacylglycerols and responsible for cleavage of fatty acids by *Y. lipolytica* cells. *Lip2* gene promotor is being activated by the presence of oleic acid [Fickers et al., 2004]. Fabiszewska et al. [2014] compared the composition of fatty acids present in olive oil and waste fish oil after the smoking process. Olive oil consisted in 80% of oleic acid in total fatty acids content. Oleic acid accounted for about 17% in waste fish oil and it also contained omega-3 fatty acids such as DHA and EPA [Fabiszewska et al., 2014]. The substrate specificity of Lip2p lipase could affect the storage lipids yield, therefore the content of microbial oil produced in MO5 medium is two times higher compared to the MF5 medium.

Cultures in mineral media were characterized by a decrease in pH value during the logarithmic phase of yeast growth (Fig. 2, 3), which could be due to the release of organic acids into the culture medium, including citric acid. Final pH values for the MO5 and MF5 medium were 1.95 and 2.05, respectively. Under conditions of limitation of nitrogen source disturbances in the Krebs cycle are observed. The resulting citric acid is not included in the metabolic cycle, and its excess is secreted to the cytosol and can be secreted further outside the cell [Ageitos et al., 2011].

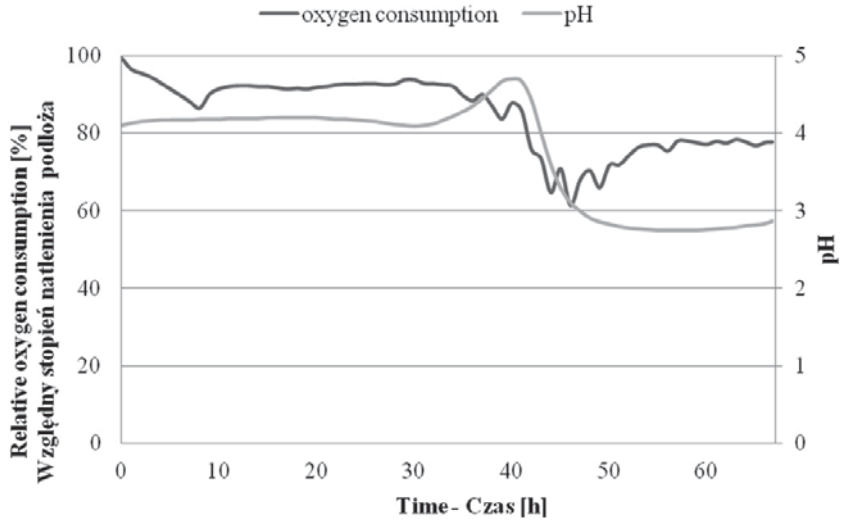

Fig. 2. Changes in pH (light line) and oxygen consumption (dark line) during a batch culture of *Y. lipolytica* KKP 379 strain in MF5 medium containing waste fish oil

Rys. 2. Zmiany pH (jasna linia) oraz stopnia natlenienia podłoża (ciemna linia) w czasie hodowli stacjonarnej szczepu drożdży *Y. lipolytica* KKP 379 w podłożu MF5 zawierającym odpadowy olej rybi

Source/Źródło: own study based/opracowanie własne.

Based on the yield of yeast biomass and determination of cellular lipids, maximum concentration of lipids produced was calculated in cultures carried out in mineral substrates (Table 2).

The highest efficiency of intracellular lipid biosynthesis by yeast strain *Y. lipolytica* KKP 379 was observed in mineral medium supplemented with 50 $g \cdot dm^{-3}$ olive oil. The lipid content reached 0.356 g per 1 g of dry biomass and the maximum concentration of lipids produced was 7.7 $g \cdot dm^{-3}$ of the culture medium. In the medium with 5% waste fish oil the lipid accumulation also occurred – there was synthesized 0.187 g per 1 g of dry biomass and was almost twice as low as that obtained in olive oil mineral medium.

Szczęsna-Antczak et al. [2010] cultured *Mucor circinelloides* T45 using various carbon substrates: vegetable oil, animal fat, glucose and corn soak. The mold strain was able to produce lipids with a maximum concentration of 6.4 to 17.0 $g \cdot dm^{-3}$. The microbial oil

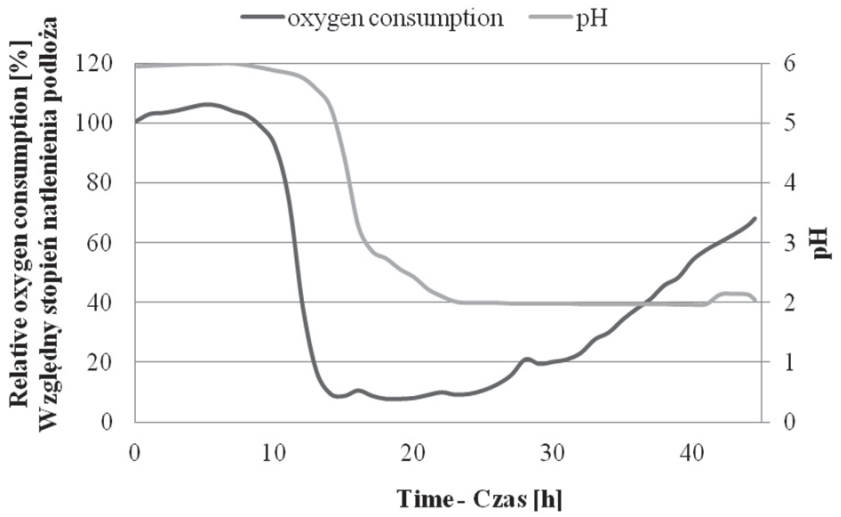

Fig. 3. Changes in pH (light line) and oxygen consumption (dark line) during a batch culture of *Y. lipolytica* KKP 379 strain in MO5 medium containing olive oil

