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RESEARCH ARTICLE

Whole seeds of *Bauhinia variegata* L. (Fabaceae) as an efficient biocatalyst for benzyl alcohol preparations from benzaldehydes*

Gabriel I. Demmel[a], Daniela L. Bordón[b], Ana M. Vázquez[a], María F. Decarlini[a], Mariángeles Díaz Panero[a], Laura I. Rossi[c,d] and Mario L. Aimar[b]

[a]Facultad de Ciencias Químicas, Cátedra de Tecnología Química, Universidad Católica de Córdoba, Córdoba, Argentina; [b]Facultad de Ciencias Exactas, Físicas y Naturales, Cátedra de Química Aplicada, Universidad Nacional de Córdoba, Córdoba, Argentina; [c]Facultad de Ciencias Químicas, Departamento de Química Orgánica, Universidad Nacional de Córdoba, Córdoba, Argentina; [d]Instituto de Investigaciones en Físico-Química de Córdoba (INFIQC-CONICET), Córdoba, Argentina

ABSTRACT

Whole seeds of *Bauhinia variegata* L. (Fabaceae) were utilized as a biological reducer to transform benzaldehyde into benzyl alcohol. The effects of some variables such as temperature, the load of substrate and co-solvent, were established to optimize the reductive process. Utilizing the optimal reaction conditions, a laboratory-scale reaction (final concentration of the substrate: 21.2 mM) was performed to obtain benzyl alcohol (conversion: 95%; isolated yield: 49%; productivity: 1.11 g L^{-1} or 0.046 g $L^{-1}h^{-1}$ of benzyl alcohol). In addition, using these optimal conditions, fourteen substituted benzaldehydes were reduced, with a conversion achieved to their corresponding benzyl alcohols ranging from 62% to >99% (isolated yields from 7% to 70%). Moreover, useful building blocks by the synthesis of the drugs and important commercial products were also obtained. The scope, limitations and advantages of this new biocatalytic synthetic method are also discussed.

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1. Introduction

Nowadays, access to chemical processes that can be carried out in a manner compatible with the environment is a necessity of the first order (Roschangar et al. 2015). For this reason, the production of chemical compounds through the use of biological processes is gaining more and more prominence, because these methodologies can be used on a sustainable basis rather than by depleting natural resources (Cordell et al. 2007).

In this sense, the biocatalytic reduction of carbonyl compounds utilized to synthesize alcohols has established itself as a very important alternative to classical chemical procedures, not only because the reaction performance is very high, but also because the reaction media is usually water, so these systems generally operate at ambient temperature with the biocatalyst being renewable raw material (Patel 2018). For these characteristics, the waste products generated at the end of the process are generally more compatible with the environment than classical chemical reactions, which clearly represents an advantage (Kumaraswamy and Ramesh 2003). For these reasons, and as a direct consequence of the philosophical conception of Green Chemistry, the synthetic community has begun to pay greater attention to the use of bioprocesses applied to the synthesis of chemical compounds.

The bioreduction processes of carbonyl compounds to obtain alcohols have been catalyzed using different biological reducers. However, it is important to note that most investigations have focussed on the search for and improvement of methods that can provide chiral secondary alcohols obtained from the biocatalytic reduction of prochiral ketones. For this reason, the bioreduction of carboxylic acids (Strohmeier et al. 2019; Tramontina et al. 2020) and aldehydes (Li et al. 2013; Torrelo et al. 2015) has been proportionally less well studied, despite the importance of primary alcohols as intermediaries in the synthesis of numerous chemical compounds with industrial applications (Akhtar et al. 2013; Tramontina et al. 2020). Furthermore, the studies to date which have reported on aldehyde reduction have been oriented in two

CONTACT Mario L. Aimar mlaimar@unc.edu.ar Facultad de Ciencias Exactas, Físicas y Naturales, Cátedra de Química Aplicada, Universidad Nacional de Córdoba, Córdoba, Argentina

*This work is dedicated to the memory and teaching career of Prof. Dr. Hector R. Juliani.

main directions (Hollmann et al. 2021). On the one hand, the bioreduction of aldehydes has been carried out on racemic mixtures of α-substituted aldehydes, which when bioreduced can provide primary alcohols with a chiral centre adjacent to the carbon atom that contains the hydroxyl group (Giacomini et al. 2007; Friest et al. 2010; Galletti et al. 2010; Gooding et al. 2010; Kalaitzakis and Smonou 2010a, 2010b). On the other hand, studies have also been carried out in order to develop chemoselective reduction of aldehydes in the presence of ketones and other functional groups (Sello et al. 2006; Suárez-Franco et al. 2010; Salvano et al. 2011; Li et al. 2013).

Whole cells of microorganisms (bacteria (Vitale et al. 2020), fungus (Decarlini et al. 2017) and algae (Mouad et al. 2011)), isolated enzymes from various resources (Petrovičová et al. 2021), and more recently plants (Bennamane et al. 2014, 2015; Pavoković et al. 2017; Atak et al. 2019) and their cell cultures (Akakabe and Naoshima 1994; Baldassarre et al. 2000; Kazici et al. 2016, 2017), are among the biocatalytic systems that have been used to carry out the bioreduction of carbonyl compounds. However, it is necessary to recognize that the utilization of vegetables for these methods is a relatively new methodology (Cordell et al. 2007; Patil 2015). This way of working has recently been termed "botanochemistry" (Vandenberghe et al. 2013), and it offers numerous advantages in terms of bioavailability, cost and time, since complex processes of preparation, extraction, purification and multiplication of the biocatalyst are not necessary, which is often the case when working with microorganisms or cell cultures.

As in the case of classical biocatalytic systems, the use of different parts of plants as biocatalysts has been mainly utilized to biocatalyze the stereoselective reduction of ketones (Cordell et al. 2007; Patil 2015). However, as mentioned above, this methodology has also been applied, but less frequently, to carry out the synthesis of primary alcohols obtained from aldehydes (*Daucus corota, Conium maculatum, Manihot esculenta, Manihot dulcis, Cocos nucífera, Zea maiz, Musa sapientum*) (Yadav et al. 2002; Machado et al. 2006 and 2008; Fonseca et al. 2009; Salvano et al. 2011; Misra et al. 2012; Luna et al. 2014; Solís et al. 2019).

The possibility of using seeds as biocatalysts, moreover, has been very little explored in the synthesis of primary alcohols. In this sense, as far as we have been able to determine, very few reports have described the utilization of seeds as a biological reducer for carrying out the bioreduction of aldehydes (*Glicine max, Phaseolus vulgaris, Ximenia Americana, Lens culinaris, Ligustrum lucidum*) (Kumaraswamy and Ramesh 2003; Bertini 2012; Alves-Ferreira, da Costa, et al. 2012; Alves-Ferreira, Da Silva, et al. 2012; Carvalho da Silva et al. 2018; Bordón et al. 2021), and as of yet, there is only one report published that has described the possibility of reducing benzaldehydes to their corresponding benzyl alcohols using whole seeds as the biocatalyst (Bordón et al. 2021).

It is important to highlight that the use of seeds as biocatalysts does not imply a restriction related to the seasonal availability of seeds, since they can be stored until being used (Bordón et al. 2021; Demmel et al. 2021). This situation clearly contributes to their utilization as a "stable bag of enzymes" capable of carrying out chemical transformations of different compounds as if they were classic chemical catalysts. Moreover, the direct utilization of whole seeds further simplifies the work methodology.

Recently, our team has reported a study on the excellent capability of whole seeds of *Bauhinia variegata* L. (Fabaceae) to carry out the stereoselective reduction of acetophenones (Demmel et al. 2021) and following on from this investigation, we describe here the results obtained using whole seeds of "Orchid tree" to reduce different aromatic aldehydes to the corresponding benzyl alcohols. In this way, the field of application of this efficient and new biocatalyst has been expanded to be able to obtain important key intermediates used in the production of drugs and chemical compounds of commercial interest.

