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Microbial transformations of chalcones to produce food sweetener derivatives

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ABSTRACT

Biotransformations of two substrates: chalcone (**1**) and 2′-hydroxychalcone (**4**) were carried out using four yeast strains and five filamentous fungi cultures. Substrate **1** was effectively hydrogenated in all of tested yeast cultures (80–99% of substrate conversion after 1 h of biotransformation) affording dihydrochalcone **2**. In the cultures of filamentous fungi the reaction was much slower, however, *Chaetomium* sp. gave product **2** in 97% yield. After 12 h of incubation a reduction of dihydrochalcone **2** to alcohol **3** was additionally observed. After 3 days of biotransformation in the culture of *Rhodotorula rubra* product (*S*)-**3** was obtained with 75% *ee* (enantiomeric excess) and 99% of conversion. Also after a 3-day biotransformation using the strain *Fusarium culmorum* product (*R*)-**3** was obtained with 98% *ee* and 97% of conversion. In most of the tested strains a change in enantiomeric excess of compound **3** during the biotransformation process was noticed. In the culture of *Rhodotorula glutinis* after 3 h of transformation alcohol (*R*)-**3** was formed with 47% *ee* and 31% of substrate conversion, whereas after 6 days the (*S*)-**3** enantiomer was obtained with 99% *ee* and 91% of conversion. In the case of 2′-hydroxychalcone (**4**), the hydrogenation proceeded much slower and led to 2′-hydroxydihydrochalcone (**5**) – in the culture of *Yarrowia lipolytica* 97% of conversion was observed after 3 days. In all cultures of the tested strains no products of 2′-hydroxydihydrochalcone reduction were detected.

1. Introduction

One of the group of flavonoids are chalcones – the compounds of a lipophilic nature, and predominantly yellow-colored [1,2]. Their characteristic structural feature is 3-carbon open fragment, the closure of which leads to flavanones [3]. In nature chalcones are present most often as aglycones (the non-sugar parts of glycosides) [4,5]. They are commonly found in plants and therefore, similarly to other flavonoids, they are a part of human diet. Chalcones and their derivatives, both natural and synthetic ones have diverse pharmacological activity [6–11].

There are reports in the literature connecting transformation of chalcones by microorganisms. In many cases the authors indicate that these transformations are similar to metabolic pathway of chalcones in plants [12,13]. They comprise mainly cyclization, hydroxylation, *O*-demethylation and glycosidation [14–16], less often mentioned is dihydrogenation [17–19], and very rarely, reduction of a dihydrochalcone to the respective alcohol is reported [20].

The biotransformation of compounds having in its structure α,β-unsaturated carbonyl group have gained growing interest with the recent "rediscovery" of Old Yellow Enzyme (OYE) [21,22] and its homologs from yeasts, bacteria, and plants [23,24]. Enoate reductases catalyze the highly stereoselective reduction of a broad variety of α,β-unsaturated compounds, affording excellent yields [21,25,26].

Compared to other flavonoids, information on biological activity of dihydrochalcones in the literature is scarce. 2′-Hydroxydihydrochalcone is used as a building block in synthesis of propaphenone, an active substance contained in antiarrhythmic drugs [27–29]. Dihydrochalcones are also known for their antimicrobial activity against a panel of pathogenic microorganisms, including gram-positive and gram-negative bacteria, and fungi [30]. Dihydrochalcone phloretin is an active inhibitor of mushrom tyrosinase [31]. Dihydrochalcones, due to their sweet taste and natural occurrence may find application as sweeteners in food industry [32,33]. Hesperetin dihydrochalcone is 300 times sweeter than sucrose and this compound was obtained from hesperitin isolated from the peels of the plant *Citrus sinensis* and *Citrus limoni* (Rutaceae) [34]. However, currently mainly sugars and their derivatives including sweet polyhydric alcohols (sorbitol, mannitol, maltitol and xylitol) are used as sweeteners. However, intense sweeteners of natural origin include phyllodulcin,

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stevioside, glycyrrhizin and osladin are also commonly applied [35,36].

2. Experimental

2.1. Materials

The substrates: chalcone (**1**) and 2′-hydroxychalcone (**4**) were obtained following the method by Yadav et al. [11]; (*E*)-4-phenyl-but-3-en-2-one (**6**) was purchased from Fluka. The microorganisms, including four strains of yeast: *Yarrowia lipolytica* KCh 71, *Rhodotorula glutinis* KCh 242, *Rhodotorula rubra* KCh 4, and *Saccharomyces cerevisiae* KCh 464 and five filamentous fungi: *Syncephalastrum racemosum* KCh 105, *Chaetomium* sp. KCh 6651, *Didymosphaeria igniaria* KCh 6670, *Absidia coerulea* KCh 93, and *Fusarium culmorum* KCh 10 were obtained from the Department of Chemistry of Wrocław University of Environmental and Life Sciences. All the strains were cultivated on a Sabouraud agar consisting of aminobac (5 g), peptone K (5 g), glucose (40 g) and agar (15 g) dissolved in 1 l of distilled water, at 28 °C and pH 5.5 and stored in a refrigerator at 4 °C.

