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ORIGINAL PAPER

Effects of Different Oil Sources and Residues on Biomass and Metabolite Production by *Yarrowia lipolytica* YB 423-12

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Abstract *Yarrowia lipolytica* is known to have the ability to assimilate hydrophobic substrates like triglycerides, fats, and oils, and to produce single-cell oils, lipases, and organic acids. The aim of the present study was to investigate the effects of different oil sources (borage, canola, sesame, *Echium*, and trout oils) and oil industry residues (olive pomace oil, hazelnut oil press cake, and sunflower seed oil cake) on the growth, lipid accumulation, and lipase and citric acid production by *Y. lipolytica* YB 423-12. The maximum biomass and lipid accumulation were observed with linseed oil. Among the tested oil sources and oil industry residues, hazelnut oil press cake was the best medium for lipase production. The *Y. lipolytica* YB 423-12 strain produced 12.32 ± 1.54 U/mL (lipase activity) of lipase on hazelnut oil press cake medium supplemented with glucose. The best substrate for citric acid production was found to be borage oil, with an output of 5.34 ± 0.94 g/L. The biotechnological production of valuable metabolites such as single-cell oil, lipase, and citric acid could be achieved by using these wastes and low-cost substrates with this strain. Furthermore, the cost of the bio-process could also be significantly reduced by the utilization of various low-cost raw materials, residues, wastes, and renewable resources as substrates for this yeast.

Keywords *Yarrowia lipolytica* · Single-cell oil · Linseed oil · Lipid accumulation · Biomass · Lipase · Citric acid

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Introduction

The current rise in agricultural and industrial production has led to an excessive generation of various low-cost by-products and/or residues/wastes. Fermentation of these products/residues/wastes for the production of high-added-value metabolites such as single-cell oils, organic acids, and lipases has an important economic significance for the industry [1–3]. The non-conventional dimorphic yeast *Yarrowia lipolytica* has the capability to assimilate hydrophobic substrates like triglycerides, fats, and oils, and to produce lipases and organic acids [1, 4, 5]. Furthermore, *Y. lipolytica* has the ability to produce bioemulsifiers and degrade fats and oils [1, 2].

Single-cell oils (SCOs) present a potential industrial interest since many types of oleaginous microorganisms are able to store lipids with either unusual composition (such as substitutes of high-value fats like cocoa butter, sal fat, and illipé fat) or conventional composition (such as biodiesel precursors) [2, 6–8]. In SCO production, the ability of oleaginous microorganisms to synthesize lipids containing medically and dietetically important polyunsaturated fatty acids has considerable interest [9]. Products that have been produced commercially include cocoa butter equivalents (from *Apiotrichum curvatum*), γ-linolenic acid (GLA, 18:3n-6) (from *Mortierella isabellina, Chlorella* and *Mucor circinelloides*), docosahexaenoic acid (DHA, 22:6n-3) (from *Crypthecodinium* and *Schizochytrium*), and arachidonic acid (AA, 20:4n-6) (from *Mortierella, Porphyridium* spp. and *Pythium*) [10–13]. Among the microbial sources, *Y. lipolytica* has been recognized as a promising yeast capable of accumulating oil, producing lipase, and living on fats and oils as carbon sources [14].

The addition of different oils to the cultivation media of *Y. lipolytica* may enhance the production of polyunsaturated

fatty acids depending on the fatty acid contents of the added oils. In previous studies, the fatty acid composition of the hydrophobic substrates affected the lipid accumulation and fatty acid composition in the cells [6, 7]. The fatty acid composition of the oil produced by yeast was very similar to the substrate oil in the cited studies. Chatzifragkou et al. [15] also concluded that the addition of oil to the culture medium favored the accumulation of saturated fatty acids (C16:0 and C18:0) by *Y. lipolytica.*

Oleaginous microorganisms are able to assimilate many carbon sources, such as by-products of the food and agricultural industry, and produce SCO rich in polyunsaturated fatty acids or high-nutritional-value fatty acids [16]. These microorganisms accumulate SCO during secondary metabolic growth in media containing high amounts of carbon and limited amounts of nitrogen. They convert residual carbon into oil when the amount of nitrogen is depleted in the media [12, 17].

Economou et al. [16, 18] used sweet sorghum extract and rice hull hydrolysate as substrates for SCO production by oleaginous fungus *Mortierella isabellina*, and developed detailed mathematical models that can predict biomass growth, SCO production, and consumption of sugar and nitrogen. In their following study, they developed a biofilm model to describe the accumulation of oil in semi-solid state fermentation using *M. isabellina* and sweet sorghum as a substrate [17].

