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Biocatalytic reductions by plant tissue - Green alternative to alcohol production

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review

Summary

The use of biocatalysts for the industrial synthesis of chemicals has been attracting much attention as an environmental friendly synthetic method. Various plants, such as apple (*Malus pumila*), carrot (*Daucus carota*), cucumber (*Cucumis sativus*), onion (*Allium cepa*), potato (*Solanum tuberosum*), radish (*Raphanus sativus*) and sweet potato (*Ipomoea batatas*) were used as biocatalysts. Enzymes that plants produce are able to perform reactions under mild conditions (pH and temperature), with remarkable chemo-, regio-, and stereoselectivity. Due to this feature the number of biocatalysts used in organic synthesis has rapidly increased during the last decades, especially for the production of chiral compounds. This review presents biotechnological processes for the production of chiral alcohols by reducing prochiral ketones with whole plant tissue. Chiral alcohols are important building blocks for the synthesis of pharmaceuticals, pesticides, pheromones, flavors, fragrances and advanced materials such as liquid crystals. Reductase-catalyzed reactions are dependent on cofactors, and therefore, one major task in process development is to provide an effective method for regeneration of the cofactors consumed. The need for expensive cofactors is eliminated by using the whole plant tissue since the plant automatically provides this requirement. Depending on the vegetable used, either enantiomer may be obtained in high yield and high enantiomeric excess (*ee*), which could be a critical factor for drug development/bioactivity evaluation perspective. In this paper, various processes carried out on laboratory scales are presented. Attention is turned to conversion, yield, enantiomeric excess (*ee*).

Keywords: biocatalysts, ketone reduction, plant tissue

Introduction

Biotransformations are a worthy alternative when trying to substitute a conventional chemical reaction by a greener method in organic chemistry (Ravía et al., 2006). There are numerous chemical and biological methodologies available to obtain chiral alcohols. Asymmetric reduction of prochiral ketones is one of the most important and practical reaction for producing chiral alcohols which can be transformed into various functionalities, to synthesize industrially important chemicals such as pharmaceuticals, pesticides, pheromones, flavors, fragrances and industrial fine chemicals (Baldassarre et al., 2000; Suárez-Franco et al., 2010; Lakshmi et al., 2011). Biocatalysis, involving either isolated oxido-reductases or living organisms is always regarded as one of the most promising method due to its remarkable enantioselectivity and mild reaction conditions. Plant cell enzymes, like those from microorganisms, are able to catalyse reduction of prochiral ketones with high regio- and stereospecificity. Faber (2011) emphasized that using enzymes as biocatalysts improves stereochemical quality and simplifies separation and disposal steps. In a green context, there are several reports about the possibility of using parts of fresh plant tissue as biocatalysts, since different oxido-reductases and the cofactor regeneration system exist in the plant cell (Yang et al., 2008; Chang et al., 2010; Liu et al., 2010). Moreover, application of comminuted tissue of ripe vegetable roots in biotransformations instead of isolated enzymes is possible due to the group of enzymes excreted to extracellular medium that are able to accept xenobiotic substrates (Mączka and Mironowicz, 2002). Recently, there are reports on asymmetric reduction of different kinds of non-natural prochiral ketones with apple (*Malu pumila*), carrot (*Daucus carota*), cucumber (*Cucumis sativus*), onion (*Allium cepa*), potato (*Solanum tuberosum*), radish (*Raphanus sativus*) and sweet potato (*Ipomoea batatas*) as the biocatalysts (Yang et al., 2008). The biotransformation procedure is very simple and can be an alternative to the procedure with baker's yeast due to the availability of plants, as well as the use of water without the addition of cosolvents even for slightly soluble substances (Bruni et al., 2002). Acetophenone is a kind of very interesting xenobiotic substrate model for bioreduction, because it may give rise to both enantiomers of 1-phenylethanol. The efficiency of biocatalysts is defined by the activity, enantioselectivity, and scope of substrates. Highly active biocatalysts exhibit high turnover number and/or turnover frequency. The enantioselective course of the biocatalysts is determined by the enantiomeric excess of the products (Ohkuma, 2010).

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This paper reviews various processes of reduction of prochiral ketones to the corresponding alcohols by fragmented parts of different plants carried out on laboratory scales.

