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RESEARCH ARTICLE

Synthesis of flavor compound ethyl hydrocinnamate by *Yarrowia lipolytica* lipases

Bartłomiej Zieniuk[a], Agata Fabiszewska[a], Małgorzata Wołoszynowska[b] and Ewa Białecka-Florjańczyk[a]

[a]Department of Chemistry, Institute of Food Sciences, Warsaw University of Life Sciences, Warsaw, Poland; [b]Analytical Department, Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Warsaw, Poland

ABSTRACT

Two biocatalysts with high lipolytic activity were obtained – freeze-dried supernatant and freeze-dried biomass of *Yarrowia lipolytica* KKP 379. The biocatalysts were compared with *Candida antarctica* lipase B in the synthesis of ethyl hydrocinnamate – a flavour compound with a floral-honey aroma. Ester synthesis was completed after 2 h when 3 grams of freeze-dried biomass was used. Using smaller amounts of biomass (1.2 and 0.6 g) also allowed to achieve high ester conversion and results were comparable with those obtained for CALB used as a catalyst. Freeze-dried supernatant showed a weaker lipase activity (22.17 U/g) compared to freeze-dried biomass (46.13 U/g), which resulted in a lower conversion of 3-phenylpropionic acid to its ethyl ester, and after 36 h a conversion peaked at around 70%, then began to decrease and finally the conversion reached 50%. Moreover, antimicrobial and antioxidant properties of the synthesised ester were evaluated. Ethyl hydrocinnamate showed no antibacterial activity and weak antioxidant activity towards DPPH radical. In contrast to bacteria, the obtained compound moderately inhibited the growth of tested yeast species, where inhibition zones ranged from 10 to 16 mm.

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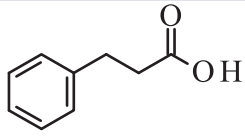
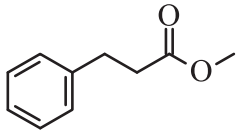
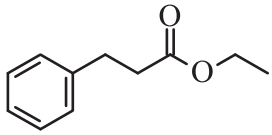
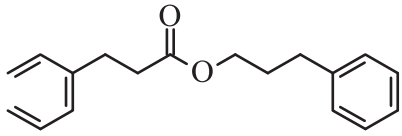
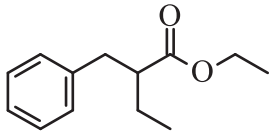
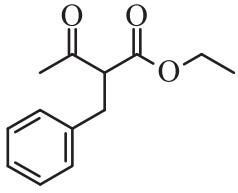
KEYWORDS
Ethyl hydrocinnamate; flavour compound; *Yarrowia lipolytica*; lipases; enzymatic synthesis

Introduction

Flavour is the sensory impression and it is associated with taste and smell perceived in food. It is considered as one of the most important attributes determining food acceptance by consumers (Canon et al. 2018). Flavourings are substances used to alter or enhance the taste of food and beverages. According to the Codex Committee on Food Additives (CCFA), these compounds can be divided into the following groups: natural, nature-identical, and artificial flavouring substances (Codex Alimentarius 2004). From the chemical point of view, compounds responsible for the taste and smell of food fall into different categories of chemical substances for instance terpenes, aldehydes and ketones, alcohols, carboxylic acids, lactones and esters (Tylewicz et al. 2017). This latter group of compounds is mainly found in food products with an intense aroma. Esters of short-chain carboxylic acids, especially acetates and butyrates have fruity fragrances and they are presented in numerous food products such as beers, wines, and dairy products. They can be formed during the production process or are added to food to enhance its quality. In the latter case, they may be isolated from products of natural origin - esters are produced by some microorganisms and plants or synthesised by chemical or biotechnological methods (Cong et al. 2019). In recent years, biotechnological methods of synthesis have gained much popularity in comparison to chemical synthesis or ester extraction from natural sources. Biotechnological production of esters is primarily related to lipase-catalysed esterification or transesterification reactions, and it can be applied more often due to higher chemo-, regio-, and stereospecificity of catalysts in comparison with conventional chemical synthesis (de Oliveira et al. 2019). Furthermore, biotechnological methods of esters synthesis allow obtaining compounds with food preservation and therapeutic potentials, e.g. phenolic acid esters that have antimicrobial and antioxidant activities and also have neuro-, cardio-, and hepatoprotective properties (Białecka-Florjańczyk et al. 2018). Moreover, consumers' expectations of food are constantly changing and there is a growing interest in food additives obtained using biotechnological methods.

