

Article

Highly Effective, Regiospecific Hydrogenation of Methoxychalcone by *Yarrowia lipolytica* Enables Production of Food Sweeteners

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Abstract: We describe the impact of the number and location of methoxy groups in the structure of chalcones on the speed and efficiency of their transformation by unconventional yeast strains. The effect of substrate concentration on the conversion efficiency in the culture of the *Yarrowia lipolytica* KCh 71 strain was tested. In the culture of this strain, monomethoxychalcones (2'-hydroxy-2'', 3''- and 4''-methoxychalcone) were effectively hydrogenated at over 40% to the specific dihydrochalcones at a concentration of 0.5 g/L of medium after just 1 h of incubation. A conversion rate of over 40% was also observed for concentrations of these compounds of 1 g/L of medium after three hours of transformation. As the number of methoxy substituents increases in the chalcone substrate, the rate and efficiency of transformation to dihydrochalcones decreased. The only exception was 2'-hydroxy-2'',5''-dimethoxychalcone, which was transformed into dihydrochalcone by strain KCh71 with a yield comparable to that of chalcone containing a single methoxy group.

Keywords: biotransformations; sweeteners; methoxychalcones; dihydrochalcones; yeast

1. Introduction

Sweet taste plays a dominant role in human food preferences [1]. It is the most important sensory feature of food products. It is not only a source of pleasure but also a basic energy stimulus for the body. Prospective studies have provided information on the correlation between excessive consumption of sugar and sugar-rich products, and an increased risk of pancreatic cancer. Given the role of hyperglycaemia and hyperinsulinaemia in the development of this cancer, it has been established that the direct cause of organ tissue death is the induction of frequent food hyperglycaemia and the increase in demand and reduction of insulin sensitivity [2]. There is also growing evidence of the role of increased sugar consumption in the development of hypertension, inflammation, and coronary artery disease [3–5].

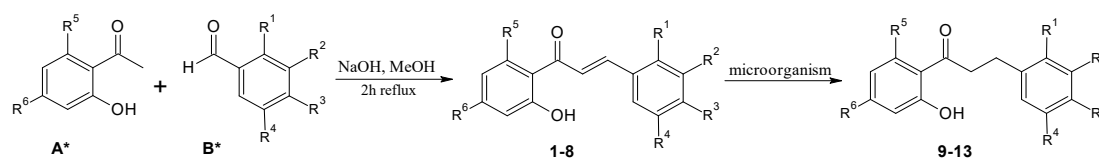
For this reason, low-energy substitutes are being sought, exhibiting physical and chemical properties comparable to sucrose, but providing additional health benefits [6]. The increased interest in a healthy lifestyle and increased incidence of diseases caused by impaired metabolism of sugar compounds enhance the popularity of products containing sweeteners with reduced caloric value [7]. Sweeteners are defined as food additives that mimic the feeling of sweet taste, similar to saccharose [8]. Dihydrochalcones interacting with the receptors of sweet taste, T1R2, are included in this group [9]. An ideal sweetener, apart from low caloric value and sweetness intensity, similar to sucrose, should be safe for the health of the consumer and also stable in various processing conditions [6,10]. The multiplicity of restrictions and disadvantages of sweetener substances available on the market has created the need to search for new compounds that are more beneficial in terms of their impact

on human health and more attractive to industry [11]. Dihydrochalcones raise significant interest in this area [12–14]. They are obtained as a result of hydrogenation of chalcones, and show great potential as synthetic sweeteners [15,16]. The best-studied compound reported as an intensely sweet substance is neohesperidin dihydrochalcone [17]. Desirable biological properties characterise most of the dihydrochalcones found in plants. Phloretin and phlorizin (found in apples) significantly reduce the risk of developing cardiovascular disease and diabetes [18]. Aspalathin is found mainly in larger quantities in the leaves of *Aspalathus linearis* (6–13%), and in the stems it is much less abundant (0.16–0.78%). It affects the reduction of oxidative stress and may slow down the ageing process [19].

The main aim of our research was to obtain, as a result of biotransformation, a bank of dihydrochalcones characterised by a sweet taste. Additionally, we characterised the ability of selected yeast strains to hydrogenate both natural and unidentified in plants chalcones. Unconventional yeasts were used as biocatalysts because of specific enzymes capable of hydrogenation of chalcones [20–23]. Eight microorganisms were chosen to perform biotransformations; however, in this work, we pay special attention to the study of the catalytic capacity of a strain of the species *Yarrowia lipolytica*, which is currently known for its use in the production of many substances essential for the food industry: organic acids [24–26], polyols [27–30], fragrance substances [31,32], hydrolytic enzymes [33–35], and biomass [36]. One of the unique features of *Y. lipolytica* is the ability to use many unconventional carbon sources for its culture, such as alkanes, glycerol, or fatty acids [36–39]. The extraordinary interest in *Y. lipolytica* yeast is due to its strong tolerance to changes in pH, salt concentration, and a wide range of carbon sources that simplify the optimisation of bioprocesses and facilitate the use of non-glucose based raw materials [40,41]. More importantly, the GRAS status (Generally Recognised As Safe) defines strains of the *Y. lipolytica* species as an attractive and environmentally friendly microbiological tool for the production of nutraceuticals, fermented foods, and dietary supplements [41–43]. Yeast of this species received marketing authorisation as a novel food in 2019 (EU Regulation 2019/760). The maximum dose used is 3 g per day for children aged 3 to 10 years. Above the age of 10, the dose is 6 g a day [44]. The ability of strains of this species to effectively hydrogenate chalcones observed in our previous studies [22,23] may, in the future, result in the development of preparations combining the nutritional values of this species' strain, and the health and sensory properties of dihydrochalcones. The combination of chemical and biological methods allows for obtaining new compounds, and the development of unique processes, will enable the development of new ways to obtain sweeteners.

