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Full Length Research Paper

Soybean *(Glycine max)* as a versatile biocatalyst for organic synthesis

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A series of aliphatic and aromatic aldehydes and ketones were reduced using plant cell preparations of *Glycine max* seeds (soybean). The biotransformation of five aromatic aldehydes in water, at room temperature afforded the corresponding alcohols in excellent yields varying from 89 to 100%. Two prochiral aromatic ketones yielded the alcohol in very low conversion, 1% and to 4%; however with good enantiomeric excess (*ee*) of 99 and 79%, respectively. Additionally, three prochiral and one cyclic aliphatic ketones produced the corresponding alcohols in moderate yields varying from 10 to 58% and *ee* varying from 73 to 99%. Hydrolysis of two aromatic esters yielded the expected carboxylic acids in 49 and 66%. Most of the obtained alcohols have commercial value as cosmetic fragrances. Although, the enzymes present in soybean (reductase/lipase) has not been defined, the reaction is an important route for the preparation of pure alcohols and carboxylic acid, with low cost and environmental impact.

Key words: *Glycine max*, biocatalysis, bioreduction, aldehydes and ketones, ester hydrolysis.

INTRODUCTION

During the last decades chemical reactions using plant cell cultures, whole plants or microorganisms as biocatalysts have received a great deal of attention. The current interest in applying biocatalysis into organic chemistry is related to the preparation of optically active compounds with high stereoselectivity under environmentally friendly conditions. The chiral alcohols obtained by plant-mediated reductions of carbonyl groups, are in great demand by various industries, since they are precursors of drugs, agrochemicals (pheromones), specialty materials (for example, liquid crystals), flavors and fragrances (Yadav et al., 2002, 2007; Ishihara et al., 2003; Caron et al., 2005).

The use of different plant species for biotransformation in particular is an increasing practice and it represents an interesting route for the synthesis of useful compounds (Giri et al., 2001; Longo and Sanromaín, 2006). General advantages of plants as reagents are their easy disposal after use, as they are biodegradable with mild reaction conditions, as well as their wide availability at low cost (Bohman et al., 2009).

In order to determine the potential source of enzymes from Brazilian northeastern plants to be used as biocatalysts, an investigation of different tropical fruits and vegetables as bioreduction agents was carried out. Recently, plant parts and microorganisms have been used directly as biocatalysts in sources of reductase activity with alcohol dehydrogenase systems, such as *Daucus carota* (Yadav et al., 2002b, 2008), *Manihot* species (Machado et al., 2006), *Saccharum officinarum* (Assunção et al., 2008), *Passiflora edulis* (Machado et al., 2008), *Cocos nucifera* (Fonseca et al., 2009), *Lentinus strigellus* (Barros-Filho et al., 2009) and *Candida tropicalis* (Vieira et al., 2010). Seeds of *Vigna unguiculata* was recently investigated as biocatalyst and showed promising agent for the reduction of carbonyl and nitro group (Bizerra et al., 2010).

Glycine max (Leguminosae) has been economically used as a source of proteins for an industrial purpose (Kumar et al., 2009). Therefore, the aim of this work was to study the biocatalytic properties of seeds from soybean

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Table 1. *Glycine max* seeds biocatalyzed reduction and hydrolysis of organic compound

Entry	Product	*c* (%)	ee_P (%)[a]	Configuration alcohol
	1a	56		
1	1b	28		
	1c	14		
2	2a	100		
3	3a	89		
4	4a	100		
5	5a	4	>99	*S*
6	6a	1	79	*S*
7	7a	47		
8	8a	49		
9	9a	10	>99	*S*
10	10a	77	73	*S*
11	11a	24	76	*S*
12	12a	58		
13	13a	100		

[a] Enantiomeric excesses (*ee*) were determined by GC using chiral column.

(*Glycine max*), as an alternative to produce important intermediates for organic synthesis. As far as we are concerned, this is the first report on the use of seeds as a biocatalyst in organic reactions.