Production of chiral alcohols by asymmetric reduction with vegetable catalyst

Biotransformations are chemical reactions that involve the use of enzymes or whole cells and organs (containing the desired enzyme or enzyme system) as catalysts (Giri et al., 2001; Milner and Maguire, 2012). Compared with microbial biocatalysts, plants have more complex metabolic pathways, which are far less understood. Various examples of reduction of prochiral ketones to chiral alcohols are reported using growing or immobilized plant cell cultures and for the last 20 years possibility to directly use parts of plants as biocatalyst has been investigated.
In order to screen potentially suitable biocatalytic enzymes for reactivity, it is crucial to understand how they function (Ishige et al., 2005). For the reduction process, the coenzymes, NADH (nicotinamide adenine dinucleotide) and NADPH (nicotinamide adenine dinucleotide phosphate) and an enzyme function together to catalyze the reaction in a way that: 1) the coenzyme and oxidized substrate bind to an enzyme, 2) substrate is reduced, while the coenzyme is oxidized, 3) the coenzyme and reduced product detach from the enzyme, and 4) the coenzyme is recycled and ready for the process to begin again (Matsuda et al., 2009), Fig. 1. Therefore, further exploration of biocatalytic pathways is a major part of the chemistry of the future. More vast assaying and implementation of these biocatalytic methods will help the industry to meet the demand for substrates such as, alcohols and amines, which are easily obtainable in biotransformations with enantiomerical purity (Fruchey, 2011). The reaction types and stereochemistry in the biotransformation depend on the functional group in the substrates and the structural moieties in the vicinity of the functional group (Ishihara et al., 2003). However, a common problem that occurs when using vegetables as biocatalysts is the effect of microbial contamination (Fruchey, 2011). Thus, microorganisms may be involved in the asymmetric reduction process when plants are used as an enzyme carrier (Matsuda et al., 2009).

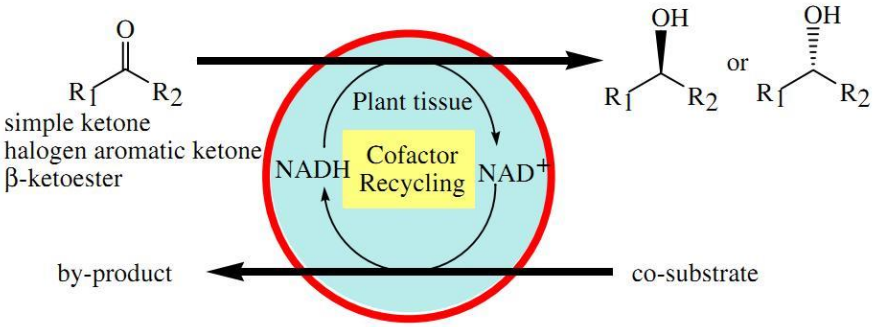


Fig.1. Asymmetric reduction of ketones catalyzed by plants tissue (Yang et al., 2010)

There are many reports describing synthesis of chiral secondary alcohols using different parts of plant tissue (Baldassarre et al., 2000; Kumaraswamy and Ramesh, 2003; Comasseto et al., 2004; Mączka and Mironowicz, 2002). Since 2000 common carrot plant has been used repeatedly as an effective agent to perform the stereoselective reduction of many ketones, while acetophenone derivates are probably the most studied substrates used for enantioselective bioreduction to the corresponding alcohols (Rodrigues et al., 2004 and 2007). In general, the reductions of acetophenone derivatives into chiral alcohols follow Prelog's rule, which predicts that hydrogen transfer to the prochiral ketone always occur from the *Re*-face where L represents a large substituent and S a small substituent adjacent to the carbonyl group, Fig. 2 (Prelog, 1964).

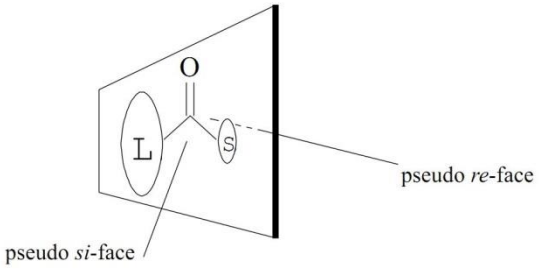
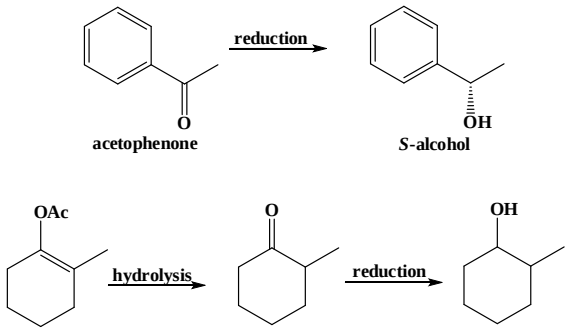