2. Results and Discussion

The main purpose of the study was to assess the capacity of various yeast strains for selective reduction of the double bond in a series of methoxychalcones obtained as a result of chemical synthesis. Additionally, the influence of the number and the position of methoxy substituents on the speed of biotransformation was checked. The selected substrates had one (1, 2, 3), two (4, 6), three (5, 7) or five (8) methoxy groups, and also the hydroxyl group at the 2' position of the A ring (crucial for natural chalcones) (Scheme 1).



Scheme 1. Synthesis of chalcones and dihydrochalcones obtained as a result of transformation by unconventional yeast strains.

Eight microorganisms (*Rhodotorula rubra* KCh 4, *Yarrowia lipolytica* KCh 71, *Rhodotorula marina* KCh 77, *Rhodotorula rubra* KCh 82, *Candida viswanathii* KCh 120, *Rhodotorula glutinis* KCh 242, *Saccharomyces cerevisiae* KCh 464, and *Candida parapsilosis* KCh 909) [13,23] were chosen based on their previously observed high regioselectivity during biotransformation, among others 3-(2''-furyl)-

and 3-(2''-thienyl)-1-(2'-hydroxyphenyl)-prop-2-en-1-one [23]. The strains have a high ability to hydrogenate the double bond between C2–C3 in compounds lacking additional functional groups [13].

Eight substrates (1–8) were obtained as a result of chemical synthesis, according to the Claisen–Schmidt reaction (Table 1). Chemically obtained chalcones (1–8) and dihydrochalcones (9–13) resulting from biotransformation were purified and then characterised by NMR (¹H NMR, ¹³C NMR, correlation spectra and long-range heteronuclear correlation—HMBC (Heteronuclear Multiple Bond Correlation)) and gas chromatography analysis (GC), and as well thin layer liquid chromatography (TLC) analysis.

Table 1. Chalcones obtained by chemical synthesis and dihydrochalcones identify as a results of biotransformations.

Chalcones	Dihydrochalcones	R1	R2	R3	R4	R5	R6
1	9	-OCH ₃	-H	-H	-H	-H	-H
2	10	-H	-OCH ₃	-H	-H	-H	-H
3	11	-H	-H	-OCH ₃	-H	-H	-H
4	12	-OCH ₃	-H	-H	-OCH ₃	-H	-H
5	13	-H	-OCH ₃	-OCH ₃	-OCH ₃	-H	-H
6	-	-H	-H	-H	-H	-OCH ₃	-OCH ₃
7	-	-H	-H	-OCH ₃	-H	-OCH ₃	-OCH ₃
8	-	-H	-OCH ₃	-OCH ₃	-OCH ₃	-OCH ₃	-OCH ₃

Five out of eight substrates (compounds 1–5), all having methoxy substituents only in the B ring, were transformed to dihydrochalcones (2'-hydroxy-2''-methoxy- α,β -dihydrochalcone (9), 2'-hydroxy-3''-methoxy- α,β -dihydrochalcone (10), 2'-hydroxy-4''-methoxy- α,β -dihydrochalcone (11), 2'-hydroxy-2'',5''-dimethoxy- α,β -dihydrochalcone (12), and 2'-hydroxy-3'',4'',5''-trimethoxy- α,β -dihydrochalcone (13)). In contrast, no conversion of substrates having methoxyl substituents also at the 4',6' positions of ring A (substrates 6, 7, and 8), to the expected product was observed, regardless of the used strain. The highest substrate conversion was observed in the culture of the *Yarrowia lipolytica* KCh 71 strain (Table 2). We observed very high conversions (88–99%) of the four used substrates—2'-hydroxy-2''-methoxychalcone (1), 2'-hydroxy-3''-methoxychalcone (2), 2'-hydroxy-4''-methoxychalcone (3), and 2'-hydroxy-2'',5''-dimethoxychalcone (4)—in the culture of this strain after just one day. At the same time, as the number of methoxyl groups in the B ring increased, the conversion efficiency to dihydrochalcones decreased. For a substrate containing three methoxyl groups in the 3'', 4'', and 5'' positions (5), after reaching a certain level, the biotransformation was stopped and, with the expiration of the time, the substrate was no longer converted.

