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Review

Yarrowia lipolytica Yeast: A Treasure Trove of Enzymes for Biocatalytic Applications—A Review

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Abstract: *Yarrowia lipolytica* is a robust yeast species that has gained significant attention as a biofactory for various biotechnological applications and undoubtedly can be referred to as a hidden treasure trove due to boasting a diverse array of enzymes with wide-ranging applications in multiple industries, including biofuel production, food processing, biotechnology, and pharmaceuticals. As the biotechnology field continues to expand, *Y. lipolytica* is poised to play a pivotal role in developing eco-friendly and economically viable bioprocesses. Its versatility and potential for large-scale production make it a promising candidate for sustainably addressing various societal and industrial needs. The current review article aimed to highlight the diverse enzymatic capabilities of *Y. lipolytica* and provide a detailed analysis of its relevance in biocatalysis, including the use of whole-cell catalysts and isolated enzymes. The review focused on wild-type yeast strains and their species-dependant properties and selected relevant examples of *Y. lipolytica* used as a host organism for overexpressing some enzymes. Furthermore, the application of *Y. lipolytica*'s potential in enantiomers resolution, lipids processing, and biodiesel synthesis, as well as the synthesis of polymers or esterification of different substrates for upgrading biologically active compounds, was discussed.

Keywords: *Yarrowia lipolytica*; lipases; whole-cell biocatalyst; biocatalysis; enzymes

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1. Introduction

Biocatalysis and biotransformation are pivotal processes in enzymatic catalysis, offering sustainable and efficient alternatives to traditional chemical methods. In simple words, biocatalysis involves the use of biological catalysts—enzymes—to facilitate chemical reactions, while biotransformation refers to the conversion of compounds by living organisms [1]. The utilization of biocatalysts presents numerous benefits over conventional chemistry, including milder reaction conditions and reduced environmental impact or energy consumption. Biocatalysts often exhibit high selectivity, resulting in fewer by-products and increased yields. And above all, they are also renewable and biodegradable [2]. Biocatalysts are classified into the following main types based on their nature and function: (a) isolated enzymes and (b) whole cells [3].

Yarrowia lipolytica is a versatile yeast species known for its broad and diverse application over the years, with its robust enzymatic capabilities and significant biocatalytic potential [4]. Moreover, the processes based on this yeast have GRAS (Generally Recognized As Safe) status, and the yeast biomass has been defined as a safe novel food by the EFSA (European Food Safety Authority), allowing it to be used as food for people over the age of 3, as well as a dietary supplement [5,6]. This species, formerly known as *Saccharomycopsis lipolytica* and *Candida lipolytica*, was named by van der Walt and von

Arx in 1980 as a way of acknowledging David Yarrow for identifying a new genus. The name "lipolytica" originates from the yeast's ability to hydrolyze lipids [7]. The history of discovering lipase in *Y. lipolytica* dates back to the early reports of lipase secretion in 1948 by Peters and Nelson [8]. Since then, lipase and esterase activities have been extensively studied in various strains of this yeast species. Moreover, yeasts of this species also biosynthesize a variety of enzymes beyond lipases, such as proteases or L-asparaginase, oxidoreductases, or enzymes of the cytochrome P450 family [9–13].

The unique enzymatic profile of *Y. lipolytica* yeasts positions them as a treasure trove of biocatalysts with immense potential for industrial applications. The current review article presents the fascinating world of biocatalysis, highlighting the importance of *Y. lipolytica* yeasts as a rich source of enzymes with great potential for driving biotransformation reactions (Figure 1).

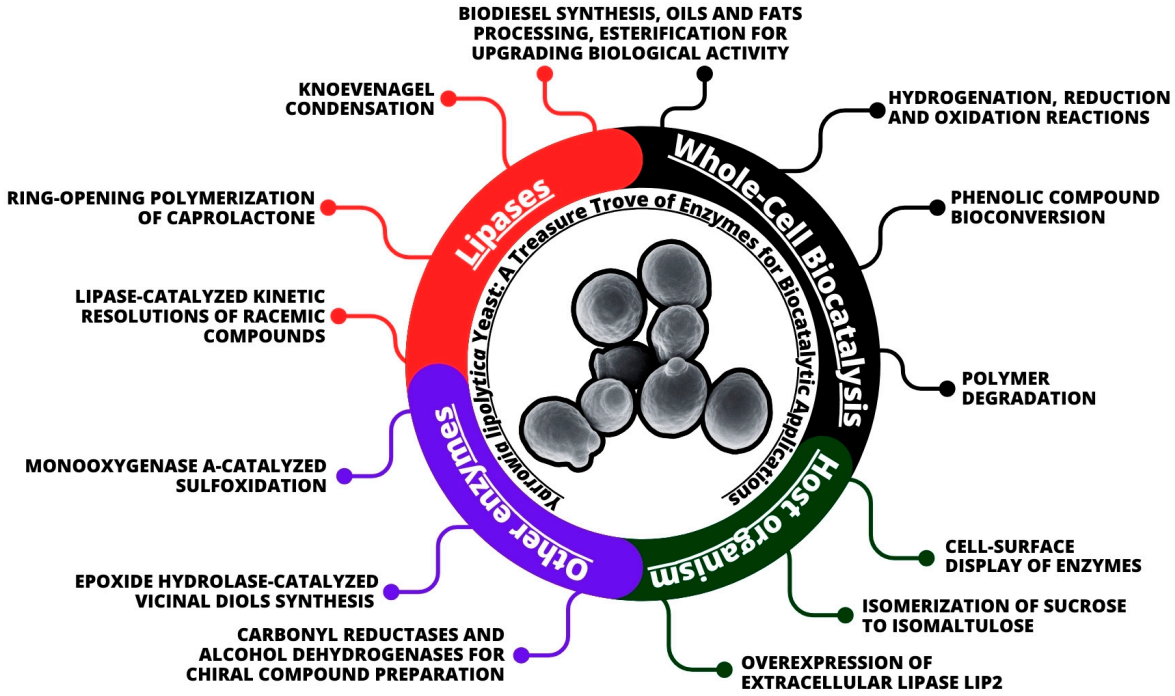


Figure 1. An overview of the biocatalytic application of *Y. lipolytica*.

This review highlights the prospects of employing biological catalysts, including whole-cell catalysts and isolated enzymes of *Y. lipolytica*. Furthermore, enantiomer resolution, lipid processing, biodiesel synthesis, and the synthesis of polymers or esterification of different groups of compounds via whole cells or certain enzymes of *Y. lipolytica* were discussed. The aim of this study was to gather existing knowledge on the enzymes produced by wild-type, unmodified strains and their applicability in biotransformations. The catalog of enzymes overexpressed in modified *Y. lipolytica* yeast cells is much broader than the examples presented in this review. This paper focuses exclusively on the applications of recombinant proteins in biotransformations, indicating the possibilities offered by the modification of strains of the yeast species in question.

2. Whole-Cell Biocatalysis with *Y. lipolytica* Cells

Whole-cell biocatalysis presents some advantages compared to other types of biocatalysis. It allows the use of the entire cell as a biocatalyst without requiring enzyme purification, significantly reducing costs. Furthermore, such a method is particularly advantageous in terms of cost-effectiveness, ease of preparation, and the ability to effectively perform several types of reactions [14]. Table 1 demonstrates the past application of whole cells of *Y. lipolytica* as a biocatalyst in various reactions, including hydrogenation, reduction, and oxidation [15–29].

Table 1. An overview of the application of *Y. lipolytica* whole-cells in the hydrogenation, reduction, and oxidation reactions.

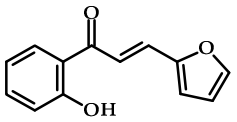
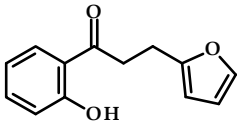
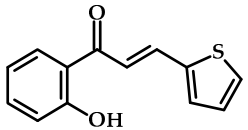
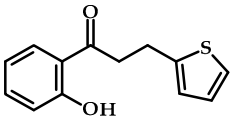
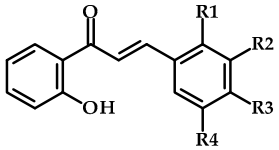
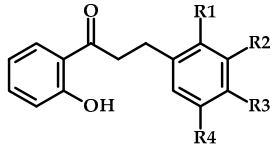
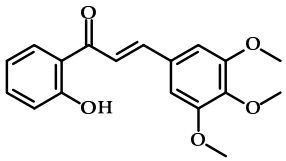
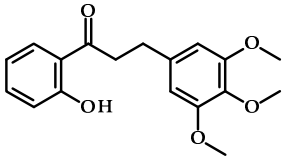
Substrate	Product	Conversion/Yield [%]	Enantiomeric Excess and Absolute Configuration	Reference
Reaction type—Hydrogenation (Bioreduction of C=C bond)				
 3-(2″-furyl)-1-(2′-hydroxyphenyl)-prop-2-en-1-one	 3-(2″-furyl)-1-(2′-hydroxyphenyl)-propan-1-one	88 (after 3 h); >99 (after 6 h)	-	[15]
 3-(2″-thienyl)-1-(2′-hydroxyphenyl)-prop-2-en-1-one	 3-(2″-thienyl)-1-(2′-hydroxyphenyl)-propan-1-one	67 (after 1 h); >99 (after 3 h)	-	
 2′-hydroxy-(di)methoxychalcones and corresponding dihydrochalcones; R1, R2, R3, and R4 = H or OCH_3		88–99 (after 1 h)	-	
 2′-hydroxy-3″,4″,5″-trimethoxychalcone	 2′-hydroxy-3″,4″,5″-trimethoxy-α,β-dihydrochalcone	7 (after 1 h); 20 (after 7 h)	-	[16]

Table 1. *Cont.*

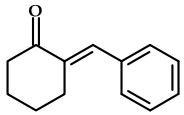
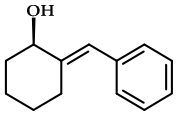
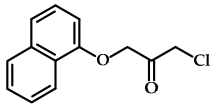
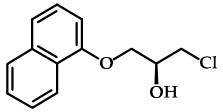
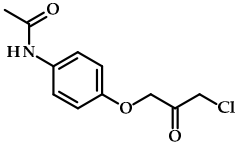
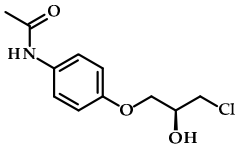
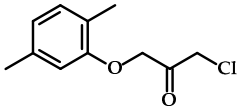
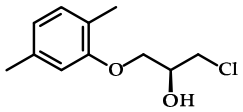
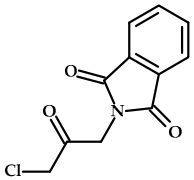
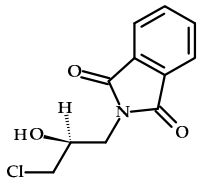
Substrate	Product	Conversion/Yield [%]	Enantiomeric Excess and Absolute Configuration	Reference
Reaction type—Reduction				
 2-benzylidenecyclohexanone	 (*R*)-2-benzylidenecyclohexanol	40 (after 24 h)	93%; *R*	[17]
 1-chloro-3-(1-naphthyloxy)propan-2-one	 (*S*)-1-chloro-3-(1-naphthyloxy)propan-2-ol	25–88 (after 48 h)	99%; *S*	[18–21]
 *N*-[4-(3-chloro-2-oxo-propoxy)phenyl]acetamide	 *N*-[4-[(*S*)-3-chloro-2-hydroxy-propoxy]phenyl]acetamide	90 (after 48 h)	90%; *S*	
 1-chloro-3-(2,5-dimethylphenoxy)propan-2-one	 (*S*)-1-chloro-3-(2,5-dimethylphenoxy)propan-2-ol	90 (after 48 h)	99%; *S*	[20]
 1-chloro-3-phthalimidopropan-2-one	 (*S*)-1-chloro-3-phthalimidopropan-2-ol	90 (after 48 h)	5%; *S*	