Fig.2. Pseudo *si*-face and *re*-face of a ketone

Bruni et al. (2002) investigated the reduction of acetophenone and the hydrolysis of 1-acetoxy-2-methylcyclohexene with plants to the corresponding *S*-carbinol and *S*-ketone, Scheme 1. Results showed that carrot (*Daucus carota*) reduced the acetophenone to the pure (*S*)-1-phenylethanol after 3 days with *ee* of 100 %. Lower yields were achieved with fennel (*Foeniculum vulgare*) and marrow (*Cucurbita pepo*), 37 and 10 %, respectively, but with high enantioselectivity (*ee* 100 %). Aubergine (*Solanum melongena*), cucumber (*Cucumis sativus*), white and red onion (*Allium cepa*), garlic (*Allium sativum*) and radish (*Raphanus sativus*) did not reduce the substrate even after 5 days of incubation. Hydrolysis of 1-acetoxy-2-methylcyclohexene with cucumber, mamey sapote (*Pouteria sapota*, fruit), banana passion fruit (*Passiflora tarminiana*, fruit), red onion, garlic and radish gives the *S*-ketone (*ee* 33-44 %). The further incubation of 2-methylcyclohexanone with such plants as carrot, cherimoya (*Annona cherimola*), wild cucumber (*Cyclanthera pedata*), giant granadilla (*Passiflora quadrangularis*), oca (*Oxalis tuberose*) afforded the enantiomercally pure *trans*- and/or *cis*-alcohol, respectively.



Scheme.1. The reduction of acetophenone and the hydrolysis of 1-acetoxy-2-methylcyclohexene (Bruni et al., 2002)

Kumaraswamy and Ramesh (2003) have investigated reduction of aromatic ketones with soaked *Phaseolus aureus L* as a biocatalyst. Soaked *Phaseolus aureus L* reduced acetophenone to give (*S*)-1-phenyletanol with good selectivity (*ee* 84 %). They also used *Phaseolus mungo L* and *Cicer arietinum L* which gave only 10 % and 6 % *ee* respectively with negligible yield of (*S*)-1-phenyletanol.

Comasseto et al. (2004) for the first time performed a biotransformation of organo-chalcogeno acetophenones to afford the corresponding chiral alcohols with *Daucus carota*. When *ortho*-methylseleno acetophenone, *ortho*-phenylseleno acetophenone and *ortho*-methylthio acetophenone were treated with carrot roots the chiral alcohols were not obtained. A possible steric effect due to the organoselenium and methylthio groups at the *ortho*-position to the keto group hinders the reaction. In contrast, the *meta*- and *para*-organo-chalcogeno acetophenones were reduced to the corresponding chiral organo-chalcogeno-α-methylbenzyl alcohols with excellent enantioselectivity (*ee* > 99 %) and high conversion.

Mączka and Mironowicz (2002) conducted the reduction of aryl methyl ketones using comminuted roots of carrots, celeriac (*Apium graveolens* L. var. *rapaceum*) and horseradish (*Armoracia lapatifolia* Gilib.). Substrates with one aromatic ring were transformed more easily and with higher enantioselectivity then molecules containing biaryl systems.

Yadav et al. (2002) used *Daucus carota* as biocatalyst for enantiselective reduction of various prochiral ketones such as acetophenones, aliphatic acyclic and cyclic ketones, β-ketoesters and α-azido aryl ketones. With acetophenone and substituted acetophenones the reduction was completed within 40-50 h and excellent yield (70-80 %) and optical purity (> 90 %) was observed. For the first time Yadav et al. (2009) used green peas *Pisum sativa* as biocatalysts in the enantioselective reduction of prochiral ketones. Various substituted acetophenones were converted into chiral secondary alcohols, the *S*-alcohols were obtained in all cases with *ee* ranging from 91-98 % and yield ranging from 55-72 %. The reduction was achieved using sprouted green peas in aqueous buffer pH 7.0 at room temperature.

Caron et al. (2005) reported on the enantioselective reduction of prochiral ketones by *Daucus carota* hairy roots. The results showed that incubation of acetophenone with hairy root cultures for 7 days gave (*S*)-1-phenylethanol in 96 % yield and > 98 % *ee*. The repetitive use of the roots culture after six consecutive reuses showed that biocatalyst still maintained theactivity providing 1-phenylethanol in the same high chemical and optical yields. Additionally, reductions of acetophenone mediated by whole cells usually require a high ratio of biocatalysts to substrate (B/S), while hairy root cultures needed a lower ratio (B/S = 4.5, dry weight at the beginning of the reaction) in relation to carrot roots (B/S = 100) or baker's yeast (B/S = 360).