The results of each reaction were analysed in two aspects: (a) strains performing effective hydrogenation of as many substrates as possible, regardless of their structure. Considering this criterion, the best biocatalyst turned out to be the *Yarrowia lipolytica* KCh 71 strain (Table 2). Similar efficiencies of this strain have also been described during biotransformation of chalcone having heteroatom in the B ring [23]; (b) most efficiently converted substrate. In this case, with the increasing number of methoxyl groups in the B ring, the conversion efficiency of the substrate decreased, while in the presence of methoxyl substituents in the A ring, the product did not occur at all. These results were noted for most of the tested biocatalysts (Table 2).

Table 2. Conversion [%] of the substrates in time.

Substrate	2'-hydroxy-2''-methoxychalcone (1)			2'-hydroxy-3''-methoxychalcone (2)			2'-hydroxy-4''-methoxychalcone (3)			2'-hydroxy-2'',5''-dimethoxychalcone (4)			2'-hydroxy-3'',4'',5''-trimethoxychalcone (5)		
	1	3	7	1	3	7	1	3	7	1	3	7	1	3	7
<i>Rhodotorula rubra</i> KCh 4	2	3	4	7	8	10	5	5	8	4	5	7	0	0	2
<i>Yarrowia lipolytica</i> KCh 71	98	99	99	96	97	98	88	95	96	99	99	99	7	14	20
<i>Rhodotorula marina</i> KCh 77	3	3	4	8	8	10	10	14	17	2	5	8	0	3	5
<i>Rhodotorula rubra</i> KCh 82	3	3	3	25	30	43	6	8	13	2	4	6	0	4	6
<i>Candida viswanathii</i> KCh 120	98	99	99	70	95	95	98	98	98	0	39	58	6	10	19
<i>Rhodotorula glutinis</i> KCh 242	2	3	14	95	95	95	43	75	94	3	6	6	4	4	6
<i>Saccharomyces cerevisiae</i> KCh 464	10	14	34	6	12	24	74	86	97	3	4	5	3	4	9
<i>Candida parapsilosis</i> KCh 909	44	68	83	33	54	73	29	75	84	12	17	48	4	6	7

Also, the methoxyl substitution position affected the rate and efficiency of the hydrogenation process. These differences are seen the best in the *R. glutinis* KCh 242 strain, in a culture whose conversion of compounds containing one methoxyl group in various positions (in the same ring) is diametrically different. Substrate **1** having a methoxyl group at the C-2'' carbon was 14% converted after seven days of incubation, whereas for substrates **2** and **3** (methoxyl group located at C-3'' and C-4'', respectively) conversion rates exceeded 90% after seven days (Figure 1A). Another preference, selectivity due to the structure of the substrate, was observed for the strain *Saccharomyces cerevisiae* KCh 464, for which a significantly higher conversion was recorded for compound **3**. Compounds with the methoxy group located at the C-2'' and C-3'' carbon demonstrate a much slower conversion in the culture of this strain (Figure 1B). This observation was surprising because strains of this species in many papers are described as effective and universal biocatalysts for the hydrogenation of double bonds, both in chalcones [13,20] and other compounds containing the enone moiety [45,46].

