Industrial Crops & Products 119 (2018) 102–110

Contents lists available at ScienceDirect

Industrial Crops & Products

journal homepage: www.elsevier.com/locate/indcrop

The bioconversion of waste products from rapeseed processing into keto acids by *Yarrowia lipolytica*

Cybulski Krzysztof, Tomaszewska-Hetman Ludwika, Rakicka Magdalena, Łaba Wojciech, Rymowicz Waldemar, Rywińska Anita*

Department of Biotechnology and Food Microbiology, Wrocław University of Environmental and Life Sciences, Chełmońskiego Str. 37, 51-630, Wroclaw, Poland

ARTICLE INFO

Keywords:
α-ketoglutaric acid
Pyruvic acid
Yarrowia lipolytica
Rapeseed oil
Glycerol
Fatty acids

ABSTRACT

Despite dynamic fluctuations observed on the oil crops market, the rapeseed production is expected to at least remain unchanged if not grow further. The production of rapeseed oil for food and for technical purposes generates large amounts of waste substances. The following paper presents a concept of utilizing substrates from rapeseed processing through bioconversion to α-ketoglutaric acid (KGA) and pyruvic acid (PA) with the use of *Yarrowia lipolytica* yeast. In a shake-flasks experiment, twenty strains of *Y. lipolytica* were examined with focus on their growth on food-quality rapeseed oil under thiamine-mediated growth limitation. Strain A-10 was selected and tested for KGA production from various oils and fatty acids and for PA production from crude glycerol in bioreactor batch and fed-batch cultures. Based on the substrate used, under conditions providing intensified KGA production, the yeast produced up to 72.0 g/L of KGA with volumetric production rate amounting to 0.52 g/Lh and specific production rate equal to 0.044 g/gh. For PA production, three types of glycerol, biotin supplementation, pH value, thiamine concentration, and C:N ratio were examined. In selected conditions, after 71.5 h of fermentation the yeast produced 48.1 g/L of PA from 100 g/L of crude glycerol. Biomass of *Y. lipolytica* A-10 obtained after the production process (from 150 g/L of pure glycerol) contained 50.52% protein and 13.36% lipids in dry weight, most of which were the unsaturated fatty acids (77.7%). Such composition of the biomass allows its use as a protein supplement in animal feeding.

1. Introduction

During the last 20 years global rapeseed (*Brassica napus*) production grew steadily and is now the most prominent among oil crops production, second only to soybean (*Glycine max*). Europe is the major rapeseed oil producing region and Poland is currently one of the biggest European rapeseed producers and processors (Carré and Pouzet, 2014). In recent years its rapeseed production share among 28 European Union countries amounted to 12% and since 2007 Poland is, interchangeably with the Great Britain, the third biggest rapeseed producer, after Germany and France. Rape seeds are used for production of rapeseed oil used in food as well as for technical purposes such as production of methyl esters (biodiesel) (Fatty Acid Alkyl Esters – FAAE). Extensive exploitation of the fossil fuels forces us to search for alternative energy sources, and as we know biodiesel is one of the most promising among renewable fuels. Biodiesel could be produced from vegetable oils (rapeseed, palm, canola, soybean), animal fats (fish oil, beef, and sheep tallow) and their waste equivalents. In Poland rape is the most important plant source of protein and oil. It also comprises a main component in methyl esters production. One of the chemical processes of biodiesel production is known as the transesterification reaction which involves triacylglycerols' reaction with a short-chain monohydric alcohol (generally methanol), normally in the presence of a catalyst and at an elevated temperature, to form FAAE and glycerol (Moser, 2009). In 2012, the production capacity of biodiesel in the European Union reached 23.5 million tons (European Biodiesel Board, 2017). Such large FAAE production causes problems with waste management.