2.2. Analytical methods

The course of biotransformation was controlled by means of TLC. Analytical TLC was carried out on silica gel G 60 F_{254} plates (Merck). Chromatograms were developed using hexane/acetone mixture (3:1, v/v) as the eluent. Compounds were detected by spraying the plates with 1% $Ce(SO_4)_2$ and 2% $H_3[P(Mo_3O_{10})_4]$ in 10% H_2SO_4. The products were separated by column chromatography using silica gel (SiO_2, Kieselgel 60, 230–400 mesh, 40–63 μm, Merck) and hexane/acetone mixture (3:1, v/v) as the developing system. Composition of biotransformation mixtures was established by gas chromatography (GC) on Agilent Technologies 7890 A GC instrument, fitted with a flame ionization detector (FID) and a chiral column Chirasil-Dex CB 25 m × 0.25 mm × 0.25 μm. To determinate the composition and enantiomeric excesses of product mixtures the following temperature programs were used: the temperature program for **1**: 90 °C/0 min, gradient 3 °C min^{-1} to 200 °C/5 min; the retention times: **2** – 24.12 min, (*S*)-**3** – 25.64 min, (*R*)-**3** – 25.87 min, **1** – 29.69 min; the temperature program for **4**: 90 °C/0 min, gradient 3 °C min^{-1} to 200 °C/10 min; the retention times: **5** – 30.16 min, **4** – 35.35 min; and the temperature program for **6**: 90 °C/0 min, gradient 5 °C min^{-1} to 140 °C, gradient 20 °C min^{-1} to 200 °C/5 min; the retention times: **7** – 6.97 min, (*S*)-**8** – 8.84 min, (*R*)-**8** – 9.01 min, **6** – 9.54 min. Reference samples of the racemic alcohols were prepared by reducing the ketones with sodium borohydride in methanol. NMR spectra were recorded on a DRX 600 MHz Bruker spectrometer and measured in $CDCl_3$. Optical rotations were measured with an Autopol IV automatic polarimeter (Rudolph). Absolute configurations of the products were determined by comparison of their optical rotation values with literature data.

2.3. Screening procedure

Erlenmeyer flasks (300 ml), each containing 100 ml of the medium consisting of 3 g glucose and 1 g aminobac dissolved in water, were inoculated with a suspension of microorganisms and then incubated for 3–7 days at 25 °C on a rotary shaker (190 rpm). After full growth of the culture 20 mg of a substrate dissolved in 1 ml of acetone was added. After 1, 3, 7, 12 h and 1, 3, 6, 9 days of incubation under the above conditions, portions of 10 ml of the transformation mixture were taken out and extracted with $CHCl_3$ (3 × 10 ml). The extracts were dried over $MgSO_4$, concentrated in vacuo and analyzed by GC. All the experiments were repeated three times.

2.4. Preparative biotransformation

The same transformations were performed on the preparative scale in 2000 ml flasks, each containing 500 ml of the cultivation medium. The cultures were incubated under the same conditions and then 200 mg of substrates dissolved in 10 ml of acetone were added to the grown cultures. After incubation the mixtures were extracted with $CHCl_3$ (3 × 300 ml), dried ($MgSO_4$) and concentrated in vacuo. The transformation products were separated by column chromatography and analyzed (TLC, GC, ^{1}H NMR and also confirmed by ^{13}C NMR and correlation spectroscopy).

2.5. Spectral data of isolated metabolites

2.5.1. 1,3-diphenylpropan-1-one (*2*)

Seven-hour transformation of chalcone (**1**) (200 mg) in the culture *Y. lipolytica* KCh 71 yielded 188 mg of compound **2** (colorless crystals); ^{1}H NMR (600 MHz) ($CDCl_3$) δ (ppm): 3.09 (t, 2H, J = 7.7 Hz, H-2); 3.29 (t, 2H, J = 7.7 Hz, H-3); 7.22 (t, 1H, J = 7.2 Hz, H-4″), 7.27 (t, 2H, J = 7.1 Hz, H-2″, H-6″), 7.31 (t, 2H, J = 7.5 Hz, H-3″, H-5″), 7.46 (t, 2H, J = 7.7 Hz, H-3′, H-5′), 7.56 (t, 1H, J = 7.4 Hz, H-4′), and 7.97 (d, 2H, J = 7.7 Hz, H-2′, H-6′).

^{13}C NMR (151 MHz) δ (ppm): 30.18 (C-2), 40.49 (C-3), 126.19 (C-4″), 128.09 (C-3″, C-5″), 128.48 (C-2″, C-6″), 128.58 (C-3′, C-5′), 128.66 (C-2′, C-6′), 133.12 (C-4′), 136.91, (C-1′), 141.35 (C-1″), and 199.27(C-1).

2.5.2. (S)-1,3-diphenylpropan-1-ol (*3*)

One-day transformation of chalcone (**1**) (200 mg) in the culture *R. rubra* KCh 4 yielded 146 mg of compound **3** (pale yellow oil); $[\alpha]_D^{23}$ = +23.6° (c = 1.2, $CHCl_3$) (78% *ee*) (lit. $[\alpha]_D^{25}$ = +28° (c = 1, CH_2Cl_2), 86% *ee* [37]. ^{1}H NMR (600 MHz) ($CDCl_3$) δ (ppm): 1.54 (s, 1H, —O*H*), 2.09 (m, 1H, W_h = 36.9 Hz, one of H-2), 2.19 (m, 1H, W_h = 36.9 Hz, one of H-2), 2.73 (ddd, 1H, J = 16.0, 9.6, 6.4 Hz, one of H-3), 2.82 (ddd, 1H, J = 15.1, 9.3, 5.1 Hz, one of H-3), 4.73 (dd, 1H, J = 7.7, 5.5 Hz, H-1), 7.24–7.28 (m, 3H, H-3′, H-5′, H-4″), 7.33–7.36 (m, 3H, H-4′, H-2″, H-4′), and 7.39–7.43 (m, 4H, H-2′, H-6′, H-3″, H-5″).