MATERIALS AND METHODS

General

All substrates were obtained from commercial suppliers. The products were obtained and the pure starting materials were analyzed by gas chromatography-mass spectroscopy (GC–MS) on a Hewlett–Packard Model 5971, using a (5%-phenyl)-methyl-polysiloxane DB-5 capillary column (30 m x 0.25 mm) with film thickness 0.1 μm; carrier gas helium, flow rate 1 mL/min with split mode. The injector temperature and a detector temperature was 250 and 200°C, respectively. The column temperature was programmed at 4°C /min from 35 to 180°C, and then at 10°C /min from 180 to 250°C. Enantiomeric excess (*ee*) was determined from chiral gas chromatography-flame ionization detector (GC-FID) analysis, as well as measuring of the optical rotations, being measured on a Perkin–Elmer 341 digital polarimeter, followed by a comparison with literature values. The products were analyzed by GC-FID on Thermo Electron GC-FID model Trace GC Ultra, using a Varian Chirasil-Dex CB capillary column (25 m x 0.25 mm x 0.25 μm). Column chromatography was run using silica gel 60 (70 to 230 mesh, Vetec), while thin layer chromatography (TLC) was conducted on precoated silica gel polyester sheets (Kieselgel 60 F_{254}, 0.20 mm, Merck). Compounds were detected by spraying vanillin followed by heating at 120°C.

Plant material

Commercial seeds from *Glycine max* were purchase in a local market and were identified by botanist at the Federal University of Ceara, Fortaleza-Ceara-Brazil.

Reduction of substrates

Seeds were rinsed with 5% of a sodium hypochlorite solution and distilled water. The seeds were tritured in blender until obtaining uniform not pieces approximately 0.5 cm and fats were removed by extraction with hexane for 24 h.

Substrates 1 to 20 (200 mg) were added to the triturated seeds (23 g) in 150 mL of water, and the reaction mixtures were incubated in an orbital shaker (175 rpm) at room temperature for 72 h, according to the literature procedures (Machado et al., 2006). The mixture was then filtered off, and the seeds were washed with water. Filtrates were extracted with EtOAc (3 x 100 mL). Then, the organic phase was dried (Na_2SO_4) and there after evaporated in a vacuum. The final products were purified by silica gel column using Hex:EtOAc (9:2, v/v) as eluent to afford reduced product: 1a, 1b and 1c (146 mg), 2a (123 mg), 3a (158 mg), 4a (142 mg), 5a (161 mg), 6a (154 mg), 7a (138 mg), 8a (164 mg), 9a (139 mg), 10a (149 mg), 11a (143 mg), 12a (158 mg), 13a (162 mg), 14a (165 mg), 15a (153 mg), 16a (144 mg), 17a (157 mg), 18a (163 mg), 19a (172 mg) and 20a (159 mg). Conversions were quantified by GC-MS, and its results are shown on Table 1.

Bioconversion versus time

In the kinetics experiments, compounds **1** was submitted to the same procedure, as previously described in the reduction of substrates. Samples were analyzed by GC-MS after 12, 24, 36, 48, 60, 72, 84, 96, 108, 120, 132 and 144 h to compound **1**. Experiments were performed in duplicate and the results are presented in Figure 1.

Acylation of compounds 9a, 10a and 11a to determination of enantiomeric excess

The enantiomeric excess of 9a, 10a and 11a were determined through the corresponding acylated derivative. The racemate alcohols were prepared by the reduction of the carbonylic

