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Epoxide hydrolase activity in the aqueous extracts of vegetable flours and application to the stereoselective hydrolysis of limonene oxide

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Abstract: The aqueous buffered suspensions of some vegetable flours were tested in the reaction with 1,2-epoxide deriving from (4*R*)- or (4*S*)-limonene as well as with the standard substrate methyl oleate epoxide and epoxide hydrolase activity was mainly detected in the flours from soybean, mung bean and red mung bean. A simple protocol was applied for obtaining a raw freeze-dried enzymatic preparation from flours, which maintained most of the activity of the fresh suspension and offered advantages in easy handling, reproducibility and stability over the time. Preferential hydrolysis of one of the diastereoisomers present in the starting (4*R*)- or (4*S*)-limonene oxide (*cis*/*trans* mixture) was observed and the reaction converged toward the formation of a predominant diol, resulting from the attack on the most (C1)- or less (C2)-substituted carbon of the oxirane ring depending on the *cis*/*trans* stereochemistry of the substrate. Biocatalyzed hydrolysis of (*S*)-limonene oxide proceeded at higher reaction rate compared to the (*R*)-isomer and with superior stereoconvergence toward a single diol product. The kinetic separation of the slow-reacting diastereoisomers of (*R*)- and (*S*)-limonene oxides was optimized by using the enzymatic preparations from red mung bean and soybean, respectively, and *trans*-(4*R*)-limonene oxide and *cis*-(4*S*)-limonene oxide were obtained in good yield and stereochemical purity.

Keywords: epoxide hydrolase; limonene oxide; vegetable flour; lyophilization

1. Introduction

Current challenges in more sustainable and renewable chemicals have driven a growing interest in natural terpenes, which are yet widely employed in food, cosmetic and pharmaceutical industry, as a valuable chemical platform for the production of high-value compounds and materials [1-4]. Limonene, for its abundance and low price, has a strong impact on the market and limonene polycarbonate is an excellent example of bio-based polymer with good mechanical properties and thermal stability [5]. A variety of chemical modifications of limonene with different methods have been investigated, with a special focus on the epoxidation of its double bond(s) [6-9] and the synthesis of limonene diols as precursor of cyclic carbonates [10-12]. In this context, biocatalyzed reactions offer additional environmental safety for the lack of metal catalysts, mild reaction conditions and the use of aqueous solvents, providing at the same time high regio- and stereoselectivity [13-15].

Bio-oxyfunctionalization of limonene by a variety of microorganism has been reported [16-19], but the toxicity of both substrate and products often results in low productivity and high cost of the fermentation processes. The use of purified enzymes can offer a valuable alternative and the epoxidation of limonene with bacterial P450 monooxygenases has been shown feasible [20-22].

Enantioconvergent hydrolysis of limonene oxide to the corresponding 1,2-diol has been reported in the presence of limonene epoxide hydrolase (LEH, EC 3.3.2.8), a highly-specific enzyme firstly isolated from limonene-grown cells of *Rhodococcus erythropolis* [23] and then identified from the genomic data of other bacterial strains [24-25], which is structurally different from the ubiquitous epoxide hydrolase (EH, EC 3.3.2.3) [26].

Although a variety of non-mammalian EH have been tested for synthetic purposes, the substrate scope is mostly limited to styrene oxides and few other epoxyalkanes [27-31]; however, it has recently shown that two novel EHs, Sibe-EH and CH65-EH, identified from metagenomic samples from hot springs in Russia and China were shown able to convert (*R*)-limonene oxide [*cis*/*trans* diastereoisomeric mixture, (4*R*)-(+)-**1**] into (1*S*,2*S*,4*R*)-limonene-1,2-diol (+)-**2** through the same enantioconvergent process previously described for LEHs [32].