^{13}C NMR (151 MHz) δ (ppm); 32.10 (C-3), 40.50 (C-2), 73.93 (C-1), 125.92 (C-4″), 126.04 (C-3″, C-5″), 127.68 (C-4′), 128.46 (C-2″, C-6″), 128.52 (C-3′, C-5′), 128.56 (C-2′, C-6′), 141.87 (C-1″), 144.57 (C-1′).

2.5.3. 1-(2-hydroxyphenyl)-3-phenylpropan-1-one (*5*)

Three-day transformation of 2′-hydroxychalcone (**4**) (200 mg) in the culture *Y. lipolytica* KCh 71 yielded 172 mg of compound **5** (colorless crystals); ^{1}H NMR (600 MHz) ($CDCl_3$) δ (ppm): 3.09 (t, 2H, J = 7.7 Hz, H-2), 3.33 (t, 1H, J = 7.7 Hz, H-2), 6.90 (td, 1H, J = 7.6, 1.0 Hz, H-5′), 7.01 (dd, 1H, J = 8.5, 0.7 Hz, H-3′), 7.25 (t, 1H, J = 7.3 Hz, H-4″), 7.28 (d, 2H, J = 7.2 Hz, H-2″, H-6″), 7.34 (t, 2H, J = 7.5 Hz, H-3″, H-5″), 7.47 (ddd, 1H, J = 8.3, 7.3, 1.6 Hz, H-4′), 7.75 (dd, 1H, J = 8.1, 1.6 Hz, H-6′), and 12.36 (s, 1H, —O*H*).

^{13}C NMR (151 MHz) δ (ppm): 30.04 (C-2), 40.07 (C-3), 118.61 (C-3′), 119.00 (C-5′), 119.36 (C-1′), 126.41 (C-4″), 128.50 (C-2″, C-6″), 128.70 (C-3″, C-5″), 129.94 (C-6′), 136.41 (C-4′), 140.84 (C-1″), 162.55 (C-2′), 205.45 (C-1).

2.5.4. 4-phenylbutan-2-one (*7*)

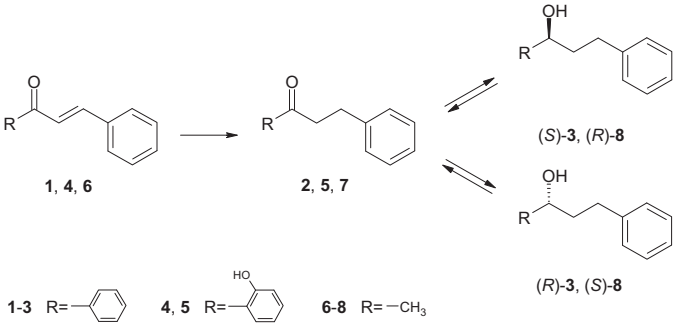
Seven-hour transformation of (*E*)-4-phenyl-but-3-en-2-one (**6**) (200 mg) in the culture *R. rubra* KCh 4 yielded 178 mg of compound **7** (pale yellow oil); ^{1}H NMR (600 MHz) ($CDCl_3$) δ (ppm): 2.09 (s, 3H, —C*H*$_3$), 2.71 (t, 2H, J = 7.3 Hz, H-2), 2.84 (t, 2H, J = 7.3 Hz, H-1), 7,11-7,16 (m, 3H, H-2′, H-4′, H-6′), and 7.20–7.25 (m, 2H, H-3′, H-5′).

^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 28.30 (C-4), 34.72 (C-2), 44.31(C-1), 126.56 and 128.42 (C-Ar), 135.13 (C-1′), and 211.54 (C-3).

*2.5.5. (R)-4-phenylbutan-2-ol (**8**)*

Seven-hour transformation of (*E*)-4-phenyl-but-3-en-2-one (**6**) (200 mg) in the culture *Chaetomium* sp. KCh 6651 yielded 168 mg of compound **8** (colorless oil); $[\alpha]_D^{25} = -33.3^\circ$ (c = 1.1, CH_2Cl_2) (99% *ee*) (lit. $[\alpha]_D^{20} = +3.4^\circ$ (c = 1.5, CH_2Cl_2), 20% *ee* [38]; ^{1}H NMR (600 MHz) ($CDCl_3$) δ (ppm): 1.21 (d, 3H, J= 6.2 Hz, —CH_3), 1.72–1.80 (m, 3H, H-2 and —O*H*), 2.60–2.80 (m, 2H, H-1), 3.81 (m, 1H, W_h = 33.0 Hz, H-3), and 7.14–7.30 (m, 5H, H-Ar).

^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 23.66 (C-4), 32.17 (C-1), 40.88 (C-2), 67.55 (C-3), 125.85 and 128.43 (C-Ar), 142.10 (C-1′).



Scheme 1. Course of transformation of chalcone (**1**), 2′-hydroxychalcone (**4**) and (*E*)-4-phenyl-but-3-en-2-one (**6**) by the selected microorganisms.

3. Results and discussion

Biotransformations of the substrates were carried out by four strains of yeast (*Y. lipolytica* KCh 71, *R. glutinis* KCh 242, *R. rubra* KCh 4, *Saccharomyces cerevisiae* KCh 464) and five strains of filamentous fungi (*S. racemosum* KCh 105, *Chaetomium* sp. KCh 6651, *D. igniaria* KCh 6670, *A. coerulea* KCh 93, and *F. culmorum* KCh 10). We have chosen the strains commonly used for research in our department and known to possess the ability to transform both low molecular weight aliphatic-aromatic ketones [39–41] and also higher molecular weight natural terpenoids [42–46].