2. Material and methods

2.1. General

Benzaldehyde (BzHO) and substituted BzHOs were purchased from Sigma-Aldrich S.A. (Argentina). The corresponding benzyl alcohols were synthesized by reduction with $NaBH_4$ (Sigma-Aldrich) in medicinal-grade ethanol and used as a standard reference in the gas chromatography (GC) analysis. Sterile deionized water was used as the reaction media, and ethyl acetate and hexane were purified by a simple distillation prior to use. GC analyses were performed using a Buck Scientific Model 910/310 instrument with a flame ionization detector (FID), and GC-mass spectrometry (MS) analyses were carried out on a Hewlett Packard HP 5890 Series II gas chromatograph equipped with a Mass Detector HP 5970. The ^{1}H nuclear magnetic resonance (NMR) and ^{13}C NMR spectra were recorded on a Bruker Avance II 400 MHz.

2.2. GC-FID and GC-MS analyses

GC separation was performed on a Restek RTX-5 capillary column (dimethyl Crosslinked diphenyl polysiloxane, 30 m, 0.25 mm, 0.25 μm film thickness). The GC general conditions used for the split analysis were: 145 mL/min; injector, 250 °C; detector FID, 250 °C; carrier gas, N_2; and head pressure, 14.5 psi. The conversion percentages of the reactions were determined by GC using normalized peak areas without a correction factor, and the GC-MS (70 eV) analyses were carried using a Hewlett Packard HP-5ms capillary column (Crosslinked 5% PhMeSiloxane, 30 m, 0.32 mm, 0.25 μm film thickness).

2.3. Biocatalyst

Seeds of *B. variegata* L. were collected during the period September–December in the city of Córdoba and identified by a botanic expert. Before use, the seeds were washed with tap water to remove traces of dirt before being disinfected by immersion in a 0.5% sodium hypochlorite solution for 15 min. The seeds were then washed again, but this time with deionized sterile water.

2.3.1. Protein content

Samples of 0.6 g of dried and crushed *B. variegata* seeds were used to determine the protein content according to the Kjeldahl method (Carvalho da Silva et al. 2018).

2.3.2. Carbohydrate content

Samples of 1.0 g of dried and crushed *B. variegata* seeds were used to determine the carbohydrate content according to the Phenol/Sulfuric acid colorimetric method (DuBois et al. 1956).

2.3.3. Lipid content

Samples of 1.7 g of dried and crushed *B. variegata* seeds were used to determine the lipid content using a Soxhlet extractor with ethyl ether as the solvent (AOAC 1980).

2.3.4. Fibre content

The solid residue after lipid content determination (Soxhlet extraction) was used to determine the fibre content by acidic and basic hydrolysis using samples of 1.4 g (AOAC 1980).

2.3.5. Moisture content

Moisture content was determined gravimetrically using samples of 4.73 g of crushed seeds of *B. variegata* which were dried in an oven at 105 °C to constant weight (AOAC 1980).

2.3.6. Ash content

Ash content was determined gravimetrically by calcination of samples of 4.73 g of crushed seeds of *B. variegata* in a muffle at 500 °C to constant weight (AOAC 1980).

2.4. General procedure for the biocatalytic reductions of BzHO

A whole seed sample of *B. variegata* (10 g) was placed in a disinfected Erlenmeyer flask (250 mL) with sterile deionized water (80 mL), and then aldehyde (100–300 mg) was dissolved in an appropriate quantity of dimethyl sulfoxide (DMSO), which was added to the suspension. The reaction was carried out by stirring on an orbital shaker (100 rpm) at 20–45 °C, with the flask being closed and protected from direct light. Finally, the crude reaction was filtered through cotton in order to remove the seeds, and the solution was extracted with ethyl acetate, with very gentle stirring performed to avoid the formation of an emulsion.

2.5. Preparative-scale reactions

For the preparative-scale reaction, 1.8 g of BzHO, 80 g of seeds of *B. variegata,* 64 mL of DMSO and 640 mL of sterile distilled water were placed in a disinfected 2 L conical flask (final concentration of the substrate: 2.56 g/L or 14.3 mM). Then, this flask was put on an orbital shaker at a shaking speed of 100 rpm at 40 °C for 24 h, after which, a sample was taken and analyzed by GC-FID (95% conversion). The crude reaction system was extracted for 24 h with ethyl acetate using a liquid-liquid extractor, and the organic solution was dried using anhydrous magnesium sulphate. Then, the organic solution was concentrated to dryness in a rotary evaporator, and the crude reaction products were filtered on a short column with silica gel (70–230 mesh) using hexane–ethyl acetate mixtures in variable proportions as the eluent. Finally, 0.78 g of pure (**1**) was isolated (yield of the isolated product: 49%).

2.6. Reduction of substituted BzHOs with whole seeds of B. variegata

The same optimal proportions used in the scaling down of BzHO were used to reduce substituted BzHOs. The final products were extracted from reaction media with ethyl acetate using a liquid-liquid

extractor. The organic layer was separated and dried using anhydrous magnesium sulphate, after which, the organic solution was evaporated and the extracted crude reaction products were filtered on a short column with silica gel (70–230 mesh) using hexane–ethyl acetate mixtures in variable proportions as the eluent.

2.7. Chromatographic and spectroscopic data

After purification, all products were identified by comparing their retention times obtained by CG-FID analysis against the control samples, and by comparing their spectra of GC-MS, ^{1}H and ^{13}C NMR with literature data (Machado et al. 2006; Assunção et al. 2008; Misra et al. 2012; Alves-Ferreira, da Costa, et al. 2012; Alves-Ferreira, Da Silva, et al. 2012; Luna et al. 2014; Solís et al. 2019).

Benzyl alcohol (**1**): GC-FID analysis conditions: T_1 = 80 °C (5 min), ΔT = 20 °C/min, T_2 = 180 °C (7 min). GC Rt: benzaldehyde: 6.12 min, benzyl alcohol: 7.65 min. ^{1}H NMR (400 M Hz, $CDCl_3$) δ (ppm) = 2.52 (s, 1H, OH), 4.68 (s, 2H, CH_2), 7.32–7.38 (m, 5H, ArH). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) = 65.26; 127.00; 127.68; 128.57; 140.85. EIMS *m/z* (rel. int. %) = 109 (M^++1, 8), 108 (M^+, 98), 107 (M^+−1, 68), 91 (18), 78 (100), 78 (11), 77 (45), 51 (19), 50 (10).

2-Nitrobenzyl alcohol (**2**): GC-FID analysis conditions: T_1 = 80 °C (5 min), ΔT = 20 °C/min, T_2 = 180 °C (7 min). GC Rt: 2-nitrobenzaldehyde: 12.64 min, 2-nitrobenzyl alcohol: 12.86 min. ^{1}H NMR (400 M Hz, $CDCl_3$) δ (ppm) = 2.92 (s, 1H, OH); 4.97 (s, 2H, CH_2); 7.45–7.49 (t, 1H, ArH); 7.67 (t, 1H, ArH); 7.74 (d, 1H, ArH); 8.08 (d, 1H, ArH). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) = 62.45; 124.98; 128.46; 129.87; 134.12; 136.81; 147.62. EIMS *m/z* (rel. int. %) = 153 (M^+, 1), 136 (M^+-17, 2), 135 (10), 107 (M^+−46, 25), 91 (30), 79 (58), 78 (22), 77 (100), 65 (13), 51 (25), 50 (13).