We have recently reported that a peroxygenase-containing preparation from oat flour efficiently catalyzes the stereospecific epoxidation of both enantiomers of limonene giving *trans*-(*R*)-limonene oxide from (*R*)-limonene or *cis*-(*S*)-limonene oxide from (*S*)-limonene with excellent diastereoselectivity (dr >98%) [33]. As a part of our study on the use of raw enzymatic preparations from vegetable flours as economical alternative to purified enzymes, we planned to test these biocatalysts in the epoxide opening of limonene oxide to give the corresponding diols and here we report the obtained results.

2. Experimental

2.1. General

(*R*)-(+)-limonene oxide, (4*R*)-(+)-**1** was obtained from Fluka as 46:54 (*cis*:*trans*) diastereoisomeric mixture (>99%) and (*S*)-(–)-limonene oxide, (4*S*)-(–)-**1** was obtained from Sigma-Aldrich (*cis:trans* 45:55 mixture, 99%). Diethyl pyrocarbonate and *N,N'*-dicyclohexylurea were purchased from Sigma-Aldrich.

Seeds from organic crops were purchased in the local supermarket. Lyophilization was carried out on a Telstar LyoQuest instrument. Centrifugation was carried out on ALC PK130 centrifuge.

GC analyses were performed on a fast GC Shimadzu 17-A instrument equipped with FID detector (GCMS-QP5050A) and a Supelco SPB-5 capillary column (15 m x 0.1 mm ID x 0.1 μm film thickness). For the analyses the following parameters were set: He total flow 230 mL/min, column pressure 300 KPa, linear velocity 45 cm/sec, split ratio 280:1, injector temperature 250 °C, detector temperature 280 °C. The oven temperature was raised from 100 °C to 280 °C at 10 °C/min for monitoring the hydrolysis of methyl oleate epoxide. Analyses of the reaction mixtures of limonene were carried out under temperature gradient from 60 °C to 180 °C at 10 °C/min.

Column chromatography was performed on silica gel (Merck, 40-63 μm) using the specified eluents. Deactivated silica gel was prepared by suspending silica (50 g) in *n*-hexane (100 mL) containing 1% (v/v) triethylamine under stirring for 15 min, then the solid was collected by decantation, rinsed with *n*-hexane and dried at 40 °C overnight. TLC analyses were performed on aluminum plates coated with silica gel and fluorescent indicator F_{254}, revealing the compounds by UV and cerium sulfate.

*2.2. Screening for EH activity in vegetable flours with (R)-(+)-**1** substrate*

Air-dried seeds from the different plants were finely grounded with a domestic blender and the obtained flour was sifted on a tea sieve. Defatted flour was prepared by suspending the powder (5 g) in diethyl ether (10 mL) and stirring the mixture for 10 min at room temperature. The suspension was then centrifuged at 2930 *g* (4000 RPM) and the solution discarded. The procedure was then repeated another two times and the final solid was dried overnight in a fume hood to give defatted flour in about 90% yield (w/w).

For test reactions with flour suspension, the defatted flour (600 mg) was suspended in 50 mM potassium phosphate buffer pH 7.5 (4 mL) and the mixture used as a whole. For test reactions on supernatant fraction, the defatted flour (600 mg) was suspended in the phosphate buffer (4 ml), mixed under magnetic stirrer for 5 min and centrifuged at 4000 RPM for 15 min. The bottom fraction was discarded and the supernatant was used for the hydrolysis reaction by adding the substrate.

When required, preliminary hydration of defatted flour was carried out by maintaining the suspension of flour in phosphate buffer under stirring for 3 h at room temperature.

Freeze-dried enzymatic preparations were obtained as follows. Defatted flour (40 g) was suspended in water (100 ml) and the suspension was sonicated for 30 min in an ultrasound cleaner (45 kHz) and left to hydrate for 3 h at 25 °C. The mixture was then centrifuged at 4000 RPM for 15 min and the supernatant collected. The procedure was repeated again on the bottom fraction of the centrifugation and the pooled supernatant fractions were freeze-dried to give 12.8 g (soybean, 32 % w/w), 6.8 g (mung bean, MB, 17 % w/w) and 6.7 g (red mung bean, RMB, 17% w/w) of flaky powders.