Table 1. *Cont.*

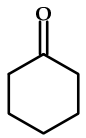
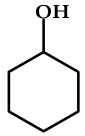
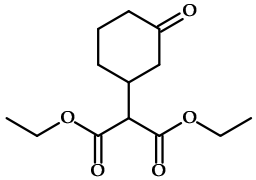
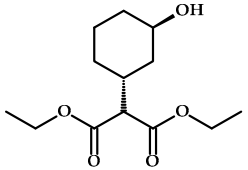
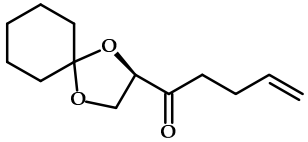
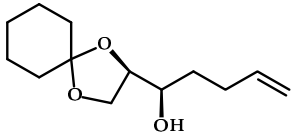
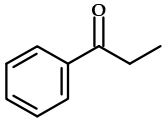
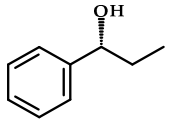
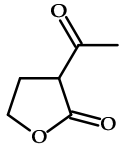
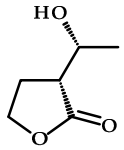
Substrate	Product	Conversion/Yield [%]	Enantiomeric Excess and Absolute Configuration	Reference
 Cyclohexanone	 Cyclohexanol	34–63 (after 48 h)	-	[21]
 diethyl 2-(3-oxocyclohexyl)malonate	 diethyl 2-[(*1R*,*3R*)-3-hydroxycyclohexyl]malonate	36 (after 9 h); 83 (after 21 h)	19% or 31%; *1R*,*3R*	[22]
 (*R*)-1,2-(cyclohexylidenedioxy)hept-6-en-3-one	 (*2R*,*3R*)-1,2-(cyclohexylidenedioxy)hept-6-en-3-ol	92 (after 48 h)	79%; *2R*,*3R*	[23]
 1-phenylpropan-1-one	 (*R*)-1-phenylpropan-1-ol	60–94 (after 9 days)	35–76%; *R*	[24]
 α-acetylbutyrolactone	 (*3R*,*1′R*)-α′-1′-hydroxyethyl-γ-butyrolactone	80–100 (after 24 h)	>99%; *3R*,*1′R*	[25]

Table 1. *Cont.*

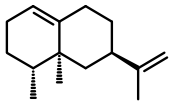
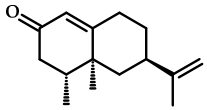
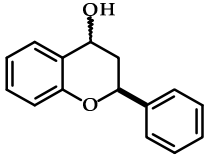
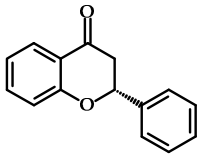
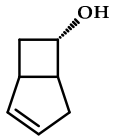
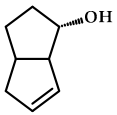
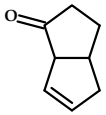
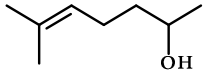
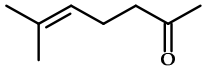
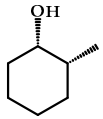
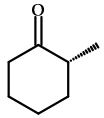
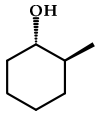
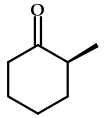
Substrate	Product	Conversion/Yield [%]	Enantiomeric Excess and Absolute Configuration	Reference
Reaction type—Oxidation				
		27.19–29.50 (after 12 days)	-	[26]
(+)-valencene	(+)-nootkatone	38.8–77.9 (after 120 h)	-	[27]
		52 (after 72 h)	85%; *R*	[28]
(±)-*trans*-flavan-4-ol	(*R*)-flavanone			
		40–43 (after 32 h)	82–100%; *1R,5S*	[29]
(*6S*)-bicyclo[3.2.0]hept-2-en-6-ol	(*1R,5S*)-bicyclo[3.2.0]hept-2-en-6-one			
		49 (after 48 h)	67%; *1R,5R*	
(*1S*)-bicyclo[3.3.0]oct-7-en-2-ol	(*1R,5R*)-bicyclo[3.3.0]oct-7-en-2-one			

Table 1. *Cont.*

Substrate	Product	Conversion/Yield [%]	Enantiomeric Excess and Absolute Configuration	Reference
 6-methylhept-5-en-2-ol	 6-methylhept-5-en-2-one	42 (after 72 h)	-	
 *cis*-2-methylcyclohexanol	 (*R*)-2-methylcyclohexanone	31–50 (after 48 h)	55–92%; *R*	[29]
 *trans*-2-methylcyclohexanol	 (*S*)-2-methylcyclohexanone	11 (after 48 h)	>95%; *S*	

Starting with hydrogenation as the first type of reaction that will be discussed, Łużny's papers [15,16] emerge at first glance on the example of dihydrochalcones synthesis—sweeteners, antiviral and antioxidant compounds. The first paper by Łużny et al. [15] explores the effective hydrogenation of 3-(2″-furyl)- and 3-(2″-thienyl)-1-(2′-hydroxyphenyl)-prop-2-en-1-one in selected yeast cultures. The authors reported a conversion of 88% after 3 h and almost 100% after 6 h when using the yeast *Y. lipolytica* for the hydrogenation of 3-(2″-furyl)-1-(2′-hydroxyphenyl)-prop-2-en-1-one. When the oxygen atom was replaced with a sulfur atom, the hydrogenation of 3-(2″-thienyl)-1-(2′-hydroxyphenyl)-prop-2-en-1-one proceeded with a similar conversion rate after 6 h. The authors reported that the discussed yeast species showed the highest conversion rate in both reactions. Moreover, these biotransformations were efficient and free from any byproducts. It is interesting to note that the results obtained were significantly higher than those achieved using *Saccharomyces cerevisiae* as a biocatalyst. This yeast is widely known for its use as a catalyst in oxidation-reduction reactions and double-bond reduction. The second paper [16] sheds light on the potential production of sweeteners with the use of *Y. lipolytica* cells via hydrogenation of methoxychalcones. The researchers found that the bioreduction of mono- and dimethoxychalcones, namely 2′-hydroxy-methoxychalcones, was successful, with conversion rates ranging from 88 to 98% depending on the substrate's chemical structure. However, the bioconversion of substrates to 2′-hydroxy-3″,4″,5″-trimethoxy-α,β-dihydrochalcone was significantly reduced to only 20% after 7 h when a third methoxyl group was present in the aromatic ring [16].

Reduction reactions are the second type of biotransformations carried out using whole-cell biocatalysts. They are usually associated with a change in the oxidation state, leading to the formation of alcohols from prochiral ketones. By using microorganism cultures, specific enantiopure forms of secondary alcohols can be achieved. Applying the aforementioned reaction in the synthesis of enantiopure compounds is a significant area of research in pharmaceutical sciences, where chiral alcohol may be produced with high optical purity [30].

The first mention of the use of *Y. lipolytica* yeast in bioreduction reactions and obtaining optically active alcohols appeared in 1997. Fuganti et al. [17] discussed the preparation of (*S*)-2-methoxycyclohexanone, a crucial intermediate in the synthesis of the antibiotic sanfetrinem. Interestingly, as the authors claimed, the most extraordinary result was achieved when *Y. lipolytica* was used, which, unlike other yeast species, allowed obtaining *R* enantiomer of 2-benzylidenecyclohexanol from 2-benzylidenecyclohexanone with a yield of 40% after 24 h and the enantiomeric excess (ee) of 93%.

1-chloro-3-(1-naphthyloxy)propan-2-one, which is an intermediate in the synthesis of propranolol, was also very often taken into consideration. The latter is a drug of the beta blocker class used as a treatment for high blood pressure. Between 2000 and 2005, four different publications focused on reducing this ketone to 1-chloro-3-(1-naphthyloxy)propan-2-ol using various yeast species. The researchers found that the reduction of the ketone to alcohol with an *S* configuration occurred for *Y. lipolytica* strains with an efficiency ranging from 25 to 88% in 48 h reactions, and also showed higher yields than *S. cerevisiae* commonly used for this reaction [18–21]. Lagos et al. [20] demonstrated also the possibility of using *Y. lipolytica* cells to obtain other compounds such as *N*-[4-[(*S*)-3-chloro-2-hydroxypropoxy]phenyl]acetamide, (*S*)-1-chloro-3-(2,5-dimethylphenoxy)propan-2-ol, and (*S*)-1-chloro-3-phthalimidopropan-2-ol from the corresponding ketones. This indicated the microorganism's usefulness in synthesizing (*S*)-adrenergic β-blockers.

Microbial enantioselective reduction of diethyl 2-(3-oxocyclohexyl)malonate was investigated by Olejniczak [22]. *Y. lipolytica* was not the most enzymatically active microorganism in this study, and it reduced the ketone to diethyl 2-[(*1R*,*3R*)-3-hydroxycyclohexyl]malonate with a yield of 83% after 21 h, but only with 31% enantiomeric excess. Among the microorganisms tested, *Absidia coerulea* AM 93 exhibited the highest enantioselectivity, and the author optimized the biotransformation process for this fungal species. In another study, *Y. lipolytica* JCM 21884 was evaluated as one of the twelve whole-cell catalysts in the reduction

of (*R*)-1,2-(cyclohexylidenedioxy)hept-6-en-3-one. The study found that *Y. lipolytica* JCM 21884, beside *Pichia angusta* and *Rhodotorula mucilaginosa*, had one of the highest conversion rates of 92% and a product ratio of (2*R*,3*S*) to (2*R*,3*R*) enantiomers of 21:79 [23].

It is worth noting how microbes transform ketones is strain-dependent. Janeczko & Kostrzewa-Susłow [24] compared 15 different fungal strains, including four strains of *Y. lipolytica* (1-101-1,31-K1, KCh 71, KCh 72, and ATCC 32-338 A). The authors found that these strains could effectively dehalogenate 3-chloro-1-phenylpropan-1-one within one hour of the reaction. The resulting yield of (*R*)-1-phenylpropan-1-ol ranged from 42% to 92%, with enantiomeric excess levels of 26% to 70%. When 1-phenylpropan-1-one was used instead of the chloro derivative, *Y. lipolytica* strains could still convert it into the alcohol mentioned above, with conversion rates between 60% and 94% observed after nine days. Mączka et al. [25] investigated the significance of stereoselectivity in the reduction of α-acetylbutyrolactone using yeasts as biocatalysts. The study found that *Y. lipolytica* P26A exhibited high enantioselectivity, producing (*3R*,*1′R*)-α'-1′-hydroxyethyl-γ-butyrolactone in just one day. Other strains, such as AM71 and AM72, could also convert the ketone functional group to a hydroxyl group, but with slightly lower conversion rates.