Crude glycerol (also known as waste glycerol) is the principal by-product of esters' production, one part of which being produced per 10 parts of biodiesel (Meher et al., 2006; Johnson and Taconi, 2007; Papanikolaou, 2009). Composition of crude glycerol depends on several factors, among which the most important are oil source and production technology. Waste glycerol is composed of 40–90% (w/w) of glycerol and several other ingredients, such as methanol, water, inorganic salts (mostly sodium chloride), and contaminants. Moreover, raw glycerol obtained from multiple feedstocks is characterized by significantly diversified content of macroelements, carbon and nitrogen (Thompson and He, 2006). Due to low purity of crude glycerol and high costs of its

* Corresponding author.
E-mail address: Anita.Rywinska@upwr.edu.pl (R. Anita).

https://doi.org/10.1016/j.indcrop.2018.04.014
Received 13 December 2017; Received in revised form 22 February 2018; Accepted 4 April 2018

Nomenclature

KGA	α-ketoglutaric acid
PA	Pyruvic acid
GLY	Glycerol
CA	Citric acid
qPA/KGA	Specific production rate of pyruvic acid / α-ketoglutaric acid (g/gh)
QPA/KGA	Volumetric production rate of pyruvic acid / α-ketoglutaric acid (g/Lh)
YPA/KGA	Yield of pyruvic acid / α-ketoglutaric acid production (g acid/g glycerol)

purification, the price of crude glycerol is relatively low, which encourages the development of its new value-added uses. Studies show that crude glycerol can be used, inter alia, as a feed ingredient for animals, in production of hydrogen, syngas, monoglycerides and green solvents for organic reactions (Yang et al., 2012). Valorisation of crude glycerol is also widely studied in biotechnology. Literature works report that raw glycerol can be applied as a carbon source in bacterial or yeast biosynthesis of desirable products e.g. citric acid, erythritol, 1,3-propanediol, SCP (Single Cell Protein) or SCO (Single Cell Oil), docosahexaenoic acid (DHA), and 1,3-dihydroxyacetone (Yang et al., 2012; Rywińska et al., 2013). Aside from glycerol, the waste materials include post-processing oil cakes and post-extraction middlings which may be utilized as protein feed for livestock. As many other member states, for 2017 and 2018 Poland plans its biofuel share in liquid fuel consumption indicator to equal to 7.8% and 8.5% respectively [Regulation of 23 July 2013]. Despite the indicator level becoming stable it is anticipated that the domestic ester production from rapeseed oil will steadily grow in the upcoming years. Such forecast is based on the expected increase in diesel expenditure in transportation as well as in expected diesel import decrease. It is estimated that in 2015–2020 Polish fuel sector's demand for esters will increase from 0.9 mln tons to about 1.5 mln tons. That is the reason behind good forecast for Polish rapeseed production and processing despite the decreasing global trend. Last year the global rapeseed production was 70.05 million tons and this year's production is estimated at 68.86 million tons (http://www.globalrapeseedproduction.com). Most likely fate of the first generation of biofuels will change significantly after 2020 and global rapeseed production will be expected to report further losses. Nevertheless, due to the evolving nutrition trends, the rape seeds' nutritional value and many more of its valuable properties it can be expected that the demand for rapeseed will remain sufficiently high to ensure the continuity of its production.

The aim of the present study was to investigate the parameters of pyruvic (PA) and α-ketoglutaric acid (KGA) production by *Yarrowia lipolytica* yeast from the rapeseed processing raw materials: rapeseed oil, crude oils, fatty acids, and crude glycerol. The research was also aimed at the nutritional characterization of *Y. lipolytica* A-10 biomass obtained after PA production process.

2. Materials and methods

2.1. Microorganisms

Screening of oil-grown yeast was carried out among 20 *Yarrowia lipolytica* strains. Strains CCY-29-26-5 and CCY-29-26-3 of *Y. lipolytica* were acquired from the Culture Collection of Yeasts. Strains ATCC 8661 and ATCC 8661UV'1 were acquired from the American Type Culture Collection. The *Y. lipolytica* N15 strain came from the collection of the laboratory of Aerobic Metabolism of Microorganisms of the Institute of Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences, Puschino, Russia (Kamzolova et al., 2008). The other strains came from the collection belonging to the Department of Biotechnology and Food Microbiology at Wrocław University of Environmental and Life Sciences, Wrocław, Poland. The wild type strains were isolated from different surroundings at different times: A-1, A-3, A-6, A-8, A-10, A-15, A-61 and A-311 were isolated in 1974 from soil in the vicinity of a garage in Wrocław, A-I-5 was isolated in 1976 from oil well soil near Jasło, S3 was isolated in 2008 from soil, SKO6 and SKO 12 were isolated in 2012 from soil of the land belonging to a tannery in Skoczów. In the presented study acetate-negative mutants were also used: Wratislavia 1.31, Wratislavia K1 and K1UV21 of *Y. lipolytica*. The Wratislavia 1.31 was isolated from wild type strain A-101 after exposure to UV radiation, Wratislavia K1 strain was isolated from Wratislavia 1.31 strain in the course of continuous citric acid production from glucose in a nitrogen-limited chemostat culture at a dilution rate of $D = 0.016\,h^{-1}$. The K1UV21 was isolated from Wratislavia K1 after exposure to UV radiation (Rywińska et al., 2012). The yeast strains were maintained on YM slants at 4 °C.