In the test for activity, defatted flour (600 mg) or the suitable enzyme preparation deriving from 600 mg of flour was suspended in 4 mL of 50 mM potassium phosphate buffer at pH 7.5. To this suspension *(4R)*-(+)-**1** (20 μl, 18.6 mg, 0.12 mmol) was added and the reaction mixture was maintained under vigorous stirring at 25 °C. The reaction progress was monitored by GC analysis of aliquots (0.2 mL) of the reaction taken at regular intervals and extracted with MeOH:AcOEt 2:8 v/v (0.3 mL); after centrifugation of the sample 3 μL of the organic solution were injected for the GC analyses.

2.3. Test for EH activity with cis-9,10-epoxystearic acid methyl ester

Defatted flour (600 mg) was suspended in 50 mM phosphate buffer pH 7.5 (4 ml) and *cis*-9,10-epoxystearic acid methyl ester (40 μL, 0.12 mmol) was added. The suspension was maintained under vigorous stirring at 25 °C and the reaction progress was monitored by GC. Aliquots (0.2 mL) of the reaction were taken at regular intervals and extracted with MeOH:AcOEt 2:8 v/v (0.3 mL); after centrifugation of the sample 4 μL of the organic solution were injected for the GC analyses.

*2.4. Biocatalyzed hydrolysis of (4R)-(+)-**1***

Commercial *(4R)*-(+)-**1** (0.49 mL, 456 mg, 3 mmol) was added to a suspension of RMB freeze dried preparation (1 gr) in 50 mM potassium phosphate buffer pH 7.5 (20 mL) under vigorous magnetic stirring. The reaction was maintained at 40 °C and the substrate conversion monitored by GC analysis. After 30 h a 52% substrate conversion was reached and the mixture was extracted with *n*-pentane (3 × 15 mL) centrifuging at 4000 RPM for 30 min to better separate the organic phase. The organic layers were collected, dried over Na_2SO_4 and taken to dryness under controlled pressure (700 mbar, 30 °C) to give a residue which contained all the (4*R*)-*trans*-**1** and low amount of the diols. The residue was purified by column chromatography on deactivated Si gel eluting with *n*-pentane:Et_2O 80:20 (v/v) to give (4*R*)-*trans*-**1** (164 mg, 1.08 mmol, *dr* 97:3, 36% yield). Further elution with Et_2O gave (1*S*,2*S*,4*R*)-(+)-**2** (15 mg, 0.09 mmol, 3% yield).

The aqueous phase of the first extraction was partitioned with EtOAc (3 ×10 mL) and the organic phase, containing only the diol products, was separated and taken to dryness. Purification of the residue by column chromatography on Si gel with *n*-hexane:EtOAc 30:70 (v/v) allowed to separate diol (1*S*,2*S*,4*R*)-(+)-**2** (202 mg, 1.18 mmol, 39% yield) and (1*R*,2*R*,4*R*)-(–)-**3** (20 mg, 0.12 mmol, 4% yield). Spectroscopic and optical data of all the isolated compounds were in agreement with literature [33-34].

*2.5. Biocatalyzed hydrolysis of (S)-(–)-**1***

Commercial *(S)*-(–)-**1** (0.49 mL, 455 mg, 3 mmol) was added to a suspension of freeze dried preparation (1 g) from soybean in 50 mM potassium phosphate buffer pH 7.5 (20 mL) under vigorous magnetic stirring. The reaction was carried out at 25 °C and monitored by GC analysis. The reaction was stopped at 57% substrate conversion (44 h) and work-up of the reaction mixture as above gave (4*S*)- *cis*-**1** (145 mg, 0.95 mmol, *dr* 96:4, 32% yield) and (1*R*,2*R*,4*S*)-(–)-**2** (18 mg, 0.10 mmol, 3% yield) from the *n*-pentane extract. The EtOAc extract was taken to dryness to give pure (1*R*,2*R*,4*S*)-(–)-**2** (256 mg, 1.5 mmol, 50% yield) without further purification. Spectroscopic and optical data of all the isolated compounds were in agreement with literature [33-34].

