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BRIEF COMMUNICATIONS

BIOTRANSFORMATION OF AROMATIC KETONES BY *Linum usitatissimum*

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Acetophenone is a component of castoreum, the exudate from the castor sacs of the mature beaver [1]. *Linum usitatissimum* L. (Linaceae), known as flax or linseed, is a good source of oil and meal, which is rich in fiber, protein, and fat. Its oil is arguably one of the richest source of polyunsaturated fatty acids, used in food and in the prevention of cardiovascular diseases [2, 3].

The use of natural catalysts offers a clean way to perform chemical process under mild reaction conditions with less or no usage of organic solvents, a high degree of selectivity due to the high chemo-, regio- and enantioselectivity of enzymes, while working under mild and environmental friendly conditions linked to the principles of green chemistry [4–8]. Thus, the biocatalysts can be an interesting alternative to prepare chiral alcohols and can also act as chiral auxiliaries in asymmetric synthesis of chiral molecules, and as a result the production of single enantiomers of molecules has become increasingly important in the food, cosmetic, and pharmaceutical industries, from the corresponding prochiral ketones [6, 9–13]. Therefore, researchers have strived to find solutions for the replacement of conventional chemical routes by application of enzymes.

Reports in the literature describe the use of different plant species for biotransformation, with several advantages such as their disposal after use, their biodegrability, as well as their wide availability at low cost [6, 8–11, 14–17].

In particular, the potential source of enzymes from Brazilian northwestern plants and their use as biocatalysts in bioreduction reactions have been related [10, 11, 14, 17, 18]. In order to contribute to the study of vegetables as natural catalyst, this paper reports the use of whole plant cell of seeds of *L. usitatissimum* as a biocatalyst for the reduction reactions of aromatic ketones.

The enzymatic reduction of acetophenone (**1**) as standard substrate prochiral ketone and derivatives **2–14** using *L. usitatissimum* as biocatalyst is reported. All reactions were carried out using 50 mg of substrate and 20 g of biocatalyst in a buffer solution (Na_2HPO_4–KH_2PO_4), pH 6.0, over a period of 72 h at 25°C without a co-solvent which are the optimal reaction conditions among the tested parameters.

The bioreduction pattern of acetophenone (**1**) by *L. usitatissimum* occurred with a bioconversion value of 70.4%, a very good enantioselectivity (93.7% *ee*), and a satisfactory yield of (*S*)-alcohol. The study of reduction enzymatic reaction of acetophenone **1** and other prochiral ketones derived from acetophenones **2** to **12**, including α-tetralone **13** and 1-acetonaphthone **14**, was performed (Scheme 1).

The results showed that *L. usitatissimum* exhibited a broad spectrum of enantionselectivity among the acetophenones and their derivatives, with a very good enantioselectivity (>75% *ee*) for acetophenone (**1**) and its derivatives **2**, **6**, **8**, **9**, and **10** (Table 1). It was observed that the best enantiomeric excess (*ee*) compared with acetophenone (**1**) was obtained from 4-methylacetophenone (**6**) and 2-methoxyacetophenone (**10**), with an *ee* of 93.8 and ≥ 99.0, respectively.

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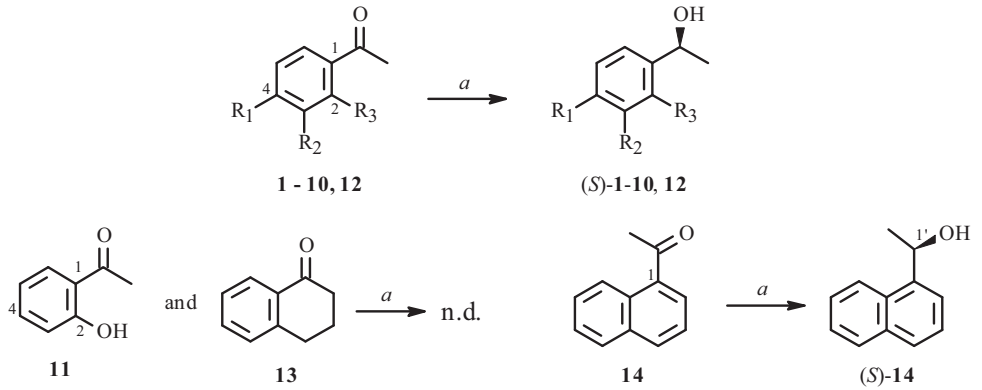
TABLE 1. *Linum usitatissimum* Seeds as Biocatalyst in Reduction of Acetophenone and Its Derivatives