Rys. 3. Zmiany pH (jasna linia) oraz stopnia natlenienia podłoża (ciemna linia) w czasie hodowli stacjonarnej szczepu drożdży *Y. lipolytica* KKP 379 w podłożu MO5 zawierającym oliwę z oliwek

Table 2. Storage lipids yield biosynthesized in a batch bioreactor culture of *Y. lipolytica* in MO3, MO5 and MF5 medium

Tabela 2. Lipidy zapasowe syntezowane w hodowli stacjonarnej drożdży *Y. lipolytica* w podłożach MO3, MO5 i MF5

	Culture medium Podłoże hodowlane		
	MO3	MO5	MF5
Dry biomass yield Plon biomasy [g d.m.·dm^{-3}]	13.2	21.6	11.7
Cellular lipids concentration Zawartość tłuszczów zapasowych [g g·d.m.$^{-1}$]	0.119	0.356	0.187
Maximum concentration of lipids produced Maksymalna zawartość syntezowanych lipidów [g·dm^{-3}]	1.6	7.7	2.2

Source/Źródło: own study based/opracowanie własne.

contained a high amount of unsaturated fatty acids such as oleic, linoleic and linolenic acid [Szczęsna-Antczak et al., 2010].

Bialy et al. [2011] used the yeast strain *Y. lipolytica* in a six-day culture in media containing waste oils after frying food products as an additional carbon source (such as meat,

fish and vegetables). The highest content of lipids in the dry matter of yeast was observed for cells cultured in media with waste oil after frying vegetables (57.89%) and control glucose medium without oil (55.63%) with the lowest biomass yield (4.36 $g \cdot dm^{-3}$). In the case of media with waste oil after frying fish and chicken meat, the researchers achieved respectively: 45.49%, 37.70% lipid content in dry matter of yeast. The observed differences corresponded to a vrious content of protein in waste oils, and thus a different content of nitrogen source in the medium [Bialy et al., 2011].

All examples cited above related to purified fish oils, extracted from parts of fish carcasses or related to post-frying oils. There is no literature reports on the production of microbial oil in media containing waste oils after the fish smoking process. The results presented in the paper are probably one of the first attempts to use *Y. lipolytica* yeast in valorization of the waste oil by ex novo lipid biosynthesis.

SUMMARY

The use of waste substrates in microbial culture can reduce the cost of producing yeast biomass and their metabolites synthesis and become a sustainable technology for the management of wastes which are difficult to utilize. The results obtained in the study are the basis for further researches into the microbiological utilization of fishery industry waste. The synthesized microbial oil accumulated in yeast cells of *Y. lipolytica* KKP 379 is likely to be used in the industry as a single cell oil source (SCO), but it is necessary to optimize the yield of its biosynthesis.

REFERENCES

Ageitos J.M., Vallejo J.A., Veiga-Crespo P., Villa T.G., 2011. Oily yeasts as oleaginous cell factories. Appl Microbiol Biotechnol. 90, 1219-1227.

Bialy H.E., Gomaa O.M., Azab K.S., 2011. Conversion of oil waste to valuable fatty acids using Oleaginous yeast. World J Microbiol Biotechnol. 27, 2791-2798.

Boguski A., 2008. Efektywność usuwania substancji tłuszczowych w układzie oczyszczania ścieków *miejskich.* Rocznik Ochrona Środowiska. 10, 481–489 [in Polish].

Fabiszewska A., Mazurczak P., Pielińska A., Zieniuk B., Nowak D., Białecka-Florjańczyk E., 2014. Próba zastosowania drożdży Yarrowia lipolytica KKP 379 w zagospodarowaniu odpadów przemysłu rybnego*.* Postępy Techniki Przetwórstwa Spożywczego. 2, 28-33 [in Polish].

Fabiszewska A., Pielińska A., Mazurczak P., Zieniuk B., Wołoszynowska M., 2017. Wpływ wybranych czynników na wydajność ekstrakcji i skład kwasów tłuszczowych otrzymywanego oleju mikrobiologicznego w komórkach drożdży *Yarrowia lipolytica.* Żywność. Nauka. Technologia. Jakość. 24, 58-68 [in Polish].

Fabiszewska A., Misiukiewicz-Stępień P., Paplińska-Goryca M., Zieniuk B., Białecka-Florjańczyk E., 2019. An insight into storage lipids synthesis by *Yarrowia lipolytica* yeast relating to lipid and sugar substrates metabolism. Biomol. 9, 685-697.