2.6. Inhibition of the enzymatic activity

To a suspension of freeze dried enzymatic preparation from soybean (192 mg) or RMB (105 mg) in 50 mM potassium phosphate buffer pH 7.5 (2 mL), the inhibitor of choice, diethyl pyrocarbonate (8 μL, 0.055 mmol) or *N,N'*-dicyclohexylurea (12 mg, 0.055 mmol), was added. After incubating the mixture for 20 min at 25 °C under vigorous magnetic stirring, (4*R*)-(+)-**1** (50 μL, 46 mg, 0.3 mmol) was added and the reaction progress monitored by GC analysis.

3. Results and discussion

Since it is known that seeds of different plants belonging to Leguminosae family display EH activity [29-31, 35-37], at the onset of our work we considered some of them as source of the enzyme and the corresponding flours were tested for the hydrolytic opening of the commercial diastereomeric mixture (*cis*:*trans* 46:54 mixture) of (4*R*)-(+)-**1** (30 mM). In a preliminary screening defatted flours from grinded seeds of soybean (*Glycine max*), peas (*Pisum sativum*), red mung bean (*Vigna radiate,* RMB), mung bean (*Vigna angularis,* MB), faba (*Vicia faba*) and grass pea (*Lathyrus sativus*) were suspended in phosphate buffer at pH 7.5 and (4*R*)-(+)-**1** was added to the whole slurry. The reaction progress was monitored by GC and, pleasantly, we detected activity for all the flours, even at different rates; in all the cases the *cis*-oxide (4*R*)-*cis*-**1** was hydrolyzed first, giving a major (1*S*,2*S*,4*R*)-diol (+)-**2** and a minor (1*R*,2*R*,4*R*)-diol (–)-**3**, both identified by comparison with authentic samples (Figure 1).

After 24 h reaction time, the highest conversion of (4*R*)-(+)-**1** (50%) into the corresponding diols was observed in the presence of RMB flour, but satisfactory amount of diols (40-45%) coupled with acceptable selectivity toward (+)-**2** was also obtained with flours from soybean and MB. Prolonging the reaction time, the rate of conversion of the substrate sensibly slowed down after all the preferred (4*R*)-*cis*-**1** diastereoisomer was consumed, suggesting the occurrence of two sequential reactions, potentially exploitable for the kinetic separation of the substrate isomers.

The observed reaction rates roughly reflect the activity shown by the same flours in the same reaction conditions with *cis*-9,10-epoxystearic acid methyl ester, considered a standard test substrate for epoxide hydrolase activity. With this fatty acid substrate, all the flours displayed increased reaction rate compared to limonene oxide and the highest activity was obtained again with flour from RMB while the extracts from soybean and MB and displayed slightly lower reaction rate. Suspensions deriving from the flours of the other plants were found about 2-3 times less active than RMB (Table SI-1).

Flours from soybean, MB and RMB, which gave the best results in the hydrolysis of (4*R*)-limonene oxide, were chosen for further experiments aimed to optimize the reaction. The reaction profiles were compared for conversion at 24 h and in this time interval *cis*-(4*R*)-(+)-**1** was preferentially hydrolyzed

over the *trans*-(4*R*)-(+)-**1** isomer. Although both diastereoisomers of (4*R*)-(+)-**1** can contribute to the formation of (+)-**2** and (–)-**3**, in the first step of the biocatalyzed reaction the ratio of the two diol products, expressed as *Regioselectivity* = ***R*** = [(+)-**2**]/(–)-**3**] in Table 1, can be mainly related to the selectivity in the hydrolytic attack on the C1 or C2 carbon of *cis*-(4*R*)-(+)-**1** (Scheme 1).