Substrate	Bioconversion, %	*ee*, %	Substrate	Bioconversion, %	*ee*, %
1	70.4	93.7 (*S*)	**8**	20.6	81.0 (*S*)
2	99.2	84.5 (*S*)	**9**	40.8	85.1 (*S*)
3	97.5	62.2 (*S*)	**10**	1.1	≥ 99 (*S*)
4	39.1	41.3 (*S*)	**11**	N.d.	
5	2.9	64.4 (*S*)	**12**	80.3	76.5 (*S*)
6	92.1	93.8 (*S*)	**13**	N.d.	
7	73.4	46.8 (*S*)	**14**	83.1	78.9 (*S*)

N.d.: not determined.



1: $R_1 = R_2 = R_3 = H$; **2:** $R_1 = Br$, $R_2 = R_3 = H$; **3:** $R_1 = F$, $R_2 = R_3 = H$; **4:** $R_1 = Cl$, $R_2 = R_3 = H$
5: $R_1 = OCH_3$, $R_2 = R_3 = H$; **6:** $R_1 = CH_3$, $R_2 = R_3 = H$; **7:** $R_1 = R_3 = H$, $R_2 = Br$
8: $R_1 = R_3 = H$, $R_2 = OCH_3$; **9:** $R_1 = R_2 = H$, $R_3 = Br$; **10:** $R_1 = R_2 = H$, $R_3 = OCH_3$
12: $R_1 = R_2 = H$, $R_3 = Ph$

a. Linum usitatissimum

Scheme 1

Additionally we observed that 2-methoxyacetophenone (**10**) was reduced to an (*S*)-alcohol with excellent *ee* (≥ 99%) but with a very low bioconversion of 1.1%. The bioconversion values and *ee* are given in Table 1. The configurations of the enantiomers **1–10**, **12**, and **14** were established as "*S*" after comparison of their specific rotation with those described in the literature, which is in agreement with the Prelog model for bioreduction [18]. The bioreduction of the 2-hydroxyacetophenone (**11**) and α-tetralone (**13**) was not observed in **11**, probably because of the *ortho*-position of the hydroxyl group in the aromatic ring, which favors the formation of intramolecular hydrogen bonds and makes the carbonyl group less reactive. In derivative **13** we can attribute this to steric hindrance, making enzyme-substrate interaction more difficult.

Values obtained from the seeds of *L. usitatissimum* were poor when compared to the use of immobilized yeast cells such as *Cryptococcus laurentii* as biocatalyst [19]. Therein a higher percentage of bioconversion and an enantiomeric excess was observed for compounds **1–7** and **9**, but there was no change in enantioselectivity of the reaction, yielding the corresponding (*S*)-enantiomer of the alcohol, which is in agreement with Prelog′s rule. This was also related to the low values of bioconversion in acetophenone derivatives containing electron-donating substituents at the *para*-position [19, 20].

In conclusion, this paper reports a novel, facile, efficient, and green biocatalyst for reducing acetophenone, a natural compound, and some of its derivatives. Seeds of *Linum usitatissimum* L. can be very effective in the stereoselective reduction of this aromatic carbonyl compound with enantioselectivity from medium to high conversions.

Commercial seeds of *Linum usitatissimum* were purchased from a local market. Botanical identification data were provided by the Celeiro Alimentos Co. (celeiro@celeiroalimentos.com.br).