Fickers P., Nicaud J.M., Gaillardin C., Destain J., Thonart P., 2004. Carbon and nitrogen sources modulate lipase production in the yeast *Yarrowia lipolytica*. J. Appl. Microbiol. 96, 742--749.

Fickers P., Marty A., Nicaud J.-M., 2011. The lipases from *Yarrowia lipolytica*: genetics, production, regulation, biochemical characterization and biotechnological applications. Biotechnol. Adv. 29 (6): 632-644.

Goncalves C., Lopes M., Ferreira J.P., Belo I., 2009. Biological treatment of olive mill wastewater by non-conventional yeasts. Bioresour. Technol. 100, 3759-3763.

Kinoshita H., Ota Y., 2001. Concentration of docosahexaenoic acid from fish oils using *Geotrichum sp. FO347-2*. Biosci. Biotechnol. Biochem. 65, 1022-1026.

Kapturowska A., Stolarzewicz I., Krzyczkowska J., Białecka-Florjańczyk E., 2012. Studies on lipolytic activity of sonicated enzymes from *Yarrowia lipolytica*. Ultrason. Sonochem. 19, 186-191.

Krzyczkowska J., Fabiszewska A., 2015. *Yarrowia* lipolytica – niekonwencjonalne drożdże w biotechnologii. Post. Mikrobiol. 54, 33-43.

Martinez E.J., Raghavan V., Gonzalez-Andres F., Gomez X., 2015. New biofuel alternatives: integrating waste managment and single cell oil production. Int. J. Mol. Sci. 16, 9385-9405.

Mazurczak P., Zieniuk B., Fabiszewska A., Nowak D., Wołoszynowska M., Białecka-Florjańczyk E., 2017. Utylizacja odpadów pochodzących z zakładów przemysłu spożywczego i paliwowego z wykorzystaniem lipolitycznych drożdży *Yarrowia lipolytica.* Zeszyty Problemowe Postępów Nauk Rolniczych 588, 15-24 [in Polish].

Papanikolaou S., Aggelis G., 2011. Lipids of oleaginous yeasts. Part I: Biochemistry of single cell oil production. Eur. J. Lipid Sci. Technol. 113, 1031-1051.

Stappen R.K., 2006. A Sustainable World is Possible. Der Wise Consensus: Problemlösungen für das 21 Jahrhundert. Impulsdokument Manuskript 1.2/2006.

Szczęsna-Antczak M., Antczak T., Bielecki S., 2010. Sposób wytwarzania oleju mikrobiologicznego. Patent nr PL 204911. Publication data: 26.02.2010.

Yahyaee R., Ghobadian B., Najafi G., 2013. Waste fish oil biodiesel as a source of renewable fuel in Iran. Ren. Sust. En. Rev. 17, 312-319.

ODPADOWY OLEJ RYBI JAKO ALTERNATYWNE ŹRÓDŁO WĘGLA W SYNTEZIE OLEJU MIKROBIOLOGICZNEGO PRZEZ DROŻDŻE Z GATUNKU *YARROWIA LIPOLYTICA*

Streszczenie. Rozwój wielu gałęzi przemysłu, w tym przemysłu spożywczego jest nieodłącznie związany ze wzrostem ilości odpadów poprodukcyjnych oraz zwiększeniem problemów dotyczących ich zagospodarowania. Dzięki bogatemu aparatowi enzymatycznemu, mikroorganizmy są zdolne do wykorzystywania nietypowych odpadowych źródeł węgla i energii z jednoczesną syntezą cennych metabolitów (waloryzacja substratów odpadowych). Mikroorganizmem cechującym się unikatowymi zdolnościami do metabolizowania cukrów oraz tłuszczów są drożdże z gatunku *Y. lipolytica*. Celem pracy była ocena możliwości wykorzystania lipidowego odpadu przemysłu rybnego pochodzącego z procesu wędzenia ryb w wyniku jego zastosowania jako źródła węgla oraz induktora syntezy enzymów lipolitycznych w hodowli szczepu drożdży *Y. lipolytica* KKP 379, jak również zbadano możliwość syntezy oleju mikrobiologicznego w podłożu zawierającym ten odpad z jednoczesną jego waloryzacją. Otrzymane wyniki pozwoliły na potwierdzenie zdolności badanego szczepu drożdży do hydrolizy triacylogliceroli zawartych w oleju po procesie

wędzenia ryb, której produkty (wolne kwasy tłuszczowe) były pobierane przez komórkę i wykorzystywane na potrzeby energetyczne oraz budowę i wzrost komórek drożdży. Mimo że najwyższą wydajność syntezy lipidów wewnątrzkomórkowych szczepu *Y. lipolytica* KKP 379 uzyskano w podłożu mineralnym zawierającym 50 $g \cdot dm^{-3}$ oliwy z oliwek, lipidy zapasowe były syntezowane także w podłożu zawierającym 5% oleju po procesie wędzenia ryb w ilości 0,187 g oleju/g s.m. drożdży.

Słowa kluczowe: lipaza, olej mikrobiologiczny, odpadowy olej rybi, *Yarrowia lipolytica*