3-Nitrobenzyl alcohol (**3**): GC-FID analysis conditions: T_1 = 80 °C (5 min), ΔT = 20 °C/min, T_2 = 180 °C (7 min). GC Rt: 3-nitrobenzaldehyde: 12.86 min, 3-nitrobenzyl alcohol: 14.50 min. ^{1}H NMR (400 M Hz, $CDCl_3$) δ (ppm) = 1.56 (s, 1H, OH); 4.83 (s, 2H, CH_2), 7.54 (t, 1H, ArH); 7.70 (d, 1H, ArH); 8.15 (d, 1H, ArH); 8.26 (s, 1H, ArH). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) = 64.02; 121.54; 122.62; 129.45; 130.89; 132.56; 142.86. EIMS *m/z* (rel. int. %) = 154 (M^++1, 3), 153 (M^+, 30), 136 (M^+−17, 33), 124 (5), 107 (M^+−46, 33), 89 (60), 77 (100), 63 (12), 51 (30), 50 (15).

4-Nitrobenzyl alcohol (**4**): GC-FID analysis conditions: T_1 = 80 °C (5 min), ΔT = 20 °C/min, T_2 = 180 °C (7 min). GC Rt: 4-nitrobenzaldehyde: 14.65 min, 4-nitrobenzyl alcohol: 15.96 min. ^{1}H NMR (400 M Hz, $CDCl_3$) δ (ppm) = 2.96 (s, 1H, OH), 4.82 (s, 2H, CH_2), 7.53–7.56 (d, 2H, ArH), 8.18–8.21 (d, 2H, ArH). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) = 63.78; 123.61; 126.95; 148.62; 207.58. EIMS *m/z* (rel. int. %) = 154 (M^++1, 3), 153 (M^+, 10), 136 (M^+−17, 5), 124 (2), 107 (M^+−46, 47), 89 (30), 77 (100), 63 (20), 51 (50), 50 (30).

2-Chlorobenzyl alcohol (**5**): GC-FID analysis conditions: T_1 = 80 °C (5 min), ΔT = 20 °C/min, T_2 = 180 °C (7 min). GC Rt: 2-chlorobenzaldehyde: 10.38 min, 2-chlorobenzyl alcohol: 12.32 min. ^{1}H NMR (400 M Hz, $CDCl_3$) δ (ppm) = 2.31 (s, 1H, OH); 4.75 (s, 2H; CH_2); 7.21–7.29 (m, 2H, ArH); 7.33–7.35 (m, 1H, ArH); 7.45–7.47 (m, 1H, ArH). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) = 62.78; 127.02; 128.72; 128.81; 129.34; 132.71; 138.19. EIMS *m/z* (rel. int. %) = 144 (M^++2, 12), 142 (M^+, 45), 125 (M^+−17, 10), 113 (12), 107 (M^+−36, 70), 105 (12), 89 (10), 79 (87), 77 (100), 63 (10), 51 (30), 50 (18).

3-Chlorobenzyl alcohol (**6**): GC-FID analysis conditions: T_1 = 80 °C (5 min), ΔT = 20 °C/min, T_2 = 180 °C (7 min). GC Rt: 2-chlorobenzaldehyde: 10.72 min, 3-chlorobenzyl alcohol: 11.93 min. ^{1}H NMR (400 M Hz, $CDCl_3$) δ (ppm) = 1.26 (s, 1H, OH), 4.68 (s, 2H, CH_2), 7.20–7.29 (m, 4H, ArH). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) = 64.57; 124.84; 126.97; 127.72; 129.81; 134.48; 142.83. EIMS *m/z* (rel. int. %) = 144 (M^++2, 20), 142 (M^+, 70), 125 (M^+–17, 10), 113 (20), 107 (M^+– 36, 83), 105 (12), 89 (10), 79 (78), 77 (100), 63 (7), 51 (20), 50 (12).

4-chlorobenzyl alcohol (**7**): GC-FID analysis conditions: T_1 = 80 °C (5 min), ΔT = 20 °C/min, T_2 = 180 °C (7 min). GC Rt: 4-chlorobenzaldehyde: 10.69 min, 4-chlorobenzyl alcohol: 11.99 min. ^{1}H NMR (400 M Hz, $CDCl_3$) δ (ppm) = 1.88 (s, 1H, OH), 4.67 (s, 2H, CH_2), 6.76–7.40–7.50 (m, 4H, ArH). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) = 64.57; 128.27; 128.69; 133.39; 139.27. EIMS *m/z* (rel. int. %) = 144 (M^++2), 142 (M^+, 28), 125 (M^+−17, 5), 113 (12), 107 (M^+– 35, 55), 79 (87), 77 (100), 63 (10), 51 (30).

2,4-diChlorobenzyl alcohol (**8**): GC-FID analysis conditions: T_1 = 80 °C (5 min), ΔT = 20 °C/min, T_2 = 180 °C (7 min). GC Rt: 2,4-dichlorobenzaldehyde: 10.05 min, 2,4-dichlorobenzyl alcohol: 11.51 min. ^{1}H NMR (400 M Hz, $CDCl_3$) δ (ppm) = 4.76 (s, 2H, CH_2), 7.28–7.52 (m, 3H, ArH). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) = 62.21; 127.18; 128.16; 129.48; 133.44; 133.89; 136.74. EIMS *m/z* (rel. int. %) = 178 (M^++2, 10), 176 (M^+, 26), 159 (M^+−17, 7), 141 (M^+−35, 41), 113 (46),111 (43), 77 (100), 75 (35), 63 (10), 50 (23).

4-Flurobenzyl alcohol (**9**): GC-FID analysis conditions: T_1 = 80 °C (5 min), ΔT = 20 °C/min, T_2 = 180 °C (7 min). GC Rt: 4-fluorobenzaldehyde: 7.96 min, 4-flurobenzyl alcohol: 9.64 min. ^{1}H NMR (400 M Hz, $CDCl_3$) δ (ppm)

= 2.50 (s, 1H, ArH); 4.66 (s, 2H, CH_2); 7.02–7.34 (m, 4H, ArH). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) = 64.65; 115.29; 115.50; 128.74; 128.72; 136.53; 161.12; 167.55. EIMS *m/z* (rel. int. %) = 126 (M^+, 100), 125 (M^+-1, 67), 109 (M^+-17, 30), 105 (30), 92 (100), 90 (27), 83 (10), 77 (30), 75 (13), 51 (14), 50 (9).

3-Hydroxybenzyl alcohol (**10**): GC-FID analysis conditions: T_1 = 80 °C (5 min), ΔT = 20 °C/min, T_2 = 180 °C (7 min). GC Rt: 3-hydroxybenzaldehyde: 11.82 min, 3-hydroxybenzyl alcohol: 13.03 min. ^{1}H NMR (400 M Hz, $CDCl_3$) δ (ppm) = 5.07 (s, 2H, CH_2); 5.74 (s, 1H, OH); 6.79–6.85 (m, 2H, ArH); 6.90 (d, 1H, ArH); 7.20–7.26 (m, 1H, ArH). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) = 66.20; 115.09; 115.33; 120.35; 129.88; 137.53; 155.96. EIMS *m/z* (rel. int. %) = 125 (M^++1, 7), 124 (M^+, 100), 123 (M^+-1, 27), 107 (M^+-17, 25), 106 (M^+-18, 21), 105 (23), 95 (98), 79 (18), 78 (50), 77 (89), 65 (23), 51 (19), 50 (9).

