

Tween[®] 20-Enhanced Bioreduction of Acetophenones Promoted by *Daucus carota* Root

Monique Rodrigues da Costa and Álvaro Takeo Omori*

Center for Natural Sciences and Humanities, Federal University of ABC – UFABC,
Av. dos Estados 5001, CEP 09210-580, Santo André, SP, Brazil

Received: May 13, 2016

Accepted: March 16, 2017

Summary

The effect of surfactants on the bioreduction of acetophenones mediated by carrot (*Daucus carota*) root was investigated. Among the tested surfactants, Tween[®] 20 slightly improved the conversion. The amount of surfactant was optimised, and the presented methodology was applied to other substituted acetophenones, which led to the preparation of their respective optically enriched 1-phenylethanols with high conversion levels and high enantioselectivities.

Key words: biocatalysis, carrot root, surfactants, chiral alcohols

Introduction

The carrot (*Daucus carota*) is one of the most reliable, affordable, and easy-to-handle biocatalysts for the enantioselective reduction of prochiral ketones. This vegetable offers an effective ketoreductase activity even in an intact plant part. Also, carrots are inexpensive, readily available, and active, regardless of their source or type (1). The reaction is very simple to set up; no aseptic procedure is needed, and the reaction is carried out in water under mild conditions. Moreover, the workup is much easier than those of other biocatalytic systems (simple filtration followed by liquid-liquid extraction). Furthermore, high conversion rates and high enantioselectivities are often achieved (2). Recently, different conditions have been explored to evaluate the catalytic potential of this edible plant in chemical synthesis. Unusual substrates (*e.g.* graphene oxide) (3), biphasic systems (4), one-pot multistep reactions (5), and even reactions in organic solvents (6) have been reported when using carrots as catalysts. However, the relatively long reaction time (up to 72 h) combined with a high biocatalyst loading (usually 100 g/L) and a low substrate concentration (1 g/L or less) can hamper approaches to reaction scale-up. Thus, an investigation to

minimise the disadvantages of bioreduction mediated by carrots is necessary.

Few reports on the effect of surfactants and/or detergents in biocatalysis have been published. Furthermore, this effect has only been investigated by using whole cells and isolated enzyme systems. For instance, Goswami *et al.* (7) observed that the enantioselectivity in the reduction of ω -bromoacetophenones by whole cells of *Rhodotorula rubra* was enhanced by the addition of sodium lauryl sulphate. Kim *et al.* (8) found that the enantioselective hydrolysis of epichlorohydrins using recombinant *Pichia pastoris* was enhanced by the addition of 5 % (by volume) Tween[®] 20. This surfactant also contributed to the better enantiomeric excess (*e.e.*) values and the improved isolated yield of (*S*)-2-octanol obtained *via* the bioreduction of the corresponding ketone using *Saccharomyces cerevisiae* (9).

To the best of our knowledge, no study has investigated the use of surfactants in plant biocatalysis. In this context, the aim of this work is to verify whether the use of surfactants could enhance the biocatalytic reduction mediated by plants, specifically by carrot roots.

*Corresponding author: Phone: +55 11 4996 0177; E-mail: alvaro.omori@ufabc.edu.br

ORCID IDs: 0000-0002-7575-2464 (da Costa), 0000-0002-0115-3485 (Omori)

Materials and Methods

Materials

All reagents and chemicals (acetophenone, 2-bromoacetophenone, 4'-bromoacetophenone, 3'-bromoacetophenone, 2'-bromoacetophenone, 4'-aminoacetophenone, 3'-aminoacetophenone and cetyl triethylammonium bromide (CTAB)) were purchased from Sigma–Aldrich (St. Louis, MO, USA) and used directly without further purification. Ethyl acetate and hexanes of reagent grade (Synth, Diadema, SP, Brazil) were used for extraction, thin layer chromatography (TLC) analysis and chromatographic purification. Commercial linear alkylbenzene sulfonate (LAS, 6–10 %) was purchased from a local market (Castelo Alimentos, Jundiaí, SP, Brazil). Healthy carrots (*Daucus carota* ssp. *sativus* var. *sativus*) were obtained from a local market and used after washing in water. It was not necessary to peel the vegetables. The carrot root was cut into thin slices (5 mm). Each slice was cut in half (one half for the reaction control and the other for the reaction with Tween® 20) or in four pieces (for determining the effect of surfactant and the volume fraction of Tween® 20 on the reduction of substituted acetophenones).