CONTACT Bartłomiej Zieniuk bartlomiej_zieniuk@sggw.pl Department of Chemistry, Institute of Food Sciences, Warsaw University of Life Sciences, 159c Nowoursynowska St., 02-776 Warsaw, Poland

Table 1. Flavouring substances permitted for use in food with 3-phenylpropionic moiety.

Name	Structure	CAS	JECFA No	CoE No	Odour profile[a]
3-Phenylpropionic acid		501-52-0	646	32	Sweet, fatty, rose, musk, cinnamon
Methyl 3-phenylpropionate		103-25-3	643	427	Honey, fruity, wine, balsam, floral
Ethyl 3-phenylpropionate		2021-28-5	644	429	Hyacinth, rose, honey, fruity, rum
3-Phenylpropyl 3-phenylpropionate		60045-27-4	–	–	–
Ethyl 2-ethyl-3-phenylpropionate		2983-36-0	1475	10587	Green, floral, fruity
Ethyl 2-acetyl-3-phenylpropionate		620-79-1	835	2241	Sweet, fruity, floral, spicy, balsamic, berry, davana, tropical fruit

JECFA: Joint FAO/WHO Expert Committee on Food Additives; CoE: The Council of Europe.
[a]Based on the data of The Good Scents Company Information System [http://www.thegoodscentscompany.com/index.html].

Hydrocinnamic acid (3-phenylpropionic acid, HCA) is the phenylpropanoid family member composed of an aromatic ring and a three-carbon chain with a carboxylic group. 3-Phenylpropionic acid and other phenylpropanoids can be produced in the human body as a result of the metabolism of phenylalanine and tyrosine by intestinal bacteria (Turlin et al. 2001). It can be also produced by bacteria of the *Clostridium* and *Streptomyces* genus (Narayana et al. 2007; Sun et al. 2016; Mordaka et al. 2018). HCA and its derivatives are applied in the synthesis of various chemical compounds used in medicine, agriculture and biology (Korneev 2013). Esters of hydrocinnamic acid occur naturally in Spanish-style green table olive aroma (López-López et al. 2018), lager beers (Xu et al. 2017), wines (Zhao et al. 2017), spirit from Spine grape (*Vitis davidii* Foex) wine (Xiang et al. 2020), or propolis essential oil (Oliveira et al. 2010). On the list of flavouring substances from the Commission Implementing Regulation (EU) No 872/2012 there are 6 chemical compounds with the 3-phenylpropionic moiety (2012). These compounds and their odour profiles are listed in Table 1.

The current study showed the possibility of using freeze-dried preparations as a catalyst for ester synthesis. The methodology applied is based on the enzymatic catalysis, which is one of the "green" methods of biotechnological production of chemical compounds. Quoted below studies also show and acknowledge that *Yarrowia lipolytica* lipases are versatile enzymes used in a variety of chemical reactions. *Y. lipolytica* lipase was involved in biodiesel synthesis (Meng et al. 2010, 2011), and more often, extracellular lipases of *Y. lipolytica* were used for enzymatic ring-opening polymerisation of ε-caprolactone (Barrera-Rivera et al. 2008, 2009) or resolution of 2-bromo-arylacetic acid esters (Cancino et al. 2008), (*R,S*)-2-octanol (Liu et al. 2013), and (*R,S*)-1-phenylethanol (Cui et al. 2015).

Two fractions of *Y. lipolytica* lipases are distinguished: extracellular and intracellular, which

additionally can be divided into enzymes found in cytosol and enzymes associated with cell wall structure (Fraga et al. 2018). Lipases and esterases of *Y. lipolytica* are encoded by the *LIP* gene family. The *LIP2* gene encodes the Lip2p lipase – an *sn*-1,3-regioselective enzyme, which is responsible for the extracellular lipolytic activity and has a high affinity for oleic acid esters, while Lip7p and Lip8p lipases (intracellular lipases) have an affinity for esters of caproic (C6) and capric (C10) acids, respectively (Fickers et al. 2011).

The aim of this study was to apply a freeze-dried supernatant or freeze-dried biomass of *Y. lipolytica* KKP 379 as a biocatalyst with lipolytic activity in the synthesis of ethyl hydrocinnamate, a flavour compound which has a floral-honey type odour. The use of whole cells is a useful strategy with several advantages over isolated enzymes because purification is usually costly and time-consuming. Therefore, whole-cell catalysis creates perspectives for convenient synthesis of additives for the food industry provided that the microorganisms have GRAS status (Lin and Tao 2017).