Citric acid (CA), which is an intermediate of the tricarboxylic acid cycle (TCA) and is used in the food and detergent industry as an acidulant, flavoring agent, antioxidant, preservative, stabilizer, and bleaching component, has important functions in the metabolism of microbial cells [4, 19–21]. Sugars, glycerol, and polysaccharides can be utilized as carbon sources by various types of microorganisms under nitrogen-limited conditions. After nitrogen depletion from the medium, growth is ceased and secondary metabolites are produced. These metabolites are organic acids (e.g., CA), lipids, and other extra-cellular compounds (e.g., polyols) depending on the microorganism [20, 22, 23]. Usually, molds such as *Aspergillus niger* and yeasts like *Y. lipolytica*, *Candida guillermondii*, and *C. oleophila* are used in microbial CA production [20, 24]. Using yeasts instead of molds for CA production is of particular interest due to higher resistance against high substrate conditions and metal ions, as well as the accumulation of lower amounts of solid and liquid wastes for the latter [25]. *Y. lipolytica* has the ability to produce organic acids such as CA, isocitric acid, and 2-ketoglutaric acid. This strain has many advantages including low sensitivity to low dissolved oxygen concentrations and heavy metals, greater tolerance to metal ions, high product yield and wide substrate range, as well as a greater resistance to high concentrations of substrate compared to other yeast strains [19, 26].

Lipases (triacylglycerol acyl hydrolases—E.C 3.1.1.3) are considered to be the most important industrial enzyme, with numerous applications reported. They are used in the production of detergents, cosmetics, pharmaceuticals, flavor enhancers, and foods [27]. Microbial lipases are used as biocatalysts, especially in food and detergent manufacture and also in other biotechnological applications. One of the most studied dimorphic lipolytic yeasts is *Y. lipolytica*, which naturally secretes the lipase enzyme [28, 29]. The lipase activity of *Y. lipolytica* was first reported by Peters and Nelson in 1948 [30, 31]. Pignede et al. [32] isolated and identified the *LIP2* gene and showed its function on the extracellular lipase activity of *Y. lipolytica.* They demonstrated that *LIP2* and *POX2* promoters were induced by oleic acid, which functions as a stabilizer-activator of extracellular lipase, probably because of an expression system of the lipase gene under the control of *LIP2* and the oleic acid-inducible *POX2*. Both genetic engineering and mutagenesis methods have been used to increase lipase enzyme production by *Y. lipolytica* strains. The expression system of the lipase enzyme contains cassettes carrying the *LIP2* gene, and this gene is expressed under the control of the oleic acid-inducible *POX2* promoter [9]. The objectives of this study were to investigate the growth, lipid accumulation, and metabolite production by the *Y. lipolytica* YB 423-12 strain with the addition of external carbon sources (oils and oil-cakes) such as linseed, sesame, borage, canola, trout oil, *Echium* spp. seed oil, olive pomace oil, hazelnut oil press cake, and sunflower seed oil cake, and to explore the possibility of producing high-value fatty acids by using low-cost oils and oil processing residues or wastes.

Materials and Methods

Microorganism and Culture Conditions

The *Y. lipolytica* YB 423-12 strain was kindly provided from the NRRL [Agricultural Research Service (ARS)] Culture Collection, Peoria, IL USA. The microorganism was kept on Potato Dextrose Agar (PDA) (Fluka) at +4 °C. During the experiments the strain was cultivated in 250-mL conical flasks at 28 °C and 150 rpm. Each flask contained 50 mL of sterile growth medium. The composition of the basal medium (in g/L) was: glucose 30.00; yeast extract 0.50; KH_2PO_4 7.00; Na_2HPO_4 2.50; $CaCl_2{\bullet}2H_2O$ 0.15; $(NH_4)_2SO_4$ 1.00; $MnSO_4{\bullet}H_2O$ 0.06; $ZnSO_4{\bullet}7H_2O$ 0.02; $FeCl_3{\bullet}6H_2O$ 0.15; $MgSO_4{\bullet}7H_2O$ 1.50 (Merck, Darmstadt, Germany). Linseed, borage, sesame, and trout oils were donated from Ramazanoğulları Ltd. Şti (Istanbul, Turkey). Canola oil was purchased from a local market in İstanbul, Turkey. *Echium* seed oil, olive pomace oil, hazelnut oil

press cake, and sunflower seed cake were kindly provided from Harke Group GmbH (Mülheim, Germany), Antgıda A.Ş. (Balıkesir, Turkey), Altaş Yağ A.Ş. (Ordu, Turkey), and Öztürkler Yem ve Yağ San. Tic. A.Ş. (Edirne, Turkey), respectively. All chemicals and reagents used in our analyses were of chromatographic or laboratory grade. Cultures were cultivated in 250-mL erlenmeyer flasks containing 50 mL of the growth medium noted above together with 10.00 g/L of oil/oil cake. The composition of the pre-culture medium was the same growth medium as described above [15, 22, 33–35].