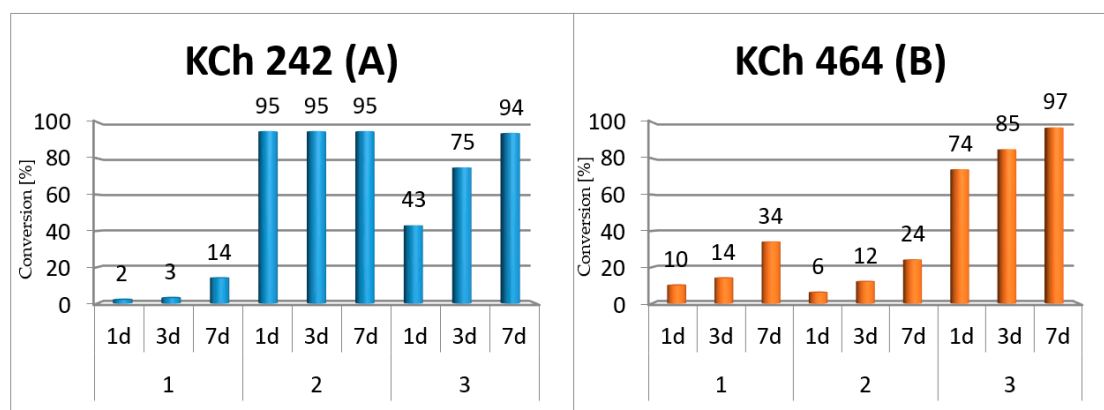


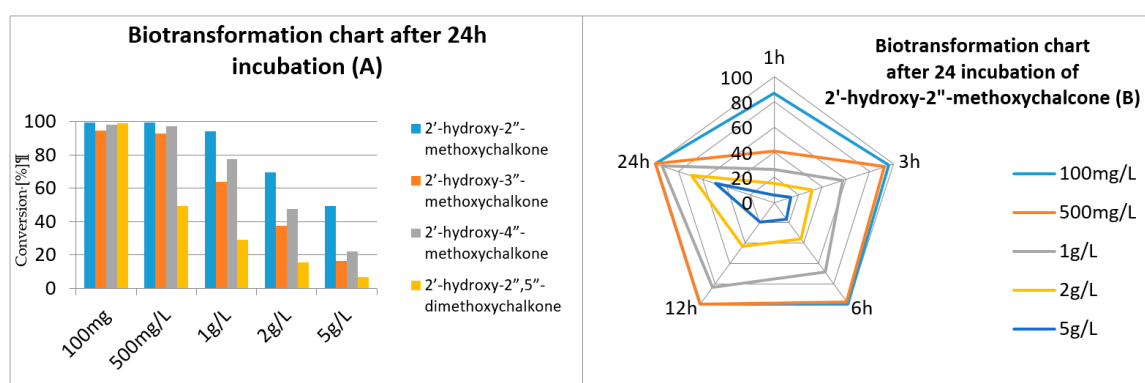
Figure 1. (A) Substrate conversion by *R. glutinis* KCh 242 strain after 1 and 7 days of incubation. (1) 2'-hydroxy-2''-methoxychalcone, (2) 2'-hydroxy-3''-methoxychalcone and (3) 2'-hydroxy-4''-methoxychalcone; (B) Substrate conversion by *S. Cerevisiae* KCh 464 strain after 1 and 7 days of incubation. (1) 2'-hydroxy-2''-methoxychalcone, (2) 2'-hydroxy-3''-methoxychalcone and (3) 2'-hydroxy-4''-methoxychalcone.

Based on screening tests, we decided to choose *Yarrowia lipolytica* KCh 71 strain for further experiments. This strain showed the highest conversion and the largest number of substrates tested after just one day of biotransformation. In addition, yeast from this species was authorised in 2019 as a novel food (EU Regulation 2019/760). The use of a strain with such properties, as a biocatalyst, minimises the possibility of extraction of any toxic metabolites with the obtained product [44]. For these reasons, we decided to check the progress of biotransformation during the first day of substrate incubation, and the ability of this strain to hydrogenate higher concentrations of tested compounds. At this stage, the four most efficiently convertible compounds were used as substrates (1–4). The standard concentration of substrate for screening tests was 100 mg/L. However, we tested and compared the efficiency of this reaction by increasing the scale of the process so that the medium contained a maximum of 5 g of substrate per 1 L of a medium, which is shown in the table (Table 3).

Table 3. Conversion of selected substrates depending on their concentration and biotransformation time, performed in triplicate.

Substrate	Concentration [g/L]	Biotransformation Time				
		1 h	3 h	6 h	12 h	24 h
1	0.1	87 ± 1.2	96 ± 3.5	100 ± 0.0	100 ± 0.0	100 ± 0.6
	0.5	41 ± 7.1	92 ± 3.0	98 ± 0.0	100 ± 0.0	100 ± 0.6
	1	26 ± 4.9	57 ± 6.7	69 ± 7.6	83 ± 7.2	94 ± 0.0
	2	15 ± 1.5	32 ± 2.1	36 ± 1.7	43 ± 4.2	70 ± 4.5
	5	6 ± 1.0	14 ± 2.0	16 ± 2.3	19 ± 2.5	49 ± 0.6
2	0.1	66 ± 8.1	91 ± 5.0	91 ± 8.1	93 ± 1.0	95 ± 1.5
	0.5	46 ± 5.8	77 ± 4.4	85 ± 3.2	90 ± 1.0	93 ± 1.0
	1	27 ± 2.0	41 ± 4.7	49 ± 4.7	60 ± 4.9	64 ± 3.5
	2	14 ± 1.5	25 ± 1.2	30 ± 2.6	31 ± 1.4	37 ± 3.2
	5	5 ± 0.6	8 ± 0.0	9 ± 0.6	10 ± 0.6	16 ± 1.2
3	0.1	74 ± 1.5	95 ± 1.5	98 ± 0.6	98 ± 0.6	98 ± 0.0
	0.5	51 ± 5.3	75 ± 2.6	88 ± 1.7	96 ± 0.6	97 ± 0.6
	1	23 ± 2.5	44 ± 1.5	51 ± 1.0	66 ± 7.0	77 ± 6.4
	2	11 ± 0.6	22 ± 1.0	26 ± 1.2	33 ± 3.2	48 ± 4.0
	5	4 ± 0.6	6 ± 1.2	8 ± 1.7	12 ± 3.0	22 ± 5.0
4	0.1	91 ± 3.5	96 ± 0.6	99 ± 0.0	99 ± 0.0	99 ± 0.0
	0.5	40 ± 3.6	50 ± 3.6	43 ± 2.6	44 ± 0.6	49 ± 1.2
	1	15 ± 1.5	26 ± 3.2	25 ± 3.5	25 ± 2.6	29 ± 2.0
	2	9 ± 1.2	13 ± 2.1	13 ± 1.5	14 ± 1.7	16 ± 2.5
	5	3 ± 0.6	4 ± 0.6	5 ± 0.6	6 ± 1.5	7 ± 1.5

In this part of the experiment, the scale of the process was changed and Riplate square wells were used instead of Erlenmeyer flasks. This test was performed in triplicate. The substrate addition was as follows: 0.3, 1.5, 3, 6, and 15 mg per well, which corresponds to concentrations: 100, 500, 1, 2, and 5 g per 1 L of culture medium, respectively. At the lower concentrations, there is no significant difference between the conversion of various substrates. As the concentration increases, 2'-hydroxy-2''-methoxychalcone (1) is transformed the most efficiently, and the conversion is shown in Figure 2B. The lowest conversion rate recorded for this substrate after 24 h was 50%, at the maximum concentration of 5 g/L.