Materials and methods

Microorganisms

In the study, the following yeast and bacterial strains were used: *Y. lipolytica* KKP 379, *Candida cylindracea* DSM-2031, *Rhodotorula mucilaginosa* EPSC001, *Saccharomyces cerevisiae* EPSC002, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213, and *Enterococcus faecalis* ATCC 29212. *Y. lipolytica* KKP 379 was purchased from the Collection of Industrial Microorganisms of Institute of Agricultural and Food Biotechnology in Warsaw. Other strains were purchased from American Type Culture Collection (ATCC, USA) and Leibniz Institute DSMZ – German Collection of Microorganisms and Cell Cultures. *R. mucilaginosa* EPSC001 and *Saccharomyces cerevisiae* EPSC002 were isolated and identified in the Department of Chemistry (WULS, Poland; data not shown). Yeasts were stored on YPD (2% peptone, 1% yeast extract and 2% glucose) agar slants at 4 °C. Bacterial strains were stored at −70 °C, prior to testing.

Materials

Candida antarctica Lipase B (CALB) immobilised on the acrylic resin was purchased from Sigma-Aldrich (Poznań, Poland). Chemicals were purchased from Sigma-Aldrich and Avantor Performance Materials (Gliwice, Poland). Components of culture media were purchased from BTL Sp. z o. o. (Łódź, Poland).

Methods

Culture media and yeast cultivation

Inoculation cultures were prepared in 100 mL of liquid YPD medium. The yeast culture was incubated at 28 °C for 24 h on a rotary shaker (140 rpm) and had a pH of 5.0. *Y. lipolytica* was then cultivated in BIOFLO 3000 bioreactor (New Brunswick Scientific Edison, NJ, USA) with a working volume of 4 L in YPO medium (2% peptone, 1% yeast extract and 2% olive oil) at 28 °C, 300 rpm of agitator speed and compressed air aeration at a flow of 105 L/h per 1 L of medium (Fabiszewska et al. 2015). Automatic measurement of the culture pH and oxygen consumption was carried out, and obtained values allowed determining yeast growth phases. Bioreactor culture was ended upon reaching relatively high activity of extracellular lipolytic enzymes (Stolarzewicz et al. 2017; Zieniuk, Wołoszynowska, et al. 2020). Post-culture fluid (supernatant) containing extracellular lipases produced by *Y. lipolytica* and yeast biomass rich in cell-bound lipases were separated by centrifugation (10 °C, 8000 rpm, 10 min).

Freeze-drying procedure

The supernatant was concentrated about 10 times using Buchi Rotavapor R-200 evaporator (Switzerland) with a bath temperature of 57 °C, the boiling point of water of 37 °C and 62 mbar of pressure. Then concentrated supernatant and yeast biomass were frozen for 2 h at −42 °C, and freeze-dried for 24 h using Christ Gamma 1–16 laboratory freeze dryer (Germany). The pressure inside the chamber was kept at 63 Pa, and the process was conducted at a shelf temperature of 10 °C.

Lipase activity assay

Extracellular and intracellular lipase activities were measured by a spectrophotometric method based on the hydrolysis of *p*-nitrophenyl laurate (Kapturowska et al. 2012). Briefly, 0.25 g of freeze-dried yeast biomass or freeze-dried supernatant was suspended in 15 mL of distilled water and added to 0.3 mmol of *p*-nitrophenyl laurate dissolved in 2 mL of heptane. Hydrolysis reaction was performed on a magnetic stirrer at 37 °C, and after 15 min, absorbance was measured in UV/Vis spectrophotometer at 410 nm. Results

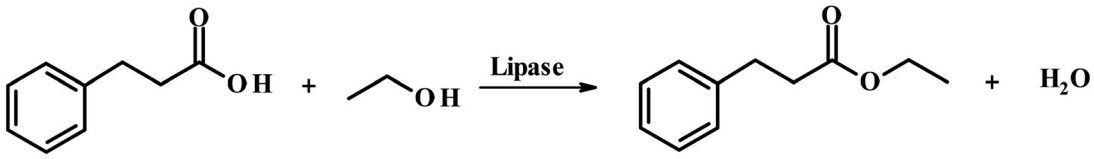


Figure 1. Lipase-catalysed esterification of hydrocinnamic acid with ethyl alcohol.

were expressed as Units of activity (the enzyme quantity that liberated 1 μmol of *p*-nitrophenol per minute under the assay conditions at 37 °C) per gram of freeze-dried supernatant or biomass.

Determination of protein content in freeze-dried biocatalysts

Protein content in freeze-dried supernatant was measured by a modified spectrophotometric Lowry's method at 750 nm (Lowry et al. 1951; Kapturowska et al. 2012). Protein content in freeze-dried biomass was determined by the Kjeldahl method (Maehre et al. 2018). Results were expressed as milligrams of proteins per gram of freeze-dried supernatant/biomass.