4-Hydroxybenzyl alcohol (**11**): GC-FID analysis conditions: T_1 = 80 °C (5 min), ΔT = 20 °C/min, T_2 = 180 °C (7 min). GC Rt: 4-hydroxybenzaldehyde: 11.78 min, 4-hydroxybenzyl alcohol: 11.97 min. ^{1}H NMR (400 M Hz, $CDCl_3$) δ (ppm) = 2.09 (s, 2H, CH_2); 5.05 (s, 1H, OH); 7.00 (d, 2H, ArH); 7.81 (d, 2H, ArH), 9.85 (s, 1H, OH). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) = 66.42; 115.49; 130.36; 132.66; 162.08. EIMS *m/z* (rel. int. %) = 125 (M^++1, 6), 124 (M^+, 88), 123 (M^+-1, 47), 107 (M^+-17, 50), 106 (M^+-18, 40), 105 (17), 95 (100), 79 (16), 78 (72), 77 (91), 65 (23), 51 (35), 50 (20).

4-Methylbenzyl alcohol (**12**): GC-FID analysis conditions: T_1 = 80 °C (5 min), ΔT = 20 °C/min, T_2 = 180 °C (7 min). GC Rt: 4-methylbenzaldehyde: 9.31 min, 4-methylbenzyl alcohol: 11.00 min. ^{1}H NMR (400 M Hz, $CDCl_3$) δ (ppm) = 2.04 (s, 1H, OH); 2.34 (s, 3H, CH_3); 4.63 (s, 2H, CH_2); 7.16 (d, 2H, ArH); 7.24 (d, 2H, ArH). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) = 21.14; 65.25; 127.13; 129.24; 137.39; 137.89. EIMS *m/z* (rel. int. %) = 123 (M^++1, 7), 122 (M^+, 86), 121 (M^+– 1, 20), 107 (M^+-15, 100), 93 (48), 91 (52), 79 (60), 77 (52), 65 (18), 51 (14), 50 (7).

Vanillyl alcohol (**13**): GC-FID analysis conditions: T_1 = 80 °C (5 min), ΔT = 20 °C/min, T_2 = 200 °C (10 min). GC Rt: vanillin: 12.45 min; vanillyl alcohol: 13.07 min. ^{1}H NMR (400 M Hz, $CDCl_3$) δ (ppm) = 3.90 (s, 3H, OCH_3), 4.60 (s, 2H, –CH_2–), 5.60 (s, 1H, OH), 6.82–6.92 (m, 3H, ArH), 7.26 (s, 1H, ArH). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) = 55.93; 65.44; 109.99; 114.29; 120.25; 132.96; 145.33; 146.69. EIMS *m/z* (rel. int. %) = 154 (M^+, 72), 153 (M^+-1, 7), 137 (32), 125 (39), 93(88), 77 (21), 65 (100), 55 (36).

Cinnamyl alcohol (**14**): GC-FID analysis conditions: T_1 = 80 °C (5 min), ΔT = 20 °C/min, T_2 = 180 °C (7 min). GC Rt: cinnamaldehyde: 11.52 min; cinnamyl alcohol: 13.22 min. ^{1}H NMR (400 M Hz, $CDCl_3$) δ (ppm) = 2.1 (s, 1H, OH), 4.31–4.33 (c, 1H), 6.33–6.64 (m, 1H), 7.24–7.44 (m, 5H, ArH). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) = 63.14; 126.47; 127.69; 128.58; 128.66; 131.22; 136.71. EIMS *m/z* (rel. int. %) = 134 (M^+, 50), 133 (M^+-1, 10), 115 (37), 105 (40), 92 (100), 91 (75), 78 (70), 77 (50), 63 (18), 55 (17), 51 (40).

Table 1. Proximate composition of *B. variegata* seeds.

Entry	Constituents	% p/p[a]
1	Proteins	5.2 ± 1
2	Carbohydrates	61.8 ± 2.2
3	Lipids	18.1 ± 0.3
4	Fibres	3.8 ± 1.3
5	Moisture	5.5 ± 0.9
6	Ash	5.6 ± 0.9

[a]All determinations were made in duplicate.

3. Results and discussion

3.1. Composition of B. variegata seeds

In order to characterize the seeds of *B. variegata*, a proximal analysis was carried out to establish the content of proteins, lipids, carbohydrates, fibres, moisture and ash, with the results shown below in Table 1.

It can be seen in Table 1 (entry 1) that the protein content was 5.2%, which is significantly lower than that observed for some other seeds used as biocatalysts. In this sense, *Ximenia americana* grains, for example, presented 19.8% protein content (Carvalho da Silva et al. 2018), while *Sinapis alba* (De Sousa et al. 2019) revealed a protein content of approximately 34.6%. In contrast, *Lens culinaris* (Alves-Ferreira et al. 2012) showed a low protein content (1.07–1.33%), which was significantly lower than that found in the seeds of *B. variegata*. Similarly, a low protein content (1.2%) was reported for *Cocos nucifera* (Fonseca et al. 2009). Because enzymes are made up of proteins, these results indicate that enzymes present in seeds may be responsible for the biocatalytic process (Almeida de Souza et al. 2019). On the other hand, a high content of carbohydrates was observed (61.8% – Table 1, entry 2), while the value of lipids, fibres, moisture and ash content (Table 1, entries 3–6) were in agreement with other results reported for the seeds of *B. variegata* (Sharma et al. 2020).

3.2. Kinetic study

Considering the results previously reported with acetophenones (Demmel et al. 2021), and in order to broaden the scope of the biocatalytic process promoted by whole seeds of *B. variegata*, the capacity of

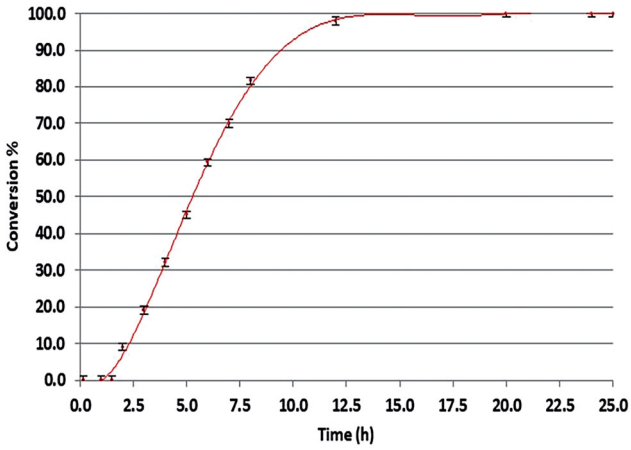


Figure 1. Kinetics of the reduction of BzHO to **1** promoted by the whole seeds of *B. variegata*.

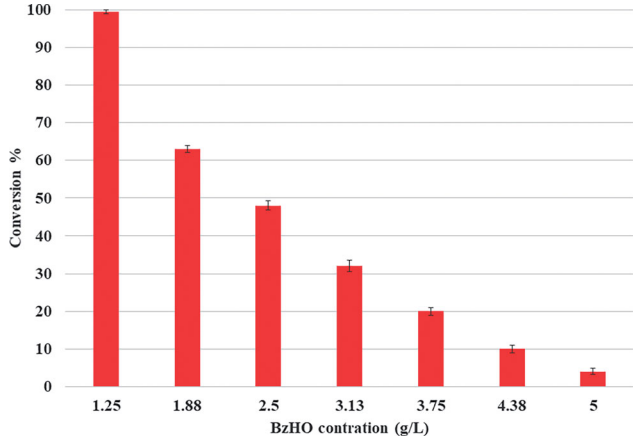


Figure 2. Effect of BzHO concentration on the formation of **1** promoted by whole seeds of *B. variegata*.

this new biocatalyst to carry out the reduction BzHO, and that of 14 substituted benzaldehydes derivatives to obtain their corresponding benzyl alcohols (**1–15**) was investigated.