Synthesis of racemic alcohols for TLC and GC standards

The racemic alcohols (*R,S*)-**2a–i** were prepared by reduction of the corresponding acetophenones **1a–i** with sodium borohydride (Sigma–Aldrich) in methanol (1 mmol of NaBH₄ per 10 mL of methanol; Synth) as previously described by Fernandes Assis *et al.* (10). Compounds **2a–i** were identified by mass spectrometry (GC/MS) and used for TLC and GC standards without further purification (Table 1).

Table 1. Compound names of alcohols 2a–i with the corresponding CAS registry numbers

Compound	Name	CAS
2a	(<i>R,S</i>)-1-(4-bromophenyl)ethanol	[5391-88-8]
2b	(<i>R,S</i>)-2-bromo-1-phenylethanol	[2425-28-7]
2c	(<i>R,S</i>)-1-(2-bromophenyl)ethanol	[5411-56-3]
2d	(<i>R,S</i>)-1-(3-bromophenyl)ethanol	[52780-14-0]
2e	(<i>R,S</i>)-1-(2-aminophenyl)ethanol	[10517-50-7]
2f	(<i>R,S</i>)-1-(3-aminophenyl)ethanol	[2454-37-7]
2g	(<i>R,S</i>)-1-(4-aminophenyl)ethanol	[14572-89-5]
2h	(<i>R,S</i>)-1-(4-hydroxyphenyl)ethanol	[2380-91-8]
2i	(<i>R,S</i>)-1-phenylethanol	[98-85-1]

Gas chromatography/mass spectrometry

Mass spectrometry (GC/MS) data were acquired on a Varian 4000 (Varian, Palo Alto, CA, USA) ion trap operating at 70 eV. Sample introduction and separation were handled by a Varian CP-3800 GC with a Varian CP-8400 autosampler (Varian) and equipped with a DB-5MS (30 m×0.250 mm) fused silica capillary column (Agilent Technologies, Santa Clara, CA, USA). Chromatographic conditions were: injector temperature 250 °C, helium 6.0 as carrier gas, front inlet pressure 60.7 kPa, column flow 1.1

mL/min and split ratio 1:20. Oven temperature program included initial temperature of 60 °C, temperature rate of 10 °C/min and final temperature of 270 °C. Mass spectrometry conditions were: flow rate 4.0 mL/min, inlet pressure 648.1 kPa and source temperature 180 °C.

Thin-layer chromatography

Analytical thin-layer chromatography (TLC) was performed on aluminium TLC plates with silica gel matrix, 60 Å medium pore diameter, 0.2 mm thickness, with fluorescent indicator for $\lambda=254$ nm (Sigma–Aldrich).

Column chromatography

Flash column chromatography was performed using high-purity grade silica gel with pore size 60 Å and 230–400 mesh particle size from Sigma–Aldrich. The yields of purified products were determined.

Chiral GC-FID

Conversion rate and enantiomeric excesses (e.e.) of biocatalysed reactions were determined using a 450-GC (Varian) equipped with a Chiralsil-Dex CB β -cyclodextrin (25 m×0.25 mm i.d.) fused silica capillary column (Agilent Technologies). Chromatographic conditions were: injector temperature 220 °C, hydrogen as carrier gas, front inlet pressure 68.9 kPa, detector temperature 220 °C, and split ratio 1:20. Oven temperature program for chiral GC analyses to determine the e.e. values of **2a–i** was adopted from the recent literature, and the respective retention times are in agreement with the expected results described by Omori *et al.* (1).