2.2. Media and culture conditions

2.2.1. Carbon sources

The following substances were used in the presented study as carbon and energy sources: edible rapeseed oil (bought in a popular grocery store), raw rapeseed oil, de-sludged rapeseed oil, fatty acids, technical grade glycerol (98% w/w), and biodiesel-production-grade glycerol (73% and 83%). Raw and de-sludged rapeseed oil are feed materials produced in accordance with Polish and EU laws. Composition and content of impurities in individual substances were given in accordance with the specification obtained from the factory. The raw oil contains a maximum of 1.75% of free fatty acids and the content of selected minerals (mg/kg) is as follows: As < 2; Cd < 1; Ni < 0.2; Cu < 0.1; Pb < 10; Hg < 0.1; Fe < 1.5. The de-sludged oil may contain traces of free fatty acids (< 0.1%). Fatty acids are meant for production of esterified oil. Free fatty acids represented 30% of the total fatty acids, up to 95% of fatty substances, up to 0.5% contaminants and up to 2% water. Content of the selected minerals (mg/kg) is as follows: As < 0.1; Cd < 0.03; Ni < 0.2; Cu < 0.1; Pb < 0.1; Hg < 0.01; Fe < 1.5. Pure glycerol (98% w/w) was from Chempur, Poland, crude glycerol 76% (76% w/w) and crude glycerol 83% (83% w/w) were from Lotos Poland and Wratislavia-Bio Poland, respectively. The unpurified crude glycerol (76% and 83%) was obtained from methyl ester production. The impurities in the crude glycerol 76% solution include NaCl 4.00%, metals measured as mg/kg (Mg 6.71, Cu 0.11, Fe 0.92, Zn 2.9 and Ca 46), heavy metals (Pb 0.02, Cd, Cr, Hg not detected), and water mass fraction 19.5%. The impurities in the crude glycerol 83% solution were NaCl 7.3%, metals measured as mg/kg (Mg 17.70, Cu 0.30, Fe 1.90, Zn 2 and Ca 13), heavy metals (Cd, Cr, Hg not detected), and water mass fraction 6.5%.

2.2.2. Media composition

Two types of inoculation media were used: mineral medium and YNB-based media (Yeast Nitrogen Base – with vitamins, Sigma-Aldrich, St. Louis, USA). The mineral medium consisted of: 20 g of rapeseed oil, 4.5 g of $(NH_4)_2SO_4$, 1 g of KH_2PO_4, 0.1 g of K_2HPO_4, 0.7 g of $MgSO_4 \cdot 7H_2O$, 0.5 g of NaCl, 0.4 g of $Ca(NO_3)_2$, and 0.2 µg of thiamine in 1 L of distilled water. The YNB-based media 0.67 and 0.067 contained 50 g of pure glycerol (98% w/w) and 0.67 or 0.067 g of YNB, per litre of distilled water, respectively.

The growth test was run in mineral medium at thiamine concentration of 1.0 µg in 1 L of distilled water. Growth tests were performed in three replicates and the results are presented as mean values.

α-Ketoglutaric acid (KGA) biosynthesis was performed in medium containing: 100–150 g of carbon source, 12 g of $(NH_4)_2SO_4$, 2 g of KH_2PO_4, 0.2 g K_2HPO_4, 1.4 g of $MgSO_4 \cdot 7H_2O$, 0.5 g of NaCl, 0.8 g of $Ca(NO_3)_2 \cdot (4H_2O)$, and 0.6–1.0 µg of thiamine per litre of tap water. The process was started at the level of 20 g/L or 30 g/L of oil or fatty acids,