Studies were previously performed to determine the optimal reaction conditions among the parameters: amount of biocatalyst (2 g, 5 g, 10 g, and 20 g), time of reaction (24 h, 48, 72, and 96 h), use of co-solvent isopropyl alcohol in the proportions (v/v) 2, 5, and 10% in distilled water and a buffer solution previously prepared from Na_2HPO_4–KH_2PO_4, with pH 6.0, 7.0, and 8.0, using 50 mg of acetophenone as pattern substrate. Therefore, the reactions were carried out in the best reaction conditions among the parameters tested, using 50 mg of substrate and 20 g of biocatalyst in a buffer solution

(Na_2HPO_4–KH_2PO_4), pH 6.0, over a period of 72 h at 25°C without a co-solvent. In these reaction conditions, 70.4% of bioconversion and an *ee* of 93.7% were obtained for pattern acetophenone. All biotransformation reactions were performed using a modified methodology proposed by Machado et al. [11]. Whole seeds of *Linum usitatissimum* L. were washed with 5% sodium hypochlorite and rinsed with sterile distilled water. Each individual carbonyl substrate, **1**–**14** (50 mg), was added to a suspension of 20 g of *L. usitatissimum* seeds in 40 mL of a buffered solution (Na_2HPO_4–KH_2PO_4), pH 6.0, and incubated at 25°C in an orbital shaker (175 rpm) for 72 h. Controls were similarly processed, except that no substrates were added. All reactions were performed in triplicate. The course of all reactions was monitored by TLC (Merck, silica gel 60 F_{254}) and the substances revealed by spraying with vanillin solution. After completion of the reaction, each suspension was filtered and washed with water, and the aqueous solutions were extracted with CH_2Cl_2 (3 × 50 mL). The organic phases were dried with Na_2SO_4 and removed in a rotator evaporator. The reaction products were purified by column chromatography on silica gel 60 VETEC with a binary mixture of hexane–ethyl acetate (8:2, v/v) as eluent to afford the (*S*)-alcohols (Scheme 1). The optical rotations were measured on a PerkinElmer 241 digital polarimeter.

All reaction products were analyzed by high-performance liquid chromatography (HPLC) using an L201147 Shimadzu pump equipped with chiral columns OB-H and OD-H, a mobile phase binary mixture of *n*-hexane–isopropyl alcohol (90:10 to 98:2) varying in composition according to the requirements of the sample, and a Shimadzu UV-Vis detector SPD-M20A. The mass spectra were obtained using a Shimadzu GC-2010 plus model gas chromatograph coupled to a Shimadzu GCMS-QP model SE 2010 mass spectrometer, using a column with a stationary phase of 95% dimethylpolysiloxane–5% diphenylpolysiloxane with a length of 30 m, internal diameter 0.25 mm, outer diameter of 0.39 mm, and 0.25 μm film thickness and a Shimadzu auto-injector, model AOC-20i. The mobile phase was helium. The levels of conversion and enantiomeric excess of biocatalytic reactions were evaluated by a calibration curve (HPLC) made in the column corresponding to the starting ketone **1–14** and its corresponding alcohol (Table 1).

(*S*)-1-Phenylethanol (1). $[\alpha]_D^{20}$–33.1° (*c* 0.01, $CHCl_3$). GC-MS *m/z* (%): 122 (M^+, $C_8H_{10}O$; 25), 107 (100), 79 (90), 43 (30); HPLC: Chiralcel OB-H, 30°C, *n*-hexane–isopropyl alcohol (95:5), 0.5 mL/min, t_R (min): 9.8 (*S*), 14.4 (*R*).

(*S*)-1-(4-Bromophenyl)ethanol (2). $[\alpha]^{20}{}_D$ –24.6° (*c* 0.015, $CHCl_3$). GC-MS *m/z* (%): 200 (M^+, C_8H_9BrO; 20), 185 (60), 43 (60), 77 (100); HPLC: Chiralcel OB-H, 30°C, *n*-hexane–isopropyl alcohol (95:5), 0.3 mL/min, t_R (min): 15.4 (*S*), 17.4 (*R*).

(*S*)-1-(4-Fluorophenyl)ethanol (3). $[\alpha]_D^{20}$–18.2° (*c* 0.02, CH_2Cl_2). GC-MS *m/z* (%): 140 (M^+, C_8H_9FO; 20), 125 (100), 97 (70), 77 (30); HPLC: Chiralcel OB-H, 30°C, *n*-hexane–isopropyl alcohol (95:5), 0.3 mL/min, t_R (min): 15.2 (*S*), 16.5 (*R*).