Initially, a kinetic study was performed in order to determine the time in which the reduction of BzHO to (**1**) promoted by whole seeds of *B. variegata* reached the maximum percentage of conversion. Reactions were carried out in duplicate using 100 mg of substrate dissolved in 80 mL of sterile milliQ water inside an Erlenmeyer (250 mL), which had been previously disinfected, and with the addition of 10 g of disinfected whole seeds (room temperature and 100 rpm). The reactions were monitored for 24 h, taking samples at regular time intervals, and their evolutions were established by GC-FID and by GC-MS analyses. The results are shown below in Figure 1.

As can be seen in Figure 1, the process of bioreduction of BzHO was completed in approximately 13 h of reaction in a highly efficient way (≈100% of conversion) for the formation of (**1**).

It is important to mention that despite the fact that the capacity of several vegetables to carry out the reduction of BzHO has been previously reported, only five studies have described the utilization of seeds as biocatalysts for carrying out this reductive process. In this sense, Bertini (2012) reported a study using ground seeds of *Glycine max* (soybean), with BzHO being transformed to (**1**) with 100% of conversion in 72 h. Moreover, Alves-Ferreira, da Costa, et al. (2012) and Alves-Ferreira, Da Silva, et al. (2012) reported the capability of ground seeds of *Lens culinaris* to reduce several carbonyl compounds, and among these, BzHO was reduced with a bioconversion of ≥99% in 72 h of reaction. On the other hand, Solís et al. (2017) described the use of five varieties of ground and soaked seeds of *Phaseolus vulgaris* to carry out the reduction of BzHO, and observed a 100% conversion for the formation of (**1**) for all varieties utilized in 24 h of reaction at 25 °C. In addition, Carvalho da Silva et al. (2018) carried out studies using shredded grains of *Ximenia americana*, with (**1**) being obtained with a conversion of >99% for their reaction conditions. Finally, Bordón et al. (2021) described the performance of whole seeds of *Ligustrum lucidum* to carry out the transformation from BzHO to (**1**) and obtained 90% of conversion in 72 h of reaction at 25 °C. It is important to note that the study reported by Bordón et al. (2021) is the only report that has described the use of whole seeds as a biological reducer being capable of transforming BzHO into (**1**).

Due to the encouraging results obtained when using whole seeds of *B. variegata* to reduce BzHO (Figure 1), it was decided to perform studies to determine the effect of some different variables on the performance of the reaction.

3.3. Effect of BzHO concentration

To determine the effect of substrate loading on the production of (**1**), tests were carried out using different concentrations of BzHO in the reaction medium. The quantities used were 100, 150, 200, 250, 300, 350 and 400 µL in 80 mL of milliQ water (BzHO concentrations ranging from 1.25 to 5.6 g/L) with 10 g of the whole *B. variegata* seeds, which were stirred at 100 rpm at room temperature. The evolution of the reaction was followed for 24 h, taking samples at different times and analyzing these by GC-FID. The results can be observed in Figure 2.

As shown in Figure 2, when the amount of BzHO in the reaction medium was increased by 50% (from 1.25 to 1.88 g/L), a pronounced decrease in the percentage

of (**1**) formation was observed, with the conversion decreasing from values >99% at a substrate concentration of 1.25 g/L to <65% when the substrate concentration was 1.88 g/L. Furthermore, the decrease in the conversion percentage was more significant when the substrate concentration was higher (with a conversion of just 6% at 5.0 g/L of BzHO), thus evidencing a strong negative effect of the increase in substrate loading on the performance of the process. Due to the results achieved in these experiments, it was decided to continue with the studies using a concentration of BzHO of 1.25 g/L in the reaction medium.

3.4. Effect of temperature

In order to determine the effect of temperature on the production of (**1**) promoted by *B. variegata* seeds, tests were carried out on working temperatures of 25, 30, 35, 40 and 45 °C. The experiments were performed using 100 µL of the substrate (1.25 g/L) in 80 mL of milliQ water, plus the addition of 10 g of whole seeds and under constant orbital shaking at 100 rpm. Under these working conditions, the kinetic profiles of each reaction were obtained by taking samples at regular intervals of time and analyzing these by GC-FID in order to establish the percentage of conversion. All reactions were performed in duplicate, and the kinetic profiles obtained are shown in Figure 3.

As can be seen in Figure 3, on increasing the temperature from 25 to 45 °C, a corresponding increase in the reaction rate was observed, and consequently, the reaction time at which the maximum percentage of conversion was reached was reduced, which decreased from 20 h (at 25 °C – lower curve) to 10 h (40 °C – upper curve).

To visualize better the effect of temperature on reaction rate, the kinetic profiles were fitted to a first-order equation using Origin Pro 8 software, and the rate constants were calculated for each temperature. The results can be seen below in Figure 4.

As can be seen in Figure 4, when the reaction temperature was higher, the rate at which BzHO was reduced to (**1**) increased. Furthermore, the increase in reaction speed was mostly observed in the temperature range 30–40 °C.

The experiments carried out at 40° and 45 °C were the ones that presented the highest reaction rates, with the experiments carried out at temperatures of 40 °C showing the maximum value. In addition, it is interesting to observe that in contrast with the results obtained for the optimization of the temperature in the stereoselective reduction of acetophenone

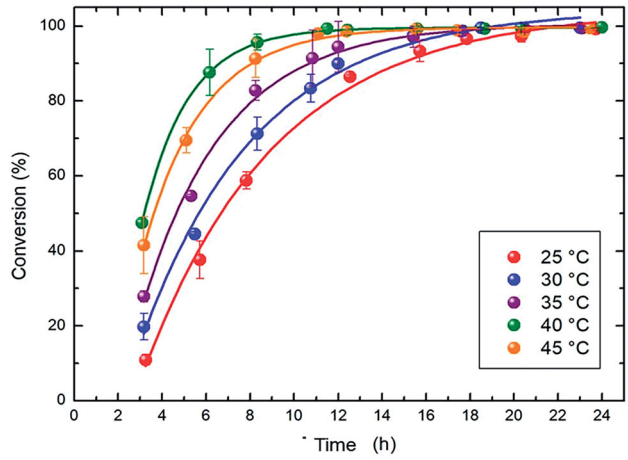


Figure 3. Kinetics of the reduction of BzHO mediated by whole seeds of *B. variegata* determined at different temperatures.

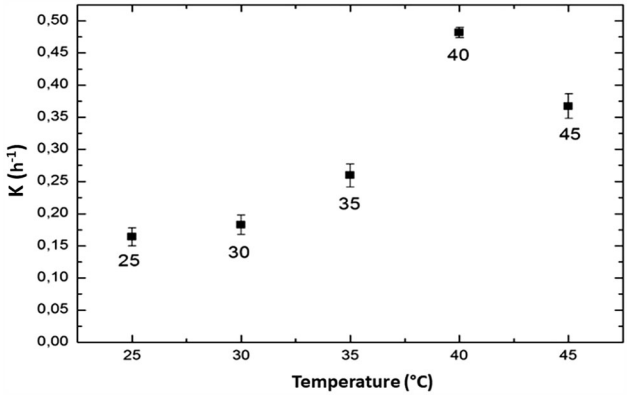


Figure 4. Rate constant (k) vs. reaction temperature.

(Demmel et al. 2021), in the reduction of BzHO the increase in the value of the calculated rate constants did not reveal a linear behaviour for the range between 25–40 °C. It is important to mention that no studies have yet been described for carrying out optimization of the temperature using seeds as biological reducers in BzHO reduction procedures. In this sense, using *Phaseolus vulgaris* (bean) seeds as a biocatalyst, the formation of (**1**) was reported for a reaction temperature at 25 °C with a total process time of 24 h (Solís et al. 2017, Solís et al. Solís et al. 2019).