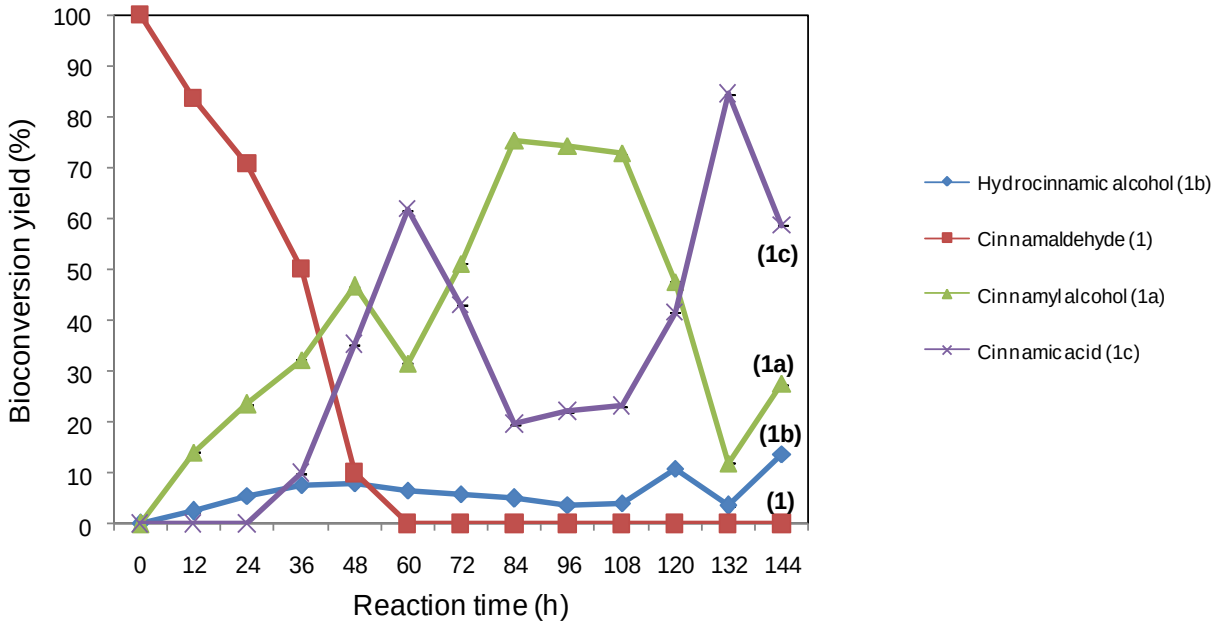


Figure 1. Bioconversion of cinnamaldehyde (**1**) to the corresponding hydrocinnamic alcohol (**1b**), cinnamyl alcohol (**1a**) and cinnamic acid (**1c**), using *Glycine max* seeds at room temperature. Error bars represent the standard error of mean.

compounds 9 to 11 with sodium borohydride in methanol. Racemate and the reaction product obtained by reduction were separately acylated with Ac_2O/ pyridine at room temperature (Almeida et al., 2010). Both acylated products were analyzed by Chiral GC and the *ee* of the bioreduction process was determined. GC conditions: 100°C, 2°C /min until 180°C.

RESULTS AND DISCUSSION

A series of simple aromatic and aliphatic, such as aldehydes, ketones and esters (Scheme 1) were treated with *Glycine max* seeds as biocatalyst.

Insert scheme 1

Crude reactions were analyzed by GC-MS and the results are presented in Table 1. All tested aldehydes (1 to 4) produced the corresponding alcohols in excellent yield, varying from 89 to 100% comparable to others previously published (Machado et al., 2008; Fonseca et al., 2009). Two aromatic ketones (acetophenone and *m*-methoxy-acephenone) 5 and 6 yielded the corresponding alcohols in very low conversion 1 and to 4%, however with good *ee* of 99 and 79%, respectively. Aliphatic ketones 9 to 12 produced the corresponding alcohols in moderate yield varying from 10 to 58% and *ee* varying from 73 to 99%.