Enantiomeric excess determination

In order to verify the enantioselectivity of the bioreduction reaction mediated by the carrot root, the enantiomeric excess (e.e.) was determined from the GC chromatograms by the following equation:

$$e.e. = \frac{(R-S)}{(R+S)} \quad /1/$$

where *R* and *S* are the respective peak areas of both enantiomers.

Absolute configuration

The absolute configuration of enantioenriched alcohols **2a–i** was determined by comparing the order of elution of enantiomers separated by chiral GC with the literature data (1,10).

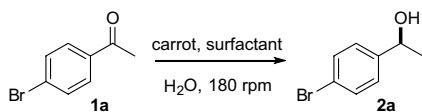
Procedure for bioreduction reactions with carrots in the presence of the surfactant

The pieces of carrot (10 g), water (40 or 100 mL), and the appropriate ketone **1a–i** (100 mg if solid compound or approx. 0.5 mmol if liquid) were added to an Erlenmeyer flask (125 or 250 mL). The appropriate surfactant was then added, and the biotransformation was carried out in an orbital shaker (rotational speed 180 rpm) at room temperature for 2 days. The progress of the reaction was monitored by GC analysis every 24 h. Sampling was done as follows: 2 mL of sample were extracted by stirring with 1 mL of ethyl acetate in a 15-mL Falcon tube. A volume of

1 or 2 μL of the organic layer was then directly analysed by GC/MS and chiral GC.

Results and Discussion

Initially, the carrot root reacted with 4'-bromoacetophenone (**1a**) (model compound) in the presence of a surfactant (Scheme 1).



Scheme 1. Bioreduction of 4'-bromoacetophenone by carrot in the presence of surfactant at room temperature

The main aim of this screening is to select the surfactant that provides the best values of conversion and e.e. Thus, the same carrot material was used for all four tested reactions and the results are summarised in Table 2.

Table 2. Surfactant effect in bioreduction of 4-bromoacetophenone with carrot root

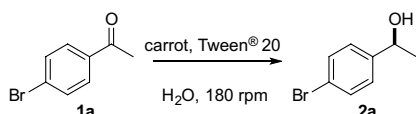
Surfactant	<i>t</i> day	Conversion rate %	e.e. %	Configuration
Control (solvent only)	1	0	>98	S
	2	34		
c(CTAB)= 0.02 mol/L	1	10	>98	S
	2	0		
c(Tween [®] 20)= 0.022 mol/L	1	7	>98	S
	2	50		
φ (LAS)= 0.9 %	1	4	>98	S
	2	0		

e.e.=enantiomeric excess

CTAB=cetyl triethylammonium bromide, LAS=linear alkylbenzene sulfonate

According to Table 2, Tween[®] 20 showed a better conversion rate after 48 h of reaction than CTAB, LAS and the reaction control. Both CTAB and LAS showed a contrary behaviour, thus indicating a possible influence in favour of the oxidation reaction. The corresponding secondary alcohol (**2a**) was obtained in excellent enantiomeric excess in all cases. This result is in agreement with previous findings (1) and therefore we observed that the surfactant has no influence on the enantioselectivity when carrot roots react with 4'-bromoacetophenone.

In the next step, the dosage of Tween[®] 20 in the enantioselective reduction of 4'-bromoacetophenone was evaluated (Scheme 2).



Scheme 2. Bioreduction of 4'-bromoacetophenone in the presence of Tween[®] 20 (dosage study) at room temperature

Four different volume fractions of the selected surfactant were tested (0.1, 0.5, 1.0 and 1.5 %) and the same piece of carrot root was divided in these four reactions. The results are shown in Table 3.