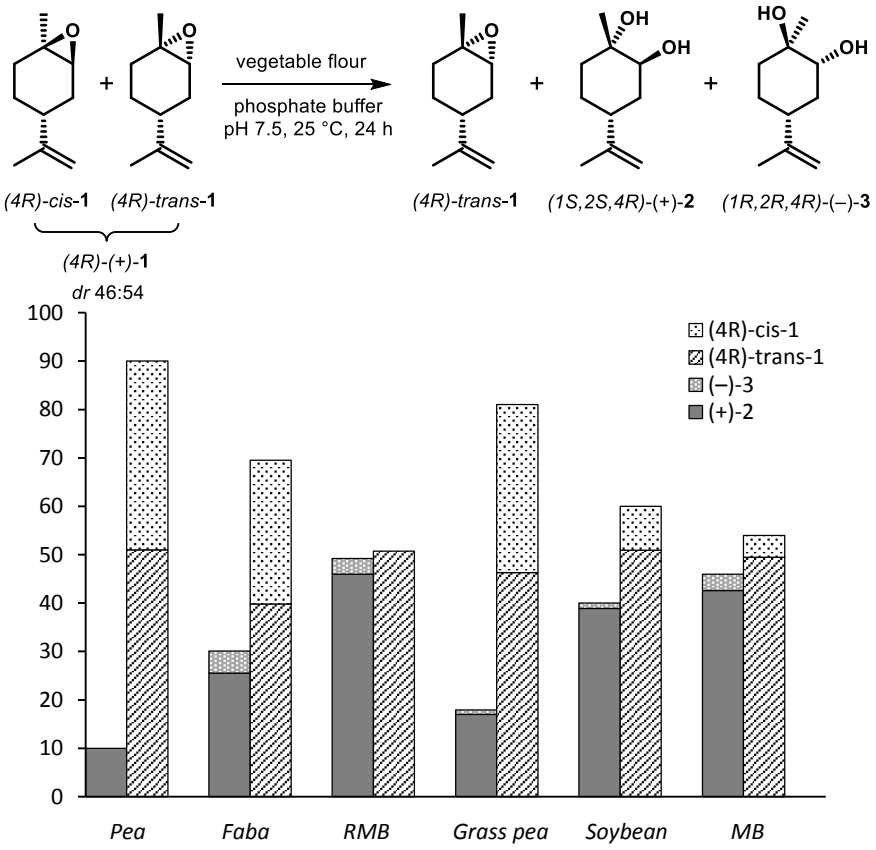
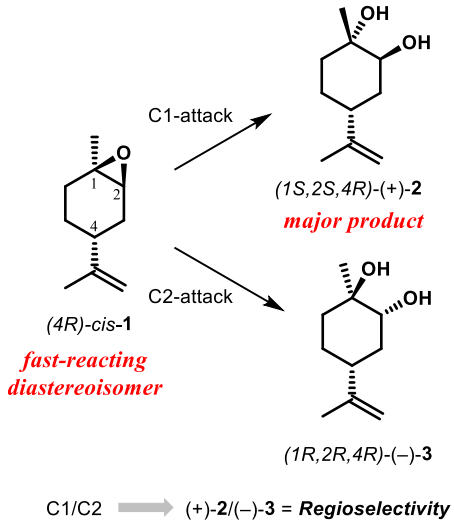


Figure 1. Composition of the reaction mixture (24 h) from biocatalyzed hydrolysis of (4*R*)-(+)-**1** with aqueous suspensions of vegetable flours



Scheme 1. Attack on the different carbons of the oxirane ring of *cis*-(4*R*)-(+)-limonene oxide leading to diols (+)-**2** and (–)-**3**