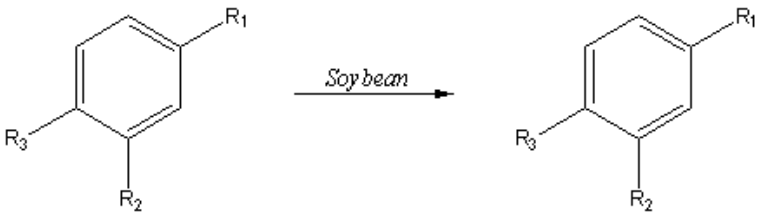
As expected, aldehydes were more reactive than ketones, and the presence of methoxy group in the *meta* position cause a decrease on the reaction yield (89%) when compared with unsubstituted benzaldehyde (100%) or benzaldehyde containing a methoxy group in the *para* position (100%). The chemoselectivity was not observed with cinnamaldehyde (1), where the reduction reaction generated three products after 72 h; first (1a, 56%) resulting from selective reduction of the carbonyl group; second (1b, 28%) related to non-chemoselective reduction of the carbonyl and olefinic bonds, as well as one minor product (1c, 14%), resulted from oxidation of the carbonyl group or alcohols to carboxylic acids. The products 1a and 1b were previously observed with *Saccharum officinarum* bioreduction (Assunção et al., 2008).

Insert Table 1

A kinetic investigation of biotransformation of cinnamaldehyde (1) was performed during seven days. Aliquots were analyzed by GC-MS with reaction times varied from 12, 24, 36, 48, 60, 72, 84, 96, 108, 120, 132 and 144 h. The results show the production of cinnamic acid (1c), hydrocinnamic alcohol (1b) and cinnamyl alcohol (1a). The results show it was important to extended reaction time in order to observe oxidation reaction. The results of kinetic studies are shown in Figure 1.

Insert Figure 1

For reduction of the aromatic ketone, 5 and 6, respectively the bioconversion afforded the corresponding



1. R_1 = CH=CHCHO; R_2= R_3= H

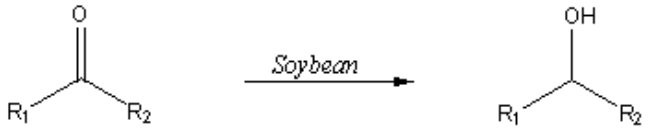
1a. R_1 = CH=CHCH$_2$OH; R_2= R_3= H
1b. R_1 = CH$_2$CH$_2$CH$_2$OH, R_2= R_3= H
1c. R_1 = CH=CHCOOH, R_2= R_3= H

2. R_1 = COH; R_2= R_3= H
3. R_1 = COH; R_2= OCH$_3$; R_3= H
4. R_1 = COH; R_2= H; R_3= OCH$_3$
5. R_1 = COCH$_3$; R_2= R_3= H
6. R_1 = COCH$_3$; R_2= OCH$_3$; R_3= H
7. R_1 = COOCH$_3$; R_2= R_3 = H

8. R_1 = COOCH$_2$CH$_3$; R_2= R_3= H

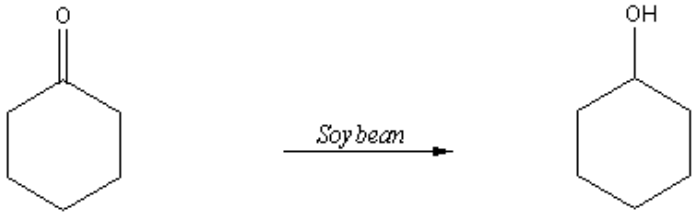
2a. R_1= CH$_2$OH; R_2=R_3= H
3a. R_1 = CH$_2$OH; R_2= OCH$_3$; R_3=H
4a. R_1 = CH$_2$OH; R_2= H; R_3= OCH$_3$
5a. R_1 = CHOHCH$_3$; R_2= R_3= H
6a. R_1 = CHOHCH$_3$; R_2= OCH$_3$; R_3= H
7a. R_1 = COOH; R_2=R_3= H

8a. R_1 = COOH; R_2= R_3= H



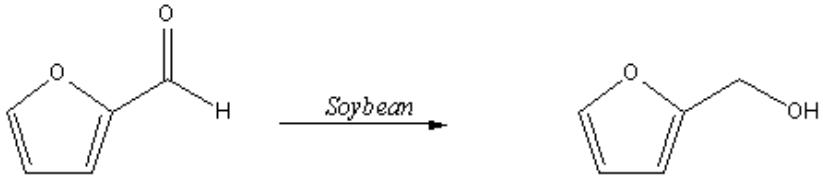
9. R_1 = CH$_3$; R_2= CH$_2$(CH$_2$)$_4$CH$_3$
10. R_1 = CH$_3$; R_2= CH$_2$COOCH$_2$CH$_3$
11. R_1= CH$_3$; R_2= CH$_2$CH$_2$CHCH$_2$