Physicochemical properties of freeze-dried biocatalysts

The dry weight of freeze-dried supernatant and yeast biomass was measured by Radwag MAC 50/NH (Poland) moisture analyser at 105 °C. The water activity of freeze-dried supernatant and yeast biomass was determined using AquaLab CX-2 (USA) water activity metre with a dew point sensor at 25 °C.

Biotransformation reaction

The reaction substrates were hydrocinnamic acid and ethyl alcohol (Figure 1) in a molar ratio 1:2 and the amount of acid was 0.0025 moles. The following catalysts were used: *Candida antarctica lipase* B (5% w/w of substrates mass), freeze-dried *Y. lipolytica* supernatant containing extracellular lipase extract (1.2 or 3 g), and freeze-dried yeast biomass (0.3, 0.6, 1.2 or 3 g). Reactions were carried out in round bottom flasks in 10 mL of isooctane at 40 °C. Liquid samples were withdrawn periodically from the reaction mixtures and analysed by gas chromatography (GC).

Gas chromatography

Samples were analysed by gas chromatography equipped with a flame ionisation detector (GC-FID). Analysis was carried out using Agilent Technologies 7820 A (USA) with HP-5 column (0.25 mm, 30 m, 0.25 μm). Nitrogen was used as carrier gas at a flow rate of 1.5 mL/min. The temperature program was as follow: 90 °C to 170 °C (10 °C/min), 170 °C for 3 min, 170 °C to 220 °C (1.5 °C/min) and 220 °C to 240 °C (40 °C/min), 240 °C for 10 min. Injector temperature: 270 °C and detector temperature: 300 °C, injection volume: 1 μl. Percentage conversion was calculated based on the area under peaks of acid and ester.

Column chromatography and confirmation of the structure of the obtained ester

Ester (ethyl hydrocinnamate) was purified using column chromatography. Silica gel 60 (0.040–0.063 mm; 230–400 mesh) was used as a stationary phase and chloroform was applied as a mobile phase. Successive eluent fractions were collected in separate flasks and then analysed by TLC. The ester-containing fractions were then dried with $MgSO_4$, filtered and the solvent was evaporated.

The ^{1}H NMR spectrum was measured using Bruker AVANCE 300 MHz (USA) and $CDCl_3$ as a solvent. Proton chemical shifts of ethyl hydrocinnamate are reported below in ppm (δ) relative to internal standard - tetramethylsilane (TMS).

^{1}H NMR (300 MHz, $CDCl_3$): δ 1.216 (3H, t, $J = 7.1$), 2.601 (2H, t, $J = 7.4$), 2.936 (2H, t, $J = 7.4$), 4.11 (2H, q, $J = 7.1$), 7.15−7.30 (5H, m).

Evaluation of antimicrobial properties

Antimicrobial activity was evaluated using the disc diffusion method with Mueller-Hinton agar (BD, Germany) or Sabouraud dextrose agar (BTL Sp. z o. o., Łódź, Poland) plates. Blank discs (Oxoid, UK) were soaked with 10 μL of the compound. Bacterial suspensions of *E. coli* ATCC 25,922 *P. aeruginosa* ATCC 27853, *S. aureus* ATCC 29213 and *E. faecalis* ATCC 29212 or yeast suspension of *Y. lipolytica* KKP 379, *Candida cylindracea* DSM-2031, *Rhodotorula mucilaginosa* EPSC001 and *Saccharomyces cerevisiae* EPSC002 in 0.9% NaCl solution at a density of 0.5 McFarland were spread over the surface of the agar plates. Then discs were placed on the Muller-Hinton/Sabouraud agar and

plates were incubated for 16–18 h at 37 °C (bacteria) or 48 h at 28 °C (in the case of yeasts). After incubation, inhibition zone diameters were measured. The diameter of the disc soaked with the selected compound is 6 mm, thus 6 mm inhibition zone means no antimicrobial activity.

Evaluation of antioxidant activity

The antioxidant activity of ethyl hydrocinnamate was evaluated using DPPH (2,2-Diphenyl-1-picrylhydrazyl) method following Zanetti et al. (2017). The percentage reduction of the DPPH radical was determined after 60 min. Moreover, the IC_{50} parameter (concentration required for 50% reduction of the DPPH radical) was determined.

Statistical analysis

Statistical analysis was performed using Statistica 13.3 software (TIBCO Software Inc., USA). The results were analysed using the one-way analysis of variance (ANOVA) and Tukey's *post-hoc* test. The significance level was $\alpha = 0.05$.