The C=C double bond of substrate **1** was hydrogenated to give the saturated ketone **2** (80–99% of the substrate conversion after 1 h of biotransformation). In the cultures of filamentous fungi the reaction was much slower, nevertheless, in transformation using the strain *Chaetomium* sp. KCh 6651 compound **2** was obtained in 97% yield (Table 1). However, in most of experiments after 3 h of biotransformation a decrease in concentration of dihydrochalcone **2** was observed. This was due to the reduction of compound **2** to the respective alcohol (**3**) (Scheme 1). As a result of the effective hydrogenation followed by the enantioselective reduction of C=O bond, after a three-day biotransformation in the culture of *R. rubra* KCh 4 alcohol (*S*)-**3** was obtained with 75% *ee* and 99% of dihydrochalcone (**2**) conversion (Fig. 1A). (*R*)-1,3-Diphenylpropan-1-ol ((*R*)-**3**) with 98% of *ee* and 97% of dihydrochalcone (**2**) conversion was also obtained in a 3-day biotransformation of **1** in the culture of *F. culmorum* KCh 10. In this case, however, the enantiomeric excess of alcohol **3** was changing in course of biotransformation and the content of the (*R*)-enantiomer was gradually increasing. In all the experiments small amounts of dihydrochalcone **2** were also observed. These results indicate that compound **2** is reduced by this strain with relatively low enantioselectivity, and the high *ee* of (*R*)-alcohol is the result of the subsequent oxidation of (*S*)-enantiomer of alcohol **3** back to dihydrochalcone **2** (Fig. 1B).

The change of enantiomeric excess of alcohol **3** during the biotransformation process was observed in the majority of tested strains. It was the most distinctive in the case of the strain *R. glutinis* KCh 242, where after 3 h of the reaction alcohol (*R*)-**3** was observed with 47% *ee* and 31% of conversion, whereas after 6 days product (*S*)-**3** was obtained with 99% *ee* and 91% of conversion (Fig. 1C).

Stereoinversion of compound **3** was also observed during incubation of substrate **1** in the culture of *D. igniaria* KCh 6670. In this case after 3 h of biotransformation 16% of alcohol (*S*)-**3** was visible in the reaction mixture with 36% of *ee*, but after 7 h it was the (*R*)-**3** enantiomer that was the dominant one. The enantiomeric excess

Table 1
Microbial hydrogenation of chalcones by selected yeasts and filamentous fungi strains.

Type of biocatalyst	Name of biocatalyst	Substrate number	Reaction time [h]	Substrate conversion [%]
Yeast	*Yarrowia lipolytica* KCh 71	**1**	1	99
		4	72	98
		6	1	62
	Rhodotorula glutinis KCh 242	**1**	1	98
		4	72	65
		6	1	100
	Rhodotorula rubra KCh 4	**1**	1	98
		4	72	63
		6	1	100
	Saccharomyces cerevisiae KCh 464	**1**	1	80
		4	72	98
		6	1	15
Filamentous fungi	*Syncephalastrum racemosum* KCh 105	**1**	1	6
		4	72	78
		6	1	11
	Chaetomium sp. KCh 6651	**1**	1	97
		4	72	76
		6	1	32
	Didymosphaeria igniaria KCh 6670	**1**	1	29
		4	72	58
		6	1	15
	Absidia coerulea KCh 93	**1**	1	31
		4	72	46
		6	1	16
	Fusarium culmorum KCh 10	**1**	1	84
		4	72	2
		6	1	31