**Figure 2.** Comparison of conversions of increased substrate concentrations: after 24 h (A); conversion of 2'-hydroxy-2''-methoxychalcone (1) by *Yarrowia lipolytica* KCh 71 at increasing concentrations of substrate (B).

As the concentration increased, significant differences between the conversion of individual compounds started to be visible (Figure 3). The expected product (dihydrochalcone) was also observed during the transformation of the highest substrate concentration but with a much lower yield compared to the screening concentration (100 mg/L). After 24 h of biotransformation, at the highest substrate

concentration (5 g/L), 2'-hydroxy-2''-methoxychalcone was converted the most efficiently—where about 50% conversion was observed (Figure 3A). Under the above conditions, the *Yarrowia lipolytica* KCh 71 strain was also able to transform the other substrates, although with much lower yield (7%–22%) (Table 3). In addition, such a high level of conversion for the tested methoxychalcones, at a concentration of 100 mg/L, is comparable to the previously described studies on the unsubstituted [13] or containing hydroxyl or methyl groups [47] chalcone derivatives.

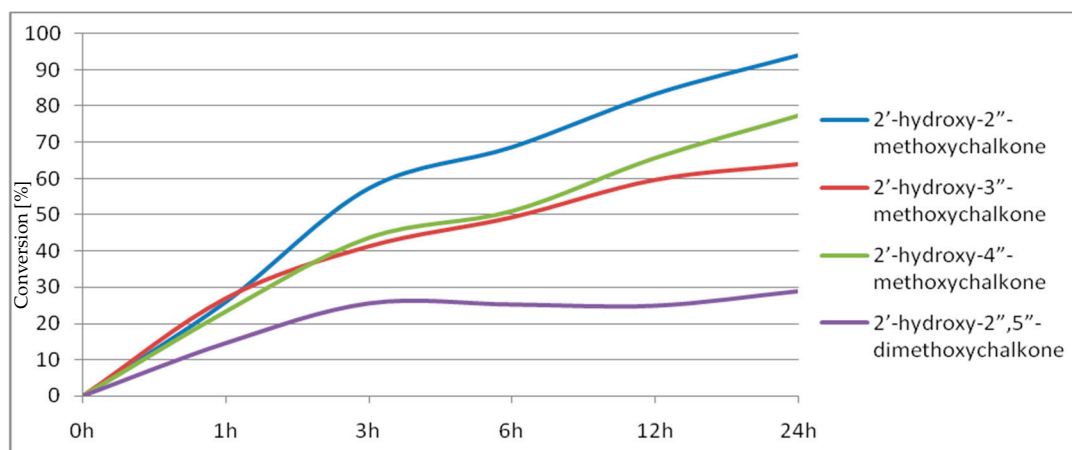


Figure 3. Comparison of conversion of selected substrates to products during 24 h incubation, for a substrate concentration of 1 g/L.

Moreover, the use of yeast from the *Yarrowia lipolytica* species is not accidental. This is a microorganism whose properties are widely used in the food industry—from the production of citric acid [25] or eicosapentaenoic acid [48] by preventing rotting of the harvest [49] and cleaning the soil from petroleum hydrocarbons [50], to the production of high-protein animal feed [51] and unconventional food for humans [44]. It is also used in the production of various types of sweeteners [41]. Moreover, yeast of this species can be successfully used to produce significant amounts of various types of dihydrochalcones. In addition, biotransformations using these microorganisms allow one to obtain with high efficiency a single compound with high purity. The only reaction products were dihydrochalcones (also observed in our previous studies) [23]. The microorganisms used were not able to produce other flavonoid derivatives (flavones, flavanones), which are found during the transformation of chalcones in cultures of filamentous fungi, bacteria, or algae [13,47,52,53].

3. Materials and Methods

3.1. Substrates

The substrates, used for biotransformation, were obtained by Claisen–Schmidt condensation reaction of 2-hydroxyacetophenone or 2'-hydroxy-4',6'-dimethoxyacetophenone (A*) with benzaldehyde containing methoxylated group(s) in appropriate positions (B*) (purchased from Sigma-Aldrich (St. Louis, MO, USA)) dissolved in methanol in an alkaline environment (Table 1) at a high temperature, according to the procedure described previously [13,23]. The resulting compounds (1–8) were used as substrates for the biotransformation and their NMR spectral data are identical to those previously published [52,54–56].