Yang et al. (2008) investigated the asymmetric reduction of different simple prochiral ketones catalyzed by various plants tissue. Acetophenone, 4'-chloroacetophenone and ethyl 4-chloroacetoacetate were chosen as the model substrates, while apple

(*Malus pumila*), carrot (*Daucus carota*), cucumber (*Cucumis sativus*), onion (*Allium cepa*), potato (*Solanum tuberosum*), radish (*Raphanus sativus*) and sweet potato (*Ipomoea batatus*) have been used as biocatalyst. Depending on the biocatalyst used, both *R*- and *S*-chiral alcohols were obtained, Table 1.

Table 1. Asymmetric reduction of acetophenone by various plants tissue (Yand et al., 2008)

	Plant tissue	50 h		100 h		Config.
		Yield / %	*ee*/ %	Yield / %	*ee*/ %	
1	Apple (*M. pumila*)	38.7±2.1	82.5±2.5	40.9±1.5	81.5±2.7	*R*
2	Carrot (*D. carota*)	78.4±2.6	95.0±2.9	79.2±2.0	96.4±1.9	*S*
3	Cucumber (*C. sativus*)	50.5±1.5	75.2±3.1	55.5±1.8	75.8±3.2	*S*
4	Onion (*A. cepa*)	52.7±1.8	74.2±2.6	54.3±1.4	73.8±2.9	*S*
5	Potato (*S. tuberosum*)	28.0±2.3	93.7±2.8	51.4±2.2	92.1±3.4	*R*
6	Radish (*R. sativus*)	71.9±1.7	70.6±3.8	82.3±2.5	72.8±2.8	*S*
7	Sweet potato (*I. batatas*)	42.5±2.4	80.0±3.4	43.5±1.9	80.2±2.9	*R*

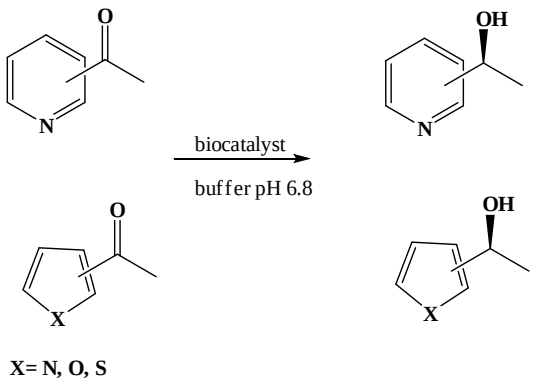
Results of 4'-chloroacetophenone reduction were more attractive in terms of the yield and *ee*, enantioselectivity and yield are ≥ 90 % and ≥ 50 % respectively to most plants and remarkably higher than that of the acetophenone reduction reaction. That indicates that halogen-containing aromatic ketone was more acceptable to plant cells than simple aromatic ketone.

Chang et al. (2010) investigated the reaction characteristics in detail by using various vegetables (apple, carrot, cucumber, onion, potato, radish, sweet potato) as biocatalysts and acetophenone as substrate. The authors determined the optimal reaction condition as follows: reaction time 50 h, substrate concentration 20 mmol/L, reaction temperature 35 °C and pH 7, and achieved 85 % yield and 95 % *ee* (to *S*-1-phenylethanol).

Liu et al. (2010) reported on the reduction of acetophenone analogues catalyzed by carrot and celeriac (*Apium graveolens* L.). The results showed that acetophenone was reduced to the corresponding chiral alcohol with excellent conversion (97-100 %) and enantioselectivity (96-99 %) with both biocatalysts, while the presence of a substituent at the aromatic ring might have reduced the conversion, especially the presence of *o*-substituent (18 %). In addition, this research also focused on the influence of reaction temperature on bioreduction of acetophenone analogues. It has been shown that the conversions at 33 °C were higher in comparision to other temperatures tested (RT, 27 °C, 30 °C and 36 °C) suggesting that these ketoreductase of whole carrot and celeriac cell systems possessed higher activity at 33 °C.

Utsukihara et al. (2006) investigated the stereoselective reduction of ketones by various vegetables. Reduction of (+) and (-)-camphorquinones by carrot, potato, sweet potato, apple, Japanese radish, cucumber, burdock and onion gave *α*-hydroxycamphor selectively. In particular, reduction of (+)-camphorquinone with burdock gave (-)-3-*S*-exo-hydroxycamphor with the highest stereoselectivity.

