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Purple carrots: Better biocatalysts for the enantioselective reduction of acetophenones than common orange carrots (*D. carota*)

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ABSTRACT

The constant and reproducible enantioselective reduction of acetophenones were investigated in several edible plants. Among them, only carrot (*Daucus carota*) acted as a reliable biocatalyst. Different carrot varieties (orange, yellow and purple carrots) in contact with substituted acetophenones gave the corresponding optically-active alcohols in good-to-excellent rates of conversion with high enantioselectivity. Comparatively, the purple carrot was a better biocatalyst than the common orange variety.

1. Introduction

In organic synthesis, chiral alcohols are important building blocks for the synthesis of several compounds, including chiral auxiliaries, pharmaceuticals and natural products. One "green" way to obtain such compounds is through biocatalytic asymmetric reduction of ketones [1,2]. Interestingly, pieces of carrot root have been successfully used for this purpose [3]; the growing attractiveness of carrots in biocatalysis is due to the observed high conversion rates and high enantioselectivities and because carrots are inexpensive, readily available, active worldwide (research group locations that reported the use of carrots in biocatalysis: Argentina [4], Brazil [5], China [6], France [7], India [8], Italy [9], Japan [10], Lithuania [11], Poland [12], South Korea [13], Sweden [14], Uruguay [15]) and require neither aseptic procedures nor buffer media. Usually, the reaction is carried out under mild conditions (water as solvent at room temperature) and has easier workup than other biocatalytic systems. Recently, different conditions have been explored to evaluate the potential of this edible plant. Unusual substrates (e.g. graphene oxide, organoselenium compounds) [14,5], biphasic systems [16], one-pot multistep reactions [17] or even reaction in organic solvents [13] were reported using carrots as catalysts. In view of the consolidated applicability of carrots in bioreduction reactions, it was observed that there is no report of carrot use involving other varieties of the same species (e.g. other colors of carrots). We investigated if other sources of biocatalysts (edible fruits) would have reproducibility similar to that of carrots. Also, the biocatalytic activities of purple and yellow carrots (*Daucus carota* ssp. *sativus* var. *atrorubens)* were compared to the orange carrot (*D. carota* ssp. *sativus* var. *sativus*).

2. Experimental

2.1. General methods

All reagents and chemicals were purchased from Sigma–Aldrich and used directly without further purification. Solvents (reagent grade) were used for extraction and TLC. GC–MS data were acquired on a Varian 4000 ion trap operating at 70 eV. Chiral GC-FID analyses were recorded on a 450-GC with a Chirasil-Dex CB-β-cyclodextrin (25 m × 0.25 mm) column using H_2 as the carrier gas.

Biocatalysts: healthy plants and fruits were obtained from a local market (cashew—*Anacardium occidental*, star fruit—*Averrhoa carambola*, strawberry—*Fragaria* spp., sugar-apple—*Annona squamosa*, kiwi—*Actinidia deliciosa*, persimmon—*Diospyros kaki*, fig—*Ficus carica*, avocado—*Persea americana*, pomegranate—*Punica granatum*, medlar—*Eriobotrya japonica*, brazilian cherry—*Eugnia uniflora*, plum—*Prunus domestica*, pear—*Pyrus* sp., tamarillo—*Solanum betaceum*, parsley—*Petroselinum crispum*, ginger—*Zingiber officinale*, fennel—*Foeniculum vulgare*, coriander—*Coriandrum sativum*, and purple carrot—*D. carota* ssp. *sativus* var. *atrorubens*).

2.2. Synthesis of racemic alcohols

Synthesis of racemic alcohols for TLC and GC standards: The alcohols (RS)-**2a–j** were prepared by reduction of the corresponding acetophenones **1a–j** with sodium borohydride in methanol [18]. Compounds **2a–j** were identified by GC–MS and used for TLC and GC standards without further purification.

(*R,S*)-1-phenylethanol (2a): CAS [98-85-1]

(*R,S*)-2-bromo-1-(4-methoxyphenyl) ethanol (2b): CAS [19922-83-9]

(*R,S*)-2-bromo-1-phenylethanol (2c): CAS [2425-28-7]

(*R,S*)-1-(2-bromophenyl) ethanol (2d): CAS [5411-56-3]

(*R,S*)-1-(3-bromophenyl) ethanol (2e): CAS [52780-14-0]