Finally, since it is possible to use *B. variegata* seeds at a higher working temperature (40 °C) than in previous reports, it was possible to carry out the reduction of BzHO in a shorter reaction time (≈10 h), with this characteristic clearly representing a comparative advantage. In fact, 40 °C was the most suitable temperature for carrying out the BzHO bio-reduction process, and for this reason, this temperature was selected to continue with our studies.

3.5. Effect of DMSO concentration

As mentioned above, biocatalytic systems are more environmentally friendly because the reaction medium is usually water. However, this situation can be a drawback when using organic substrates that are insoluble or poorly soluble in the reaction media. Although this limitation can be solved, in part, by using co-solvents that facilitate the solubilization of the substrates, the effect of the cosolvents must be established, with the aim of preventing negative effects on the enzymatic activity, and consequently, reducing the performance of the reaction. As a counterpart, some cosolvents can sometimes have a beneficial effect on the regeneration of cofactors (Du et al. 2014).

As demonstrated in previous studies using the whole seed of *B. variegata* (Demmel et al. 2021), DMSO was the only cosolvent that did not interfere with the enzymatic activity in the reductive transformation of acetophenone. Furthermore, a small improvement in the performance of the reaction was observed when using DMSO as a cosolvent.

In order to determine the effect of the concentration of DMSO in the reaction medium, essays with different concentrations of DMSO (0.5–10 mL for each 80 mL of water) were carried out. Additionally, an experiment was also made without the addition of a cosolvent (control test). All the tests were carried out at 40 °C using 100 µL of BzHO with 10 g of whole seeds of *B. variegata*, at 100 rpm for 24 h (substrate concentration 1.25 g/L with 125 g/L of seeds). Samples were taken at the end of each reaction and analyzed by GC-FID to determine the percentage conversion. These results are shown below in Figure 5.

As can be seen in Figure 5, the use of different DMSO concentrations in the reaction medium did not have a significant effect on BzHO formation, and in all the tests a percentage of conversion >98% was obtained.

It is important to mention that although BzHO is relatively soluble in water, the results achieved in our experiments provide a solution for substrates that have a very low or zero solubility in the reaction media and require appreciable amounts of a cosolvent to carry out their solubilization. Due to the results obtained in these studies, the addition of DMSO at a final concentration of 9% v/v was selected as being the optimal amount of cosolvent to use in the essays that are shown in the following sections.

3.6. Effect of BzHO concentration

As a consequence of the results obtained in the previous essays, a new substrate loading study was again performed, but this time using the addition of DMSO as a cosolvent in order to determine if the tolerance of the whole seeds of *B. variegata* could be improved. To carry out this study, concentrations ranging between 1.25 g/L and 5.6 g/L of BzHO were used with 10 g of whole seeds, 80 mL of milliQ water, and the addition of 8 mL of DMSO. The system was maintained at 40 °C with orbital shaking (100 rpm) for 24 h. At the end of the reaction, samples were taken, which were

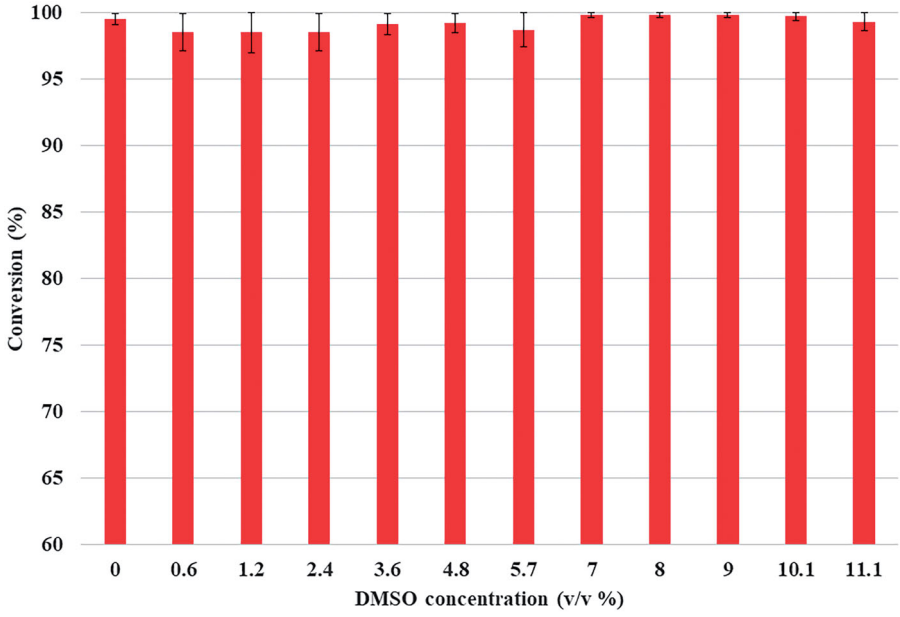


Figure 5. Effect of different amounts of DMSO on the reduction of BzHO promoted by whole seeds of *B. variegate*.

analyzed by GC-FID to determine the conversion percentage. These results are shown below in Figure 6.

As can be seen in Figure 6, when the BzHO concentration was increased from 1.25 to 2.5 g/L, the conversion percentage only decreased slightly due to the addition of DMSO at 9% v/v, while for higher concentrations (>2.5 g/L), the conversion decreased more markedly. Thus, it is clear that concentrations higher than 2.5 g/L of the substrate can cause an inhibitory effect on the catalytic activity of the enzymatic system in the seeds of *B. variegata*. For this reason, 2.5 g/L of BzHO was selected as the optimal concentration as the loading ratio of the substrate per 112 g/L of seeds.

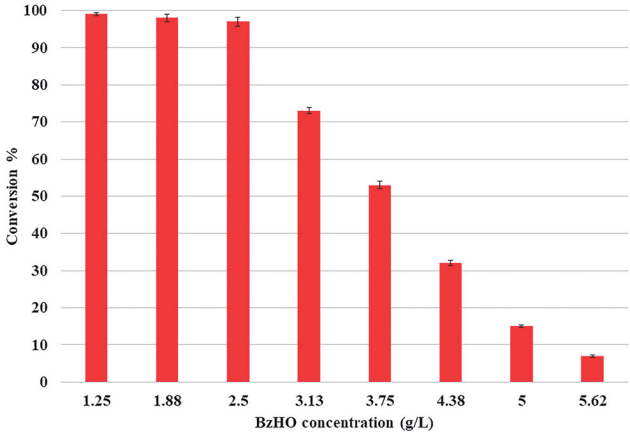


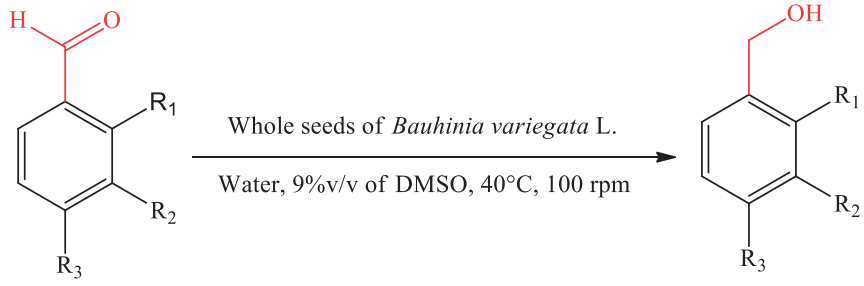
Figure 6. Effect of the concentration of BzHO in the reaction media on the production of (1) promoted by the seeds of *B. variegata* with the addition of DMSO at 9% v/v.

Finally, it should be mentioned that essays to optimize the pH of the reaction media were not carried out, because our laboratory team (Demmel et al. 2021) had reported that the use of a buffer to regulate the concentration of hydrogen ions was detrimental to the performance of the reaction of reduction of acetophenone, probably due to a negative effect on the enzymatic system caused by the modification of the ionic strength of the medium.