Chiral heteroaryl alcohols have numerous applications as important intermediates in the synthesis of biological active molecules. There are many reports describing synthesis using biocatalysts obtained from microbial/different parts of plant tissues, however, most of these processes have limitation in commercial application due to long incubation time, low substrate loading, poor isolated yields and enantioselectivity. Lakshmi et al. (2011) studied the chiral reduction of substituted heteroaryl prochiral ketones using *Daucus carota*, Scheme 2, Table 2. They demonstrated that dehydrogenases present in *Daucus carota* selectively reduced substituted aromatic heterocyclic methyl ketones to the corresponding single chiral secondary alcohols in good yields (60-95 %) and high enantioselectivity (76-99 %). The bioreduction of different heteroaryl methyl ketones (pyridyls and pyrrole) to corresponding chiral alcohols showed exclusively *S* configuration thus following common Prelog's rule, whereas furan and thiophene derivatives have shown *R* configuration according to anti Prelog's rule.



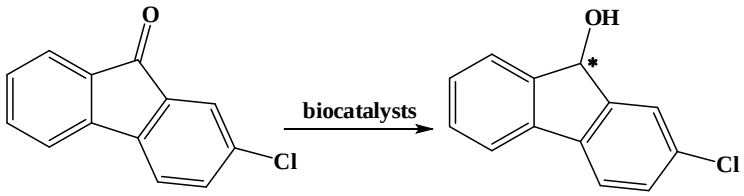
Scheme 2. Chiral reduction of substituted heteroaryl pro chiral ketones by *Daucus Carota* (Lakshmi et al., 2011)

Table 2. Asymmetric reduction of substituted heteroaryl ketones by *Daucus carota* (Lakshmi et al., 2011)

Product	Time / h	Conversation %	Isolated yield %	*ee* %	Conf.
2-(1-hydroxyethyl)pyridine	48	100	95	99	*S*
3-(1-hydroxyethyl)pyridine	56	90	94	98	*S*
4-(1-hydroxyethyl)pyridine	52	100	94	92	*S*
2-(1-hydroxyethyl)-6-chloro-pyridine	60	80	74	89	*S*
2-(1-hydroxyethyl)-6-bromo-pyridine	65	75	78	92	*S*
2-(1-hydroxyethyl)-4-methyl-pyridine	55	95	80	96	*S*
2-(1-hydroxypropyl)pyridine	65	95	63	90	*S*
2-(1-hydroxyethyl)pyrrole	72	65	65	90	*S*
2-(1-hydroxyethyl)furan	76	55	60	76	*R*
2-(1-hydroxyethyl)tiophene	70	50	55	7	*R*

Ferraz et al. (2008) carried out the bioreduction of a series of substituted α-tetralones using *Daucus carota* root which afforded the corresponding homochiral α-tetralols in variable conversions (9-90 %) and excellent enantiomeric excesses.

Xie et al. (2009) used different fresh fruits and vegetables such as apple (*Malus pumila*), banana (*Musa balbisiana*), orange (*Citrus reticulate*), potato (*Solanum tuberosum*), strawberry (*Fragaria ananassa*), scallion (*Allium fistulosum*), plum (*Prunus* spp.), garlic (*Allium sativum*), onion (*Allium cepa*), cherry (*Prunus pseudocerasus*), jujube (*Zizyphus jujube*), topinambur (*Helianthus tuberosus*) and grape (*Vitis* spp.) as biocatalysts for fluorenones enantioselective reductions. Among the tested plants, the grape exhibited the best results with *ee* 99 % and 97 % conversion. In addition, all other plant species were able to reduce 2-chloro-fluorenone except garlic which was inactive under the reaction conditions used. However, the *ee* values and conversion rates varied a lot, Scheme 3, Table 3. The absolute configuration of the reduced product was *R*, the biocatalytic reduction followed anti-Prelog's rule.



Scheme 3. Enantioselective reduction of 2-chlorofluorenone using biocatalysts (Xie et al., 2009)

Table 3. Enantioselective reduction of 2-chlorofluorenone using biocatalysts (Xie et al. 2009)

Species	Conversion / %	*ee*/ %
Apple (*Malus pumila* Mill.)	69	90
Banana (*Musa balbisiana* Colla)	30	46
Orange (*Citrus reticulata* Blanco.)	15	43
Potato (*Solanum tuberosum* L.)	7	50
Strawberry (*Fragaria ananassa* Duch.)	37	93
Scallion (*Allium fistulosum* L. var.)	13	72
Plum (*Prunus* spp.)	100	50
Garlic (*Allium saticum* L.)	-	-
Onion (*Allium cepa* L)	8	26
Cherry (*Prunus pseudocerasus* Lindl.)	4	87
Jujube (*Zizyphus jujube* Mill.)	88	28
Topinambur (*Jerusalem artichoko*)	100	76
Grape (*Vitis* spp.)	97	>99

Lacheretz et al. (2009) used *Daucus carota* to reduce cyclic aminoketones (piperidin-3-ones) in high yields and enantiomeric excess. When piperidin-3-one ammonium chloride and *N*-benzyl-piperidin-3-one were treated with carrot, the recovery of corresponding alcohol was very low (0-11 %) probably due to the high solubility of product in water. To avoid extraction problems they protected the amino group of the piperidin-3-one with electron-withdrawing groups (benzoyl, tosyl, trifluoracetyl, acetyl and *tert*-butoxycarbonyl). After protection piperidin-3-ones were transformed to the corresponding piperidin-3-ols in good yield (66-78 %) and good enantiomeric excess (75-95 %).