The aqueous suspension of flours from MB and RMB, which belong to the same plant genus (*Vigna*), promoted the hydrolysis of (4*R*)-(+)-**1** with comparable reaction rate, higher than that of soybean, which instead was found markedly more selective in giving (+)-**2** (Table 1, compare entries 1, 6 and 9). Since epoxide hydrolase is known as a cytosolic enzyme in plants [36-37], the whole aqueous suspension of flours was centrifuged at 2930 *g* (4000 RPM) and the supernatant fractions were tested. The supernatant fraction from soybean and MB displayed halved activity with respect to the whole suspension (Table 1, compare entries 1-2 and 9-10) suggesting that a not negligible fraction of the enzyme could be localized in high-density cell fractions or not sufficiently solubilized. The loss of enzymatic activity in these fractions was partially mitigated by preliminary hydration of the flour before centrifugation (Table 1, compare entries 2 with 4 and 10 with 12), while the enzymatic performances for RMB were not significantly affected by this pretreatment.

Table 1. Hydrolysis of the diastereoisomeric mixture of (4*R*)-(+)-**1** with different enzymatic preparations[a] from vegetable flours[b]

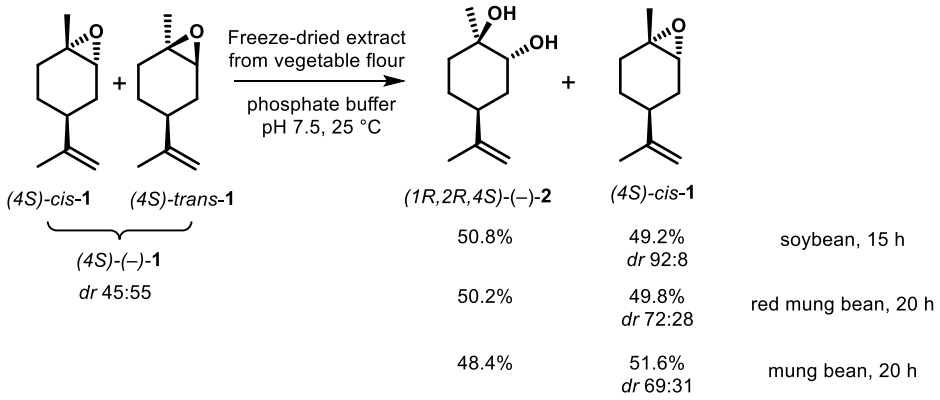
Entry	Flour	Tested fraction	Conv. %[b]	*Cis-(+)-**1*** %[c]	*Trans-(+)-***1** %[c]	*Dr (cis:trans)*	**(+)-2** %[c]	**(–)-3** %[c]	***R*[d]**
1	Soybean	suspension	44.4	8.4	47.1	15:85	43.2	1.2	35.4
2		supernatant	22.5	27.1	50.3	35:65	21.8	0.7	31.3
3		suspension[e]	44.1	8.6	47.2	15:85	42.7	1.4	29.5
4		supernatant[e]	33.4	17.8	48.8	27:73	32.5	0.9	35.9
5		LIO[e]	34.6	18.4	47.0	28:72	33.6	1.0	33.1
6	RMB	suspension	53.7	0.5	45.9	1:99	50.2	3.5	14.4
7		supernatant	41.7	10.9	47.5	19:81	39.0	2.7	14.5
8		LIO	43.3	9.5	47.2	17:83	40.5	2.8	14.6
9	MB	suspension	50.5	4.1	45.3	8:92	46.9	3.7	12.5
10		supernatant	27.5	25.0	47.5	34:66	25.6	1.8	14.0
11		suspension[e]	50.4	5.7	43.9	11:88	46.4	4.1	11.4
12		supernatant[e]	48.7	6.7	44.6	13:87	44.8	4.0	11.3
13		LIO[e]	39.9	19.0	41.2	31:69	37.0	2.9	12.7

[a] Suspension*:* defatted flour suspended in phosphate buffer; Supernatant: the solution obtained after centrifugation of the suspension at 2930 *g* (4000 RPM) for 15 min; LIO: freeze dried preparation; [b] Reaction conditions: defatted flour (600 mg) or the suitable enzyme preparation deriving from 600 mg of flour, 50 mM phosphate buffer pH 7.5 (4 ml), (4*R*)-(+)-**1** (0.12 mmol, 20 μl), 25 °C, 24 h. [c] Determined by GC analysis. [d] R = [(+)-**2**]/(–)-**3**] [e] Defatted flour was hydrated at 25 °C for 3h before the use (see experimental details).