9a. R_1 = CH$_3$; R_2= CH$_2$(CH$_2$)$_4$CH$_3$
10a. R_1 = CH$_3$; R_2= CH$_2$COOCH$_2$CH$_3$
11a. R_1= CH$_3$; R_2= CH$_2$CH$_2$CHCH$_2$



12.

12a.



13.

13a.

Scheme 1. Reaction of *Glycine max* seeds with aromatic and aliphatic carbonyl compounds (aldehydes, ketones an esters).

secondary alcohols in low yields (4% of 5a and 1% of 6a). However, excellent and moderate enantioselectivity were obtained, with 99% *ee* for the (S)-isomer (5a) and 79% *ee* for the (S)-isomer (6a). Bioreduction of aliphatic ketones (9 and 11) were carried out producing the corresponding alcohols at low yields and excellent to

moderate enantioselectivity [10% (ee 99%) and 24% (ee 76%), respectively]. A moderated bioconversion (58%) was obtained with cyclohexanone 12, but no reaction was observed for menthone (14), carvone (15) α,β-unsaturated ketone and cyclopentanone (16). The reductase enzyme system present in soybean was also evaluated for effects on the β-keto-ester 10, having two different carbonyl groups. Complete chemio- and enantio-selectivity were observed through the exclusive reduction of the keto group at C-3 yielding 3*S*(+)-hydroxy-ethyl-butyrate 10a (77% yield), as a product showing an *ee* value of 73%.

Finally, the enzymatic reaction was extended to other functionable compounds: esters (methyl benzoate, 7 and ethyl benzoate, 8), an acid carboxylic (benzoic acid, 17), one nitrile (benzonitrile, 18), an amide (benzamide, 19), and a nitro derivative (nitrobenzene, 20). The aromatic ester produced the corresponding carboxylic acid (47% for 7a and 49% for 8a).

No reaction product was observed for the benzoic acid, benzamide, benzonitrile and nitrobenzene.

Conclusion

In summary, this work duly demonstrated that *Glycine max* seeds can act as a promising stereoselective biocatalyst to reduce carbonyl compounds to the corresponding alcohols with medium or high conversions suggesting that this vegetable may have interesting potential as a cheap sustainable alternative. It is encouraging to use the vast abundance of Brazilian biodiversity biocatalysts, calling an attention for selectivity and simplicity.

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REFERENCES

Almeida MCS, Alves LA, Souza LGS, Matos MC, Oliveira MCF, Lemos TLG, Braz-Filho R (2010). Flavonoides e outras substâncias de *Lippia sidoides* e suas atividades antioxidantes. Quim. Nova. 33:1877-1881.

Assunção JCC, Machado LL, Lemos TLG, Cordell GA, Monte FJQ (2008). Sugar cane juice for the bioreduction of carbonyl compounds. J. Mol. Cat. B: Enzyme, 52:194-198.

Barros-Filho, BA, de Oliveira MCF, Lemos TLG, de Mattos MC, Gonzalo G, Gotor-Fernández V, Gotor V (2009). *Lentinus strigellus*: a new versatile stereoselective biocatalyst for the bioreduction of prochiral ketones. Tetrahedron. Assym. 20: 1057-1061.

Bizerra AMC, Gonzalo G, Lavandera I, Gotor-Fernadez V, de Mattos MC, de Oliveira MCF, Lemos TLG, Gotor V (2010). Reduction processes biocatalyzed by *Vigna unguiculata*. Tetrahedron. Assym. 21: 566-570.