Javidnia et al. (2013) reported on asymmetric reduction of 8 prochiral ketoesters mediated by *Daucus carota* and *Brassica rapa* fresh plant roots. They investigated the role of endophytic microorganisms in bioreduction by conducting the bioreduction in two sterile and non sterile media. They observed that in non-sterile conditions *Brassica rapa* was a better biocatalyst due to high chemical and optical yield achieved. However, in sterile condition, *Daucus carota* exhibited better conversion yields than *Brassica rapa*. The reported data support the hypothesis that endophytic microorganisms are important being involved in the biotransformation. Therefore it seems that sterilization prior to the biotransformation with plants is important for exclusion of the effect of endophytic microorganisms as well as microbial contamination.

Aldabalde et al. (2007) reported on the reduction of methyl heteroaryl ketones using plant tissue as the only biocatalyst and reducing agent. The incubation of methyl heteroaryl ketones with fresh carrot fragments *Daucus carota* in water afforded, in most cases, the corresponding alcohols, in very good to excellent enantiomeric excess (93-98 %), the *S*-alcohols were obtained in all cases. They performed reactions under various conditions in order to optimize the procedure in terms of reaction time, yield and optimal mass of carrot. They tested the ability of other common plants such as: pumpkin (*Curcubita maxima*), marrow (*Cucubita maxima*), zucchini (*Cucurbita pepo*), fennel (*Foeniculum vulgare*), beet (*Beta vulgaris*), potato (*Solarium tuberosum*), eggplant (*Solarium melongena*), sweet potato (*Ipomoea batatas*), lemon (*Citrus X limon*) and onion (*Allium cepa*) to perform the reduction of 4-acetylpyridine using the reaction condition optimized for *Daucus carota*. Only pumkin and fennel measured up to carrot in case of *ee* (98 %). However the isolated yield of the reactions were lower, pumkin rendered the product 4-pyridylethanol in 27 % yield and fennel in 23 % yield. Eggplant, sweet potato, lemon and onion did not give the corresponding alcohol.

Fruchey (2011) explored the biocatalytic activity of a variety of pea seeds (*Cajanu scajan*, *Phaseolus vulgaris*, *Cicer arietinum*, *Pisum sativum*, *Vigna unguiculata*) in the reductions of an assortment of ketones, acetophenone, 4'-chloroacetophenone, 4'-methoxyacetophenone, 4-phenyl-2-butanone, 2-octanone, 1-indanone. All of the peas provided high *ee* values (70-99 %), but certain peas were more successful than others depending on the substrate being reduced. Furthermore, it was determined that the resulting stereochemistry is strictly connected with a pea type. It was also evident that the use of a phosphate buffer in the reactions increased the conversion. Without the use of the buffer, the reaction mixture became fairly acidic and it was found that the enzymes responsible for the reduction were not fully activated below pH of 5. Optimal pH determined for these reactions ranged from 5.5 to 7.5.

Majewska and Kozłowska (2013) used carrot, celeriac (*Apium graveolens* L. var. *rapaceum*) and beetroot (*Beta vulgaris* L. subsp. *Vulgaris*) for biotransformation of *trans*-4-phenylbut-3-en-2-one. (*S*)-*trans*-4-phenylbut-3-en-2-ol has been obtained in excellent yields and with high *ee* in isooctane as solvent. Whole cell biocatalysis in an organic solvent has some limitation due to the toxic effect of the solvent on the plant cells. Also, organic solvents change the membrane fluidity and mitigate in substrate uptake resulting in activity retention. The highest yield was achieved with carrot (96 %), and lower with celeriac (78 %) and beetroot (71 %) but only the carbonyl group was reduced. The results they obtained may offer new possibilities for the reduction of carbonyl compounds that are insoluble in water. Namely, most of the problems in connection with enzymes acting in an aqueous environment can be solved by changing an aqueous medium to an organic solvent because non-aqueous solvents can increase the substrate solubility, the kinetics of the reaction can be improved, higher yields achieved, as well as regio- and chemo-selectivity of enzymes controlled (Milner and Maguire, 2012).