The oxidation reactions, which are opposite to reduction, were also studied using *Y. lipolytica* as a whole-cell biocatalyst. The reactions investigated were quite diverse. For instance, Palmerín-Carreño et al. [26] conducted a study on allylic oxidation. They examined the bioconversion of valencene, a sesquiterpene found in orange essential oil, into (+)-nootkatone, which is widely used as an active repellent, food additive, and cosmetic and pharmaceutical agent. Interestingly, the authors applied the surface cultures of six fungal strains, among which three were able to oxidize the substrate. Pure (+)-valencene, orange essential oil, and a biphasic system containing equal volumes of essential oil and phosphate buffer were tested, and no significant differences in the bioconversion were observed when the cultures of *Y. lipolytica* were evaluated.

The next step in the research on obtaining this valuable sesquiterpene concerned the bioconversion of the described substrate in bioreactor culture conditions [27]. The authors explained their approach through the low permeability of (+)-valencene across the yeast membranes and the observed subjection to product inhibition. To overcome such drawbacks of surface cultures, Palmerín-Carreño et al. [27] elaborated a three-phase partitioning bioreactor, i.e., aqueous–organic–air; hence, the orange essential oil was used as substrate and dispersed phase, and further extractant for the bioconversion product. Moreover, cell membrane permeabilization with cetyltrimethylammonium bromide (CTAB) and cofactor NADH regeneration with niacin improved the bioconversion. The essential oil was added after 33 h of yeast cultures at 30 °C, 200 rpm, and 0.5 vvm. Subsequently, after 120 h, the bioconversion peaked at 77.9% [27] compared to 27.19–29.50% after 12 days using surface cultures [26].

A unique performance of *Y. lipolytica* KCh 71 was observed by Janeczko et al. [28]. It was the only strain that did not perform the reduction of flavanone. On the other hand, only this strain effectively oxidized (±)-*trans*-flavan-4-ol to (*R*)-flavanone with a 52% yield and an ee of 85% after three days. Finally, Fantin et al. [29] described the possibility of kinetic resolution of racemic secondary alcohols through their oxidation to corresponding ketones with whole-cell biocatalysts based on *Y. lipolytica* cells. Both alicyclic and aliphatic secondary alcohol oxidations were screened within 11 *Y. lipolytica* strains. The YL2 yeast strain showed astonishing activity compared to the other strains, indicating the strain-depending properties. The following alcohols were oxidized to their ketones: 6-methylhept-5-en-2-ol, *cis*- and *trans*-2-methylcyclohexanols, (*1S*)-bicyclo[3.3.0]oct-7-en-2-ol, and (*6S*)-bicyclo[3.2.0]hept-2-en-6-ol (Table 1). The authors of the study highlighted that the oxidation process resulted in the kinetic resolution of the alcohols screened in the experiment. Although the yields of some ketones were meager (6–10%), the tested strains still managed to produce certain enantiomeric forms of alcohol [29].

Botezatu et al. [31] investigated the ability of different yeast strains to catalyze the synthesis of indolizine derivatives. The compounds of interest possess heterocyclic ring

cores and may exhibit precious biological activities. *Y. lipolytica* strains showed the slightest inhibition among the tested yeasts, which were *Candida robusta*, *C. tropicalis*, *C. utilis*, *S. cerevisae*, and *S. fibuligera*. Subsequently, three tested strains were applied in the "one-pot" biosynthesis with cycloaddition into bis-indolizines from 4,4′-bipyridine, ethyl propiolate, and varied 2-bromoacetophenones (Figure 2). The reactions were carried out in buffer solution at room temperature with the two forms of biocatalysts, i.e., biomass or cell-free supernatant.

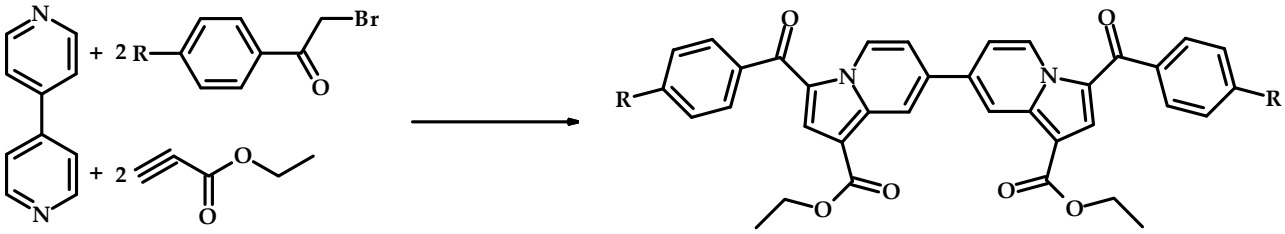


Figure 2. "One-pot" bis-indolizines biosynthesis with the use of *Y. lipolytica* biomass or cell-free supernatant [31]. R = H, OCH_3, or NO_2.

Interestingly, the highest bioconversion yield was observed in the presence of the cell-free supernatant of *Y. lipolytica* MIUG RD 14, which allowed us to obtain 1,1′-diethyldicarboxylate-3,3′-benzoyl-7,7′-bis(indolizine) with a yield of 77%. The authors claimed that the results may be correlated with the high extracellular lipase production of the yeast strain and the potential role of lipases in the biosynthesis of such compounds. In the case of the remaining strains, the bioconversion yields were significantly lower. At the same time, the application of biomass revealed that it can be used as a biocatalyst, but its reactions were weaker than the supernatant. Furthermore, the presence of methoxyl or nitro substituents impacted the final yields of bis-indolizines, which were about 50% [31]. On the other hand, Zieniuk et al. [32] investigated freeze-dried *Y. lipolytica* biomass and freeze-dried supernatant in ethyl hydrocinnamate synthesis. Contrary to the results cited, effective ester synthesis was conducted with the presence of a whole-cell biocatalyst, which suggested that the source of the enzyme determines the type of isoenzymes and enzyme natural immobilization, affecting the final conversion rate.

Y. lipolytica yeasts are well known for metabolizing, detoxifying, and degrading a dozen potentially harmful substances [33–35]. Since this review article concerns the biosynthesis of valuable compounds for use in various industries, the authors' attention is more focused on the metabolism of other groups of compounds, such as proteinogenic amino acids. In the research of Serra et al. [36], the strain *Y. lipolytica* DSM 8218 conducted a biotransformation of L-phenylalanine to 2-phenylethanol and phenylacetic acid (Figure 3). Under low aeration conditions, the yield of the latter two was 16.1%, while the concentration of phenylacetic acid was 0.47 g/L. However, increasing the volume of the Erlenmeyer flask up to 1 L and shaking it at 140 rpm resulted in a remarkable rise in yield to 53%. The titer of phenylacetic acid was almost 2 g/L with a ratio of <1:99 referring to alcohol and acid, respectively.

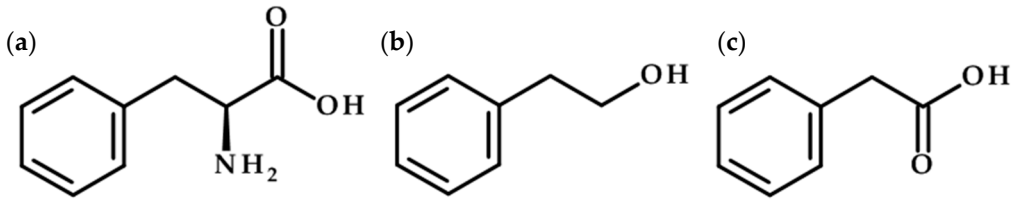


Figure 3. Chemical structures of (**a**) L-phenylalanine, (**b**) 2-phenylethanol, and (**c**) phenylacetic acid.

In addition, such an ability was also observed in other strains of *Y. lipolytica*, as well as within the genus of Yarrowia, including *Y. deformans*, *Y. yakushimensis*, *Y. bubula*, *Y. phangngaensis*, and *Y. brassicae*, both in flask and bioreactor cultures with yield ranging from 24%

up to 75%. It is worth noting that switching the L-enantiomer to the racemic mixture or D-phenylalanine caused a significant decrease in phenylacetic yield during the culturing of *Y. lipolytica* DSM 8218. Moreover, the authors also took into account two other aromatic amino acids, i.e., L-tyrosine and L-tryptophan. The biotransformation of the former amino acid led to obtaining of an analogous derivative and thus 4-hydroxyphenylacetic acid, while the application of L-tryptophan in the concentration of 5 g/L resulted in the biosynthesis of 2-aminobenzoic acid (anthranilic acid, 33.6%), indole-3-lactic acid (16%), and indolacetic acid (1.3%) [36].

Phenolic compounds are undoubtedly one of the most frequently studied biologically active substances. This group of compounds contains more than 5000 representatives, and within this group, a few classes can be distinguished, such as phenolic acids, stilbenes, lignans, or flavonoids [37]. The latter, and especially naringenin, became the subject of research, and in nature, the most abundant sources of naringenin are lemons and other citrus fruits. In line with the belief that biological activity, especially antioxidant activity, depends on the number and arrangement of hydroxyl substituents in the B ring of flavonoids, scientists are looking for simple, sustainable, and green methods of naringenin modification. In the study of Hernández-Guzmán et al. [38], in both surface and liquid cultures, *Y. lipolytica* 2.2ab grew and metabolized varying concentrations of naringenin in the culture medium (100–6000 mg/L). Two crucial enzymes of *Y. lipolytica* took part in the biomodification of naringenin. The first one, cytochrome P450 monooxygenase (CYP450), is an enzyme responsible for, e.g., hydroxylation, as well as reducing or oxidizing particular substrate. The second one involved within the described mechanism was the 2-oxoglutarate-dependent dioxygenase, possessing the activity of flavone synthase conducting the formation of C=C bonds. Comparing two types of cultures, six new compounds were identified in the surface approach, while when the authors applied the liquid cultures, one additional compound was present. The compounds obtained were arranged in order of their concentration as follows: apigenin > ampelopsin ≃ myricetin > aromadendrin > eriodictyol > luteolin > quercetin [38]. The suggested flavonoid pathway for bioconversion of naringenin is presented in Figure 4.

Halolactone biotransformations were studied by Wińska et al. [39]. In accordance with the argument that halogen compounds have a negative impact on the environment and living organisms, researchers were looking for a microbiological method of transforming these compounds along with the potential upcycling of these substances, i.e., their biotransformation into new compounds with interesting properties. Chloro-, bromo-, and iodolactones (Figure 5) were subjected to potential transformation in the presence of about twenty yeast and mold species. Among tested strains, only seven were able to modify some of the lactones, and two of them, i.e., *Fusarium equiseti* AM22 and *Y. lipolytica* AM71, exhibited the highest biotransformation efficiency with the observed production of hydroxylated derivative (2-hydroxy-6-methyl-9-oxabicyclo[4.3.0]nonan-8-one) of halolactones. Interestingly, the application of *Penicillium vermiculatum* AM30 led to the formation of new compounds with another hydroxylation pattern without the dehalogenation step. In the case of *Y. lipolytica*, it turned out that the yield of biotransformation depended on the halogen substituent, and conversions of 86.7, 59.3, and 27.4% were achieved for bromo-, iodo-, and chlorolactones, respectively [39].

As previously mentioned, the specific epithet of the described yeast species refers to its lipase production. Lipases are known for their hydrolytic activity, but unlike in an aqueous environment, these enzymes also catalyze reactions in organic solvents. Discovering this phenomenon is attributed to Zaks and Klibanov [40], who observed the possibility of lipase application in esterification, as well as amidation or thioesterification. It is also worth mentioning that the mechanism of enzymatic reactions catalyzed by lipases, the kinetics of these reactions, and the hydrolytic and synthetic activity of lipolytic enzymes were researched by Ernest Sym (1893–1950), a Polish biochemist, in the 1930s [41]. The application of purified lipases of *Y. lipolytica* will be described in detail in the next section, and the following examples refer to the use of whole-cell catalysts or cell-free supernatants.