3.2. Microorganisms

The research was carried out on eight strains of yeast from the species: *Rhodotorula rubra* (KCh 4 and KCh 82), *Rhodotorula marina* KCh 77, *Rhodotorula glutinis* KCh 242, *Yarrowia lipolytica* KCh 71, *Candida viswanathii* KCh 120, *Saccharomyces cerevisiae* KCh 464, and *Candida parapsilosis* KCh 909 were obtained from the collection of the Department of Chemistry, Wrocław University of Environmental

and Life Sciences (Wrocław, Poland). Whose storage and biocatalytic capacity have been previously described [22,23,57].

3.3. Screening

Erlenmeyer flasks of 300 mL were used for biotransformation on an analytical scale, each containing 100 mL of Sabouraud culture medium (3% glucose, 1% aminobac). Used microorganisms were incubated for three days at 24 °C on a rotary shaker (144 rpm) (Eppendorf AG, Hamburg, Germany). After this time, 10 mg of the substrate was dissolved in DMSO (dimethyl sulfoxide) and added. Samples were collected after 1, 3, and 7 days. Portions of 10 mL of the transformation mixture were taken out and extracted with ethyl acetate. The extracts were dried over MgSO_4 , concentrated *in vacuo*, and analysed by gas chromatography (GC) and thin-layer chromatography (TLC) (SiO_2 , DC Alufolien Kieselgel 60 F254 (0.2 mm thick), Merck, Darmstadt, Germany).

3.4. Gas Chromatography

GC analysis was performed using an Agilent 7890A gas chromatograph, equipped with a flame ionisation detector (FID) (Agilent, Santa Clara, CA, USA). The capillary column DB-5HT (30 m $\times$ 0.25 mm $\times$ 0.10 μm) was used to determine the composition of the product mixtures. The temperature programme was applied as follows: 80–300 °C, the temperature on the detector: 300 °C, injection 1 μL , flow 1 mL/min, flow H2: 35 mL/min, airflow; 300 mL/min, time of analysis: 18.67 min. The retention times of the substrates and products are described in Table 4.

Table 4. Compounds mass after extraction (preparative scale) [mg]—in relation to 100 mg of substrate used and retention times based on GC.

Substrate	Product	Isolated Yield [%]	Substrate Retention Time [min]	Product Retention Time [min]
2'-hydroxy-2''-methoxychalcone (1)	9	47	11.93	10.58
2'-hydroxy-3''-methoxychalcone (2)	10	33	11.90	10.87
2'-hydroxy-4''-methoxychalcone (3)	11	40	12.24	11.03
2'-hydroxy-2'',5''-dimethoxychalcone (4)	12	54	13.36	12.54
2'-hydroxy-3'',4'',5''-trimethoxychalcone (5)	13	5	14.17	13.46
2'-hydroxy-4',6'-dimethoxychalcone (6)	-	-	13.83	-
2'-hydroxy-4',6',4''-trimethoxychalcone (7)	-	-	14.69	-
2'-hydroxy-4',6',3'',4'',5''-pentamethoxychalcone (8)	-	-	16.01	-

3.5. Preparative Scale

Preparative biotransformations were performed in 2 L Erlenmeyer flasks, each containing 500 mL of culture medium (3% glucose, 1% peptone). The transferred microorganisms were incubated for three days at 24 °C on a rotary shaker. After this time, 100 mg of the substrate dissolved in 2 mL of DMSO was added. After three days, the product was isolated by triple extraction with ethyl acetate (3 extractions with 300 mL), dried with anhydrous magnesium sulfate, and concentrated *in vacuo*. The transformation products were separated by preparative TLC and analysed (TLC, GC, NMR).

3.6. TLC and NMR Analysis

The course of biotransformation was monitored using TLC plates (SiO_2 , DC Alufolien Kiesel gel 60 F254 (0.2 mm thick), Merck, Darmstadt, Germany). Products were separated using preparative TLC plates (Silica Gel GF, 20 $\times$ 20 cm, 500 μm , Analtech, Newark, DE, USA) and a cyclohexane: ethyl acetate mixture (9:1, *v/v*) as an eluent, according to the method described previously [58]. The product was observed (without additional visualisation) under the UV lamp at the wavelength of 254 nm.

NMR analysis was performed using a DRX 600 MHz Bruker spectrometer (Bruker, Billerica, MA, USA). The prepared samples were dissolved in deuterated chloroform CDCl_3 . The performed analyses include ^1H NMR, ^{13}C NMR, HMBC (two-dimensional analysis) HMQC (heteronuclear correlation) and COSY (correlation spectroscopy) (Supplementary Materials).

3.7. Increasing the Concentrations of Tested Substrates

Transfer (scaling) of the biotransformation scale was carried out in Riplate square wells, 24 wells, to which 3 mL of culture medium with an inoculum of *Yarrowia lipolytica* KCh 71 was added. Microorganisms were incubated for three days at 24 °C on an oscillating shaker (190 rpm)(ELMI, Riga, Latvia). Screening was performed for five substrate concentrations—100 mg/L, 500 mg/L, 1 g/L, 2 g/L, and 5 g/L. The substrate was dissolved in DMSO. A total of 100 µL of the prepared substrate solution was added to each well. The experiment was carried out in triplicate. Samples (500 µL each) were collected after 1, 3, 6, 12, and 24 h of substrate incubation, then extracted with ethyl acetate, centrifuged, and analysed with GC.