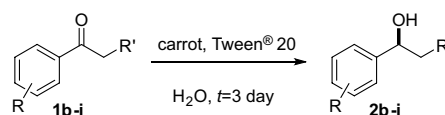
Table 3. Effect of the volume fraction of Tween[®] 20 on the bioreduction of 4-bromoacetophenone

φ (Tween [®] 20) %	<i>t</i> day	Conversion rate %	e.e. %	Configuration
0.1	1	26	92	S
	2	28		
0.5	1	47	94	S
	2	43		
1.0	1	35	97	S
	2	49		
1.5	1	23	96	S
	2	67		

e.e.=enantiomeric excess

According to Table 3, the bioreduction of **1a** using 0.1 % Tween[®] 20 led to the formation of (S)-**2a** with low conversion (28 %) and a good e.e. (96 %). On the other hand, we obtained higher e.e. and conversion when higher doses of the surfactant were used. For example, using 1.5 % of Tween[®] 20 led to (S)-**2a** with 67 % of conversion and 96 % e.e. This result suggests that the surfactant may have an important role in the bioreduction reaction. Both conversion and e.e. differ slightly when compared to the results of the first experiment (Table 2). Since a different carrot root was employed in this screening, different sources (and probably different growing conditions) may present different enzymatic systems (11). Therefore, low variations in e.e. and conversion are often observed. More important is the fact that in carrot root the mechanism of the reduction of prochiral ketones to the corresponding secondary alcohols in high enantioselectivity is reproducible. A rapid analysis of Table 3 suggests that a Tween[®] 20 dosage of 1.5 % (by volume) produces a higher conversion rate.

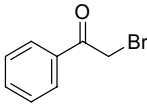
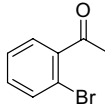
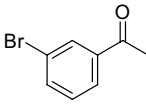
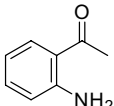
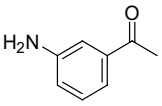
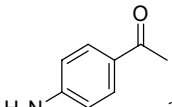
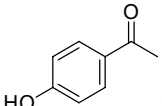
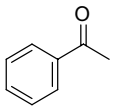
The enhanced activity of carrots in bioreduction of 4'-bromoacetophenone in the presence of Tween[®] 20 led us to investigate whether other acetophenones can undergo the same process. To this end, carrots and 1.5 % Tween[®] 20 in water were added to a series of substituted acetophenones (**1b-i**) (Scheme 3).



Scheme 3. Bioreduction of substituted acetophenones catalysed by carrot root in the presence of Tween[®] 20 at room temperature

Bioreduction of each substrate was carried out with a control reaction (without surfactant) and the piece of carrot root was split evenly between the reactions (in the presence or absence of Tween[®] 20). The results are summarized in Table 4.

Table 4. Bioreduction of substituted acetophenones using pieces of carrot root and 1.5 % Tween® 20

Substrate	Tween® 20				Control			
	Conversion rate %	Yield %	e.e. %	Configuration*	Conversion rate %	Yield %	e.e. %	Configuration
 1b	90	43	>98	S	66	13	28	S
 1c	78	66	>98	R	75	40	>99	R
 1d	94	88	90	S	79	23	87	S
 1e	–	–	–	–	–	–	–	–
 1f	82	48	>98	S	80	54	50	S
 1g	8	–	50	S	–	–	–	–
 1h	–	–	–	–	–	–	–	–
 1i	31	10	>98	S	11	–	92	S

e.e.=enantiomeric excess

Good reduction activities were observed in almost all the tested acetophenones, with the exception of 2'-aminoacetophenone (**1e**), 4'-aminoacetophenone (**1g**) and 4'-hydroxyacetophenone (**1h**).

In the case of the reduction of bromo-substituted acetophenones (**1b–d**), we observed a notable influence of the Tween® 20 on the yield and the e.e. of the corresponding alcohols (**2b–d**). After two days of reaction, the yields of **2b–d** without surfactant varied from 13 to 40 %, whereas the same reaction with the surfactant gave yields ranging from 43 to 88 %. Also, the e.e. is somehow influenced by the presence of the surfactant. The e.e. varied greatly from 28 to 99 % without the surfactant, whereas the enantioselectivity remained high when Tween® 20 was present.