Each flask was inoculated with 1 mL (2 % inoculum volume fraction) of 24 h exponential pre-culture (containing approximately 3×10^8 microorganisms per mL) and incubated in an orbital shaking waterbath (New Brunswick Scientific Co., Inc. Classic C76, Enfield, Connecticut, USA) operating at 150 rpm and 28 °C. The incubation period was 5 days, since the latter optimal period for oil accumulation and metabolite production by *Y. lipolytica* strains was reported by several researchers [7, 15, 20, 22, 35–37]. Control experiments were conducted using the same medium with no carbon source.

Proximate Analysis

Proximate analysis of the residues was performed according to the methods of AOAC (1984) [38]: crude protein was analyzed by the Kjeldahl method after acid digestion; crude fat was found by hexane extraction in a Soxhlet system; ash was determined via combustion at 550 °C in a muffle furnace for 12 h; moisture was examined with a model M0C63u Unibloc moisture analyzer from Shimadzu (Tokyo, Japan).

Determination of Biomass

Cells were harvested by centrifugation (Sigma 2-16 PK, Laborzentrifugen GmbH, Osterode, Germany) performed at 3,305 g for 20 min. In order to remove the remaining residual extracellular oils on the surface, all residual cells were washed successively with ethanol, hexane, and distilled water, and then centrifuged. For verification, lipid analysis was performed on the supernatant of the last wash [6, 39, 40]. Biomass content expressed as dry matter per liter of fermentation broth was determined after drying centrifuged cells at 105 °C for 24 h [2]. All experiments were performed in triplicate, and the average results were reported.

Lipid Extraction and Methylation

Total intracellular lipid content was extracted with a chloroform: methanol (2:1; v/v) mixture [41, 42], and solvents were removed by a rotary evaporator (Buchi Rotavapor RII, Flawil, Switzerland) at 40 °C. Lipid yields and lipid contents were expressed as grams of lipid per liter of fermentation broth and grams of lipid per grams of dry biomass, respectively. Lipids were converted to methyl esters for further fatty acid composition analysis [43].

Fatty Acid Composition Analysis

Fatty acid compositions of methyl esters were analyzed by capillary gas chromatography (GC), using a Thermo Quest Trace GC 2000 (Milan, Italy) equipped with a flame-ionization detector. DB-wax capillary column (30 m × 0.32 mm ID × 0.25 μm film thickness) (J&W Scientific Folsom, CA) was used for the analysis. The injector and detector temperatures were held at 250 °C and 260 °C, respectively. The conditions used were: the oven temperature was initially held at 150 °C for 3 min, and was then programmed to 225 °C for 10 min at a rate of 10 °C/min. Helium was used as a carrier gas at a flow rate of 1.5 mL/min and a split ratio of 1:70. A 1-μL sample was injected into the GC.

Enzyme Activity Assay

Lipase activity was analyzed by titrimetric assay according to an olive oil emulsion method with slight modifications [4]. The substrate emulsion was formed with olive oil and polyvinyl alcohol at 40 % (v/v) and 2 % (w/v), respectively. The solution was emulsified with an Ultra-Turrax homogenizer (Janke and Kunkel, IKA, Staufen, Germany) to obtain the dispersion of oil particles into the aqueous phase. The assay sample (500.00 μL) containing culture medium and cells were added to the substrate emulsion (5.00 mL). Subsequently, 0.05 M phosphate buffer (4.50 mL) was added to the mixture. The total mixture (10.00 mL) was incubated for 1 h in a water bath (New Brunswick Scientific Co, Inc. Classic C76, Enfield, Connecticut, USA), at 28 °C and 180 rpm. The effect of pH on lipase activity was studied at a broad range of pH (from 5 to 9). The optimal pH for lipase activity was found to be 8 (in accordance with Refs. [2, 4]). The reaction was terminated by adding 4 mL of acetone-ethanol (50:50 v/v) mixture to the medium. Lipase activity was determined by titration of the fatty acid released with 50 mM sodium hydroxide (up to a final pH of 9). One activity unit of lipase was defined as the amount of enzyme, which released 1 μmol of fatty acids per minute under assay conditions at 28 °C. Lipase activity was measured by the equation below, where V_1 and V_2 are the volume (mL) of NaOH (aq) for the sample and blank, respectively, C is the normality of NaOH (aq), t is time, and E is the volume taken from the fermentation medium:

$$\text{Specific activity} = (V_1 - V_2) \times C \times 1,000/t \times E$$

CA Determination

Spectrophotometric analysis of citric acid (CA) was performed using the method of Marrier and Boulet [44]. Filtered aliquots of the culture liquid were used for the analysis[45–47]. In this method, citric acid esters were created by the reaction between acetic anhydride and citric acid, and spectrophotometric measurement was carried out. Supernatants were harvested by centrifugation operated at 3,305 g for 20 min. One mL of supernatant was mixed with 1.30 mL of pyridine and 5.70 mL of acetic anhydride, which are then incubated in a water bath (New Brunswick Scientific Co, Inc. Classic C76, Enfield, Connecticut, USA) at 32 °C for 30 min. The citric acid content was measured by spectrophotometer (Optima SP3000 nano Tokyo, Japan) at 420 nm wavelength [44].