Results and discussion

Production, physicochemical characteristics and application of freeze-dried biocatalysts in the enzymatic esterification of hydrocinnamic acid with ethanol

Previous work carried out by Białecka-Florjańczyk et al. (2012) using *Y. lipolytica* yeast in the synthesis of food additives showed that raw yeast biomass could be a catalyst for the 2-phenylethyl acetate synthesis reaction, with the conversion similar to those achieved with the commercial lipase preparation from *C. antarctica* (CALB). In the current work freeze-dried supernatant and biomass of *Y. lipolytica* KKP 379 were used as catalysts in the esterification reaction of hydrocinnamic acid with ethanol.

In the first stage of the experiment, it was necessary to obtain high lipolytic activity of yeast in bioreactor culture. In the research of Stolarzewicz et al. (2017) extracellular lipases production by *Y. lipolytica* KKP 379 was increasing up to the 46th hour of the culture during the log growth phase. Based on the course of cultures performed by the team of Stolarzewicz et al. (2017), where the same yeast strain, cultivation medium and conditions, as well as the same laboratory bioreactor were used, in the current study controlling the changes in pH and dissolved oxygen concentration (Figure 2) allowed to finish the culture after 40 h, when the extracellular lipolytic activity was the highest. At the end of the culture, the pH value was 7.15 and almost all of the dissolved oxygen in the medium was used by yeasts, which proved their intensive growth. The supernatant and biomass were freeze-dried and characterised by dry matter, water activity, protein content and lipase activity (Table 2).

Moisture and water activity of the freeze-dried supernatant were 3.5% and 0.235, respectively, and for yeast biomass the following values were obtained: 2.5% and 0.090. The effect of the water content in enzymatic reactions is a frequently discussed topic in literature. A certain level of water is necessary to ensure the natural conformation of enzymes and thus the activity of these biocatalysts (Halling 1994). But in

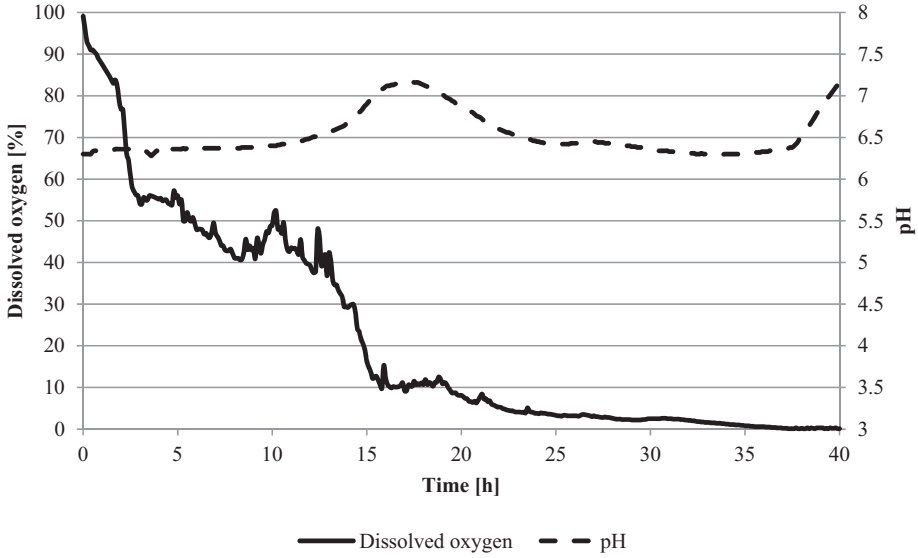


Figure 2. Changes in pH and dissolved oxygen during a batch culture of *Y. lipolytica* KKP 379.

Table 2. Characteristics of freeze-dried biocatalysts from *Y. lipolytica* KKP 379.

Material	Dry matter [%]	Moisture [%]	Water activity	Protein content [mg/g]	Lipase activity [U/g]	Specific activity [U/mg]
Freeze-dried supernatant	96.5 ± 1.1^{a}	3.5 ± 1.1^{a}	0.235 ± 0.001^{a}	336 ± 28^{a}	22.17 ± 1.20^{a}	0.066
Freeze-dried biomass	97.5 ± 0.2^{a}	2.5 ± 0.2^{a}	0.090 ± 0.006^{b}	531 ± 1^{b}	46.13 ± 2.00^{b}	0.087

The values with the same letter in a column did not differ significantly ($\alpha < 0.05$).