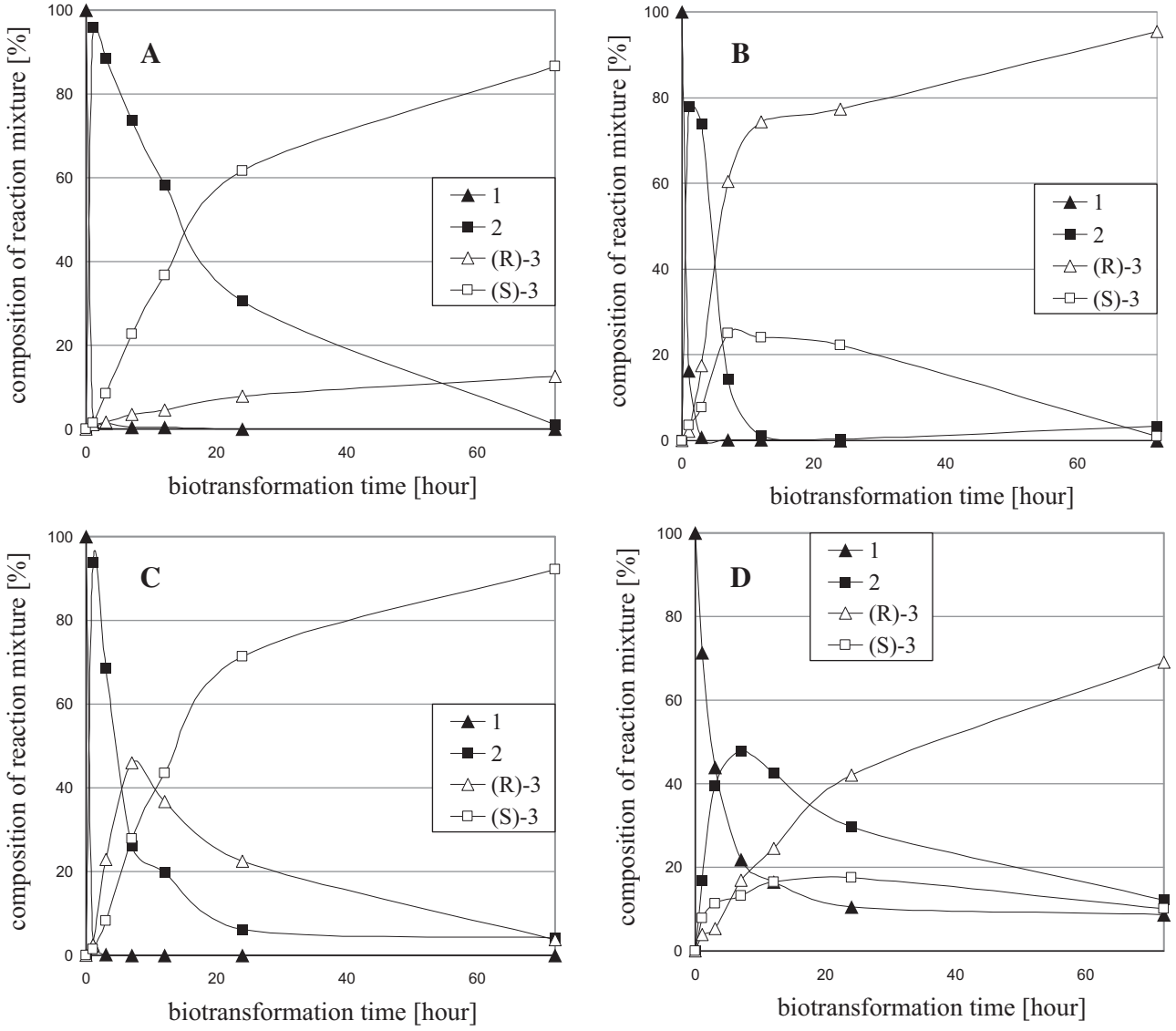


Fig. 1. Time dependence of the transformation of chalcone (**1**) in the culture of: (A) *Rhodotorula rubra* KCh 4; (B) *Fusarium culmorum* KCh 10; (C) *Rhodotorula glutinis* KCh 242; and (D) *Didymosphaeria igniaria* KCh 6670.

of (*R*)-1,3-diphenylpropan-1-ol ((*R*)-**3**) constantly increased with time and finally, after 9 days, reached the level of 90%. Moreover, this strain showed the lowest capability of hydrogenation of substrate **1** – only 9% after 3 days of the incubation. Among tested microorganisms comparably low rate of conversion was observed only for *S. racemosum* KCh 105, which after 3 days showed 7% of conversion (and after 12 h 86% of unreacted substrate **1** was still present in the reaction mixture). These lead to the conclusion that *S. racemosum* possesses substrate-induced enoate reductases of double bond. Formation of (*S*)-**3** was observed as late as in the 6th day of biotransformation in the culture of this strain (72% of the reaction mixture and 47% of *ee*).

In the culture of *Chaetomium* sp. KCh 6651 the efficient hydrogenation of **1** was observed, however, initially the reduction of dihydrochalcone (**2**) proceeded slowly (only 13% after 12 h), and the resulting alcohol was of low enantiomeric excess (20% *ee* of (*R*)-**3**). But after one day of biotransformation a considerable increase in the reduction rate was noted (59% of conversion of **2** and 10% *ee* of (*S*)-**3**). Further increase in the effectiveness of the reduction and inversion of configuration led to the (*S*)-**3** alcohol with 90% of conversion and 51% *ee*, after 3 days of incubation. A similar transformation course was observed for the strain *A. coerulea* KCh 93, where the effective conversion of **1** after 7 h was of 94%, however, in this case after 3 days it was only 7% of alcohol (*R*)-**3** present in the reaction mixture. After nine days of biotransformation compound (*R*)-**3** was obtained with 13% of conversion and 83% *ee*.

The next two strains – *Y. lipolytica* KCh 71 and *Saccharomyces cerevisiae* KCh 464 – performed the effective hydrogenation (Table 1), but the reduction of ketone **2** proceeded with even lower conversion efficiency than for the strains *S. racemosum* KCh 105 and *Chaetomium* sp. KCh 6651, described earlier. Moreover, in these cases longer incubation time did not improve the effectiveness of the reduction.

Hydrogenation of 2′-hydroxychalcone (**4**) (Scheme 1) proceeded much slower than of chalcone (**1**). After 1 h of incubation only the strain *Y. lipolytica* KCh 71 showed moderate efficiency of conversion – 46% (Fig. 2B), whereas in the culture of *Chaetomium* sp. KCh 6651 only 10% of conversion was observed and in other tested microorganisms the substrate conversion did not exceed 5% (Fig. 2C).