Microscopic observations

Yeast morphology was detected by microscopic observations at × 100 and × 800 using a light microscope (Olympus Corporation, Shinjuku, Tokyo, Japan) after the cells were stained with methylene blue.

Statistical Analysis

The MINITAB program was used to perform statistical analysis of the data, employing an analysis of variance (ANOVA) using the General Linear Model, as well as Tukey's test with a 95 % confidence index (CI).

Results and Discussion

The *Y. lipolytica* YB 423-12 strain was cultivated in media containing glucose and various oils and oil industry residues as carbon and energy sources. The effects of different oils and residues on the growth and the production of lipids and metabolites from *Y. lipolytica* were determined by measuring dry biomass, lipid yield, fatty acid profile, citric acid concentration, and lipase activity. According to the findings, the protein contents of hazelnut oil and sunflower seed oil cakes were found to be quite high. However, sunflower seed oil cake contained low amounts of lipids when compared to hazelnut oil press cake (Table 1).

Table 1 Proximate analysis of residues for press cakes

Proximate analysis	Hazelnut oil press cake	Sunflower seed cake
Crude protein	30.0 ± 1.4	36.0 ± 1.8
Crude lipid	25.7 ± 0.4	1.3 ± 0.2
Ash	4.8 ± 0.5	6.8 ± 0.5
Moisture	5.1 ± 0.4	9.1 ± 0.3

All data (%) are mean values ± standard deviations of triplicate measurements

Table 2 Biomass and lipid yield results for different substrates

Substrate (oil)	Biomass (g/L)	Lipid (g/L)	Lipid yield (%, g/g)
Control[b]	14.40 ± 0.01	4.61 ± 0.06	31.99 ± 0.09
Trout oil[ab]	20.48 ± 0.03	11.02 ± 0.01	53.82 ± 0.01
Borage oil[ab]	22.57 ± 0.16	11.63 ± 0.07	51.55 ± 0.05
Sesame oil[ab]	23.91 ± 0.04	11.30 ± 0.06	47.28 ± 0.06
Rapeseed oil[ab]	25.14 ± 0.03	12.06 ± 0.17	47.97 ± 0.17
Echium oil[a]	26.14 ± 0.01	14.86 ± 0.03	56.85 ± 0.02
Linseed oil[a]	26.67 ± 0.09	16.45 ± 0.22	61.67 ± 0.26

All data are mean values ± standard deviations of triplicate measurements

Differences between substrates are indicated with lettering according to the results of statistical experiments

Growth and Morphology of *Y. lipolytica* on Various Substrates

The effects of different oils such as linseed, sesame, borage, canola, trout, and *Echium* were investigated for biomass yield. According to the findings, the biomass content varied between 14.40 and 26.67 g/L (Table 2). As can be seen from Table 2, the addition of oils to the medium caused a significant increase in the dry biomass of the strain ($p < 0.05$). The maximum dry biomass yield was obtained with linseed oil, and the minimum biomass yield was obtained with the control and trout oil.

Biomass and lipid production of *Y. lipolytica* strains have been analyzed by different researchers [2, 3, 7, 33, 46, 48–50]. Lipid production was found to be 7.90 mg/mL when industrial derivative of tallow was added to the culture medium [2]. Higher levels of biomass yields such as 9.73 and 8.30 g/L were obtained in the presence of casein and olive oil in the medium, respectively [3]. The maximum biomass was reported by Papanikolaou et al. [7] as 11.40 g/L in the medium containing 10 g/L stearin and 34 g/L glycerol. Papanikolaou et al. [33] studied the cultivation of *Y. lipolytica* on olive-mill wastewater-based media, and the biomass content was found to be in the range of 5.90–7.30 g/L. In the presence of glucose (28.10 g/L concentration), biomass yield was determined to be 7.30 g/L [33]. On the other hand, Aguirre et al. [49] determined the maximum biomass production with the *Y. lipolytica* S26 strain to be 25 g/L with the medium containing 40 g/L molasses and 5 g/L yeast extract. Different biomass results may be due to the differences between the strains and medium components. Lipid accumulation in the