3.7. Bioreduction of BzHO to laboratory scale

In order to demonstrate the capacity of the developed biocatalytic system, a reaction was carried out using the optimized working conditions but on a larger scale. To accomplish this, 1.6 g of BzHO, 80 g of seeds of *B. variegata*, which had been previously washed and disinfected, 64 mL of DMSO, and 640 mL of sterile distilled water were placed in a disinfected 2 L conical flask (final concentration of the substrate: 21.2 mM). The reaction was carried out for 24 h, keeping the reaction system at 40 °C under constant orbital shaking of 100 rpm, after which, a sample was taken and analyzed by GC-FID (95% conversion). The reaction crude was filtered and the water reaction media was extracted for 24 h with ethyl acetate, using a liquid-

Table 2. Ability of whole seeds of *B. variegata* to reduce different substituted BzHOs.



Entry	Substrate/product			Time (days)	Conversion (%)[a]	Yield (%)[b]
	R_1	R_2	R_3			
1[c]	H–	H–	H–	1	95	49
2	NO_2–	H–	H–	1	90	71
3	H–	NO_2–	H–	1	98	42
4	H–	H–	NO_2–	1	94	54
5	Cl–	H–	H–	2	>99	70
6	H–	Cl–	H–	2	92	45
7	H–	H–	Cl–	2	91	47
8	Cl–	H–	Cl–	2	97	30
9	H–	H–	F–	2	>99	41
10	HO–	H–	H–	2	nr[d]	
11	H–	HO–	H–	3	>99	50
12	H–	H–	HO–	1	94	41
13	H–	H–	CH_3–	2	>99	25
14	H–	CH_3O–	HO–	4	73	7
15	C_6H_5–CH = CH–			3	62	61

[a]Conversion (%) determined by GC-FID. [b]Isolated yield by chromatographic column. [c]Scale bioreduction reaction of BzHO. [d]nr = No reaction.

liquid extractor. The organic solution was evaporated and the residue was purified by a chromatographic column, with 0.78 g of pure (**1**) being isolated (yield: 49%). This correlates to the productivity of 1.11 g L^{-1} or a space–time yield of 0.046 g $L^{-1}h^{-1}$. It is important to note that both the extraction and purification processes were not optimized.

3.8. Advantages, limitations and scope of using whole seeds of B. variegata as a biocatalyst in the preparation of substituted benzylic alcohols

After achieving the optimization of the main variables of the bioreduction process of the BzHO to synthesize the alcohol (**1**), a study of the biocatalytic reduction of substituted BzHOs was carried out. With this objective in mind, several substituted BzHOs derivatives with different substituents (fluorine, chlorine, nitro, hydroxy, methyl, and methoxy) on the benzene ring were selected in order to establish the advantages, limitations and scope of using the whole seeds of *B. variegata* as a biological reducer. In addition, vanillin and cinnamaldehyde were also used as substrates. These results are shown below in Table 2.

As can be seen in Table 2 (entries 1–13), the whole seeds of *B. variegata* very efficiently reduced substituted benzaldehydes to their corresponding alcohols, presenting conversion percentages higher than 90%, except for vanillin and cinnamaldehyde, whose corresponding alcohols were obtained in moderate conversions. The only exception, with a lack of reactivity, was 2-hydroxybenzaldehyde, which could not be reduced under our working conditions (Table 2, entry 10). Also in Table 2 (entry 1) are shown the results obtained for a scale bioreduction of BzHO, included as a reference. Nevertheless, it can be seen from the results obtained that no significant differences in reactivity were observed between substrates containing electron-withdrawing substituents and those with electron-donating substituents (Table 2, entries 3, 5, 8 and 9 versus 11–13). As a counterpart, it is important to mention that Solís et al. (2019) carried out studies using different varieties of *Phaseolus vulgaris* seeds, and observed that both the reducing activity and the degree of conversion to the corresponding alcohol of the substituted BzHOs studied depended on the activating/deactivating effects, electrophilicity of the substituents and steric hindrance, as well as on their position on the benzene ring with respect to the carbonyl group. Furthermore, similar results using *Aloe vera* leaves were observed by Leyva et al. (2012), while Bertini (2012), using *Glicine max* seeds as a reducing agent, reported better reactivities for those benzaldehydes that presented substitutions in the *para* position.

When the seeds of *B. variegata* were used to reduce benzaldehydes with nitro substituents (Table 2, entries 2–4), the transformation was chemoselective, in addition to being efficient (conversions >90%), since no parallel reduction of the nitro to amine groups was observed. In line with our results and working with different vegetables, similar findings were observed by Suárez-Franco et al. (2010) for this type of substituted BzHOs. However, although Leyva et al. (2012) also observed chemoselectivity in the reduction of nitrobenzaldehydes, the conversions to the corresponding nitrobenzylic alcohols (**3**) and (**4**) were significantly lower (60 and 65% conversions respectively) when compared to the conversion percentages obtained using whole seeds of *B. variegata* as the biocatalyst (Table 2 entries 3–4; 98 and 94% respectively).

It is also important to mention that Andrade et al. (2006) observed when using *Manihot esculenta*, *Colocasia esculenta*, *Allium schoenoprasum* and *Arracacia xanthorrhiza* as biocatalysts to carry out stereoselective reductions of nitroacetophenones, that the nitro groups were reduced to amine in addition to the formation of the corresponding alcohol. Furthermore, *Colocasia esculenta* produced a 100% reduction of nitro groups after six days of reaction. In contrast, Alves-Ferreira, Da Silva, et al. (2012) found that the seeds of *Lens culinaris* were capable of reducing substituted nitrobenzenes to their corresponding anilines, but with different degrees of efficiency.

A special mention should be made of the results obtained in the bioreduction of benzaldehydes containing chlorine as substituents. In this sense, the conversion percentages were excellent (91 to >99%) (Table 2, entries 5–7). However, and unlike that observed for the seeds of *B. variegata*, when Leyva et al. (2012) investigated the same substrates working with the extract of *Aloe vera* leaves, the percentages were significantly lower (65%). Moreover, when Solís et al. (2019) reported the same reduction reactions using one of the varieties of *Phaseolus vulgaris* seeds, they obtained a 70% conversion in the formation of (**5**), 16% for (**6**), and 97% for (**7**) (Table 2 entries 5–7).

The results achieved by reducing chlorine substituted benzaldehydes should be highlighted as being important since both (**7**) and (**8**) are key intermediaries in the synthesis of the commercial antifungals econazole and miconazole (Cuevas et al. 2004). Furthermore, (**8**) is also used as an intermediate in the synthesis of oxiconazole (Soltani et al. 2009).

In another study, an excellent result was reported in the reduction of 4-flurobenzaldehyde (Table 2, entry 9) to obtain the fluorine derivative (**9**), which is important as this product is one of the key raw materials in the synthesis of the recently patented anticancer agent monofluorobenzyl ester of norcantharidin carboxylate (Zhao et al. 2018; Li et al. 2020). Similarly, the 3-hydroxy derivative (**11**) (Table 2, entry 11; >99%) is one of the necessary intermediates for the synthesis of insecticides such as permethrin and phenothrin (Naumann 1990), and the *p*-hydroxy derivative (**12**) (Table 2, entry 12; 94%) is a key intermediate in the synthesis of the commercial drug bisoprolol, which is a cardioselective β_1-adrenergic blocking agent used in treating high blood pressure (Soloviev et al. 2001).

In particular, an excellent result was achieved when 4-methylbenzaldehyde (Table 2, entry 13) was reduced under our reaction conditions (conversion percentage >99% for 2 days of reaction). In addition, 4-methylbezyl alcohol is used in perfumery (Villa et al. 2008). Although there are some reports in the literature that have described the reduction of this aldehyde for the formation of (**13**), such efficiency was not achieved (Leyva et al. 2012).

