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.
Materials and Methods

Materials

All reagents and chemicals (acetophenone, 2-bromoacetophenone, 4'-bromoacetophenone, 3'-bromoacetophenone, 2'-bromoacetophenone, 4'-aminoacetophenone, 3'-aminoacetophenone, 2'-aminoacetophenone, 4'-hydroxyacetophenone and cetyl trimethylammonium 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) in four pieces (for determining the effect of surfactant and the volume fraction of 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>
<thead>
<tr>
<th>Compound</th>
<th>Name</th>
<th>CAS</th>
</tr>
</thead>
<tbody>
<tr>
<td>2a</td>
<td>(R,S)-1-(4-bromophenyl)ethanol</td>
<td>[5391-88-8]</td>
</tr>
<tr>
<td>2b</td>
<td>(R,S)-2-bromo-1-phenylethanol</td>
<td>[2425-28-7]</td>
</tr>
<tr>
<td>2c</td>
<td>(R,S)-1-(2-bromophenyl)ethanol</td>
<td>[5411-56-3]</td>
</tr>
<tr>
<td>2d</td>
<td>(R,S)-1-(3-bromophenyl)ethanol</td>
<td>[52780-14-0]</td>
</tr>
<tr>
<td>2e</td>
<td>(R,S)-1-(2-aminophenyl)ethanol</td>
<td>[10517-50-7]</td>
</tr>
<tr>
<td>2f</td>
<td>(R,S)-1-(3-aminophenyl)ethanol</td>
<td>[2454-37-7]</td>
</tr>
<tr>
<td>2g</td>
<td>(R,S)-1-(4-aminophenyl)ethanol</td>
<td>[14572-89-5]</td>
</tr>
<tr>
<td>2h</td>
<td>(R,S)-1-(4-hydroxyphenyl)ethanol</td>
<td>[2380-91-8]</td>
</tr>
<tr>
<td>2i</td>
<td>(R,S)-1-phenylethanol</td>
<td>[98-85-1]</td>
</tr>
</tbody>
</table>

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:

\[
e.e. = \frac{(R-S)}{(R+S)} / 1/
\]

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

Absolute configuration

The absolute configuration of enantiomeriched 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 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 λ=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-Alrich. 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).

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
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](image)

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>
<thead>
<tr>
<th>Surfactant</th>
<th>Conversion rate (%)</th>
<th>e.e. (%)</th>
<th>Configuration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (solvent only)</td>
<td>1</td>
<td>0</td>
<td>&gt;98</td>
</tr>
<tr>
<td>c(CTAB)=0.02 mol/L</td>
<td>1</td>
<td>10</td>
<td>&gt;98</td>
</tr>
<tr>
<td>c(Tween® 20)=0.022 mol/L</td>
<td>1</td>
<td>7</td>
<td>&gt;98</td>
</tr>
<tr>
<td>ϕ(Tween® 20)=0.9 %</td>
<td>1</td>
<td>4</td>
<td>&gt;98</td>
</tr>
</tbody>
</table>

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

e.e.=enantiomeric excess

CTAB=cetyl trimethylammonium 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](image)

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

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](image)

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.
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 hydroxyl 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

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References