4. Conclusions

On account of the constantly growing requirement for sweeteners, new solutions for their production are still being sought, preferably as cheaply as possible, biotechnological and ones that simultaneously fulfil the assumptions of the “green source” theory. The use of microorganisms, which is approved as safe and even allowed for consumption, seems to be an interesting solution. *Y. lipolytica* KCh 71 adapted for biotransformation turned out to be the best of the tested microorganisms to transform methoxychalcones. The most efficiently transformed compounds were chalcones containing single methoxyl groups in the B ring. Interestingly, the substrate with 2'-hydroxy-2''-methoxychalcone even at a scale increased to 5 g substrate per 1 L of medium was convertible with up to 50% yield after 24 h. At the same time, a very high conversion, from 66 to 91% depending on the substrate, after one hour of incubation (Table 2) indicates that the ene-reductase catalysing this process is a constitutive enzyme. However, identification of which group of enzymes catalyzes this reaction requires the use of molecular biology methods.

With the increase in the number of methoxyl groups, the efficiency of the process decreased. For compounds that contain methoxyl substituents also in the A ring, the transformation did not occur at all, including commonly described flavokavain B (2'-hydroxy-4',6'-dimethoxychalcone).

The challenge facing this type of research is to limit the number of solvents used when extracting products—or to eliminate extraction. This solution would increase the efficiency of the process and, at the same time, would allow the production of a supplement containing the pro-health microorganism as well as a sweet flavonoid product with a significantly different spectrum of activity. Moreover, the use of created dihydrochalcones as sweeteners could have a positive effect on the human body, while enriching our diet and reducing the need to use sucrose and preventing various types of civilisation diseases.

Sweeteners play a significant role in the human diet. Through their palatability, they improve the taste of sweets, drinks, or snacks. Replacing some of the popular sweeteners (e.g., acesulfame) with other, less caloric compounds lacking side-effects is an increasingly common food market practice. The placement of dihydrochalcone, following the dihydrochalcone neohesperidin, in food products could have a more positive effect on the reduction of civilisation diseases than the use of sweeteners without health-promoting properties.

Supplementary Materials: Supplementary materials can be found at <http://www.mdpi.com/2073-4344/10/10/1135/s1>. Figure S1: ¹H NMR spectral of 1-(2'-hydroxyphenyl)-3-(2''-methoxyphenyl)-2-propan-1-one (9) (CDCl₃, 600 MHz), Figure S2: Part of the ¹³C NMR spectral of 1-(2'-hydroxyphenyl)-3-(2''-methoxyphenyl)-2-propan-1-one (9) (CDCl₃, 600 MHz), Figure S3: ¹³C NMR spectral of 1-(2'-hydroxyphenyl)-3-(2''-methoxyphenyl)-2-propan-1-one (9) (CDCl₃, 151 MHz), Figure S4: Part of the ¹³C NMR spectral of 1-(2'-hydroxyphenyl)-3-(2''-methoxyphenyl)-2-propan-1-one (9) (CDCl₃, 151 MHz), Figure S5: COSY spectral of 1-(2'-hydroxyphenyl)-3-(2''-methoxyphenyl)-2-propan-1-one (9) (CDCl₃, 151 MHz), Figure S6: Part of the COSY spectral of 1-(2'-hydroxyphenyl)-3-(2''-methoxyphenyl)-2-propan-1-one (9) (CDCl₃, 151 MHz), Figure S7: HSQC spectral of 1-(2'-hydroxyphenyl)-3-(2''-methoxyphenyl)-2-propan-1-one (9) (CDCl₃, 151 MHz), Figure S8: HMBC spectral of 1-(2'-hydroxyphenyl)-3-(2''-methoxyphenyl)-2-propan-1-one (9) (CDCl₃, 151 MHz), Figure S9: ¹H NMR spectral of 1-(2'-hydroxyphenyl)-3-(3''-methoxyphenyl)-2-propan-1-one (10) (CDCl₃, 600 MHz), Figure S10: Part of the ¹³C NMR spectral of 1-(2'-hydroxyphenyl)-3-(3''-methoxyphenyl)-2-propan-1-one (10) (CDCl₃,