With regard to obtaining (**14**) (Table 2, entry 14), it should be mentioned that the reaction was carried out with a longer reaction time than that carried out on BzHO (4 days vs. 1 day) to obtain the corresponding alcohol (Table 2 entry 14 *vs.* 1). Furthermore, the conversion percentage was also lower (73%). However, the efficiency of this reaction was similar to the values reported by Luna et al. (2014) when working with the leaves of *Zea mays* (64%) and also to the value reported by Carvalho da Silva et al. (2018) using *Ximenia americana* grains (51%). On the other hand, Leyva et al. (2012) did not observe any formation of (**14**) when the extract of *Aloe vera* leaves was used as a biocatalyst. However, when the biocatalyst was *Saccharum officinale* juice, the conversion percentages obtained were approximately 90% (Assunção et al. 2008).

Salvano et al. (2011) carried out similar studies aimed at reducing vanillin using roots of *Conium maculatum*. On this occasion, although (**14**) was formed, it was as a secondary product (27%) since the reaction mainly transformed the substrate into 2-methoxyphenol (guaiacol – 73%), possibly by the decarboxylation of vanillin. A similar situation was observed by Bordón et al. (2021) using whole seeds of *Ligustrum ludidum*. However, in this study, (**14**) was formed as a majority product (80%), while guaiacol was formed as a secondary product (20%).

It should be highlighted that alcohol (**14**) is used in the treatment of some neurodegenerative diseases such as Parkinson's, due to its pharmacokinetic characteristics and its bioactivity (Hsu et al. 2008), in addition to it having anticonvulsant and anti-inflammatory effects (Hsieh et al. 2000). Furthermore, (**14**) is a potential chemical platform for the production of thermoset epoxy resins obtained from renewable raw materials (Hernandez et al. 2016).

Although the conversion percentage obtained during the synthesis of (**15**) was only moderate (Table 2 entry 15; 62%), the results achieved were higher than those described by Machado et al. (2008) working with *Manihot* species. When Luna et al. (2014) carried out the reduction of cinnamaldehyde with *Passiflora edulis,* 41% of (**15**) was obtained, while by using *Zea mays* leaves as a biocatalyst a conversion of 50% was recorded for the formation of the same alcohol. In addition, it should be mentioned that when Bordón et al. (2021) used whole seeds of *Ligustrum lucidum*, a very good percentage of conversion was obtained with reaction times similar to those observed with the seeds of *B. variegata* (3 days). Furthermore, it is important to highlight that the reductive process carried out on cinnamaldehyde using whole seeds of *B. variegata* as a biological reducer was chemoselective (Table 2 entry 15), and therefore, the double bond remained unaltered in the final product. Related to this, Assunção et al. (2008), when carrying out the same transformation but using the juice of *Saccharum offincinale*, not only obtained the product of interest, but also the reduction of unsaturation in addition to the concomitant oxidation of the aldehyde group to carboxylic acid. Due to this fact, the end product turned out to be a mixture of (**15**), 3-phenylpropyl alcohol, and 3-phenyl propionic acid. The reduction of cinnamaldehyde is important because (**15**) is used as a key intermediate in the synthesis of fluoxetine, atomoxetine, and nisoxetine (Gao and Sharpless 1988), in addition to dapoxetine (Sasikumar and Nikalje 2012) and reboxetine (Henegar and Cebula 2007), which are all used as commercial antidepressants.

It is important to mention that, as far as we have been able to determine, the isolation and characterization, using a plant-catalyzed methodology, of the group of enzymes responsible for the bioreduction processes of carbonyl groups has not been described in detail. However, it is probable that the enzyme alcohol dehydrogenase – ketoreductases (AD-KR), which is dependent on the nicotinamide cofactors as

redox equivalents (Blanchard and Van De Weghe 2006; Xie et al. 2009) , is involved in the realization of this transformation. In this sense, it is worth mentioning that our research group has recently reported evidence (Demmel et al. 2021) that *Bauhinia variegata* seeds use NADPH as a redox cofactor during the biocatalysis process, which is lost by washing when the seeds are recycled for use in consecutive reactions. Furthermore, Xie et al. (2009) examined the bioreduction of alkyl aryl ketones employing a pre-concentrated enzymatic system derived from Adzuki beans and showed that glucose may be employed as an efficient cosubstrate in the NADPH recycling process. In light of both findings, it is possible that some of the sugars that are part of the high carbohydrate content (61.8% – Table 1, entry 2) found in *B. variegata* seeds are involved in the NADPH recycling process, which is employed by the enzyme system of these seeds as the redox equivalent to carry out the transformations detailed in our research. These observations support a mechanistic hypothesis via AD-KR enzymes (Xie et al. 2009). Nevertheless, it is clear that further studies are needed to establish the specific characteristics of the enzyme system and, in line with this, studies are currently being carried out with the objective of isolating and characterizing the enzymes present in *B. variegata* seeds.

Finally, the yield of the products, obtained after the corresponding purification, showed differences with respect to the conversion percentage determined by GC-FID. This situation may be attributed to difficulties experienced during the product extraction process, due to the formation of emulsions that notably complicated the isolation procedure of the final product, which may be explained by the relatively high lipid content (18.1% – Table 1, entry 3) observed in the determinations made during characterization of seeds, and could be the reason why the formation of stable emulsions was observed. Thus, it is necessary to emphasize that the purification method used to obtain the synthesized benzyl alcohols has not been optimized.

4. Conclusions

Our studies were performed with the objective of identifying efficient environmentally friendly synthesis methods and established the ability of *B. variegata* seeds to carry out the reduction of different benzaldehydes to their corresponding benzyl alcohols. In this sense, it should be mentioned that the developed methodology was shown to be little influenced by the nature of the substituents on the aromatic ring of the benzaldehydes used as substrates and, in general, the conversion percentages for the formation of the products were excellent in most cases. In addition, the methodology employed allows DMSO to be used as a co-solvent at an appreciable concentration in the reaction medium, which is a definite advantage when working with substrates of poor or no solubility in water.

It is noteworthy that the enzymatic systems present in the *B. variegata* seeds turned out to be chemoselective, which permitted the substituted benzaldehydes to be obtained with some functional groups undergoing a biotransformation process, under similar reaction conditions to those reported in the literature.

Currently, sustainability in chemical processes is being increasingly looked upon as a necessity in order to eliminate or minimize the impact on the environment of the productive procedures used. Thus, the biocatalytic processes promoted by whole seeds of vegetables could be considered a valuable tool that easily permits, in a cheap and compatible way with the environment, valuable products to be obtained with different applications. In this sense, the results described in this work, using the whole seeds of *B. variegata* as a biological reducer, allow us to offer new biocatalytic environmentally friendly strategies for the production of benzyl alcohols, which are used as key intermediates for obtaining important bioactive molecules with industrial application. Moreover, the simplicity, efficiency, fairly wide scope, and the observed chemoselectivity of this biocatalytic approach are its most advantageous features.

Finally, we believe that it is necessary to develop procedures that use renewable inputs, in order to evolve from a linear economy, consuming large amounts of non-renewable natural resources and consequently degrading the environment, to a resource-efficient circular economy that is waste-free by design. In line with this concept, further investigations using whole seeds of *B. variegata* are currently ongoing in order not only to understand the mechanism by which this type of transformation occurs and in this way, try to improve the productivity of the methodology but also to try to expand the applications of this novel, efficient, inexpensive, renewable and environmentally friendly biocatalytic agent.

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Disclosure statement

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ORCID

Laura I. Rossi http://orcid.org/0000-0003-1670-6529
Mario L. Aimar http://orcid.org/0000-0001-8547-7198

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