Bohman B, Cavonius LR, Unelius CR (2009). Vegetables as biocatalysts in stereoselective hydrolysis of labile organic compounds. Green Chem. 11:1900-1905.

Caron D, Coughlan AP, Simard M, Bernier J, Piché Y, Chênevert R (2005). Stereoselective reduction of ketones by *Daucus carota* hairy root cultures. Biotechnol. Lett. 27: 713-716.

Fonseca AM, Monte FJQ, de Oliveira MCF, de Mattos MC, Cordell GA, Braz-Filho R, Lemos TLG (2009). Coconut water (*Cocos nucifera* L.) - A new biocatalyst system for organic synthesis. J. Mol. Cat.. B: Enzyme, 57: 78-82.

Giri A, Dhingra V, Giri CC, Singh A, Ward OP, Narasu ML (2001). Biotransformation Using Plant Cells, Organ Cultures and Enzyme Systems: Current Trends and Future Prospects. Biotechnol. Adv. 19: 175-199.

Ishihara K, Hamada H, Hirata T, Nakajima N (2003). Biotransformation using plant cultured cells. J. Mol. Cat. B: Enzyme, 23: 145-170.

Kumar S, Dwevedi A, Kayastha AM (2009). Immobilization of soybean (*Glycine max*) urease on alginate and chitosan beads showing improved stability: Analytical applications. J. Mol. Cat. B: Enzyme, 58: 138-145.

Longo MA, Sanromaín MA (2006). Production of Food Aroma Compounds: Microbial and Enzymatic Methodol. Food Technol. Biotechnol. 44: 335-353.

Machado LL (2004). Estudo Fitoquimico e Atividade Biológicaos dos Frutos de Passiflora edulis e Redução Seletiva de Aldeídos e Cetonas Aromáticas Utilizando a Casca da Passiflora como Biocatalisador. MS Thesis, Edições UFC, Fortaleza, Brazil, 2003.

Machado LL, Monte FJQ, de Oliveira MCF, de Mattos MC, Lemos TLG, Gotor-Fernández V, Gonzalo G, Gotor V (2008). Bioreduction of aromatic aldehydes and ketones by fruits' barks of *Passiflora edulis*. J. Mol. Cat. B: Enzyme, 54:130-133.

Machado LL, Souza JSN, Mattos MC, Sakata SK, Cordell GA, Lemos TLG (2006). Bioreduction of aldehydes and ketones using *Manihot* species. Phytochemistry, 67: 1637-1643.

Maciel CDG, Poletine JP, Pereira JP, Mondini ML (2005). Avaliação da qualidade de sementes de soja (*Glycine max* (l.) Merrill) cultivar IAC-18.Revista Científica Eletrônica de Agronomia 7: 1-5.

Vieira GAB, Araujo DMF, Lemos TLG, de Mattos MC, de Oliveira MCF, Melo VMM, De Gonzalo G, Gotor-Fernández V, Gotor V (2010). *Candida tropicalis* CE017: a new Brazilian enzymatic source for the bioreduction of aromatic prochiral ketones. J. Braz. Chem. Soc. 21: 1509-1516.

Yadav JS, Reddy GSKK, Sabitha G, Krishna AD, Prasad AR, Rahaman HUR, Rao KV, Rao AB (2007). *Daucus carota* and baker's yeast mediated bio-reduction of prochiral ketones. Tetrahedron. Asymm. 18: 717-723.

Yadav JS, Nanda S, Thirupathi R, Bhaskar R (2002a). Efficient enantioselective reduction of ketones. J. Org. Chem. 67: 3900-3903.

Yadav JS, Nanda S, Thirupathi R, Bhaskar R (2002b). Efficient enantioselective reduction of ketones. J. Org. Chem. 67: 3900-3903.

Yadav JS, Reddy BVS, Sreelakshmi GC, Kumar GGKSN, Rao AB (2008). Enantioselective reduction of substituted tetrahydropyran-4-ones using *Daucus carota* plant cells. Tetrahedron. Lett. 49: 2768-2771.