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1381-1177/

Table 1
Retention times of substituted 1-phenylethanols **2.**

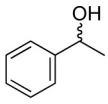
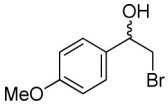
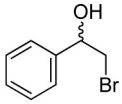
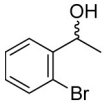
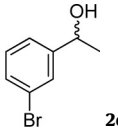
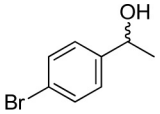
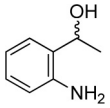
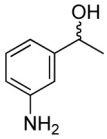
Alcohol	Literature			Ref.	Experimental		
	Conditions	t_R (min)	t_S (min)		Conditions	t_R (min)	t_S (min)
 **2a**	110 °C–3 °C/min until 180 °C	6.2	6.6	[19]	120 °C–1 °C/min until 175 °C	5.9	6.3
 **2b**	110 °C–20 min. then 20 °C/min until 180 °C	25.8	25.7	[20]	110 °C–3 °C/min until 180 °C	15.9	14.4
 **2c**	110 °C–20 min. then 20 °C/min until 180 °C	24.2	24.0	[20]	110 °C–1 °C/min until 135 °C (10 Psi)	13.8	13.5
 **2d**	110 °C–3 °C/min until 155 °C	12.0	13.7	[19]	110 °C–3 °C/min until 155 °C	15.1	15.5
 **2e**	110 °C–3 °C/min until 155 °C	12.1	12.7	[19]	110 °C–3 °C/min until 155 °C	14.3	14.7
 **2f**	110 °C–3 °C/min until 155 °C	12.5	13.2	[19]	140 °C–20 min. then 20 °C/min until 180 °C	11.7	12.7
 **2g**	–	–	–	–	140 °C–30 min. then 20 °C/min until 180 °C	13.3	13.7
 **2h**	–	–	–	–	140 °C–30 min. then 20 °C/min until 180 °C	18.5	19.1

Table 2
Active edible plants for asymmetric reduction of acetophenone.

Biocatalyst	Observed (literature)				
	Days	Conv. (%)	*ee* (%)	Config.[a]	Ref.
Coriander (*C. sativum*)	7 (3)	0 (67)	0 (36)	– (*R*)	[21]
Ginger (*Z. officinale*)	7 (10)	92 (99)	>98 (>98)	*R* (*S*)	[21]
Fennel (*F. vulgare*)	5	93 (–)	86 (–)	*S* (–)	–
Carrot (orange) (*D. carota*)	3 (3)	93 (100)	>98 (100)	*S* (*S*)	[9]

[a] Absolute configuration determined by comparing the order of elution of the enantiomers separated by chiral GC to the values reported in the literature [19,22].

(*R*,*S*)-1-(4-bromophenyl) ethanol (2f): CAS [5391-88-8]
(*R*,*S*)-1-(2-aminophenyl) ethanol (2 g): CAS [10517-50-7]
(*R*,*S*)-1-(3-aminophenyl) ethanol (2 h): CAS [2454-37-7]
(*R*,*S*)-1-(4-aminophenyl) ethanol (2i): CAS [14572-89-5]
(*R*,*S*)-1-(4-hydroxyphenyl) ethanol (2j): CAS [2380-91-8]

2.3. General procedure for biocatalyzed reactions

The plants were washed with water and then cut into small, thin slices (5 mm). The plants (10 g), water (40 mL), and the appropriate ketones **1a–j** (0.5 mmol) (if solid compound: isopropanol (1 mL) was used as the cosolvent were added to an Erlenmeyer flask (125 mL). The biotransformation was carried out in an orbital shaker (160 rpm) at room temperature for 3 days. The progress of the reaction was monitored by GC analysis every 24 h. Sampling: 2 mL of sample were extracted with 1 mL of ethyl acetate in a 15-mL Falcon tube. The organic layer was then directly analyzed by TLC (generally 4:1 hexane:ethyl acetate, visualized by UV and stained with sulphuric vanilin), chiral GC, and GC–MS.

2.4. Analytical data of the products

General GC conditions: Chirasil-Dex CB-β-cyclodextrin column; carrier gas: H_2; 10 Psi; injector 200 °C; detector 220 °C; Chiral GC analyses for determination of *ee* of **2a–j** (Table 1).

3. Results and discussion

Initially, thirteen fruits (cashew—*A. occidental*, star fruit—*A. carambola*, strawberry—*Fragaria* spp., sugar-apple—*A. squamosa*, kiwi—*A. deliciosa*, persimmon—*D. kaki*, fig—*F. carica*, avocado—*P. americana*, pomegranate—*P. granatum*, medlar—*E. japonica*, brazilian cherry—*E. uniflora*, plum—*P. domestica* and pear—*Pyrus* sp., tamarillo—*S. betaceum*) and five roots (parsley—*P. crispum*, ginger—*Z. officinale*, fennel—*F. vulgare*, coriander—*C. sativum* and orange carrot—*D. carota* ssp. *sativus* var. *sativus*) were acquired from a local market and evaluated as possible biocatalysts for the reduction of a model compound (acetophenone). In this screening, we also evaluated the repeatability of some vegetables already applied as biocatalysts in a previous report [21].

No reduction activity (by TLC analysis with 1-phenylethanol) was observed with any of the fruits: only TLC analysis of ginger,