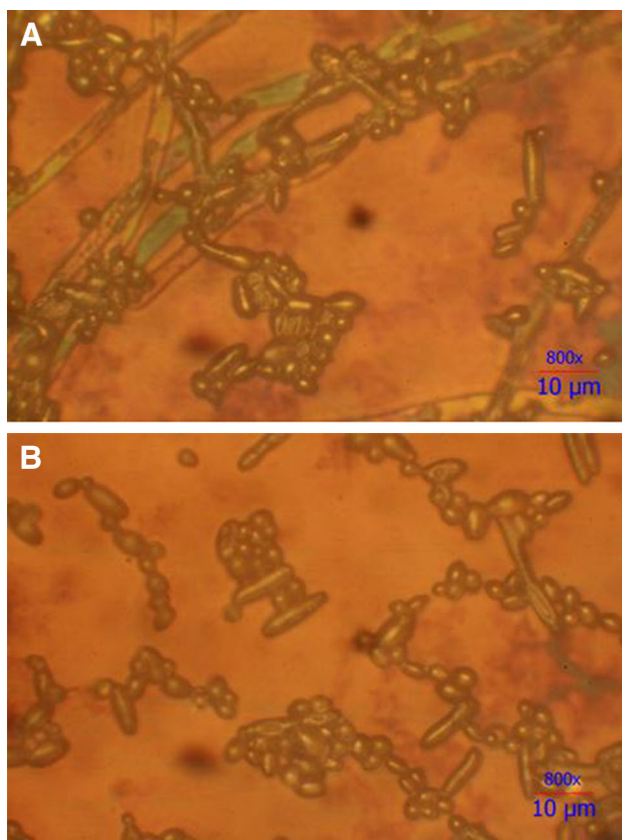

Fig. 1 Photographed yeast cells **a** after incubation for 5 days with linseed oil medium at 800× magnification, and **b** after incubation for 5 days with glucose medium (control) at 800× magnification

Y. lipolytica LGAM S(7)1 strain cultivated on glucose (9–46 g/L) and glycerol (15–120 g/L) was 5–9 %, and biomass yield was 5.20–8.00 g/L [46]. With the same strain, biomass yield was observed to be 14.5 g/L in the medium containing 25 g/L glucose and citrus oils [50]. The results indicated that, in the presence of linseed oil, biomass and lipid yields increased up to 85 and 93 %, respectively.

Yeast morphology was observed after a 5-day incubation period with medium containing linseed oil and glucose, respectively, and photographed cells are represented in Fig. 1. The typical mycelium morphology of the yeast observed in pre-culture is shown in Fig. 2. Mycelia forms like those seen in both figures have also been observed in all medium containing different substrate oils (photographs not shown). The mycelium form develops at a very early stage of fermentation and remains during nearly the entire fermentation period. In a similar manner, true mycelia form occurred with linseed oil, probably due to the morphological transition in the presence of the oil in the medium. It was stated that the presence of oil in the medium enhances the mycelium form of the *Y. lipolytica* strains [2]. According to the other investigations, a unicellular form of

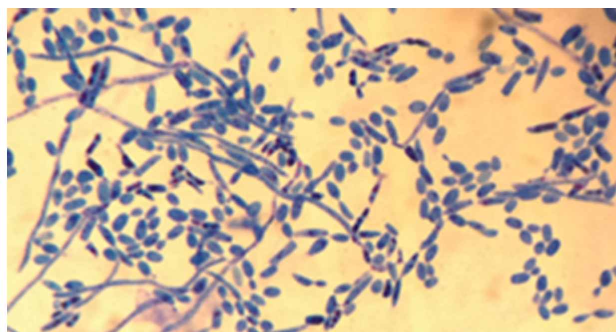

Fig. 2 Typical mycelium morphology of yeast observed in pre-culture (100× magnification)

the *Y. lipolytica* strains occurred at low glucose concentrations, while at high concentrations almost all strains were in mycelium form at the end of 40 h of inoculation, and a mycelium-to-yeast transition occurred due to the depletion of glucose concentration in the medium. After 120–140 h of inoculation, nearly all strains were in unicellular (yeast-like) morphology [23, 45, 51].

Microscopical observations were similar to the results of Makri et al. [34], who observed that the true mycelia form was dominant during the exponential growth phase. After that, large cells with lipid globules appeared in the early stationary phase (lipogenic phase), and decreases in the size of the lipid globules were observed in the late stationary phase, as can be seen in Figs. 1 and 2 [34].

Lipid Accumulation and Composition of Cellular Lipids

Cellular lipid yields were in the range of 31.99–61.67 g/100 g of dry biomass. The increase in lipid content compared to the control was 92.78 % in the linseed oil medium. The effects of linseed and *Echium* spp. seed oil sources were found to be statistically important in the lipid production of *Y. lipolytica* ($p < 0.05$). *Y. lipolytica* exhibited the highest cell growth and lipid production in the presence of linseed oil. Previous studies indicated that the fatty acid composition of the hydrophobic substrate affected the lipid accumulation in the cells, and lipid accumulation increased with the increased concentration of stearin in the culture medium [6, 7, 52, 53]. It was indicated that *Y. lipolytica* accumulated lipids at high amounts when C18:0-rich media was used, and also grew better in the presence of fatty acid mixtures [6, 54]. If lipid accumulation in the cell was high, the microorganism consumed these lipids for cellular activities instead of external lipid sources [55]. Furthermore, another reason for the low level of transportation of external oil sources to the cell was due to the decreased biological activity of the membranes [6]. It is well-known that the fatty acid profiles of the produced lipids are

affected by the fatty acid content of the oils in the substrate, which is consistent with the observations of this study. Chatzifragkou et al. [15] concluded that the addition of essential oil to the medium affected the cellular lipid composition and increased the saturation of fatty acids.