600 MHz), Figure S11: ^{13}C NMR spectral of 1-(2'-hydroxyphenyl)-3-(3''-methoxyphenyl)-2-propan-1-one (**10**) (CDCl_3 , 151 MHz), Figure S12: Part of the ^{13}C NMR spectral of 1-(2'-hydroxyphenyl)-3-(3''-methoxyphenyl)-2-propan-1-one (**10**) (CDCl_3 , 151 MHz), Figure S13: COSY spectral of 1-(2'-hydroxyphenyl)-3-(3''-methoxyphenyl)-2-propan-1-one (**10**) (CDCl_3 , 151 MHz), Figure S14: Part of the COSY spectral of 1-(2'-hydroxyphenyl)-3-(3''-methoxyphenyl)-2-propan-1-one (**10**) (CDCl_3 , 151 MHz), Figure S15: HSQC spectral of 1-(2'-hydroxyphenyl)-3-(3''-methoxyphenyl)-2-propan-1-one (**10**) (CDCl_3 , 151 MHz), Figure S16: HMBC spectral of 1-(2'-hydroxyphenyl)-3-(3''-methoxyphenyl)-2-propan-1-one (**10**) (CDCl_3 , 151 MHz), Figure S17: ^1H NMR spectral of 1-(2'-hydroxyphenyl)-3-(4''-methoxyphenyl)-2-propan-1-one (**11**) (CDCl_3 , 600 MHz), Figure S18: Part of the ^{13}C NMR spectral of 1-(2'-hydroxyphenyl)-3-(4''-methoxyphenyl)-2-propan-1-one (**11**) (CDCl_3 , 600 MHz), Figure S19: ^{13}C NMR spectral of 1-(2'-hydroxyphenyl)-3-(4''-methoxyphenyl)-2-propan-1-one (**11**) (CDCl_3 , 151 MHz), Figure S20: Part of the ^{13}C NMR spectral of 1-(2'-hydroxyphenyl)-3-(4''-methoxyphenyl)-2-propan-1-one (**11**) (CDCl_3 , 151 MHz), Figure S21: COSY spectral of 1-(2'-hydroxyphenyl)-3-(4''-methoxyphenyl)-2-propan-1-one (**10**) (CDCl_3 , 151 MHz), Figure S22: Part of the COSY spectral of 1-(2'-hydroxyphenyl)-3-(4''-methoxyphenyl)-2-propan-1-one (**11**) (CDCl_3 , 151 MHz), Figure S23: HSQC spectral of 1-(2'-hydroxyphenyl)-3-(4''-methoxyphenyl)-2-propan-1-one (**11**) (CDCl_3 , 151 MHz), Figure S24: HMBC spectral of 1-(2'-hydroxyphenyl)-3-(4''-methoxyphenyl)-2-propan-1-one (**11**) (CDCl_3 , 151 MHz), Figure S25: ^1H NMR spectral of 1-(2'-hydroxyphenyl)-3-(2'',5''-dimethoxyphenyl)-2-propan-1-one (**12**) (CDCl_3 , 600 MHz), Figure S26: Part of the ^{13}C NMR spectral of 1-(2'-hydroxyphenyl)-3-(2'',5''-dimethoxyphenyl)-2-propan-1-one (**12**) (CDCl_3 , 600 MHz), Figure S27: ^{13}C NMR spectral of 1-(2'-hydroxyphenyl)-3-(2'',5''-dimethoxyphenyl)-2-propan-1-one (**12**) (CDCl_3 , 151 MHz), Figure S28: Part of the ^{13}C NMR spectral of 1-(2'-hydroxyphenyl)-3-(2'',5''-dimethoxyphenyl)-2-propan-1-one (**12**) (CDCl_3 , 151 MHz), Figure S29: COSY spectral of 1-(2'-hydroxyphenyl)-3-(2'',5''-dimethoxyphenyl)-2-propan-1-one (**12**) (CDCl_3 , 151 MHz), Figure S30: Part of the COSY spectral of 1-(2'-hydroxyphenyl)-3-(2'',5''-dimethoxyphenyl)-2-propan-1-one (**12**) (CDCl_3 , 151 MHz), Figure S31: HSQC spectral of 1-(2'-hydroxyphenyl)-3-(2'',5''-dimethoxyphenyl)-2-propan-1-one (**12**) (CDCl_3 , 151 MHz), Figure S32: HMBC spectral of 1-(2'-hydroxyphenyl)-3-(2'',5''-dimethoxyphenyl)-2-propan-1-one (**12**) (CDCl_3 , 151 MHz), Figure S33: ^1H NMR spectral of 1-(2'-hydroxyphenyl)-3-(3'',4'',5''-trimethoxyphenyl)-2-propan-1-one (**13**) (CDCl_3 , 600 MHz), Figure S34: Part of the ^1H NMR spectral of 1-(2'-hydroxyphenyl)-3-(3'',4'',5''-trimethoxyphenyl)-2-propan-1-one (**13**) (CDCl_3 , 600 MHz), Figure S35: Part of the ^1H NMR spectral of 1-(2'-hydroxyphenyl)-3-(3'',4'',5''-trimethoxyphenyl)-2-propan-1-one (**13**) (CDCl_3 , 600 MHz), Figure S36: ^{13}C NMR spectral of 1-(2'-hydroxyphenyl)-3-(3'',4'',5''-trimethoxyphenyl)-2-propan-1-one (**13**) (CDCl_3 , 151 MHz), Figure S37: Part of the ^{13}C NMR spectral of 1-(2'-hydroxyphenyl)-3-(3'',4'',5''-trimethoxyphenyl)-2-propan-1-one (**13**) (CDCl_3 , 600 MHz), Figure S38: HMBC spectral of 1-(2'-hydroxyphenyl)-3-(3'',4'',5''-trimethoxyphenyl)-2-propan-1-one (**13**) (CDCl_3 , 151 MHz).

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Abbreviations

MDPI	Multidisciplinary Digital Publishing Institute
DOAJ	Directory of open access journals
TLA	Three letter acronym
LD	linear dichroism

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