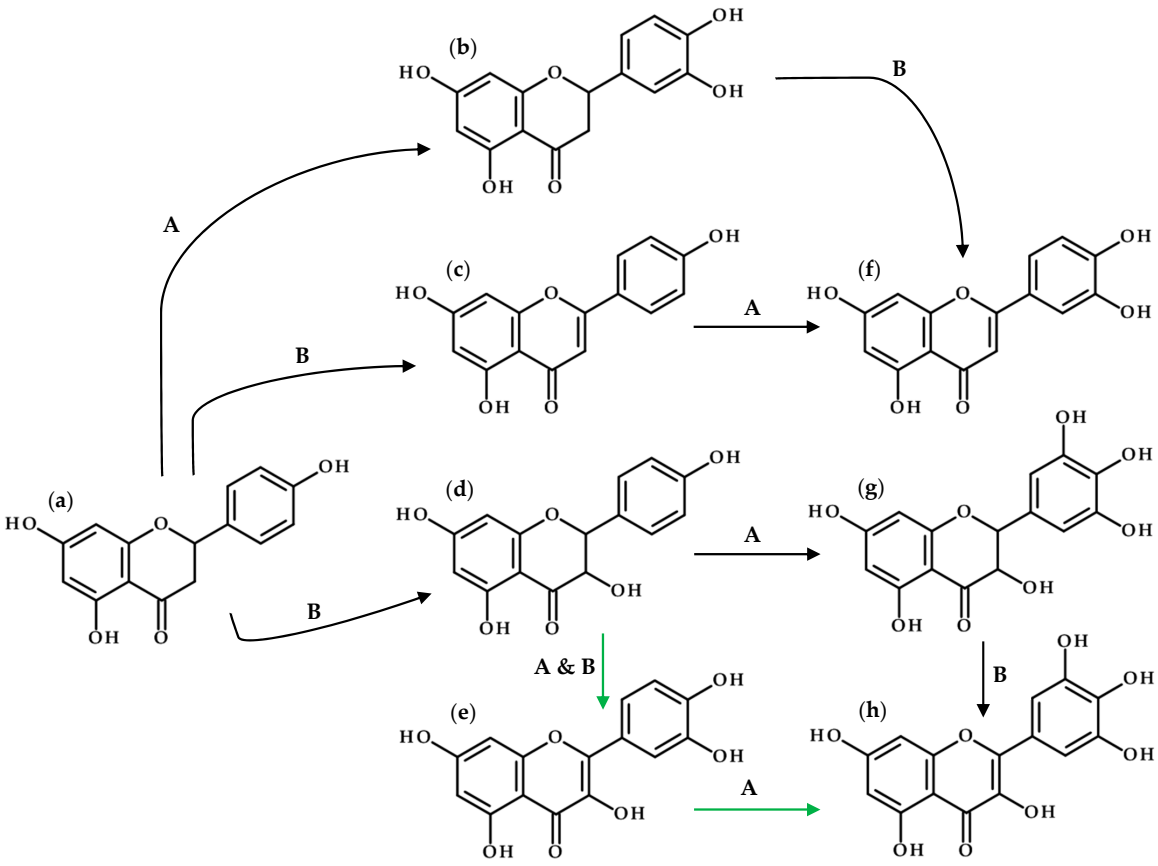


Figure 4. Proposed simplified pathway for naringenin bioconversion by *Y. lipolytica* 2.2ab (adapted and modified from [38]). Explanations: (a–h)—compounds, A–B—enzymes, i.e., (a)—naringenin, (b)—eriodictyol, (c)—apigenin, (d)—aromadendrin, (e)—quercetin, (f)—luteolin, (g)—ampelopsin, (h)—myricetin, (A)—CYP450 (cytochrome P450 monooxygenase), (B)—2-oxoglutarate-dependent dioxygenase. Green arrows indicated the process in liquid culture only.

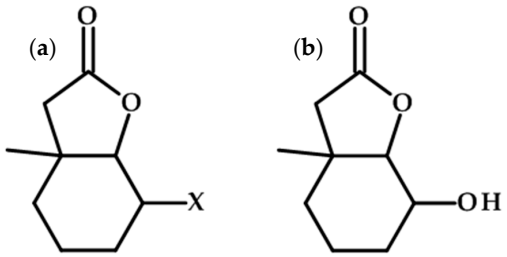


Figure 5. Chemical structures of (**a**) halolactones and (**b**) 2-hydroxy-6-methyl-9-oxabicyclo[4.3.0] nonan-8-one. X = Cl, Br, or I.

Over the last 30 years, *Y. lipolytica* yeast has been utilized for various applications, including the production of biodiesel [42], oils and fats processing [43], biosynthesis of aromas, antimicrobial or antioxidant esters [32,44–49], as well as polymer degradation [50,51].

Biodiesel is a type of fuel that is derived from renewable sources such as vegetable oils, animal fats, or recycled cooking oils. Typically, it is a mixture of methyl or ethyl esters of fatty acids that is synthesized by transesterifying triacylglycerols with a specific alcohol in the presence of a catalyst. In this process, *Y. lipolytica* yeast is commonly used in the form of whole-cell or extracellular lipase synthesized by the yeast. A study by da Silva et al. [42] developed a low-cost biocatalyst for the esterification of specific fatty acids with ethanol. The authors produced the biocatalyst by carrying out solid-state fermentation of soybean bran with *Y. lipolytica* IMUFRJ 50682, confirmed the lipase activity, and freeze-dried the

cultures. This preparation was then used for biodiesel synthesis based on the esterification of palmitic, stearic, oleic, and linoleic acids with ethanol. All tested fatty acids had a conversion yield of 79–90%. Furthermore, the authors demonstrated the possibility of reusing the biocatalysts for up to six reaction cycles [42].

Y. lipolytica is a source of both extracellular and intracellular hydrolytic enzymes, as well as cell-bound lipases. After ultrasonic pretreatment, the precipitated and centrifuged biomass, also known as cell debris, was used to hydrolyze milk fat. The lipolysis of anhydrous milk fat produced free fatty acids, including oleic, palmitic, and stearic acids, with a yield of around 28% after six hours of the process and applying 0.5 g of cell debris. The authors highlighted that the resulting biocatalyst has reduced production costs since post-frying oil can be used as the carbon source in the bioreactor culture, and immobilization is unnecessary [43].

Conventional methods for synthesizing or hydrolyzing organic esters typically involve strong acids like sulfuric acid. Alternatively, enzymes and microorganisms can be utilized for these purposes. In 1996, Molinari et al. [44] demonstrated that freeze-dried microorganisms, including bacteria and fungi, could effectively hydrolyze and esterify both linear and branched butyric acid esters. Of the 12 microorganisms tested, *Y. lipolytica* CBS 599 achieved the highest molar conversion rate of 95% during the hydrolysis of 2-octyl butyrate. However, during ester synthesis, *Aspergillus niger* and *Y. lipolytica* were not able to catalyze the synthesis of secondary alcohol esters. Additionally, *Y. lipolytica* exhibited alkyl chain-dependent synthetic activity. When comparing alcohols with 4 to 8 carbon atoms in their molecules, the molar conversion rate decreased with the increasing number of carbon atoms. The yields ranged from 58% for 1-butanol to 3% for 1-octanol at 60 °C [44].

Over the last dozen years, research carried out in the Department of Chemistry at the Warsaw University of Life Sciences demonstrated the applicability of *Y. lipolytica* biomass, either raw or freeze-dried, and lyophilized cell-free supernatant in the synthesis of aroma esters. The research has shown that isooctane-pretreated raw biomass of *Y. lipolytica* KKP 379 can be used as a biocatalyst in the synthesis of 2-phenylethyl acetate, a compound known for its pleasant rose aroma. The conversions of 72–78% were achieved within 2 h of reaction, regardless of the acyl donor used, whether ethyl acetate or vinyl acetate [45]. In another study by Zieniuk et al. [32], a compound with a floral–honey aroma, ethyl hydrocinnamate, was synthesized using freeze-dried biomass or supernatant. However, the latter exhibited significantly lower activity. The research team continued to study the application of this yeast species' biomass and revealed an unusual feature. *Y. lipolytica* biomass could not catalyze the esterification of phenylacetic acid and its hydroxyl derivative compared to *Candida antarctica* Lipase B. In the same paper, it was observed that *Y. lipolytica* biomass was more efficient in the synthesis of butyl 3-phenylpropanoate and butyl 3-(4-hydroxyphenyl)propanoate. Interestingly, the latter compound exhibited antioxidant and antifungal properties [48].

Two papers by Sales et al. [50,51] presented an alternative way to manage the urgent environmental problem of polyethylene terephthalate (PET) waste. PET is a petroleum-based synthetic polymer used as a packaging material in the beverage industry and is characterized by its rigidity and hardness. PET waste is a major environmental concern, with more than 30 million metric tons of annual global production [52]. In the study, a cell-free extract of *Y. lipolytica* was used for the biodegradation of PET. *Y. lipolytica* IMUFRJ 50682 was cultivated using solid-state fermentation in soybean bran supplemented with apple peels (source of cutin), commercial cork (source of suberin), or post-consumer PET as potential inducers of lipase and esterase production. The authors found that a 20% addition of cork or PET to the culture medium resulted in significantly increased activity of hydrolases. The source of suberin, i.e., cork, allowed for efficient bis(2-hydroxyethyl) terephthalate (BHET) hydrolysis, while PET biodegradation proceeded effectively when it was supplemented in the medium [50]. The subsequent study revealed that submerged cultures of *Y. lipolytica* resulted in better performance in PET depolymerization. Hydrolases were intensively synthesized when the media contained tryptone, and scaling up the

experiment to bioreactor cultures allowed for a 121% higher concentration of terephthalic acid in comparison with the flask cultures [51].

3. Exploiting the Catalytic Abilities of *Y. lipolytica* Lipases

The previous section discussed the reactions carried out using whole-cell biocatalysts. In many cases, it is known or suspected which class of enzymes is responsible for the reaction mechanism. This section specifically covers the application of purified lipases, mainly using Lip2p lipase as the biocatalyst. Lip2p is the primary extracellular enzyme of *Y. lipolytica*, consisting of 301 amino acids with a molecular mass of around 38 kDa. The proenzyme gene LIP2 was isolated and characterized in 2000 by Pignede et al. [53]. Along with Lip2p, other intracellular, extracellular, and cell-bound lipases, such as Lip7p, Lip8p, Lip9p, Lip11p, Lip12p, Lip14p, Lip18p, YlTgl4, and YlTgl3 have been identified over the past 20 years [48,49].

Enzymes are known for their specificity and ability to catalyze specific reactions. However, scientists worldwide are now searching for enzymes that can perform versatile or even promiscuous functions. The term "promiscuity" is commonly used in reference to lipolytic enzymes due to their unique potential for application, as highlighted in several review articles [54]. This potential is also evident in the lipase enzyme from *Y. lipolytica*, which has been found to catalyze, e.g., aldol condensation reactions, particularly the Knoevenagel condensation. This reaction is one of the most fundamental reactions in organic chemistry.

The authors have confirmed the synthesis of two compounds, namely ethyl (*Z*)-2-cyano-3-phenyl-prop-2-enoate and 2-benzylidenepropanedinitrile, which were produced via enzymatic Knoevenagel condensation [55]. The first compound was synthesized by reacting benzaldehyde with hydrolyzable ethyl 2-cyanoacetate, whereas the second compound was synthesized using non-hydrolyzable malononitrile (as shown in Figure 6).