After 7 h of incubation in the culture of *Y. lipolytica* KCh 71 the conversion of substrate **4** reached 86%, in *Chaetomium* sp. KCh 6651 – 50%, and in *Saccharomyces cerevisiae* KCh 464 – 10%. The efficiency

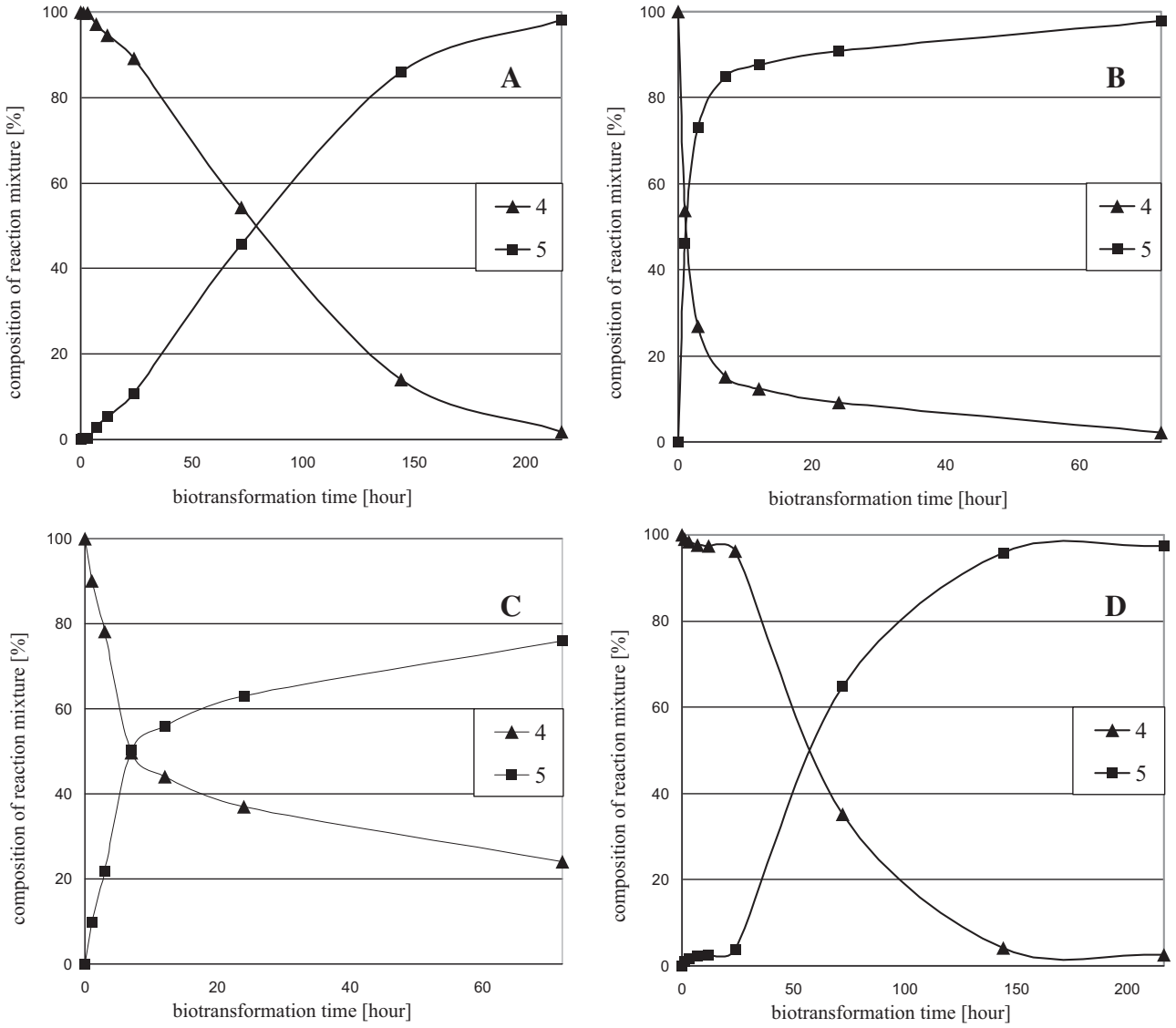


Fig. 2. Time dependence of the transformation of 2′-hydroxychalcone (**4**) in the culture of: (A) *Absidia coerulea* KCh 93. (B) *Yarrowia lipolytica* KCh 71; (C) *Chaetomium* sp. KCh 6651; and (D) *Rhodotorula glutinis* KCh 242.

of 2′-hydroxychalcone (**4**) conversion for all tested biocatalysts after 3 days of biotransformation is presented in Table 1. Unlike in the case of substrate **1**, for this substrate no products of reduction of the resulting 2′-hydroxydihydrochalcone (**5**) were observed in all tested microorganisms.

For most of the biocatalysts the course of biotransformation was similar to the one for *A. coerulea* KCh 93, presented in the graph (Fig. 2A). A different reaction course was observed for *Y. lipolytica* KCh 71, which is shown in Fig. 2B. This strain is capable of effective hydrogenation of the substrate after short incubation time. The data presented in Fig. 2B lead to the conclusion, that it possesses constitutive enoate reductase, involved in hydrogenation of the double bond of selected enones. Similar properties were observed for strain belonging to filamentous fungi – *Chaetomium* sp. KCh 6651 (Fig. 2C). However, in this case the hydrogenation was visibly inhibited and even after 9 days of incubation the conversion of substrate **4** did not exceed 90%.

A different course of hydrogenation of substrate **4** was observed in the culture of *R. glutinis* KCh 242 (Fig. 2D). In this case after the first day of biotransformation a small amount of product **5** was observed (4%). However, after longer incubation time its content distinctively increased, which indicates that this strain in the presence of the substrate produces a proper induced enzyme.