The results indicated that medium containing GLA favored the production of microbial oil with GLA content. A similar result was observed for trout oil regarding the eicosapentaenoic acid (EPA, 20:5n-3) and DHA content in the accumulated oil. Fatty acid compositions of added oils and the fatty acid compositions of the oils after the incubation period are given in Tables 3 and 4, respectively. Fatty acid compositions of the oils are in accordance with the literature [56–59]. Maximum lipid accumulation was observed as 16.45 and 14.86 g/L with linseed and *Echium* oils, respectively (after a 5-day incubation period), as seen in Table 2. The lowest lipid accumulation was observed as 11.02 g/L in trout oil medium. In all of the experiments, the C18:1 fatty acid was notably higher than other FAs in the accumulated lipid.

The free fatty acid content of the produced lipid was also analyzed and found to be 63.4 %, which was also consistent with the previous studies for *Y. lipolytica* strains. [6, 14, 60]. The high free fatty acid content was in accordance with the high lipase activity of the strain, as discussed below.

Table 3 Fatty acid profiles of oil substrates

Fatty acids	Trout	Borage	Linseed	Rapeseed	*Echium*	Sesame
C16:0	21.5 ± 0.21	11.7 ± 0.64	9.9 ± 0.14	5.5 ± 0.14	5.9 ± 0.14	10.8 ± 0.42
C18:0	4.4 ± 0.21	4.3 ± 0.42	10.1 ± 0.35	1.8 ± 0.35	2.9 ± 0.35	5.9 ± 0.78
C18:1(n-9)	22.3 ± 2.76	19.4 ± 1.13	25.7 ± 0.42	62.8 ± 0.64	13.9 ± 0.07	43.5 ± 2.83
C18:2(n-6) LA	2.7 ± 0.78	35.7 ± 1.27	16.1 ± 0.49	20.9 ± 2.47	13.9 ± 0.49	39.5 ± 2.62
C18:3(n-6) GLA	0.5 ± 0.49	17.7 ± 0.64			16.2 ± 0.21	
C18:3(n-3) ALA	0.05 ± 0.04		37.8 ± 1.70	6.6 ± 1.27	30.6 ± 1.27	
C18:4(n-3) SDA	1.6 ± 0.28	1.2 ± 0.49			13.1 ± 0.99	
C20:0	1.4 ± 0.35	0.5 ± 0.14			0.09 ± 0.01	
C20:5(n-3) EPA	10.2 ± 0.64					
C22:6(n-3) DHA	16.2 ± 0.21					
Others[a]	19.15 ± 1.35	9.50 ± 0.38	0.40 ± 0.05	2.40 ± 0.36	3.41 ± 0.27	0.30 ± 0.04

All data are mean values ± standard deviations of duplicate measurements

[a] Other fatty acids detected include C16:1, C20:1, C20:2, and C22:1

Table 4 Fatty acid profiles of oils accumulated by the *Y. lipolytica* YB 423-12 strain using different oil substrates

Fatty acids	Control	Trout	Borage	Linseed	Rapeseed	*Echium*	Sesame
C14:0		8.0 ± 0.02					
C15:0		1.1 ± 0.09					
C16:0	20.7 ± 1.06	24.8 ± 1.22	8.6 ± 0.78	6.7 ± 0.41	18.8 ± 0.92	10.8 ± 0.49	13.2 ± 0.35
C16:1	9.1 ± 2.32	6.1 ± 0.57	1.0 ± 0.57	2.8 ± 0.21			
C17:0		1.9 ± 0.47					
C17:1		1.4 ± 0.03					
C18:0	20.3 ± 0.07	5.9 ± 0.26	4.7 ± 0.49	10.2 ± 1.20	10.7 ± 0.78	5.7 ± 0.35	5.1 ± 0.14
C18:1(n-9)	37.4 ± 1.48	28.9 ± 1.18	40.5 ± 2.38	28.6 ± 1.63	49.9 ± 2.02	17.6 ± 2.46	54.5 ± 1.98
C18:2 (n-6) LA	12.5 ± 0.67	5.3 ± 0.08	37.2 ± 6.08	15.6 ± 1.43	14.7 ± 0.57	18.2 ± 0.57	27.2 ± 0.85
C18:3 (n-6) GLA		2.0 ± 0.27	7.9 ± 0.28			9.5 ± 0.14	
C18:3 (n-3) ALA		0.9 ± 0.06		35.9 ± 4.47	5.9 ± 1.28	29.1 ± 3.04	
C18:4 (n-3) SDA						9.0 ± 0.58	
C20:1		0.8 ± 0.15					
C20:2		0.35 ± 0.01					
C22:1		0.35 ± 0.13					
C20:5 (n-3) EPA		3.6 ± 0.01					
C22:6 (n-3) DHA		7.2 ± 0.28					