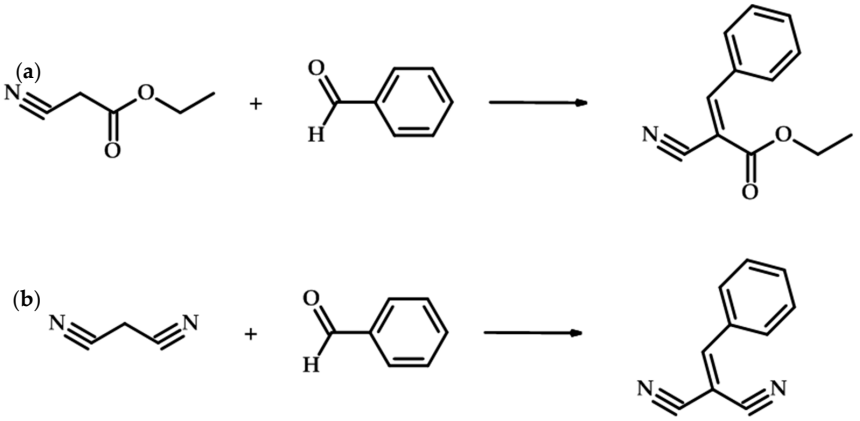


Figure 6. *Yarrowia lipolytica* lipase-catalyzed Knoevenagel condensation of (**a**) ethyl 2-cyanoacetate and benzaldehyde to ethyl (*Z*)-2-cyano-3-phenyl-prop-2-enoate or (**b**) malononitrile and benzaldehyde to 2-benzylidenepropanedinitrile.

The authors explained that the hydrolysis process was not involved in the mechanism of the enzymatic aldol condensation. Researchers used Lip2p from *Y. lipolytica* purified using ion exchange chromatography and hydrophobic interaction chromatography, as well as Amano lipase M from *Mucor javanicus* and porcine pancreas lipase as biocatalysts in a water–ethanol solvent system to catalyze the reaction between aldehyde and compounds with active hydrogen. The reactions achieved high yields, and the researchers found that the water content was crucial in determining the final yield. Depending on the reaction, a water content of 10–50% or 10–25% was necessary for high conversion [55].

Enzyme immobilization and solvent-free environments are beneficial for various industrial applications. The main benefits of enzyme immobilization include the ability to reuse and recover enzymes, increased stability, and more straightforward process control.

Solvent-free reactions are generally more efficient than solvent-based reactions and can simplify the purification process by eliminating the need to remove solvents. They can also reduce costs by avoiding the use of solvents and are more environmentally friendly by reducing waste and pollution. Meng's team used both in the enzymatic biodiesel synthesis [56,57]. *Y. lipolytica* lipase was immobilized on fabric membranes and applied in the esterification of oleic acid with ethanol. The authors revealed that the water content affected the final yield of ethyl oleate, and the stepwise addition of ethanol improved the enzyme performance. Under the optimal conditions, Meng et al. [56] produced 82 biodiesel batches with conversion rates of over 66.3%. The second study used a two-step protocol for biodiesel synthesis from soybean oil due to the presence of by-product glycerol during the direct transesterification of oil to fatty acid ethyl esters. In the first step, lipase was used to hydrolyze the soybean oil to obtain free fatty acids. The same immobilized lipase was used to esterify the free fatty acids to produce biodiesel in the second step. The reaction was carried out at 30 °C for 3 h, resulting in an 85% esterification yield. The process was repeated for 25 batches, and an over 82% esterification yield was maintained throughout all the batches [57]. He et al. [58,59] demonstrated the possibility of obtaining biodiesel via enzymatic transesterification with *Y. lipolytica* Lip2 lipase in the reaction with increased water content. To achieve higher yields within unfavorable conditions, the authors proposed to use a deep eutectic solvent based on choline chloride and glucose. Moreover, the authors applied an ultrasonic reactor, and the combined ultrasounds, deep eutectic solvents, and lipase-catalyzed reactions allowed for attaining 99% biodiesel conversion after 6 h and approximately 90% after 5 batch reactions. Cao et al. [60] carried out fundamental research for the potential commercialization of the enzymatic synthesis of biodiesel in the pilot scale. Firstly, the authors applied the Box–Behnken design and response surface methodology to optimize the reaction conditions. The application of five factors with three levels for each led to the employment of the following conditions: *Y. lipolytica* lipase dosage of 40 IU/g oil, a temperature of 39.4 °C, a water content of 1.95%, stepwise methanol addition (11.5% in 6 steps), and glucose addition in a ratio of 1:1.05 with lipase. With the optimized condition, the methanolysis of waste cooking oil containing more than 80% free fatty acids yielded 91.2% FAMEs, and the application of glucose increased the final yield by 10% compared to the control. Interestingly, computer-aided simulations have revealed that glucose acted as an agent that prevented methanol diffusion to the lipase and protein denaturation. Finally, the authors scaled up the enzymatic biodiesel synthesis to a 5-ton reactor. The FAME contents in the subsequent batch reactions were 91.4%, 89.5%, 83.4%, 82.5%, and 76.2%, respectively [60].

Lipases are versatile biocatalysts that enable the production of a wide range of structured lipids with tailored fatty acid compositions and positional distributions, which have various functional and health benefits. The choice of lipase used depends on its specificity, activity, and ability to catalyze the desired reactions. Numerous attempts to produce structured lipids have been made thus far regarding the Lip2p lipase. Structured lipids are synthesized through acidolysis reactions, and the source of free fatty acids is necessary for conducting such reactions. Fernandez-Lorente et al. [61] investigated the release of omega-3 fatty acids from sardine oil using different fungal and bacterial lipases immobilized on hydrophobic support octyl-Sepharose and Cyanogen bromide-activated-Sepharose. The Lip2p immobilized on octyl-Sepharose was the most unique biocatalyst due to its sevenfold increase in activity and tenfold increase in selectivity compared to the second carrier, and eicosapentaenoic acid (EPA) was released remarkably faster than docosahexaenoic acid (DHA) [61]. The specificity of lipases towards shorter and less unsaturated fatty acids played a crucial role in the production of DHA ethyl esters-rich concentrates. A study by Casas-Godoy et al. [62] revealed that Lip2p was selective for DHA release from tuna oil ethyl esters. The lipase easily hydrolyzed most of the esters (ethyl palmitate, stearate, oleate, and linolenate) with a conversion rate exceeding 90%. This resulted in an ethyl esters concentrate composed of 90% omega-3 and 77% DHA esters. Medium-chain triglycerides are naturally occurring in coconut and palm kernel oils. These are triacylglycerols with

at least one fatty acid residue of a maximum of 12 carbon atoms. This natural occurrence, coupled with their potential nutritional and pharmaceutical properties, underscores their safety and potential benefits. Furthermore, their synthesis can be accomplished with the use of lipases, which was presented in the study of Casas-Godoy et al. [63]. The study used immobilized *Y. lipolytica* Lip2p lipase as a biocatalyst for the acidolysis process of virgin olive oil with caprylic and capric acids. The researchers optimized the bioprocesses using response surface methodology approaches. They achieved incorporation yields of 25.6 mol% and 21.3 mol% for C8 and C10 fatty acids, respectively, after 48 h of reaction at 40 °C with a fatty acid to triacylglycerol ratio of 2:1 [63]. Additionally, the extracellular lipase of *Y. lipolytica* displays regioselectivity at the *sn*-1,3 position. The confirmation of these reactions was observed through nuclear magnetic resonance spectroscopy (^{13}C NMR) [64]. In a study conducted by Akil et al. [64], it was noted that the extracellular lipase was able to incorporate capric and lauric acids into the structure of triolein or olive oil when used as a biocatalyst. The study also revealed that increasing the ratio of free fatty acids to triacylglycerols positively affected the incorporation rate. Moreover, lipases immobilized in chitosan-alginate beads were able to perform up to five cycles without a significant decrease in activity [64]. In addition, the *sn*-1,3-regioselectivity of *Y. lipolytica* lipase made human milk fat substitute production possible. Milk fat is characterized by the presence of palmitic acid in the *sn*-2 position in the glycerol backbone, therefore, Zheng et al. [65] conducted the acidolysis of tripalmitin with oleic acid. The content of 1,3-dioleoyl-2-palmitoylglycerol reached 46.5%, and such a product may have a potential application in infant formulas.

Lipases produced by the yeast *Y. lipolytica* are extensively used in the kinetic resolution of racemic mixtures to obtain pure optically active compounds. These enzymes are responsible for the stereoselective reactions in which one enantiomer of a racemic mixture is converted into a product while the other enantiomer remains unchanged. The primary reactions catalyzed by these enzymes include hydrolysis or esterification. Over the years, researchers have used *Y. lipolytica* lipases to perform kinetic resolution on various substances. Some of these substances include 1-phenylethanol [66–68], 1-phenylethylamine [69], 2-bromo-arylacetic acid esters [70–74], (±)-perillyl alcohol [75], 2-octanol [76–79], and non-steroidal anti-inflammatory drugs such as ibuprofen and other profen esters [80,81].

In the process of kinetic resolution of (*R*, *S*)-1-phenylethanol, researchers discovered that extracellular lipase Lip2 showed a preference for the (*R*)-enantiomer. This led to the conversion of the (*R*)-enantiomer to its ester form while leaving the (*S*)-enantiomer unconverted. Zhou et al. [66] carried out transesterification using vinyl acetate with heptane as a solvent at 50 °C for 18 h. They used lipase in the immobilized form with bamboo charcoal as support and achieved a yield of 50% via resolution. Cui et al. [67] suggested using solvent engineering to enhance the enantiomeric excess in the resolution of this secondary alcohol after the esterification of caprylic acid. They tested six different co-solvents in small amounts of 1% (*v*/*v*) and discovered that while the use of cyclohexane, benzene, toluene, *tert*-butanol, and 1,4-dioxane negatively impacted the conversion yield, they significantly improved the enantiomeric excess and ratio. The concentration of 1,4-dioxane (0.8% *v*/*v*) was optimized, and the final enantiomeric excess increased to 99.1%, whereas in pure hexane, it was around 66% [67]. Syal and Gupta [68] compared the enantioselectivity of several other lipases of *Y. lipolytica*. They cloned and expressed the lipase genes *YLIP4*, *YLIP5*, *YLIP7*, *YLIP13*, and *YLIP15* in *E. coli*. These genes are also responsible for producing extracellular enzymes but are not as well-studied as Lip2. During the study on esterification reactions of capric acid with 1-phenylethanol, it was observed that lipases encoded by *YLIP4*, *YLIP5*, and *YLIP7* genes did not show any enantioselectivity. However, the remaining enzymes showed distinct properties. For instance, Lip13 exhibited (*S*)-enantioselectivity with 41% conversion and 53% ee, whereas Lip15 was selective for (*R*)-enantiomer and achieved a conversion of 63% and 36% ee [68]. The authors observed that changing the functional group from alcohol to the amino group did not affect the behavior of the Lip2 lipase in the resolution of 1-phenylethylamine. They applied co-solvent addition

to enhance the final enantiomeric excess. They found that the presence of 3% DMSO in hexane improved the enantiomeric excess of the product from 35% to 96% [69].