After nine days of incubation of substrate **4** in the cultures of all tested microorganisms, only in three cases the conversion was lower than 97%: for *R. rubra* KCh 4 and *Chaetomium* sp. KCh 6651 (89% and 90%, respectively), and for *F. culmorum* KCh 10 (only 5%). The last result was especially unexpected, because substrate **1** was transformed by this strain with the conversion of above 99% after 3 h of incubation. Because of observed significant differences in conversion rates of substrates **1** and **4**, arising from the difference in polarity associated with the presence of a hydroxyl group in compound **4**, we subjected to biotransformation another substrate – (*E*)-4-phenyl-but-3-en-2-one (**6**).

After 1 h of biotransformation substrate **6** was still present in most of the reaction mixtures, except for *R. glutinis* KCh 242 and *R. rubra* KCh 4 (Table 1). After 3 h the substrate was present in only two of the tested microorganisms. In the culture of *F. culmorum* KCh 10 after 3 h of incubation only 9% of the substrate was determined, which was fully consumed after 7 h. The slowest hydrogenation was observed in the culture of *Saccharomyces cerevisiae* KCh 464, where substrate **6** was completely transformed no sooner than after 3 days. (*E*)-4-Phenyl-but-3-en-2-one (**6**) similarly to substrate **1**

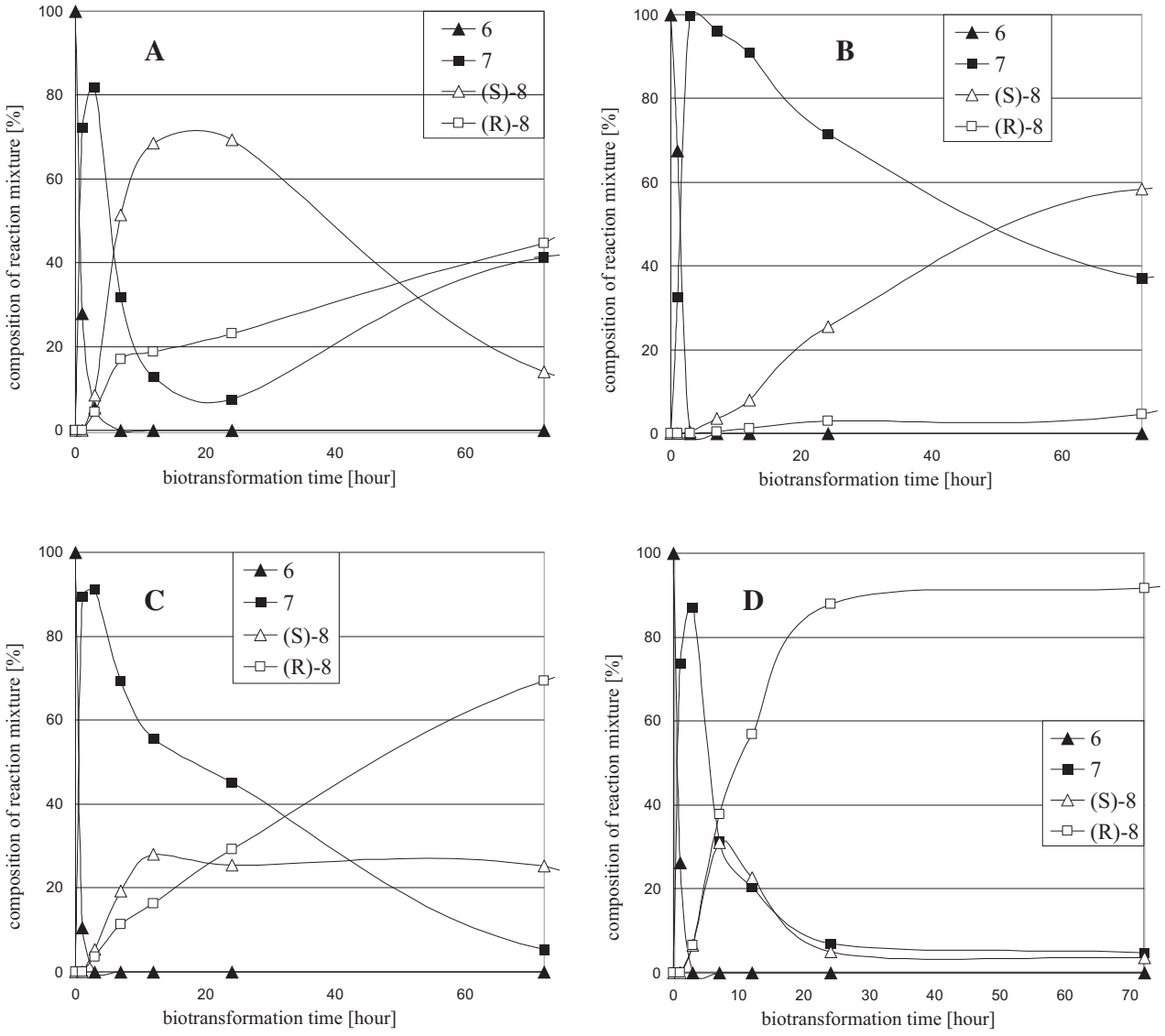


Fig. 3. Time dependence of the transformation of (*E*)-4-phenyl-but-3-en-2-one (**6**) in the culture of: (A) *Fusarium culmorum* KCh 10; (B) *Syncephalastrum racemosum* KCh 105; (C) *Yarrowia lipolytica* KCh 71; and (D) *Chaetomium* sp. KCh 6651.

underwent an effective hydrogenation in the cultures of almost all tested biocatalysts, except for *Saccharomyces cerevisiae* KCh 464. Similarly to substrate **1**, the hydrogenation was followed by the reduction of the resulting ketone **7**, to give the respective alcohol **8**. The reduction proceeded with a noticeable enantiospecificity and enantiomeric excess of the stereoisomeric alcohols gradually changed during the incubation time (Fig. 3).