Suárez-Franco et al. (2010) investigated reduction of aromatic aldehydes and ketones as substrates using the vegetable homogenates of broccoli (*Brassica oleracea* var. *italic*), cauliflower (*Brassica oleracea* var. *botrytis*), beet (*Beta vulgaris*var.*cicla*) and spinach (*Spinacia oleraceae*). By using *Brassica oleracea* var. *italic* and *Brasica oleracea* var. *botrytis* homogenates the maximum bioconversation yields were obtained within short reaction times. Ketone 1-

phenylethanone was reduced after 72 hours of reaction to (*S*)-1-phenylethanol with high enantioselectivity (> 90 % *ee*) by all the vegetables tested: broccoli (62 % yield), cauliflower (35 % yield), beet (12 % yield), spinach (8 % yield). Similarly to 1-phenyl-ethanone the best results in the benzoyl formic acid ethyl ester reduction were obtained by using *Brassica oleracea* var. *italic*, followed by *Brassica oleracea* var. *botrytis* homogenate, followed by results of reduction by *Beta vulgaris* and *Spinacia oleraceae.* The main product obtained was ethyl (*S*)-(+) mandelate.

Phukan and Devi (2012) performed the reduction of aldehydes and ketones using tomato fruit (*Lycopersicum esculentum*) soaked in deionised water and observed high enantioselectivity (92-99 %) with excellent chemical yields (70-95 %). Compared to the work by Yadav et al. (2009), the reaction time was reduced and was completed within 25-35 h.

The selectivity of *Daucus carota* root and baker's yeast in the enantioselective reduction of γ-nitroketones was investigated by Scarpi et al. (2005). The enantioselective reduction of ketones of a series of aromatic γ-nitroketones was achieved by *Daucus carota* roots in water, which afforded the corresponding (*S*)-alcohols with *ee* ranging from 73 % to 100 %. More interesting, methoxy-substituted compound was slowly converted in buffer to the corresponding alcohol with conversions never exceeding 12 %. Although a lower number of substrates were reduced, *Daucus carota* is always being more enantioselective than baker's yeast. The authors reported that the stereoselectivity of the reduction was influenced by the reaction conditions, and the reductions in plain water gave the best results.

Baldassarre et al. (2000) reported that the enzymatic reduction of (±)-2-methylcyclohexanone with fresh carrot root as biocatalyst occurred in a complete diastereoisomeric way giving a 1:1 mixture of enantiomerically pure (1*S*,2*R*)- and (1*S*,2*S*)-2-methylcyclohexanol. The corresponding reaction carried out on the racemic 2-hydroxycyclohexanone afforded a 1:2 mixture of (1*S*,2*R*)- and (1*S*,2*S*)-1,2-cyclohexanediol with an enantiomeric excess > 95 %.

Asymmetric reductions by germinated plants obtained from commercially available radish seed have been investigated by Matsuo et al. (2008). They chose radish sprout since the germination of radish is very easy. Germinated radish (*Raphanus sativus*) reduced aromatic ketones, α,α,α-trifluoroacetophenone and *o*-chloroacetophenone to corresponding (*S*)-alcohol obtained in 30-40% yield with 90-100 % *ee*. Additionally, *o*-chloroacetophenone was reduced by adding a sucrose at the cultivation time and afforded corresponding (*S*)-alcohol in > 99 % *ee*. It was noticed that the addition of sugar influenced the growth of the plant as well as bioconversion of the aromatic ketone. The yield of the reduction increased from 40 % (without addition of sucrose) to 100 % (with addition 2 g of sucrose). The addition of sucrose at the reaction time did not affect the chemical yield or the enantioselectivity of the reaction regardless of the pre-treatment of the biocatalyst. Orden et al. (2009) used *Raphanus sativus* L. hairy roots in the anti-Prelog stereoselective reduction of a series of prochiral alkyl-aryl-ketones. The observed enantioselectivity was not in accordance with Prelog's rule affording the *sec*-alcohol with the *R* configuration. These results were partially in contrast with those reported by Matsuo et al. (2008) since the authors observed the opposite enantioselectivity in the reduction of *o*-chloroacetophenone. The experiments were performed in three different reaction times (2, 4 and 7 days). At lower incubation times either yield or *ee* were moderate, however, when reaction was carried out for seven days, both conversion rate and optical purity of (*R*)-1-phenylethanol were excellent.