Since we have previously shown [38] that lyophilization of aqueous extracts from vegetable flours offers advantages in terms of handling, enzyme stability and reproducibility, the supernatant fractions were then freeze-dried to give flaky powders in 32% (w/w) yield with respect to the starting flour for soybean and 17% (w/w) yield for MB and RMB. The enzymatic preparations from soybean and RMB maintained the performances of the original supernatant fractions (Table 1, compare entries 4-5 and 7-8) and were found stable over 6 months by storage at –20 °C. A slight decrease of activity was

instead observed for the freeze-dried preparation from MB compared to the freshly prepared surnatant fraction (Table 1, compare entries 12-13).

The lyophilized preparations were also tested on (4*S*)-(–)-**1** (*cis*/*trans* 45:55 mixture) and the monitoring of the reaction progress revealed a preferential hydrolysis of (4*S*)-*trans*-**1** diastereoisomer followed by the conversion of the slow-reacting (4*S*)-*cis*-**1** substrate, coupled with complete stereoconvergence resulting in the exclusive formation of (–)-**2** (Scheme 2). All the reactions proceeded with higher reaction rate compared to (4*R*)-(+)-**1** and the best discrimination of the two diastereoisomers of (4*S*)-(–)-**1** was observed in presence of the enzymatic preparation from soybean.



Scheme 2. Biocatalyzed hydrolysis of (4*S*)-(–)-**1** in the presence of freeze-dried enzymatic preparations from vegetable flours

The formation of a single diol, (+)-**2** or (–)-**2**, starting from a diastereoisomeric mixture of limonene oxide implies a different regioselectivity in the attack on the most (C1)- or less (C2)-substituted carbon of the oxirane ring of the *cis*- or *trans*-isomers of (4*R*)-(+)-**1** and (4*S*)-(–)-**1** (see Scheme 1 for (4*R*)-(+)-**1**). However, it has been nicely demonstrated by DFT calculation that a rationale for stereoconvergence in the hydrolysis of limonene oxides by LEH from *Rhodococcus erythropolis* (*Re*LEH) can be derived from the half-chair conformation of the substrates, driven by the configuration of the isopropenyl substituent, rather than to the different orientation of the substrate in the active site of the enzyme [39].

Our results are in agreement with those reported for *Re*LEH for that concerns the stereoconvergence of the hydrolytic reaction as well as the stereopreference towards the (1*R*,2*S*)-configuration of the oxirane ring in the fast-reacting diastereoisomers of (4*R*)-(+)-**1** and (4*S*)-(–)-**1**. Conversely, the higher reaction rate here observed for (4*S*)-(–)-**1** is opposite to that reported [23, 34] and the hydrolysis of (4*R*)-(+)-**1** with these plant EHs shows a slightly decreased selectivity, leading to the formation of diol (–)-**3** as side product.

All the above described reaction could be exploited for the synthesis of (+)-**2** starting from (4*R*)-(+)-**1**, considering that the diastereoisomeric side-product (–)-**3** is easily separable by column

chromatography, or the synthesis of (–)-**2,** which is obtained as single product from (*S*)-(–)-**1**. However, complete conversion of the starting epoxides required very long reaction time (>3 days at 30 mM substrate concentration) due to the marked slowdown of the reaction rate after the preferred isomer of substrate is consumed. Since also the chemical hydrolysis of (4*R*)- or (4*S*)-limonene oxides occurs with high selectivity [40], major interest in the EH-catalyzed hydrolysis could reside in the kinetic separation of the *cis*- and *trans*-isomers of the starting substrate [34].