Extensive research is being conducted on 2-bromo-arylacetic acid esters because of their high value in the pharmaceutical industry. These compounds are used as precursors in the synthesis of painkillers and other drugs, which makes them essential for drug development. Since the first report in 2004 [70], scientists have been working to improve the enzymatic resolution of these compounds mediated by lipases of *Y. lipolytica*. One of the ideas was to use molecular biology methods, i.e., site-directed mutagenesis, and the amino acids forming the substrate binding site of the primary *Y. lipolytica* lipase were substituted. During the study, researchers obtained one and double-substituted variants of Lip2, which were successfully used to resolve 2-bromo-arylacetic acid esters. While most variants had similar hydrolytic activity to the wild-type lipase, their enantioselectivities were significantly altered. Cancino et al. [71] reported a 10-fold increase in resolution for 2-bromo-*o*-tolylacetic acid ethyl ester upon substitution of valine 232 with alanine, whereas the use of leucine instead of alanine led to the inversion of the enantiopreference in the lipase (from (*S*) to (*R*)) [72]. It was found that aspartic acid at position 97 is the second amino acid residue that significantly improves lipase properties. Cambon et al. [73] created six variants of *Y. lipolytica* Lip2 lipase, each with two substitutions. The most promising mutations were those where alanine and phenylalanine were substituted in positions 97 and 232, respectively. The authors achieved a reversed enantioselectivity and a 350% improvement in the activity towards kinetic resolution of (*R*, *S*)-2-bromo-phenylacetic acid octyl ester racemate [73].

Researchers often use enzyme immobilization, aggregation, interfacial activation, bioprinting techniques, solvent engineering, and extraordinary solvents like ionic liquids to improve the separation of racemic mixtures. The kinetic resolution of (*R*, *S*)-2-octanol was studied by Liu et al. [76], and a dozen modified biocatalysts based on *Y. lipolytica* lipase were applied in this process. Promoting activity approaches were used within this study, including the application of nonionic surfactants Tween-80 and Triton-X-100, polyethylene glycol 400 (PEG400) as a way of interfacial activation, and the addition of *R*-2-octanol as the imprinted molecule to induce conformational changes in the enzyme. Subsequently, some enzyme preparations were immobilized, and all were compared in the transesterification of 2-octanol with vinyl acetate. It was revealed that combining all methods resulted in the highest transesterification activity and enantioselectivity and allowed for at least ten reuse cycles [76]. The use of ionic liquids, which have an ionic composition, high viscosity, and low vapor pressure, is an effective method in enzyme-mediated processes for biocatalytic applications and they are considered eco-friendly solvents. The ionic liquid-dependent activity of *Y. lipolytica* lipase was observed during (*R*, *S*)-2-octanol resolution. The tested lipase was active in 9 out of 15 ionic liquids used, where the application of 1-ethyl-3-methylimidazolium tetrafluoroborate ([EMIM]BF_4) led to the highest observed transesterification activity, while 1-methyl-3-octylimidazolium tetrafluoroborate ([OMIM]BF_4) was found to affect the highest enantioselectivity. A co-solvent strategy with an equal ratio of both ionic liquids resulted in 45% conversion with 99% ee and enantioselectivity of 501 [77]. Ibuprofen ((*R*, *S*)-2-(4-(2-methylpropyl)phenyl)propanoic acid) is a well-known chiral, nonsteroidal, anti-inflammatory drug, which (*S*)-enantiomer is approximately 160-fold more active than the (*R*)-enantiomer. An attempt to obtain an optically active form of the drug was proposed by Liu et al. [80], where the proper (*S*)-enantiopreference was observed when immobilized lipase of *Y. lipolytica* was applied. Then, in the hydrolysis of ethyl esters of ibuprofen, naproxen, and ketoprofen, Lip2p showed significantly higher activity compared to *C. rugosa* lipase. The low selectivity towards the preferred enantiomers was resolved by site-directed mutagenesis, where Gérard et al. [81] found that substituted amino acid residues 232 and 235 remarkably improved the enantioselectivity and initial rates of hydrolysis.

Lipases from the discussed yeast species were often catalysts in the synthesis of esters with biological properties and potential food and pharmaceutical applications (Figure 7). Esters of isosorbide are commonly used as surfactants, emulsifiers, and plasticizers in the

detergent and cosmetic industry. Because these compounds are biodegradable, scientists are trying to find sustainable methods for synthesizing them. Interestingly, the enantioselectivity of lipases hampered the synthesis of diesters, which was the focus of research conducted by Cui et al. [82]. The syntheses of isosorbide dioleate (Figure 7a) were carried out with immobilized lipases from *Y. lipolytica*, *C. antarctica* (Novozym 435), and *Rhizomucor miehei*. Tested lipases exhibited (*R*)-enantioselectivity, and in the case of Lip2 and *R. miehei* lipases, mainly monoesters were obtained, while the application of Novozym 435 led to comparable production of (*R*)-ester and diester of isosorbide. It is worth noting when Lip2 and Novozym 435 were used sequentially as catalysts, the diester content reached 77.4%, which was significantly higher than when either catalyst was used individually [82].

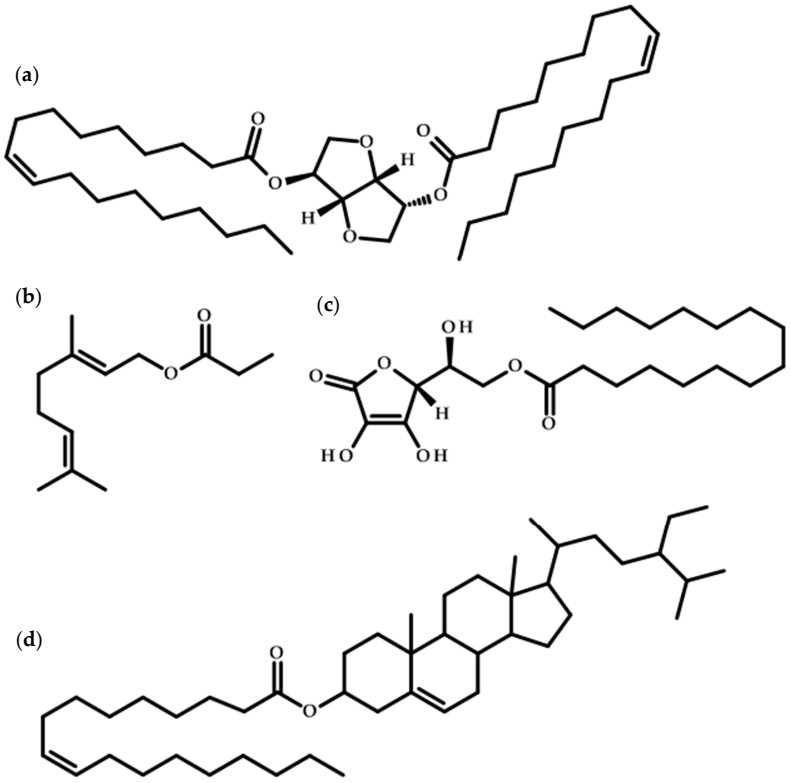


Figure 7. Examples of the compounds synthesized through *Y. lipolytica* lipase-catalyzed esterification: (**a**) isosorbide dioleate, (**b**) geranyl propionate, (**c**) L-ascorbyl palmitate, (**d**) phytosterol oleate.

The benefits of enzymatic processes can also be helpful for the cosmetics industry in obtaining fragrance compounds that are not cost-effective to extract from natural sources due to their complex compositions or low concentration in plant material. On the other hand, some of the substrates in the enzymatic reactions, such as short-chain fatty acids, may affect lipase activity. To overcome such difficulties and to improve the final yield of the enzymatic process, Tang et al. [83] applied a continuous stirred tank reactor for the lipase-catalyzed synthesis of geranyl propionate (Figure 7b). The authors initially chose the optimal solvent (isooctane) and reaction time (3.5 h) for the biocatalytic reaction. Afterward, they modified the reaction setup from batch to continuous and utilized immobilized Lip2 lipase as a biocatalyst. This modification resulted in an approximately 72% conversion yield. Notably, the steady state was maintained for at least 12 h [83].

Research on the synthesis of value-added compounds for food and nutrition applications was also undertaken. Ping et al. [84] studied the biosynthesis of L-ascorbyl palmitate (Figure 7c). Vitamin C is a commonly used antioxidant agent in varied products, but its hydrophilic nature limits its use in lipid-rich matrices. Fatty acid esters of L-ascorbic acid are commercially available lipophilic derivatives of this molecule. Enzymatic lipophilization of L-ascorbic via esterification of palmitic acid was accomplished through the use of

the extracellular lipase of *Y. lipolytica*. The authors compared the wild-type strain to one subjected to mutation breeding after implantation of the low-energy ion beam. The activity of the obtained strain was increased 5.5-fold. Similarly, its application in the synthesis turned out to be successful, with a product concentration of 27.5 g/L in comparison with the wild-type lipase (14.8 g/L) [84].

Sterol and fatty acid esters are other essential compounds with improved solubility in fats and oils. They exhibit health benefits by lowering cholesterol levels in the blood. Their biosynthesis in a large-scale 5 L bioreactor was studied by Cui et al. [85]. The esterification of the phytosterol mixture and oleic acid was conducted in a solvent-free system with either free or immobilized Lip2 lipase. Among five tested inorganic supports, celite significantly improved the reaction yield of phytosterol oleate (Figure 7d) to 98.8% and decreased reaction time to 78 h compared to free lipase (89.1% after 110 h).

Barrera-Rivera's team conducted extensive research on using lipases from *Y. lipolytica* to synthesize various polymers [86–93] (Table 2). The yeast *Y. lipolytica*, specifically its extracellular lipases, showed promising catalytic activity in the poly(ε-caprolactone) synthesis by ring-opening polymerization of ε-caprolactone. When Barrera-Rivera et al. [86] applied freeze-dried *Y. lipolytica* supernatant with high lipase activity, temperature-dependent conversions were observed. A temperature of at least 55 °C had to be used to achieve complete monomer conversion. The polymers obtained in heptane at 60–70 °C after 15 days of reaction were characterized by low molecular weights (660–975 Da), melting points of 50.7–59.58 °C, and high crystallinity in the range of 54–78.6% [86]. It is worth emphasizing that the green chemistry method, i.e., using ionic liquids instead of organic solvents, significantly increased the molecular weight of the obtained polymers while shortening the reaction time. Of the five tested ionic liquids, 1-butylpyridinium tetrafluoroborate ([BuPy][BF_4]) turned out to be the best medium for carrying out the polymerization, resulting in the production of polymer with a molecular weight exceeding 8000 Da after 24 h at 60 °C [87]. Barrera-Rivera and Martinez-Richa [88] also studied the production of amphiphilic polymers bearing an isosorbide headgroup. This time, lipases were subjected to immobilization on varied supports, and then, temperature and the addition of isosorbide were optimized. Two macroporous resins, i.e., Lewatit VP OC 1026 and Lewatit K-2629, were found to be the most suitable supports for the highest reaction rates. The polymer synthesized with the use of *Y. lipolytica* lipase immobilized on VP OC 1026 had a molecular weight of 1068 Da, crystallinity of 60.83%, and crystallization temperature of approximately 9 °C, and it was confirmed that the isosorbide headgroup was present in the structure of the obtained polymer [88]. Furthermore, immobilized *Y. lipolytica* lipase was used to synthesize dendritic polymers, i.e., dendrimeric and hyperbranched polymers composed of adipic acid and glycerol. Synthesized polymers could find their application in medicine, and their synthesis via enzymatic polymerization does not require protection and deprotection steps compared to conventional methods and is realized as a one-pot reaction [89]. Then, the chemoenzymatic method for the synthesis of polyester–urethane polymers was elaborated. Within the study, Barrera-Rivera et al. [90] studied the preparation of poly(ε-caprolactone) diols firstly, with diethylene glycol or PEG of different molecular weights (200, 400, 1000). The fully enzymatic process allowed the synthesis of varied polymers, differing in molecular weight ranging from 836 to 2504 Da, resulting in the production of soft waxes and hard solid polymers. Urethane copolymers were then produced by the reaction of particular polycaprolactone diols with hexamethylene diisocyanate and tin 2-ethyl hexanoate as a catalyst [90]. Researchers continuing their research in the subsequent work [91] demonstrated the possibility of using 1,3-propanediol to produce modified polycaprolactone. They then made a urethane copolymer with excellent elastomeric and mechanical properties [91]. Interestingly, the highest molecular weight ever observed in the studies of Barrera-Rivera's team was 10685 Da (measured by ^{1}H NMR). *Y. lipolytica* lipases were immobilized on Lewatit VP OC 1026, which led to the successful synthesis of poly(ε-caprolactone) in decane as a solvent. The reaction was carried out for 60 h at 90 °C, resulting in a 100% conversion [93].