Although the lack of reduction of **1h** is contradictory to a previous report (12), these results are in line with other reports (13,14). It is well known that amino and hy-

droxyl groups play a significant role in the activity of the catalyst. For example, Soni and Banerjee (15) attribute the poor conversion of 4'-hydroxy and 4'-aminoacetophenone to the activation of the aromatic system by the electron-releasing groups. In contrast, Gröger *et al.* (16) claim both the nucleophilicity of the hydroxyl and amino groups and the ability to form hydrogen bond interactions as possible reasons of the low reactivity of **1g** and **1h** in bioreduction reactions. As observed in a previous report by our group (1), the 4'-aminoacetophenone (**1g**) was also inactive when it was in contact with carrots (only traces of the corresponding alcohol **2g** were detected when the surfactant was present).

Considering the high conversion rate of 3'-aminoacetophenone (**1f**), the activating effect seems to be more critical in bioreduction reactions mediated by carrots, although other possible reason for the inactivity of 2'-ami-

noacetophenone can be related to an intramolecular hydrogen-bonding between the amino group and the carbonyl oxygen (17).

In general, the presence of Tween® 20 showed the best results of both conversion and e.e. among most of the tested acetophenones. We believe that the higher conversion values are associated with the increased solubility of the organic substrate in water due to the presence of surfactant. Interestingly, the enantiomeric ratio of the corresponding alcohols (**2b–i**) (and, thus, the enzyme activity) was also affected by the Tween® 20. Further, a controversial anti-Prelog selectivity was observed with the *ortho*-bromoacetophenone **1c**. This uncommon result may prolong the debate about the dehydrogenases responsible for the bioreduction reaction (18).

Conclusions

In this work, we observed the favourable influence of a non-ionic surfactant (Tween® 20) on both the conversion rate and enantioselectivity in the bioreduction of acetophenones catalysed by carrots. The surfactant also positively affected the chemical yield of optically active secondary alcohols. To the best of our knowledge, this is the first study to compare the effects of surfactants in asymmetric reductions mediated by plants. These experimental findings give insights to solving the solubility problems in biocatalytic reactions in water. Current and ongoing investigations will aim to make this methodology more scalable and useful.

Acknowledgements

The authors are grateful to the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP 2014/25659-8) and UFABC for financial support.