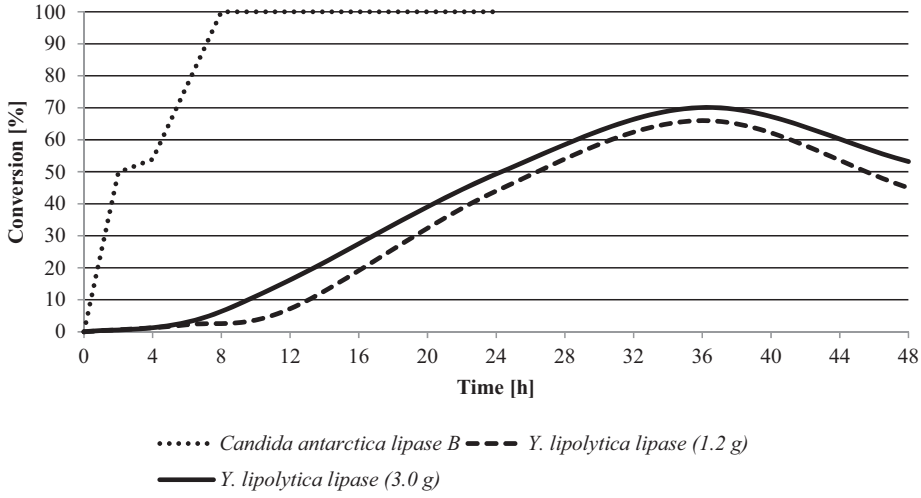


Figure 3. Percentage conversion of ethyl hydrocinnamate catalysed by *C. antarctica* lipase B (dotted line), or freeze-dried supernatant of *Y. lipolytica* KKP 379 (1.2 g – dashed line; 3 g – solid line).

the case of esterification, a higher water content disadvantageously changes the equilibrium state of the reaction. Both Shimada et al. (1999) and Iso et al. (2001) showed that increasing the water content in the biodiesel synthesis reaction reduces the amount of formed fatty acid alkyl esters. Gayot et al. (2003) increased the conversion yield of enzymatic synthesis of naringin palmitate by drying solvent and acyl acceptor. Therefore, the use of freeze-dried biocatalysts seemed advantageous. The moisture of the freeze-dried material referring to the water content and water activity, significantly affect the activity of lipolytic biocatalysts in reactions in organic solvents. On the other hand, enzyme drying may cause loss of its activity, because water is essential for the three-dimensional structure of the enzyme.

The protein content in the freeze-dried supernatant was 336 mg/g, which means that over 30% of the preparation were proteins. Among the proteins present in the freeze-dried biocatalyst, there were proteins from the culture medium (peptone) as well as yeast proteins including enzymes, such as extracellular lipases. The lipase activity of freeze-dried supernatant was 22.17 U/g and the concentration of proteins was 336 mg/g. In the case of freeze-dried biomass, the protein content was 1.5 times higher (531 mg/g) and the biomass was twice as active as supernatant (46.13 U/g). Two of the freeze-dried preparations differed in lipase activity but also in type of enzyme entrapment. In supernatant, there were present free proteins versus cell wall-bounded and present in cytosol in biomass preparation. Presence of yeast cell wall structures could additionally protect the enzymes during the freeze-drying of biomass and therefore, these enzymes could be more active than the extracellular enzymes present in post-culture supernatant. It can be also seen that the catalytic proteins in the supernatant were in a smaller amount than in biomass. Using the values of protein content and enzyme activity, it was possible to calculate the specific activity (the activity of enzyme per milligram of total protein), which was 0.066 U/mg for supernatant and 0.087 U/mg for freeze-dried biomass.

The freeze-dried supernatant and biomass were used in the synthesis of ethyl hydrocinnamate (Figure 1) and compared to the very popular catalyst – lipase B from *C. antarctica*. Conversion of hydrocinnamic acid to ethyl hydrocinnamate using three catalysts is presented in Figures 3 and 4.

In the case of CALB as a catalyst, the ester content after 2 h was almost 50%, and after 8 h the reaction was completed. In comparison with CALB, freeze-dried *Y. lipolytica* extracellular lipase extract was not such an efficient catalyst. Using smaller quantities of freeze-