In the culture of *F. culmorum* KCh after 12 h of biotransformation 4-phenylbutan-2-ol (**8**) made up 93% of the reaction mixture and with 50% *ee* was determined. After longer reaction time the content of the (*S*)-alcohol decreased, along with an increase in the (*R*) enantiomer content, and additionally, more 4-phenylbutan-2-one (**7**) was observed in the reaction mixture (Fig. 3A). After nine days of biotransformation (*R*)-4-phenylbutan-2-ol (**8**) was obtained with 99% *ee* and 60% of ketone **7** conversion.

Transformation of substrate **6** in the cultures of both strains of the genus *Rhodotorula* proceeded in a similar way. The effective hydrogenation was followed by the reduction of compound **7** to the (*S*)-alcohol (for *R. rubra* KCh 4 with 8% of conversion and 64% *ee* after 7 h, and for *R. glutinis* KCh 242 with 16% of conversion and 83% *ee* after 3 h). In both cultures, however, after 3 days the (*R*)-alcohol was the dominant one (for *R. rubra* KCh 4 with 30% of conversion and 50% *ee*, for *R. glutinis* KCh 242 with 30% of conversion and 30% *ee*). After longer time the reaction equilibrium was shifted towards the oxidation, which in the culture of *R. glutinis* KCh 242 after 9 days led to 98% of 4-phenylbutan-2-one (**7**) in the reaction mixture. The strain *R. rubra* KCh 4 after 9 days gave 83% of ketone **7** in the reaction mixture, whereas (*R*)-4-phenylbutan-2-ol (**8**) was characterized with 78% *ee*.

The effective reduction of **7** to the respective (*S*)-alcohol (63% of conversion, 85% *ee*) was observed also in the culture of *S. racemosum* KCh 105 (Fig. 3B). However, similarly to the strains of the genus *Rhodotorula*, longer reaction time resulted in a decrease in both effectiveness of conversion and enantiomeric excess of product **8** (17% *ee* after 6 days). The strain *D. igniaria* KCh 6670 gave (*S*)-4-phenylbutan-2-ol (**8**) with 83% *ee* and 93% of substrate conversion, but also in this case the enantiomeric excess of **8** gradually decreased.

The least effective reduction of **7** was observed for the strains *A. coerulea* KCh 93 and *S. cerevisiae* KCh 464. In the culture of *A. coerulea* KCh 93 in the result of initial reduction of substrate **7** to alcohol (*S*)-**8** (45%) with 30% *ee* were identified, but then it was

re-oxidized back to ketone **7**, which content in the reaction mixture reached 99% after 9 days of transformation.

In the case of *S. cerevisiae* KCh 464 the maximal observed conversion of **7** was 13%, affording (*S*)-**8** with 43% *ee*. The course of biotransformation of **6** in the culture of *Y. lipolytica* KCh 71 was different than in the case of substrate **1**. Except for the effective hydrogenation, an efficient reduction of ketone **7** was observed (Fig. 3C), which after 7 h gave the (*S*)-alcohol with 31% of conversion and 27% *ee*. Longer biotransformation time resulted in a considerable increase of the (*R*)-enantiomer content and finally led to (*R*)-4-phenylbutan-2-ol (**8**) with 91% of conversion and *ee* > 99%.

A nine-day incubation of **6** in the culture of the strain *Chaetomium* sp. KCh 6651 afforded pure (*R*)-4-phenylbutan-2-ol (**8**) as a single biotransformation product (Fig. 3D). This proceeded via a non-enantioselective reduction of ketone **7**, followed by an enantioselective oxidation of (*S*)-4-phenylbutan-2-ol (**8**). The analogous processes were observed for this strain during reduction of β-tetralone and chromanones [47,48].

4. Conclusions

Hydrogenation of chalcone **1** and its selected derivatives was faster in the cultures of yeasts. The reduction of dihydrochalcone is specific for each of the tested biocatalysts. This specificity was particularly evident when comparing the course of transformation of different substrates by the same biocatalyst. These results indicate that the tested biocatalysts are characterized with a very high substrate specificity. Changes in the substrate structures may activate different groups of enzymes of a catalyst used. However, in any of the biotransformation conducted, there was no formation of allyl alcohols. Two enzymes are involved in transformation process: enoate reductase which catalyzes the hydrogenation of C=C double bond and dehydrogenase converting the saturated ketone to alcohol. This second reaction is reversible and the enantioselective oxidation is observed resulting in the formation of enantiomerically enriched alcohol. A similar behavior has been described for the biocatalyst mediated reduction of α,β-unsaturated ketones, aldehydes and carboxylic esters [49–51].

As a result of our research we found an effective way to obtain 2′-hydroxydihydrochalcone (**5**) and dihydrochalcone (**2**). As a biocatalyst we employed the strain which is commonly used in food industry – *Y. lipolytica* KCh 71. Additionally, enantiomeric 1,4-diphenylpropan-1-ols (**3**) were obtained with excellent both conversion and enantiomeric excess. (*S*)-1,3-Diphenylpropan-1-ol ((*S*)-**3**) was obtained with 99% *ee* and 91% of conversion using the strain *R. glutinis* KCh 242, whereas the strain *F. culmorum* KCh 10 afforded (*R*)-1,3-diphenylpropan-1-ol ((*R*)-**3**) with 98% *ee* and 97% of conversion.

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