Table 2. An overview of polymers and their properties synthesized using *Y. lipolytica* lipases.

Synthesized Polymer	Immobilization Support	Reaction Conditions		Yield (%)	Molecular Weight (Da)	Degree of Crystallinity (%)	Reference
Poly(ε-caprolactone)	-	3 mmol of ε-caprolactone and 100 mg of lipase; heptane as a solvent	360 h, 60 °C	100	620/975 [a]	78.6	[86]
			120 h, 65 °C	100	608/734 [a]	76.0	
			264 h, 70 °C	100	540/660 [a]	54.0	
		17.52 mmol of ε-caprolactone and 100 mg of lipase; [BuPy][BF_4] as a solvent	24 h, 60 °C	100	8000/8158 [b]	74.0	[87]
		13.14 mmol of ε-caprolactone and 100 mg of lipase; [BuPy][BF_4] as a solvent	16 h, 100 °C	100	1808/2340 [b]	64.0	
		35 mmol of ε-caprolactone and 100 mg of lipase; [EMIM][BF_4] as a solvent	16 h, 100 °C	100	837/1823 [b]	76.0	
		22 mmol of ε-caprolactone and 100 mg of lipase; [BMIM][BF_4] as a solvent	16 h, 100 °C	100	1377/1758 [b]	55.0	
		35 mmol of ε-caprolactone and 100 mg of lipase; [BuPy][CF_3COO] as a solvent	16 h, 100 °C	100	1158/1734 [b]	66.0	
		43.8 mmol of ε-caprolactone and 100 mg of lipase; [EMIM][NO_3] as a solvent	24 h, 90 °C	100	1172/2843 [b]	68.0	
		43.8 mmol of ε-caprolactone and 100 mg of lipase; [BuPy][CF_3COO] as a solvent	24 h, 90 °C	100	1300/2603 [b]	71.0	
		52.57 mmol of ε-caprolactone and 100 mg of lipase; [BuPy][BF_4] as a solvent	6 h, 150 °C	100	3250/3788 [b]	56.0	
		35.04 mmol of ε-caprolactone and 100 mg of lipase; [BMIM][BF_4] as a solvent	6 h, 150 °C	100	2699/3092 [b]	58.0	
		52.57 mmol of ε-caprolactone and 100 mg of lipase; [EMIM][BF_4] as a solvent	6 h, 150 °C	100	2426/2787 [b]	55.0	
		52.57 mmol of ε-caprolactone and 100 mg of lipase; [BuPy][CF_3COO] as a solvent	6 h, 150 °C	100	2693/2953 [b]	53.0	
Poly(ε-caprolactone) with an isosorbide headgroup	Lewatit 1026	1 mmol of ε-caprolactone, 0.125 mmol of isosorbide and 12 mg of lipase	94 h, 80 °C	100	1068 [c]	60.8	[88]
	Lewatit K2629		24 h, 90 °C	100	ND	30.1	
Poly(ε-caprolactone)	Lewatit	1.08 mmol of ε-caprolactone and 10% (*w*/*w*) of lipase	6 h, 150 °C	74	1358 [d]	ND	[89]
	Accurel			3	653 [d]	ND	
Dendritic polymers composed of adipic acid and glycerol	Lewatit	40 g/L of adipic acid and glycerol, 5 mL of *tert*-butanol as a solvent, 100 mg of lipase	48 h, 50 °C	ND	ND	ND	

Table 2. *Cont.*

Synthesized Polymer	Immobilization Support	Reaction Conditions		Yield (%)	Molecular Weight (Da)	Degree of Crystallinity (%)	Reference
Poly(ε-caprolactone) diols with diethylene glycol	Lewatit 1026	10 mmol of ε-caprolactone, 1 mmol of diethylene glycol, and 12 mg of lipase	6 h, 120 °C	ND	4321/1363/836 [e]	ND	[90]
		10 mmol of ε-caprolactone, 0.5 mmol of diethylene glycol, and 12 mg of lipase			5101/1978/1305 [e]	ND	
		10 mmol of ε-caprolactone, 0.25 mmol of diethylene glycol, and 12 mg of lipase			7426/2429/1780 [e]	ND	
PEG-ε-caprolactone copolymers		10 mmol of ε-caprolactone, 1 mmol of PEG200, and 12 mg of lipase	6 h, 120 °C	ND	3817/974/1066 [e]	ND	
		10 mmol of ε-caprolactone, 1 mmol of PEG400, and 12 mg of lipase			4083/1120/1211 [e]	ND	
		10 mmol of ε-caprolactone, 1 mmol of PEG1000, and 12 mg of lipase			4481/971/2504 [e]	ND	
Poly(ε-caprolactone) diols with 1,3-propanediol	Lewatit 1026	10 mmol of ε-caprolactone, 0.5 mmol of 1,3-propanediol, 12 mg of lipase	6 h, 120 °C	100	5475 [f]	ND	[91]
		10 mmol of ε-caprolactone, 0.25 mmol of 1,3-propanediol, 12 mg of lipase		100	5922 [f]	ND	
	Lewatit K2629	10 mmol of ε-caprolactone, 1 mmol of 1,3-propanediol, 12 mg of lipase		100	3755 [f]	ND	
		10 mmol of ε-caprolactone, 0.5 mmol of 1,3-propanediol, 12 mg of lipase		100	4099 [f]	ND	
PEG-ε-caprolactone copolymers	Lewatit VPOC K2629	10 mmol of ε-caprolactone, 1 mmol of PEG200, 12 mg of lipase	12 h, 120 °C	~100	845/2610 [b]	ND	[92]
	Amberlyst15			~100	856/3892 [b]	ND	
Poly(ε-caprolactone) diols with diethylene glycol	Lewatit VPOC K2629	10 mmol of ε-caprolactone, 0.5 mmol of diethylene glycol, 12 mg of lipase		~100	553/3602 [b]	ND	
	Amberlyst15	10 mmol of ε-caprolactone, 1 mmol of diethylene glycol, 12 mg of lipase		~100	631/2478 [b]	ND	
Poly(ε-caprolactone) diols with ethylene glycol	Lewatit VPOC K2629	10 mmol of ε-caprolactone, 1 mmol of ethylene glycol, 12 mg of lipase		~100	617/2719 [b]	ND	
	Amberlyst15			~100	743/4005 [b]	ND	

[a]—obtained by MALDI-TOF/^{1}H NMR; [b]—obtained by ^{1}H NMR/GPC-MALLS; [c]—obtained by MALDI-TOF; [d]—obtained by ^{1}H NMR; [e]—obtained by GPC/MALDI-TOF/^{1}H NMR; [f]—obtained by GPC; Abbreviations: MALDI-TOF—Matrix-Assisted Laser Desorption Ionization-Time Of Flight, ^{1}H NMR—Proton Nuclear Magnetic Resonance, GPC-MALLS—Gel Permeation Chromatography with Multi-Angle Light Scattering Detection, ND—No Data, [BuPy][BF_4]—1-butylpyridinium tetrafluoroborate, [BuPy][CF_3COO]—1-butylpyridinium trifluoroacetate, [BMIM][BF_4]—1-butyl-3-methylimidazolium tetrafluoroborate, [EMIM][BF_4]—1-ethyl-3-methylimidazolium tetrafluoroborate, [EMIM][NO_3]—1-ethyl-3-methylimidazolium nitrate.

4. Versatile Biocatalytic Potential—Other Enzymes

Leaving the topic of lipases and considering other enzymes of the yeast *Y. lipolytica* with high biocatalytic potential, reductases, dehydrogenases, monooxygenases, and epoxide hydrolases certainly need to be mentioned. Three of the enzymes are oxidoreductases, i.e., catalyzing oxidation/reduction reactions [94–99], and the fourth one is epoxide hydrolase, which, as its name suggests, catalyzes the hydrolysis of epoxide residue, resulting in the formation of diols [100,101].

NADPH-dependent carbonyl reductase from *Y. lipolytica* was an efficient biocatalyst to synthesize chiral alcohols from aldehydes, ketoesters, and ketones, primarily substituted acetophenones. In the case of aldehydes, the highest activity was observed when hexanal was used as a substrate, and decreasing the carbon chain of aldehydes resulted in decreased activity of the enzyme. Moreover, α-ketoester were more susceptible to the carbonyl reductase action than β-ketoester with the highest activity towards ethyl pyruvate. Substituents of the acetophenone derivatives deeply impacted the enantiopreference and the activity of the used enzyme. Furthermore, carbonyl reductase from *Y. lipolytica* was effectively applied in the biosynthesis of (*R*)-2-chloro-1-phenylethol and ethyl (*S*)-4-chloro-3-hydroxybutanoate, key intermediates in the synthesis of chiral drugs [94,95]. Zhang et al. [96] identified and characterized two different carbonyl reductases from *Y. lipolytica* yeast. Both presented *S*-enantiopreference towards tested ketones, but one of them displayed slightly higher activity regarding acetophenone derivatives. Interestingly, only one of the identified reductases was able to produce chiral alcohols from 7-chloro-1,2,3,4-tetrahydro-benzo[B]azepin-5-one and 3-(2-bromoacetyl)-5-chloro-2-thiophenesulphonamide [96].

Napora-Wijata et al. [97] studied two oxidoreductases: Zn-dependent alcohol dehydrogenase (ADH) and short-chain dehydrogenase/reductase (SDR). Either enzyme preferred secondary alcohols over the primary alcohols in the oxidation reactions. In addition, different enantioselectivities were observed. When comparing the bioreduction of 2-octanone, the ADH enzyme produced (*S*)-2-octanol, while the SDH enzyme produced (*R*)-2-octanol [97]. Other studies performed by Jang and Choi [98] confirmed the expression of the ADH enzyme in *E. coli* cells and enabled the successful biotransformation of 1-dodecanol into dodecanal and dodecanoic acid.

Baeyer-Villiger monooxygenases represent a fascinating group of enzymes worth exploring. They catalyze the oxidation of ketones to esters or lactones, hence catalyzing the insertion of an oxygen atom between a carbon-carbon bond. *Y. lipolytica* was investigated as another source of these monooxygenases. The *Yarrowia* monooxygenase A from the other seven putative monooxygenases enabled unusual activities over other known oxygenases. No activity was observed towards ketones, but the enzyme catalyzed sulfoxidation reactions with the formation of sulfones from DMSO and other aromatic sulfides and sulfoxides [99].