(*S*)-1-(4-Chlorophenyl)ethanol (4). $[\alpha]_D^{20}$ –19.6° (*c* 0.01, CH_2Cl_2). GC-MS *m/z* (%): 156 (M^+, C_8H_9ClO; 30), 141 (100), 113 (40), 77 (100); HPLC: Chiralcel OB-H, 30°C, *n*-hexane–isopropyl alcohol (98:2), 0.8 mL/min, t_R (min): 9.4 (*S*), 10.5 (*R*).

(*S*)-1-(4-Methoxyphenyl)ethanol (5). $[\alpha]_D^{20}$ –20.1° (*c* 0.01, CH_2Cl_2). GC-MS *m/z* (%): 152 (M^+, $C_9H_{12}O_2$; 30), 137 (100), 109 (50), 77 (30); HPLC: Chiralcel OB-H, 30°C, *n*-hexane–isopropyl alcohol (92:8), 0.8 mL/min, t_R (min): 10.5 (*S*), 14.0 (*R*).

(*S*)-1-(4-Methylphenyl)ethanol (6). $[\alpha]_D^{20}$ –23.7° (*c* 0.012, CH_2Cl_2). GC-MS *m/z* (%): 136 (M^+, $C_9H_{12}O$; 40), 121 (100), 93 (80), 77 (35); HPLC: Chiralcel OB-H, 30°C, *n*-hexane–isopropyl alcohol (98:2), 0.5 mL/min, t_R (min): 17.0 (*S*), 19.0 (*R*).

(*S*)-1-(3-Bromophenyl)ethanol (7). $[\alpha]_D^{20}$ –29.2° (*c* 0.011, $CHCl_3$). GC-MS *m/z* (%): 200 (M^+, C_8H_9BrO; 20), 185 (50), 77 (100), 43 (60); HPLC: Chiralcel OB-H, 30°C, *n*-hexane–isopropyl alcohol (95:5), 0.5 mL/min, t_R (min): 10.4 (*S*), 14.0 (*R*).

(*S*)-1-(3-Methoxyphenyl)ethanol (8). $[\alpha]_D^{20}$–26.1° (*c* 0.01, $CHCl_3$). GC-MS *m/z* (%): 152 (M^+, $C_9H_{12}O_2$; 50), 135 (70), 109 (100), 77 (50); HPLC: Chiralcel OB-H, 30°C, *n*-hexane–isopropyl alcohol (92:8), 0.8 mL/min, t_R (min): 8.6 (*S*), 11.5 (*R*).

(*S*)-1-(2-Bromophenyl)ethanol (9). $[\alpha]_D^{20}$ –24.7° (*c* 0.013, $CHCl_3$). GC-MS *m/z* (%): 200 (M^+, C_8H_9BrO; 10), 185 (80), 77 (100), 43 (30); HPLC: Chiralcel OB-H, 30°C, *n*-hexane–isopropyl alcohol (95:5), 0.5 mL/min, t_R (min): 7.2 (*S*), 11.9 (*R*).

(*S*)-1-(2-Methoxyphenyl)ethanol (10). $[\alpha]_D^{20}$ –22.4° (*c* 0.02, $CHCl_3$). GC-MS *m/z* (%): 152 (M^+, $C_9H_{12}O_2$; 30), 137 (100), 107 (70), 77 (30); HPLC: Chiralcel OB-H, 30°C, *n*-hexane–isopropyl alcohol (95:5), 0.5 mL/min, t_R (min): 10.2 (*S*).

(*S*)-1-(2-Biphenylyl)ethanol (12). $[\alpha]_D^{20}$–22.4° (*c* 0.015, $CHCl_3$). GC-MS *m/z* (%): 198 (M^+, $C_{14}H_{14}O$; 10), 107 (70), 92 (100), 79 (60); HPLC: Chiralcel OB-H, 30°C, *n*-hexane–isopropyl alcohol (95:5), 1 mL/min, t_R (min): 7.0 (*S*), 8.1 (*R*).

(*S*)-1-(1′-Napthyl)ethanol (14). $[\alpha]_D^{20}$–16.1° (*c* 0.02, CH_2Cl_2). GC-MS *m/z* (%): 172 (M^+, $C_{12}H_{12}O$; 30), 157 (30), 129 (100), 43 (10); HPLC: Chiralcel OD-H, 30°C, *n*-hexane–isopropyl alcohol (90:10), 0.5 mL/min, t_R (min): 11.5 (*S*), 15.8 (*R*).

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