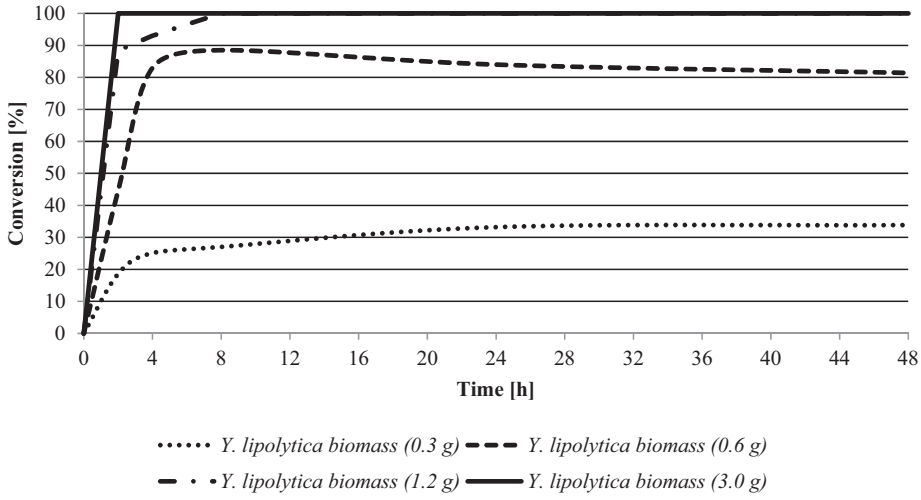


Figure 4. Percentage conversion of ethyl hydrocinnamate catalysed by freeze-dried biomass of *Y. lipolytica* KKP 379 (0.3 g – dotted line; 0.6 g – dashed line; 1.2 g – dash-dotted line; 3 g – solid line).

dried supernatant (0.3 and 0.6 grams) the reaction proceeded slightly, and traces of ester were produced (data not shown). Larger amounts of catalyst (1.2 and 3 g) allowed to obtain 50% conversion after 24 h, and 3 grams of freeze-dried supernatant instead of 1.2 g slightly improved the efficiency of the reaction. After 36 h a conversion was the highest, and it was about 70%.

The amount of ester decreased after 48 h to the level of 50%. This was due to the formation of water molecules produced in the esterification reaction, which affect the equilibrium position of the reaction towards hydrolysis. Additionally, higher water content and higher water activity in freeze-dried supernatant in comparison with freeze-dried biomass adversely affect the equilibrium position favouring the ester hydrolysis. Similar results in enzymatic synthesis of ethyl hexanoate were obtained by Musa et al. (2018). The equilibrium of the reaction may be shifted by water removal, which can be achieved by using a Dean–Stark apparatus or in a simpler way by using molecular sieves (Gayot et al. 2003).

Differences in the conversion of the reactions catalysed by immobilised lipase B from *C. antarctica* (80 U/g; Zieniuk, Fabiszewska, et al. 2020) and freeze-dried supernatant of *Y. lipolytica* (22.17 U/g) may be due to the fact that CALB is a pure enzyme immobilised on a carrier, making it more stable, without omitting the difference in the activity of those biocatalysts. Free lipases are not favoured in industrial applications because it is difficult to recover the molecules for their reuse, and they have low stability. These drawbacks could be overcome by using cell-bound enzymes or by immobilisation of enzymes on appropriate supports (Stolarzewicz et al. 2017). Moreover, freeze-dried supernatant is a crude post-culture fluid with both: lipases and other proteins, which may have a negative effect on lipase activity.

Table 3. Zone of inhibition of ethyl hydrocinnamate.

Microorganism	Inhibition zone [mm]
E. coli	6 ± 0^{a}
P. aeruginosa	6 ± 0^{a}
E. faecalis	6 ± 0^{a}
S. aureus	6 ± 0^{a}
Y. lipolytica	12 ± 1^{c}
R. mucilaginosa	16 ± 1^{d}
S. cerevisiae	12 ± 1^{c}
C. cylindracea	10 ± 1^{b}

The values with the same letter in a column did not differ significantly ($\alpha < 0.05$).

The experiment was also carried out using the same amounts of freeze-dried biomass of *Y. lipolytica* as for the supernatant (0.3; 0.6; 1.2 and 3.0 g of biomass; Figure 4). Increasing the amount of a catalyst had a positive effect on the production of ester. The use of the lowest amount of biomass (0.3 g) resulted in a conversion of about 30%. The ester content in the mixture reached about 45% after a 2 h of reaction when 0.6 g of catalyst was used, after 4 h ester content was over 80% and for a further reaction time it was stabilised at a similar level. The use of 1.2 and 3.0 g of freeze-dried biomass allowed the substrate to react completely after 8 h and 2 h, respectively.

Similar studies were performed by Priya and Chadha (2003). Researchers used *Pseudomonas cepacia* lipase and obtained high yields (72–83%) for enzymatic esterification of hydrocinnamic acid with primary and secondary alcohols, as well as unsaturated, cyclic and aromatic alcohols. The lowest yield was obtained in reaction with methanol. Similar to the current results, Priya and Chadha obtained 72% yield of

esterification with ethanol after 48 h. Furthermore, microwave-assisted transesterification of ethyl 3-phenylpropanoate was studied by Yadav and Pawar (2012) using various immobilised lipases. CALB was the most active enzyme and achieved a conversion range 51–92% for primary, secondary and aromatic alcohols used for transesterification. Ethyl hydrocinnamate and other similar compounds were used by Weitkamp et al. (2006) in enzymatic synthesis of medium- and long-chain alkyl cinnamates, hydrocinnamates and hydroxycinnamates under partial vacuum.