References

- Omori AT, Gonçalves Lobo F, Gonçalves do Amaral AC, de Oliveira CS. Purple carrots: better biocatalysts for the enantioselective reduction of acetophenones than common orange carrots (*D. carota*). *J Mol Catal B: Enzym.* 2016;127:93–7. <https://doi.org/10.1016/j.molcatb.2016.02.009>
- Blanchard N, van de Weghe P. *Daucus carota* L. mediated bioreduction of prochiral ketones. *Org Biomol Chem.* 2006;4:2348–53. <https://doi.org/10.1039/B605233A>
- Kuila T, Bose S, Khanra P, Mishra AK, Kim NH, Lee JH. A green approach for the reduction of graphene oxide by wild carrot root. *Carbon.* 2012;50:914–21. <https://doi.org/10.1016/j.carbon.2011.09.053>
- Xiong W, Wang X, Kong L. Design and application of a biphasic system that enhances productivity of *Daucus carota*-catalyzed asymmetric reduction. *Biotechnol Lett.* 2015;37:1703–9. <https://doi.org/10.1007/s10529-015-1838-9>
- de Oliveira CS, de Andrade KT, Omori AT. One-pot chemo-enzymatic synthesis of chiral disubstituted 1,2,3-triazoles in aqueous media. *J Mol Catal B: Enzym.* 2013;91:93–7. <https://doi.org/10.1016/j.molcatb.2013.03.004>
- Majewska E, Kozłowska M. Regio- and stereoselective reduction of trans-4-phenylbut-3-en-2-one using carrot, celeriac, and beetroot enzyme systems in an organic solvent. *Tetrahedron Lett.* 2013;54:6331–2. <https://doi.org/10.1016/j.tetlet.2013.09.041>
- Goswami A, Bezbaruah RL, Goswami J, Borthakur N, Dey D, Hazarika AK. Microbial reduction of ω -bromoacetophenones in the presence of surfactants. *Tetrahedron: Asymmetry.* 2000;11:3701–9. [https://doi.org/10.1016/S0957-4166\(00\)00341-4](https://doi.org/10.1016/S0957-4166(00)00341-4)
- Kim HS, Lee JH, Park S, Lee EY. Biocatalytic preparation of chiral epichlorohydrins using recombinant *Pichia pastoris* expressing epoxide hydrolase of *Rhodotorula glutinis*. *Biotechnol Bioprocess Eng.* 2004;9:62–4. <https://doi.org/10.1007/BF02949324>
- Xian'ai S, Jinlei R, Yongning Y, Yanghao G, Minhua Z. Effect of surfactant on asymmetric bioreduction of 2-octanone catalyzed by whole cell of *Saccharomyces cerevisiae*. *J Biotechnol.* 2008;136:S383. <https://doi.org/10.1016/j.jbiotec.2008.07.882>
- Fernandes Assis L, Kagohara E, Omori AT, Comasseto JV, Andrade LH, Porto ALM. Deracemization of (RS)-1-[(4-methylselanyl)phenyl]ethanol and (RS)-1-[(4-ethylselanyl)phenyl]ethanol by strains of *Aspergillus terreus*. *Food Technol Biotechnol.* 2007;45:415–9.
- Reineccius G. *Source book of flavours*. Dordrecht, The Netherlands: Springer Science & Business Media; 1994. pp. 73–4.
- Yadav JS, Nanda S, Reddy PT, Rao AB. Efficient enantioselective reduction of ketones with *Daucus carota* root. *J Org Chem.* 2002;67:3900–3. <https://doi.org/10.1021/jo010399p>
- Yang W, Xu JH, Xie Y, Xu Y, Zhao G, Lin GQ. Asymmetric reduction of ketones by employing *Rhodotorula* sp. AS2. 2241 and synthesis of the β -blocker (R)-nifenalol. *Tetrahedron: Asymmetry.* 2006;17:1769–74. <https://doi.org/10.1016/j.tetasy.2006.05.019>
- Singh A, Basit A, Banerjee UC. *Burkholderia cenocepacia*: a new biocatalyst for efficient bioreduction of ezetimibe intermediate. *J Ind Microbiol Biotechnol.* 2009;36:1369–74. <https://doi.org/10.1007/s10295-009-0622-z>
- Soni P, Banerjee UC. Enantioselective reduction of acetophenone and its derivatives with a new yeast isolate *Candida tropicalis* PBR-2 MTCC 5158. *Biotechnol J.* 2006;1:80–5. <https://doi.org/10.1002/biot.200500020>
- Gröger H, Hummel W, Rollmann C, Chamouveau F, Hüskén H, Werner H, et al. Preparative asymmetric reduction of ketones in a biphasic medium with an (S)-alcohol dehydrogenase under in situ-cofactor-recycling with a formate dehydrogenase. *Tetrahedron.* 2004;60:633–40. <https://doi.org/10.1016/j.tet.2003.11.066>
- Shimada H, Nakamura A, Yoshihara T, Tobita S. Intramolecular and intermolecular hydrogen-bonding effects on photophysical properties of 2'-aminoacetophenone and its derivatives in solution. *Photochem Photobiol Sci.* 2005;4:367–75. <https://doi.org/10.1039/B416284F>
- Rodríguez P, Barton M, Aldabalde V, Onetto S, Panizza P, Menéndez P, et al. Are endophytic microorganisms involved in the stereoselective reduction of ketones by *Daucus carota* root? *J Mol Catal B: Enzym.* 2007;49:8–11. <https://doi.org/10.1016/j.molcatb.2007.06.011>