Villa et al. (1998) investigated the ability of several plant species to perform the asymmetric reduction of model substrates such as 2-pentanone, acetophenone and ethyl acetoacetate. The results showed that reduction yields in phosphate buffer with added glucose were higher than in the transformations performed in the Gamborg, Schenk and Hindebrandt media. *Daucus carota* did not reduce 2-pentanone and acetophenone in Gamborg medium, while yields up to 20 % were obtained in phosphate buffer with glucose. The reduction ability of *Daucus carota* for ethyl acetoacetate significantly increased in the presence of glucose and the *ee* was much higher under these conditions. Obviously, the presence of glucose is required for the regeneration of co-factors. *Daucus carota* and *Ribes rubrum* gave yields of 100 % after 1 day. *Cucumis melo*, *Nasturtium officinale*, *Olea europea*, *Polygonum persicaria* and *Vitis vinifera* also converted the ketone to alcohol with yields higher than 50 %. In addition, *Daucus carota* and *Ribe srubrum* showed the highest reducing capacities for these compounds, while other species gave low conversions. The *S* enantiomer was again most abundant with *ee* higher than 98 %. Only *Actinidia chinesis* produced the *R* enantiomer but in low yields.

Machado et al. (2006) examined bioreduction of a series of aliphatic and aromatic aldehydes and two ketones with plants used as food in the Brazilian northeast. *Manihot esculenta* and *Manihot dulcis* roots

reduced the substrate in excellent yields (80-96 %), and *ee* ranging from 94-98 %, with exception for vanillin. Machado et al. (2008) for the first time used fruits' barks of *Passiflora edulisin* for bioreduction of ketones and aldehydes (acetophenone, benzaldehyde, furfuralaldehyde and cinnamaldehyde derivatives). The reduced products were obtained in very good yields but low to moderate enantiomeric excesses were reached.

Andrade et al. (2006) evaluated bioreduction of acetophenone derivatives and also biooxidation of (*RS*)-1-phenylethanol using burdock roots (*Arctium lappa* L.), sweet white potato tubers (*Ipomoea batatas* L. Lam.), sweet red potato tubers (*I. batatas* L. Lam.), potato tubers (*Solanum tuberosum* L.), beet roots (*Beta vulgaris* L.),yam tubers (*Dioscorea alata* L.), chive roots (*Allium schoenoprasum* L.), coriander roots (*Coriandrum sativum* L.), ginger roots (*Zingiber officinale Roscoe*), taro tubers (*Colocasia esculenta* L. Schott), lotus roots (*Nelumbo nucifera Gaertn*.), manioc roots (*Manihot esculenta Crantz*), arracacharoots (*Arracacia xanthorrhiza Bancroft*), turnip roots (*Brassica rapa* L.), radish roots (*Raphanus sativus* L.) andyacon roots (*Polymnia sonchifolia*) as biocatalysts. They observed high enantioslectivities (> 90 % *ee*) and moderate values of acetophenone derivatives reduction (44-57 %) when *Arracacia xanthorrhiza*, *Coriandrum sativum* and *Dioscoea alata* were used for bioreduction. High optical purity was obtained with *Allium schoenoprasum*, *Raphanus sativus* and *Zingiber officinale*, but the conversion of the reaction was low.

Fonseca et al. (2009) used coconut juice (*Cocos nucifera*) to reduce a series of aliphatic and aromatic aldehydes and ketones. The juice of the coconut species was very effective, selectively reducing a range of aromatic and aliphatic compounds with carbonyl groups showing substantial regio- and enantioselectivity towards the substrates. 1-Phenylethanol was formed from acetophenone with conversation of 79 % as the unique enantiomer with the *S* configuration, in accordance with Prelog's rule, and with the *ee* from 95 %.

Conclusions

Asymmetric reduction of prochiral ketones is an essential transformation in organic synthesis, it affords optically active secondary alcohols that serve as useful intermediates for the synthesis of biologically active compounds such as drugs, perfumes and agrochemicals. In the context of developing green and sustainable chemical processes as part of the transition towards a more biobased economy, biotechnologies are attractive alternatives. Biochemical reduction performed in aqueous media have traditionally involved isolated alcohol dehydrogenase coupled with a reduced nicotinamid cofactor. A limitation of these bioreductions is the necessity to recycle the oxidized cofactor during the reaction. Recently the use of whole plants cell obtained directly from cut portions of plants has emerged. The use of whole plant cells has many advantages. First of all, a large array of taxonomically different plants is available from local markets. The whole cells also ensure the recycling of oxidized cofactors. The stereochemical outcome of the bioreduction can be predicted based on a model proposed by Prelog. Different plants have been studied for their alcohol dehydrogenase activity, for example carrot (*Daucus carota*), cucumber (*Cucumis sativus*), onion (*Allium cepa*), potato (*Solanum tuberosum*), radish (*Raphanus sativus*) and sweet potato (*Ipomoea batatas*).

Finally, the number of biocatalysts for potencial industrial applications is increasing rapidly associated to the demand of green chemistry and it will be the main goal for many companies in order to increase process efficiency.

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