Antimicrobial and antioxidant properties of ethyl hydrocinnamate

Food spoilage can be connected with the spoilage microorganisms as well as with the oxidation food components. Ethyl hydrocinnamate was examined as an antimicrobial and antioxidant agent, which has not been previously studied in the literature. The obtained compound did not display antibacterial activity. Zones of inhibition were 6 mm of diameters for all four tested bacteria (Table 3), which means that synthesised ester did not inhibit the growth of those microorganisms. On the other hand, synthesised ester inhibited yeast growth. Inhibition zones ranged from 10 to 16 mm, where 10 mm was obtained for *C. cylindracea* and 12 mm for *S. cerevisiae* and *Y. lipolytica*. The highest inhibition zone was observed for *R. mucilaginosa*, which means that this yeast species was the most sensitive to this compound.

Narayana et al. (2007) showed that 3-phenylpropionic acid had antimicrobial activity. The minimum inhibitory concentration (MIC) of 3-PPA ranged between 10 and 100 μg/mL for tested bacteria and fungi. On the contrary, Cueva et al. (2010) showed that phenylpropionic acid was able to inhibit *E. coli* ATCC 25922 and *E. coli* CECT 5947 only in the highest concentration (1000 μg/mL). Inhibition of other bacteria growth was performed only in concentrations higher than 250 μg/mL, and growth of *L. fermentun* LPH1 and *P. aeruginosa* PA01 was not inhibited. Moreover, substituted phenylpropionic acids demonstrated weaker antimicrobial activity than non-substituted acid. There is a lack of studies in the literature relating to the antimicrobial activity of phenylpropionic acid esters. Referring to phenolic acids (where 3-phenylpropionic acid can be considered as a phenolic acid analog), Shi et al. (2018) showed that alkyl esters of ferulic acid, mainly butyl and hexyl esters possess higher antibacterial activity than ferulic acid, but further chain elongation (octyl, decyl, and dodecyl esters) lowered antimicrobial activity. Similarly, Merkl et al. (2010) found that esterification of phenolic acids caused increasing in antimicrobial activity of synthesised compounds.

Ethyl hydrocinnamate showed 7.25% ± 0.10% scavenging activity towards DPPH after 60 min, and IC_{50} value was 199 mg/mL (data not presented on any figure). Hydrocinnamic acid (phenylpropionic acid), likewise its dehydrogenated derivative, cinnamic acid had no antioxidant activity. The presence of a hydroxyl group in the aromatic ring is responsible for high antioxidant activity, hence caffeic and ferulic acids show high radical scavenging activity. Moreover, additional hydroxyl or methoxyl group in the phenolic ring enhances compounds activity because the more hydroxyl groups a compound has, the greater its antioxidant activity (Białecka-Florjańczyk et al. 2018). In case of phenolic acid derivatives, the presence of double bond in the hydrocarbon chain have stabilising effect on the phenoxyl radical, which is formed in the reaction of oxidants and free radicals with phenolic hydroxyl groups (Sova 2012).

Conclusion

The observed differences in the activity of freeze-dried supernatant and freeze-dried biomass suggest that the source of the enzyme (supernatant containing extracellular lipases and biomass with intracellular lipases) determines the type of isoenzymes, affecting the conversion of performed reactions. It should be also taken into account that enzymes incorporated into cell structure may be deactivated to a lesser extent during the freeze-drying. Therefore, further research could be carried out on searching for new biocatalysts and use of appropriate purification and/or immobilisation processes may improve the activity and stability of enzymes.

The use of freeze-dried *Y. lipolytica* biomass resulted in effective ester synthesis. Synthesised ethyl hydrocinnamate, a compound with a floral-honey aroma had antifungal activity, where among 4 tested yeasts species *R. mucilaginosa* showed the highest sensitivity. In contrast, ester had no antibacterial activity and showed weak antioxidant activity. Still, this whole cell bioprocess creates the opportunity for a promising way to obtain a valuable additive used in food, beverages and cosmetic field.

Human and animal studies

This study does not involve research on human participants and/or animals.

Disclosure statement

The authors declare that they have no conflict of interest.

ORCID

Bartłomiej Zieniuk http://orcid.org/0000-0001-6344-8695

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