Incubation conditions: 150 rpm/28 °C and 120 h, 1 % carbon source addition

All data are mean values ± standard deviations of duplicate measurements

Y. lipolytica is very efficient in degrading hydrophobic substrates such as fats, oils, alkanes, and fatty acids. Triglycerides can be utilized by *Y. lipolytica* as a carbon source. Lipolytic enzymes (lipases) hydrolyze these triglycerides into free fatty acids that are taken up by the cell, whereas alkanes enter directly into the cell [1].

According to the investigations, selective fatty acid uptake of *Y. lipolytica* can be explained by two different chain-length selective transport systems: one carrier system is specific for C12 and C14 fatty acids, while the second one is specific for C16 and C18 fatty acids [3]. The fatty acids of C12, 14, and 16 are used for growth, while C18 is accumulated as a reserve lipid and stored [5, 6]. This can be explained by the fact that fatty acids will either be used for growth or biotransformation processes, to produce new fatty acids that did not exist in the substrate previously [55].

Papanikolaou et al. found that *Y. lipolytica* produced a highly saturated cellular lipid that contained C18:0 and C16:0 at 78–80 % and 17–20 % (w/w) of total lipids, respectively [2]. Generally, *Y. lipolytica* strains tend to accumulate mono-unsaturated and saturated fatty acids when cultivated on fatty materials [2, 5–7]. The growth and lipid accumulation by *Y. lipolytica* has been found to be increased in the media containing saturated fatty acids (C16:0 and C18:0), and the microbial lipid was found to contain high amounts of stearic acid [7, 54]. The microbial lipid was affected by the fatty acid composition of the substrate used, so it was possible to produce some fat substitutes with the desired fatty acid composition by using selected strains of *Y. lipolytica, Rhodotorula* sp., *Mucor* sp., *Candida* spp., and *Rhodosporidium toruloides* [8, 39, 40, 42]. Furthermore, modeling approaches can be used to select the strains or growth parameters of oleaginous microorganisms [54].

CA Concentration and Lipase Activity Analysis

Citric acid can be produced from alkanes, oils, glycerol, ethanol, molasses, and starch hydrolysates [1, 33, 48]. In this study, the effect of different oils and oil industry residues wastes on the lipase and CA production of *Y. lipolytica* YB 423-12 was investigated. The effects of different carbon sources in fermentation medium on citric acid content and lipase activity produced by *Y. lipolytica* YB 423-12 were tabulated in Table 5.

The highest CA concentration (5.34 ± 0.938 g/L) was observed in medium containing borage oil. *Echium* seed, trout, canola, linseed and sesame oils, and sunflower seed oil cake also had statistically important effects in CA production. In contrast, lipase production in medium containing borage oil was lower. Makri et al. studied the growth of *Y. lipolytica* ACA-DC 50,109 on glycerol, and three distinct phases—namely a biomass production phase, lipogenic phase, and citric acid production phase—were identified during the growth cycle. The results indicated that significant amounts of lipids were produced on glycerol. When citric acid is secreted into the medium, a low amount of the lipid is synthesized, whereas during the lipid turnover period, citric acid production was suppressed [34]. Anastassiadis et al. [20] concluded that nitrogen content and its exhaustion are important factors in citric acid formation by yeasts. There is a negative correlation between citric acid formation and growth rate, because nitrogen limitation increases the production rate of organic acids while decreasing cellular growth and glucose uptake [20]. Nitrogen limitation directed the metabolism towards the citric acid production [22, 46]. When glycerol was used as substrate for *Y. lipolytica* strains, high-value products such as SCO, CA, and mannitol were produced [22, 34].

A high concentration of glucose favored the accumulation of lipids and citric acid. The production rates of organic acids and growth rates on glycerol were very similar with the results on glucose. The microbial lipids are initially accumulated, and thereafter are degraded in favor of citric acid production [22, 23, 46]. It was reported that microbial lipids were principally composed of unsaturated C16 and C18 fatty acids; likewise, in *Y. lipolytica*, oleic acid was the major cellular fatty acid [20, 23, 26, 34].