With this aim, the enzymatic preparations from RMB and soybean, which had given the highest reaction rate and selectivity in the preliminary experiments with (4*R*)-(+)-**1** and (4*S*)-(–)-**1**, respectively, were chosen for further optimization of the productivity of the biocatalyzed hydrolysis. A series of reactions was then carried out with RMB by increasing the concentration of (4*R*)-(+)-**1** in buffered water as well as in the presence of acetone cosolvent (10% v/v) and the reaction rate was found to decrease almost linearly with the increasing substrate concentration. Comparable results in the reaction rate were obtained even in the presence of acetone, but the selectivity toward the (4*R*)-*cis*-**1** isomer was affected negatively. Also 10% acetonitrile- and 10% dioxane-buffer mixtures were tested, but unsatisfactory results in terms of reaction rate/selectivity were observed so that the use of the cosolvent was not further investigated. Although the enzymatic activity was maintained for over 6 d, a good compromise between substrate concentration and reaction time was found by using 150 mM (4*R*)-(+)-**1** and 1:2 (w/w) ratio of substrate to lyophilized enzymatic preparation. Increasing temperature at 40 °C resulted in almost doubled reaction rate without lowering the diastereoselectivity, so allowing to reach the optimal conditions for the kinetic separation of the slow-reacting isomer of (4*R*)-(+)-**1** in 24-30 h. In a preparative reaction, indeed, after 30 h (52% conversion) the unreacted substrate was isolated as (4*R*)-*trans*-**1** (*dr* 97:3) in 36% yield together with the expected diols (1*S*,2*S*,4*R*)-(+)-**2** and (1*S*,2*S*,4*R*)-(–)-**3** in 42% and 4% yield, respectively.

The enzyme preparation from soybean well tolerated a concentration of (4*S*)-(–)-**1** substrate up to 150 mM, but was partially deactivated by increasing the reaction temperature. In a preparative reaction, after 44 h at 25 °C (57% conversion) the unreacted (4*S*)-*cis*-(–)-**1** (*dr* 96:4) was obtained in 32% yield together with (1*R*,2*R*,4*S*)-(–)-**2** as single diol (53% yield).

As far as we know, this is the first report of the ability of a non-bacterial epoxide hydrolase to catalyze the stereoselective biotransformation of 1,2-limonene oxide and inhibition experiments (Table SI-2) suggested that the active enzymes in our preparations from soybean and RMB are epoxide hydrolases belonging to the α,β-hydrolase fold superfamily. Indeed, the enzymatic activity was effectively reduced in the presence of the imidazole-modifying compound diethylpyrocarbonate, a well-known inhibitor of α,β-hydrolase folded epoxide hydrolases [41, 42] for its ability to modify the histidine residue present in their active site [43]. Some inhibitory effect on our enzymatic preparations was

also observed with *N,N'*-dicyclohexylurea, a potent specific inhibitor of the human cytosolic epoxide hydrolase [44] whose binding to the enzyme has been associated to the presence of catalytic tyrosines (Tyr381 and Tyr465) involved in substrate activation [45].

4. Conclusions

Raw enzymatic preparations from some vegetable flours were found to display epoxide hydrolase activity and those deriving from red mung bean and soybean were applied to the stereoselective and stereoconvergent hydrolysis of (4*R*)- and (4*S*)-limonene-1,2-oxide, respectively. A kinetically preferred hydrolysis of one of the two diastereoisomers present in the starting substrates was observed, which allowed to isolate the unreacted (4*R*)-*trans*- or (4*S*)-*cis*-limonene-1,2-oxide in good optical purity by choosing the suitable reaction time. Stable freeze-dried preparations from flours of red mung bean and soybean offer a valid alternative to the use of purified epoxide hydrolases, most of which are isolated from animal tissues or bacterial cultures, and further investigation on their potential in organic synthesis is currently underway.

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Declaration of interests

The authors declare that they have no conflict of interests.

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