Glycidyl ethers, e.g., phenyl and benzyl ones, and 1,2-epoxyoctane may be resolved by the epoxide hydrolases identified in *Y. lipolytica*. Bendigiri et al. [100] demonstrated the kinetic resolution of glycidyl ethers, important intermediates in organic synthesis, and reported the production of vicinal diols with opposite enantiopreference regarding the corresponding glycidyl derivatives. Therefore, (*S*)-3-benzyloxy-1,2-propanediol and (*R*)-3-phenoxy-1,2-propanediol were obtained in a short time and high enantioselectivity. The production of (*R*)-1,2-octanediol from 1,2-epoxyoctane was studied by Godase et al. [101]. The Response Surface Methodology optimized the reaction conditions for the epoxide moiety hydrolysis to achieve a yield of 47.4%. Then, choline chloride and glycerol-based deep eutectic solvent additionally improved the catalytic performance of the epoxide hydrolase from *Y. lipolytica* to report a final yield of 60.5%.

5. Overexpressing of Enzymes in *Y. lipolytica* and Their Application in the Synthesis of Value-Added Chemicals

Y. lipolytica is a promising candidate for biotechnology applications. It has become a versatile host for expressing genes and secreting recombinant proteins or displaying them

on its surface. With the aid of various molecular and genetic tools, *Y. lipolytica* is now a well-established system for manufacturing heterologous proteins. As researchers have gained a better understanding of this yeast's genome and transcriptome, they have begun to consider modifying its metabolic pathways to make it a functional whole-cell factory for various bioconversion processes. An excellent review of *Y. lipolytica* yeast as a tool in heterologous gene expression was conducted by Madzak [102,103]. This section outlines the most important applications of modified Yarrowia strains in biocatalysis. The section focuses on oxidation biocatalysis with recombined oxygenases, e.g., peroxidase, the cytochrome P450 enzymes, and delta-12 desaturase. It also shows some examples of biotransformations catalyzed by recombined lipases and hydroperoxide lyase and applications of recombined isomerases, e.g., for isomaltulose synthesis.

An enzyme called soybean seed coat peroxidase (EC 1.11.1.7) has been successfully expressed and displayed on the cell surface of the *Y. lipolytica* Po1h strain. This enzyme can be used for the oxidation of a wide range of organic and inorganic compounds, with hydrogen peroxide acting as an electron acceptor. Unfortunately, obtaining this enzyme from plant material poses a number of problems; hence, Wang et al. [104] used the pMIZY05 integrative surface display expression vector for heterologous biosynthesis of peroxidase along with the production of whole-cell biocatalysts. Moreover, through genetic engineering, a laccase enzyme from *Trametes versicolor* was obtained in the yeast *Y. lipolytica*. Contrary to peroxidases, laccases are commonly used in the biodegradation of aromatic compounds, whose mechanism covers the simultaneous reduction of molecular oxygen to water. The study of Theerachat et al. [105] demonstrated that combining random and site-directed mutagenesis can increase laccase activity. Additionally, the supernatants that showed laccase activity were utilized to decolorize various dyes, such as azo, anthraquinone, and triarylmethane synthetic pigments, whereas the highest decolorization rate was observed for Amaranth dye.

Other important monooxygenases to be considered include cytochrome P450 enzymes. Whole cells can overcome the limitations of using cytochrome P450 monooxygenases alone as biocatalysts for large-scale industrial applications. These applications are often hampered by instability and the need for expensive cofactors. Such approaches using *Y. lipolytica* as a host organism were undertaken for the expression of the benzoate-*para*-hydroxylase encoding gene and the biosynthesis of *p*-hydroxybenzoic acid from its non-substituted analog [106,107] or human liver cytochrome P450 genes for progesterone biotransformation [108].

Since plants are a rich source of enzymes, attempts are often made to clone them in microorganisms. This was the case with hydroperoxide lyase, which helps produce green-note aroma compounds, volatile aldehydes, and alcohols by breaking down fatty acid hydroperoxides. Santiago-Gomez et al. [109] expressed hydroperoxide lyase from green bell pepper in *Y. lipolytica*, confirming its applicability in cleaving 13-hydroperoxide of linoleic acid in biphasic aqueous-organic solvent systems, preferably isooctane or hexane. Another way to modify fatty acids was proposed by Zhang et al. [110]. The authors expressed the delta-12 desaturase gene from *Mortierella alpina* and the linoleic acid isomerase gene in *Y. lipolytica*. By using this approach along with the freeze-thawing technique for cell permeabilization, it was possible to produce *trans*-10,*cis*-12 conjugated linoleic acid. After a 40 h reaction with 100 g of permeabilized wet cells, 15.6 g/L of conjugated linoleic acid was achieved with a yield of 62.2% following the isomerization of linoleic acid [110].

As previously mentioned, Lip2 is the primary extracellular lipase of *Y. lipolytica*. Yuzbasheva et al. [111] decided to anchor the enzyme in the cell wall of *Y. lipolytica* Po1f. Such an approach may be an alternative for lipase immobilization, and whole-cell catalysts may be used instead in varied reactions. There are two methods that are commonly used to display a target protein on the cell wall. The first method involves fusing the protein to the C-terminal domains of different cell wall proteins. Using C-terminal fusions, a glycoprotein containing the glycosylphosphatidylinositol takes part and allows the protein to bind covalently to the β-1,6-glucans of the cell wall. The N-terminal method is preferred when the active site is near the C-terminus and involves using the Flocculin and Pir protein families of cell walls. Using Pir proteins and N-terminal fusion, the authors increased the

enzyme's thermostability and operational stability in a broader range of organic solvents and detergents. Moreover, the obtained freeze-dried whole-cell catalyst bearing Lip2 high activity was used in the methanolysis of soybean oil. The results showed that two batch reactions had high yields of methyl esters, precisely 84.1% and 71.0%, respectively [111].

The production of biodiesel through microbiological synthesis is not just a theoretical concept but also has a real potential for commercialization. This process also addresses the issue of glycerol management; hence, genetic engineering techniques have been employed to enhance this process further. The engineered *Y. lipolytica* strain with *T. lanuginosus* lipase displayed on the cell surface utilized crude glycerol from biodiesel production as the carbon source, enabling the sustainable and cost-effective production of FAMEs and other oleochemicals. Interestingly, the application of whole-cell catalyst in the form of raw biomass allowed simultaneous methanolysis of waste cooking oil and conversion of by-product glycerol into yeast biomass. Applying such an approach led to the formation of FAMEs with a 91% yield compared to the resting cell system, which produced 81% biodiesel. Moreover, the reusability of yeast cells was observed with a yield of more than 80% FAMEs during 12 consecutive batches [112]. A dual biocatalytic system for biodiesel synthesis in *Y. lipolytica* was elaborated by Wei et al. [113]. It was found that during the cultivation of oleaginous yeasts, the addition of ethanol results in the production of small amounts of fatty acid ethyl esters (FAEEs). By expressing wax ester synthase and acetyl-CoA synthetase genes and deleting a major peroxisomal matrix protein gene (*PEX10*), the intra- and extracellular production of FAEEs from sugars and oils as carbon sources was significantly improved. In addition, the authors overexpressed the gene encoding Lip2 lipase, which further increased esters yields to 12.5–13.5 g/L, depending on the carbon source used. The presence of extracellular lipase caused the hydrolysis of triacylglycerols, leading to their conversion into ethyl esters through esterification or directly into FAEEs through transesterification. This process ultimately increased the yield of the reaction and provided glycerol for yeast growth [113].

Finally, one possible application of Y. lipolytica is also the isomerization of sucrose. Isomaltulose (6-*O*-α-D-Glucopyranosyl-D-fructose) is a sucrose substitute, a sweetener approved by the US FDA. It has several health benefits, including slower digestion, prolonged energy release, and lower insulin levels. It is primarily produced from sucrose by bacterial fermentation, but using pure sucrose isomerase is more practical for isomaltulose production (Figure 8). Unfortunately, wild strains lack the required enzyme activity, and expressing sucrose isomerase genes in *E. coli* requires expensive downstream isolation processes. In their study, Zhang et al. [114] applied *Y. lipolytica* as a host for overexpressing sucrose isomerase from *Pantoea dispersa*. After purifying the recombinant enzyme and immobilizing it onto polyvinyl alcohol-alginate beads, the yield of isomaltulose production was optimized, resulting in 620.7 g/L and 0.96 g/g. Immobilization allowed for more than ten high-yield batches to be produced [114]. In a work published two years earlier by Li et al. [115], an enzyme from the same Gram-negative bacterium was displayed on the cell surface of *Y. lipolytica* through the Pir1 anchor protein. Such an approach allowed for an isomerization yield of 93%, high specificity towards the reaction product, and increased stability in a wide range of pH and temperature [115].

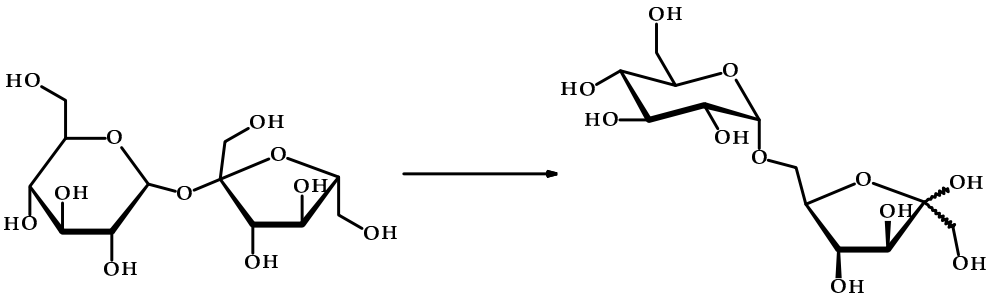


Figure 8. A scheme for the enzymatic synthesis of isomaltulose from sucrose.

6. Conclusions

The review has highlighted the diverse repertoire of enzymes derived from *Y. lipolytica*, including lipases, hydrolases, and oxidoreductases, which have found applications in various sectors such as food, pharmaceutical, and chemical industries. As the research on *Y. lipolytica* continues to advance, further optimization of enzyme production, engineering of novel biocatalysts, upgrading immobilization techniques and exploration of untapped enzymatic activities will undoubtedly expand the biotechnological potential of this yeast. In conclusion, the exploration of *Y. lipolytica* yeast enzymes for biocatalytic purposes opens up exciting avenues for sustainable and eco-friendly chemical transformations. By harnessing the enzymatic prowess of these microorganisms, researchers can unlock novel pathways toward greener and more efficient bioprocesses.

Despite a great understanding of the mechanisms of enzyme-mediated biocatalysis, it is still not possible to determine unequivocally the substrate specificity of enzyme proteins based on the characteristics of the microorganism that produces it. Molecular modeling techniques and, consequently, protein engineering can come to the rescue here. These techniques, although targeted and effective, are very labor-intensive and still revert to classical methods of selecting enzymes of microbial origin on the basis of a preliminary analysis of the properties and a study of the reaction excitability under model conditions. Progress in screening the substrate specificity of enzymes is certainly important for the further development of biocatalysis. Until then, experimental research into the properties and behavior of enzymes in biotransformations is an essential part of improving the efficiency of these reactions.

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