The optimal pH for lipase activity was found to be 8.0 in accordance with the findings of Kamzolova et al [4]. The highest lipase activity (12.32 ± 1.538 U/mL) was achieved with the hazelnut oil press cake medium, as seen in Table 5. This result can be explained by the high oleic acid content of the hazelnut oil press cake since the extracellular lipase requires oleic acid as a stabilizer/activator. Increasing the amount of polyunsaturated fatty acids containing C18 fatty acids in the medium increased lipase activity as well [61, 62]. Hazelnut oil press cake includes 25.7 % oil and 52.2 % oleic acid (C16:0, 3.3 %; C18:2, 26.7 %; C18:3n6, 6.1 %). The lipase gene was expressed under the control of the oleic acid-inducible *POX2* promoter [32]. The results indicated that sunflower seed oil cake also significantly increased lipase production ($p < 0.05$). Papanikolaou et al. [63] studied lipid accumulation with glycerol under nitrogen-excess conditions and concluded that 1.6-fold higher quantities of lipids were produced in nitrogen-excess conditions compared with nitrogen-limited culture. Therefore, in this study the presence of high amounts of metabolites in the media containing residues may be explained by excess nitrogen levels, since hazelnut oil press cake and sunflower seed cake contain 4.80 and 5.76 % nitrogen, respectively.

Lipase activities of different *Y. lipolytica* strains tested were reported to be between 1.9 and 45.5 IU/mL [2, 4]. In

Table 5 Lipase activity and citric acid production of the *Y. lipolytica* YB 423-12 strain

Fermentation medium	Lipase activity (U/mL)[A]	Citric acid (g/L)[A]
Control G (30 g/L)	1.59 ± 0.13[e]	0.68 ± 0.04[c]
HOM[B] (10 g/L) + G (30 g/L)	12.32 ± 1.54[a]	2.55 ± 0.66[abc]
HOM (40 g/L)	3.62 ± 0.60[bc]	1.94 ± 0.60[bc]
HOM (40 g/L) + G (15 g/L)	ND	4.78 ± 0.1[ab]
SOC[C] (10 g/L) + G (30 g/L)	3.81 ± 0.21[b]	3.23 ± 0.25[abc]
SOC (40 g/L)	1.78 ± 0.17[e]	4.85 ± 0.13[a]
OPO[D] (40 g/L) + G (30 g/L)	2.20 ± 0.08[de]	4.30 ± 1.20[abc]
OPO (10 g/L) + G (30 g/L)	2.17 ± 0.26[de]	2.92 ± 0.15[abc]
OPO (40 g/L)	1.84 ± 0.09[e]	2.49 ± 0.59[abc]
Echium oil (10 g/L) + G (30 g/L)	2.76 ± 0.41[bcde]	5.19 ± 0.20[a]
Linseed oil (10 g/L) + G (30 g/L)	2.34 ± 0.35[cde]	4.58 ± 0.83[ab]
Borage oil (10 g/L) + G (30 g/L)	2.03 ± 0.22[e]	5.34 ± 0.94[a]
Rapeseed oil (10 g/L) + G (30 g/L)	2.02 ± 0.18[e]	4.61 ± 0.19[ab]
Sesame oil (10 g/L) + G (30 g/L)	1.95 ± 0.17[e]	4.49 ± 0.66[ab]
Trout oil (10 g/L) + G (30 g/L)	1.85 ± 0.09[e]	4.62 ± 0.14[ab]

Incubation conditions: 150 rpm/28 °C and 120 h, 1 % carbon source addition

[A] All data are mean values ± standard deviations of triplicate measurements

[B] *HOM* Hazelnut oil press cake medium

[C] *SOC* sunflower seed oil cake

[D] OPO Olive pomace oil

Differences between fermentation mediums are indicated with lettering according to the results of statistical experiments

another study conducted by Nicaud et al. [61], lipase activity was increased to be as high as 60,000 IU/mL by gene amplification. It was indicated that lipase activity increased with the increasing level of C18 fatty acids (C18:1 and C18:n) in the substrate [62, 64].

It was observed that carbon sources in the fermentation medium also affect CA and lipase production considerably. Different carbon sources with equal masses present different energy capacities. Darvishi et al. [3] found that CA production by *Y. lipolytica* DSM3286 was found to be lower when yeast was cultivated with different oils than when it was cultivated with glucose. Glucose concentration increased citric acid production due to excess carbon conditions [20].

Conclusions

The results showed that hazelnut and sunflower oil industry residues, as well as *Echium* spp. seed and linseed oils appear to be promising substrates for *Y. lipolytica* YB-423-12 growth and metabolite production. Further studies should be carried out in order to produce high-value-added, tailor-made lipids and metabolites with this strategy. Since *Y. lipolytica* is able to accumulate lipids with a similar structure to vegetable oils containing unsaturated fatty acids in the 2-acylglycerol position [6–9], it may also be possible to produce cocoa butter substitutes or other functional oils using various hydrophobic wastes/by-products as substrates.

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